Cross-Sectional Analysis of Frailty Status and Heart Rate Variability at Rest and During Exercise in Older Adult Females

by

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Abstract

Frailty is related to an imbalance of homeostasis within the body, accompanied by an accumulation of health deficits. A possible way to measure this imbalance, is with a non-invasive measure of the autonomic nervous system through heart rate variability (HRV). This study utilized a secondary, cross-sectional analysis of data collected in the Women's Advanced Risk-Assessment in Manitoba (WARM) Hearts study. The purpose was to determine if there is a difference in HRV depending on frailty level measured by the WARM Hearts HRV Frailty Index (WH-HRV FI) and the Standardized Frailty Phenotype measured at rest and during moderate exercise; and to determine if there is an association between HRV and frailty when measuring frailty on a continuous scale both at rest and during moderate exercise. Data analysis included 125 females in the resting cohort, and 102 in the exercise cohort. Frailty was measured using two separate methods (WH-HRV FI and the Standardized Frailty Phenotype). HRV was measured using a Polar H7 heart rate monitor with a 5-minute sample at rest and a 2-minute sample during exercise. This analysis included various HRV indices including mean RR intervals (Mean RR), standard deviation of normal-normal intervals (SDNN), root mean square of successive intervals (RMSSD), low frequency (LF), high frequency (HF), total power (TP), low frequency to high frequency ratio (LF/HF), and approximate entropy (ApEn). Resting analysis revealed a trend towards a reduction in $SDNN_{log}$, TP_{log}, and LF/HF_{log} and a significant positive association in LF_{log} in those who were frail with no changes of $RMSSD_{log}$ or HF_{log} indicating an impaired sympathetic nervous system (SNS). Exercise analysis revealed a significant difference and positive association between frailty and mean RR_{log}, and an inverse relationship between frailty and TP_{log}. This indicates a reduced ability to adapt to changes and a reduced global HRV for both frailty measures. There were inconsistent results regarding the direction of the association between LF_{log} and $SDNN_{log}$ between frailty tools. Overall, there may be an impairment in SNS activity at rest and a reduced ability

to adapt, along with decreased global HRV (TP_{log}) during exercise in those who have increased levels of frailty.

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Chapter 1: Literature Review

This literature review will cover the definition of frailty along with a description of the autonomic nervous system (ANS). Methods of identifying individuals who are frail will be discussed along with methods to measure the activity of the ANS. It will then discuss the association between the ANS and frailty. Lastly, this literature review will discuss the knowledge gap based on the various frailty measures and how the measurement of the ANS may help identify at risk or vulnerable individuals.

1.1 Defining Frailty

With an aging population there is an increasing number of individuals who are living longer with a diverse number of health conditions. A term often associated with the aging population is frailty. While there is currently no operational definition for frailty, there is a general consensus that frailty is a condition that leads to the decreased function of multiple physiological systems, along with a decreased ability to respond to the outside stresses of life.[1,2] This may involve a decline in the complexity of physiological systems and can lead to the loss of homeostatic capability resulting in further vulnerabilities to adverse outcomes.[3] These adverse health outcomes can include myocardial infarction, heart disease and mortality.[4] Frailty is a state that can vary among people of the same age and was introduced as a way to identify those who have a particularly fragile health balance and are at risk of rapid health deterioration.[5] Further, the physiological changes that accompany frailty may not achieve disease status, which means that some individuals can be frail without having a life-threatening disease.[6]

1.2 Assessing Frailty

There is currently no universally accepted, "gold standard" definition of frailty. A comparative analysis done by Aguayo et al.[5] identified 67 different frailty scores in the literature. These frailty scores can fall into one of four categories, including a phenotype of frailty, multidimensional frailty, accumulation of deficits and a disability model of frailty.[5] Two of the most common methods used throughout the literature are Fried's frailty phenotype[3] and Rockwood's frailty index[2] (FI; which is also known as the accumulation of deficits model).[7]

1.2.1 Fried Phenotype Model

Historically, one of the main obstacles in providing care for individuals who were at high-risk for adverse health outcomes was the absence of a standardized measure to identify frailty in these individuals.[3] Linda Fried proposed a definition of frailty based on clinical outcomes that were common among frail individuals. This frailty score identifies frailty as the presence of three or more of five specific criteria. These criteria include exhaustion, weight loss, diminished activity, slow walking speed and weak grip strength.[3] With the five criteria, a score of 1 is given if the variable is present, a score of 0 is given if it is absent in an individual. A total score of 0 represents robust individuals, 1-2 is pre-frailty and a total score of ≥3 indicates the presence of frailty.[3] A variety of ways to measure each of these criteria have been used throughout the literature with some of the most popular including self-reported exhaustion levels, self-reported weight loss, the International Physical Activity Questionnaire for activity levels, 5 m gait test for walking speed and a dynamometer for grip strength.[7–9] A major criticism of the phenotype model is that it adopted a unidimensional approach for a condition that is a complex and multi-dimensional concept.[10]

1.2.2 Frailty Index

A FI accounts for the accumulation of health deficits in an individual. The more health deficits a person has, the more likely they are to be frail. The FI approach provides a continuous value indication where an individual can then be classified on a scale of robust or not-frail to most frail.[11] While multiple versions of this model have been created and studied, the association with mortality remains robust when variables are included that cover a broad range of systems as this will account for the multifactorial and dynamic condition that is frailty.[1] Searle et al.[2] identified five criteria that a deficit must satisfy in order to be included in an FI. The criteria include: 1) a deficit must be related to health status, 2) the prevalence must increase with age, 3) but must not saturate too early, 4) the deficits must cover a wide range of health systems and 5) the same items must be used when testing individuals more than once.[2] A FI will normally contain approximately 30-40 deficits; however, the more deficits that are included the more precise the frailty estimate will become.[2] The number of health deficits present in an individual will be divided by the total number of deficits considered within the FI. A cut point of 0.25 has been validated to identify the presence of frailty in older adults living within the community.[2]

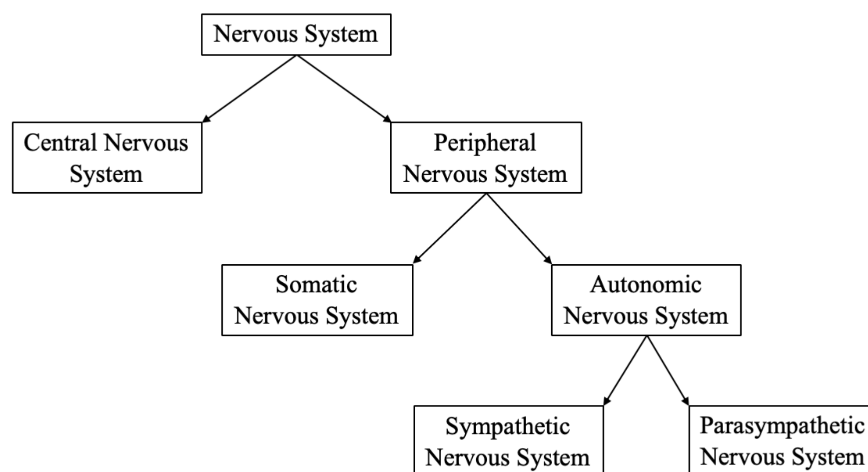
Frailty is a complex concept in which we have a limited understanding of the underlying physiological mechanisms, including how the considered deficits scale up to be clinically visible.[12] The FI has a stronger association to self-reported health and health care utilization compared to the phenotype model. The FI can also be better at discriminating in the lower to middle end of the frailty continuum compared to the phenotype model.[7] For example, a study done by Blodgett et al.[7], found that all participants who were identified as frail with the phenotype model were also frail with the FI; however, some participants that were identified as robust with the phenotype were found to be vulnerable (30.8%) or frail (13.4%) with the FI.[7] This ability to better discriminate at the lower levels of frailty has suggested that a FI may be a more sensitive measure of frailty compared to the phenotype

model of frailty.[7] This may be due to the vast number of deficits that are considered, along with the continuous scale that is used rather than the specific categories that are used with the phenotype model.

1.3 Divisions of the Nervous System

It has been suggested that measuring the activity of the nervous system may give valuable information regarding the resilience or lack of ability to maintain homeostasis to adverse events such as increased risk of frailty.[13] This is because the human nervous system plays a major role in cell signalling and maintaining homeostasis. There are two main branches of the human nervous system, as seen in Figure 1, including the central nervous system that supports the brain and spinal cord and the peripheral nervous system, which contains the nerves that communicate between the central nervous system and the rest of the body. The peripheral nervous system then breaks down into two branches which includes the somatic nervous system and the autonomic nervous system (ANS). The somatic nervous system plays a role in our senses where the ANS serves as the visceral or involuntary nervous system.[14] The ANS acts as an internal signalling system for the bodies unconscious actions and plays a key role in regulating changes in the cardiovascular system.[15] For example, it will regulate heart rate and blood pressure (BP) in the short term to cope with everyday situations.[15]

Figure 1. *Divisions of the Nervous System*



1.3.1 Autonomic Nervous System

The ANS innervates many different tissues and organ systems in the body making it a key player in maintaining homeostasis.[14] The ANS also breaks into two separate branches which are the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). Both of the SNS and PNS are tonically active and can increase or decrease the frequency of their discharge of neurons to the bodies tissues at all times.[14] These two systems are thought to be antagonist to each other where one branch will have opposing effects on a tissue compared to the other. For example, the SNS will initiate the fight and flight response, while the PNS is responsible for restorative effects on heart rate, blood pressure and contractility.[16]

As mentioned, the SNS serves as the bodies fight or flight response system. It is responsible for preparing the body for action. In relation to the cardiovascular system, the SNS will control the increase in rate and force of contraction of the heart itself along with dilate vessels within skeletal muscle. This system has alpha- and beta-adrenergic receptors and will predominate during “fight or flight” and exercise.[14,16]

The PNS deals with the rest and digest functions of the body and has opposing effects of the SNS. This means that the PNS deals with the maintenance of restorative and energy saving functions including decreasing or slowing heart rate and blood pressure.[17] A main difference between the PNS and SNS is that the effects of the PNS tend to be more discrete and targeted with only a specific tissue being stimulated, where the SNS tends to have a more diffuse, overall dischange.[14] The PNS has muscarinic receptors and will predominate when the body is in resting conditions.[14]

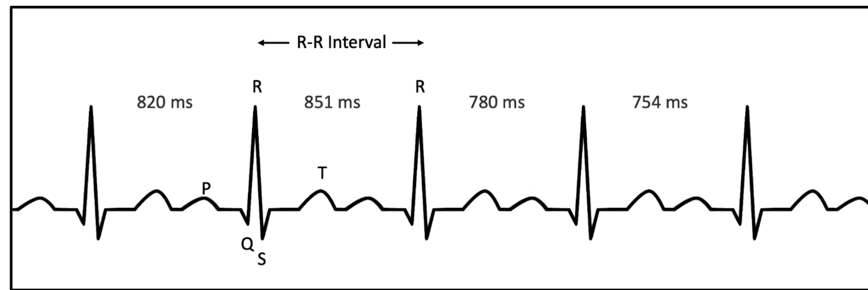
The SNS and PNS systems play an important role in our health and respond or make adjustments to our body as stressors occur. In a healthy individual, the SNS and PNS will respond dynamically to the body’s needs; however, when homeostasis is challenged one of the SNS or PNS remains more active than the other, which can result in an increased risk of morbidity and all-cause mortality.[18] For example, if the sympathetic branch dominates for a long period of time, energy

demands become excessive and cannot be met, this can eventually result in death.[18] For some individuals, an impairment in the ANS could lead to the loss of consciousness (syncope) which is due to a drop in blood pressure caused by sympathetic withdrawal, and a reduced heart rate due to increased parasympathetic activity.[19] In an aging population the consequences of losing consciousness can be extreme when frailty is present as this could lead to the accumulation of further deficits including broken bones. Further, those in the aging population are at an increased risk of hypertension, and when this is due to increased sympathetic activity this could result in more serious problems such as a haemorrhage if unmanaged.[19] When there are continuous impairments that include adrenergic activation and cardiac dysfunction this puts an individual at an increased risk for congestive heart failure as there are often functional reorganizations of the ANS that result in alterations of neural processing.[20] These disruptions within the ANS hinders the body's ability to maintain homeostasis and may be a link that accelerates the presence of frailty and increases the risk of adverse events.

1.4 Heart Rate Variability

A non-invasive measure of ANS activity is heart rate variability (HRV) which is the variation of time between consecutive heart beats or the variation in time between R-R intervals of the QRS complex (Figure 2). HRV provides information on how the body fluctuates between the sympathetic and parasympathetic nervous systems.[21] In a healthy individual, there will be higher variation in HRV as the ANS is continually adjusting to its physiological needs. In individuals with a reduced variability (R-R intervals are equal), the balance between the PNS and SNS may be failing which would result in a decreased ability to maintain homeostasis.[22] This reduction in HRV is associated with many adverse conditions such as cardiac abnormalities, diabetic neuropathies, myocardial infarction, and insulin resistance among many other factors related to a risk of cardiovascular disease.[23]

Figure 2. ***R-R Interval***



Most often, HRV is measured with the use of a Holter monitor to measure ECG over an extended period of time. The ECG will show a wave form of the electrical activity in the heart.[24] This allows for the time between each consecutive heart beat to be calculated. However, technological developments have created less expensive and more feasible assessment devices for HRV, including the use of a heart rate monitor worn on a chest strap to collect a single-lead ECG with the application of an algorithm.[25] A single lead ECG has become the typical way to determine HRV.[26] A Polar H7 heart rate monitor has an almost perfect correlation to a 12-lead ECG (Cosmed Quark T12x) when measuring HRV ($r = 0.99$)[27]. Two other studies, including Weippert et al.[28] and Porto et al.[29], have also been able to reproduce HRV data using conventional ECG methods and a heart rate monitor.

The analysis of HRV presents a range of indices that can be used to represent different aspects of cardiac activity and the ANS (see Table 1). Time and frequency domain indices are the traditional methods and most commonly used indices. For the time domain, the most common are the root mean square of successive RR intervals (RMSSD) and the standard deviation of normal beat-to-beat intervals (SDNN). For the frequency domain measures, there are five indices including very low frequency (VLF), low frequency (LF), high frequency (HF), total power (TP) and low frequency to high frequency ratio (LF/HF). Lastly, there are non-linear measures of which approximate entropy for a heart rate time series (ApEn) is often used.

Table 1. ***Heart Rate Variability Indices***

HRV Indices	Definition
Time Domain Indices	
SDNN (Standard deviation of normal beat-beat intervals)	Global index encompassing both sympathetic and parasympathetic nervous systems[30]
RMSSD (Root mean square of successive RR intervals)	Represents parasympathetic nervous system activity[30]
Frequency Domain Indices	
VLF (Very Low Frequency) (<0.04 Hz)	Reflects parasympathetic modulation of heart rate but is also influences by the renin-angiotensin system[31]
LF (Low Frequency) (0.04-0.15 Hz)	Reflects both sympathetic and parasympathetic modulation[31]
HF (High Frequency) (0.15-0.4 Hz)	Primarily reflects heart rate fluctuations due to parasympathetically mediated respiratory sinus arrhythmia, although it can also be influenced by erratic rhythms from non-respiratory sinus arrhythmia (Has been linked to vagal activity)[30]
Total Power	Overall heart rate variance across all frequency bands[31]
LF/HF Ratio	Been proposed as a marker of sympathetic-parasympathetic balance or sympathovagal balance[9,31]
Non-Linear Indices	
ApEn (Approximate entropy for a heart rate time series)	A non-linear statistic that quantifies the regularity of heart rate fluctuations over time[31]

1.5 Heart Rate Variability in People who are Frail

A systematic review by Parvaneh et al.[4] synthesized published evidence that the ANS is impaired in frail individuals when compared to those who are robust in six scientific articles that looked at frailty and cardiac autonomic control.[4] The ANS impairment was indicated by a reduction in HRV in those who had a higher level of frailty.[4] All six of the articles included in the review used the phenotype model to characterize the frailty level of participants and used a variety of methods to determine the influence of frailty status on cardiac autonomic function.

Two of the six articles included in the review used other measures of cardiac function instead of HRV. The article by Weiss et al.[32] used measures of heart rate reserve, rate-pressure product, heart rate recovery and chronotropic index. The results were inconclusive on whether or not frailty status may modify physiological functions that influence exercise capacity.[32] Second, the article by Romero-Ortuno et al.[33] used measures of orthostatic systolic blood pressure, diastolic blood pressure and heart rate to determine whether frailty influenced these cardiac functions. It reported that heart rate appeared to be impaired in those who were frail, for example they found that frailty was an independent predictor of baseline heart rate.[33] Because these two studies did not include HRV as a measurement, which is a key component of this dissertation, they have not been included in the table summarizing our current knowledge of HRV and frailty that I have created (Table 2).

Out of the four articles that measured both HRV and frailty status, there was a variety of HRV indices that were used. Chaves et al.[31] used approximate entropy for a heart rate time series (ApEn) which is a non-linear statistic of HRV. It was found that lower ApEn was associated with a higher likelihood of being frail (OR = 1.99, 95% confidence interval = 1.1-3.7;P=0.03) along with traditional time and frequency domain indices.[31] The second article by Varadhan et al.[34] strictly used traditional time and frequency domain indices with a decrease in SDNN, VLF, LF and LF/HF all being associated with an increased risk of frailty.[34] Takahashi et al.[8] found that those who were frail or pre-frail had higher levels of ApEn when compared to those who were robust. Lastly, Katayama et al.[9] found that when using frequency domain indices there was an impairment in the autonomic nervous system in those who were frail seen through sympathetic predominance (found a decreased HF and increased LF and LF/HF).[9]

Overall, there was a range of findings from the systematic review by Parvaneh et al.[4] While some of the articles reported traditional time and frequency domain indices, others used non-traditional non-linear methods including ApEn. All four of the articles that used HRV and frailty status found significant differences within frailty levels; however, there was some inconsistencies regarding how

some HRV variables were impacted in the different studies. For example, Katayama et al.[9] observed an increase in LF and LF/HF and a decrease in HF in the frail participants, which was different than what Chaves et al.[34] found as they had a decrease in LF and LF/HF but no differences in HF. Further, there was an increased ApEn in the frail group in the Takahashi et al.[8] study, but a decreased ApEn in the Chaves et al.[34] study. A difference between the three studies that may account for these differences is the length of HRV measurement. Both Takahashi et al.[8] and Katayama et al.[9] used short-term recordings (5 minutes), whereas Chaves et al.[34] used a measurement lasting 2-3 hours which can have an influence on the complexity of HRV that is seen.[4,22]

Table 2. *Articles on frailty and HRV identified in the Parvaneh et al. systematic review*						
Article	**Objective:**	**Population**	**Frailty Assessment**	**HRV Indices**	**Statistics Used**	**Results**
Chaves, et al. 2008[34]	To assess whether less physiological complexity underlying regulation of heart rate dynamics is associated with frailty.	Women's Health and Aging Study 389 community dwelling women (with moderate-severe disability) 130 were classified as frail	Fried (three or more of the five criteria): 1. Shrinking (lost >10% weight since age 60) 2. Slowness (4m walk speed) 3. Weakness (grip strength) 4. Exhaustion (self-report using a visual analog scale) 5. Low energy expenditure (self-report physical activity in past 2 weeks)	Non-Linear Indices: ApEn Time Domain: SDNN, SDANN, RMSSD Frequency Domain: VLF, LF, HF, Total Power, LF/HF *HRV measured using an ECG (2-3 hours)	Multiple logistic regression models Frequency bars and chi-square statistics	Median ApEn was lower in frail than in robust subjects ($P = .02$) Lower ApEn was associated with significantly higher likelihood of being frail in models that adjusted for age Lower time/frequency indices were associated with higher probability of frailty ($P < .05$) with the exception of HF and RMSSD
Varadhan, et al. 2009[34]	(1) Demonstrate that cardiac autonomic function (HRV), is impaired in frail older adults (2) Develop a novel, integrative index of HRV that aggregates the traditional time and frequency domain measures	Women's Health and Aging Study 276 community dwelling women (with moderate-severe disability)	Fried (three or more of the five criteria): 1. Shrinking (lost >10% weight since age 60) 2. Slowness (4m walk speed) 3. Weakness (grip strength) 4. Exhaustion (self-report using a visual analog scale) 5. Low energy expenditure (self-report physical activity in past 2 weeks)	Time Domain: SDNN, RMSSD, pNN50 Frequency Domain: VLF, LF, HF, LF/HF Ratio *HRV measured using an ECG (2-3 hours)	Principal Component Analysis Regression Analysis Akaike information criterion (AIC) Area under the receiver operating characteristic (AU-ROC) Partial predictive score (PPS)	Decreases in SDNN, VLF, LF, and LF/HF were associated with an increased risk of frailty Increased LF/HF ratio was associated with decreased risk of frailty Among individual indices, VLF was the strongest predictor of frailty

Takahashi, et al. 2014[8]	To evaluate the short-term complexity of HR dynamics in resting supine and standing positions and its associated with frailty	80 participants between the ages of 60-94 years of age (56 women) 38 robust, 36 pre-frail, 6 frail	Fried (three or more of the five criteria): 1. Non-intentional weight loss 2. Self-reported exhaustion 3. Hand grip strength 4. Gait slowness 5. Low levels of physical activity (Minnesota Leisure Time Activity Questionnaire)	Non-Linear Indices: ApEn CE_{HR} * HRV measured using a 12-lead ECG	Chi Square test One-way ANOVA Kruskal Wallis	Frail and pre-frail individuals have a higher ApEn than those who are robust ($P < .05$)
Katayama, et al. 2015[9]	To study the association of cardiac autonomic modulation with frailty status	23 community dwelling elderly women 8 robust, 8 pre-frail, 7 frail	Fried (three or more of the five criteria): 1. Shrinking (4.5 kg or 5% weight loss in the last year) 2. Weakness (grip strength) 3. Poor endurance performance and energy (Center for Epidemiological Studies – Depression Scale) 4. Slowness (4.6m gait speed test) 5. Physical activity levels (Minnesota Leisure Time Activity Questionnaire)	Time Domain: SDNN, RMSSD Frequency Domain: pLF, pHF, LF/HF Non-Linear Domain: SE_{HR} *Measured using a Polar heart rate monitor	One-way ANOVA Kruskal Wallis Chi Square test	No statistical differences in time domain indices Decreased power of HF in frail compared to not frail ($P < .05$) Increased power of LF and LF/HF ratio in frail compared to not-frail ($P < .05$)

1.6 Heart Rate Variability and Age

Although frailty can impact people of any age, the proportion of adults who are frail increases with age.[35] With age playing a large role in frailty, it is important to know whether or not HRV is also influenced by aging. In a study done by Voss et al., it was reported that there was significant variation in HRV by age in almost all HRV domains, including a lower HRV complexity in people of ages 50-74 years when compared to those aged 25-49 years.[36] However, when the participants were further separated into additional age groupings, differences in HRV disappeared within the oldest two groups (aged 55-64 and 65-74).[36] A second article by Zhang[37] determined that older age groups (aged 50-80) had consistently lower HRV when compared to younger groups (aged 10-20) including a decrease in SDNN, LF and HF ($P < .05$). Age will need to be considered when doing data analysis of HRV and frailty status.

1.7 Effects of Exercise on HRV

The ANS plays a role in how our bodies adjust to the demands of physical activity. For example, when we initiate a single bout of physical activity there is a rapid increase in heart rate, which is mediated by a reduction of the PNS and an activation of the SNS.[38] In this scenario, the PNS operates as a "rapid responder" as exercise intensity increases there will be a withdrawal of the PNS; whereas, the SNS works as a "tone setter."[38]

In healthy individuals, when HRV is measured at rest both frequency and time domain measures are complex, which is representative of a healthy cardiovascular response to autonomic control.[39] HRV frequency and time domain measures are then greatly reduced and less-complex during exercise in healthy populations.[40,41]

HRV parameters in populations with disease states differ from those of healthy individuals. For example, Dewey et al.[39] reported that an increase in the complexity of RMSSD during exercise was a

marker for increased risk of all-cause and cardiovascular related mortality (hazard ratio of 1.4, 95% CI 1.2-1.7, $P < .001$). Further, it was found in a study by Bartels et al.[42] that there was an increase in both the HF and LF frequency measures during exercise in individuals who were diagnosed with COPD. This was shown by an increase in the HF measure of 9.9 ms^2 at rest to 10.7 ms^2 during exercise ($P < 0.01$), and an increase in the LF measure of 10.9 ms^2 to 11.5 ms^2 during exercise ($P < 0.01$).[42] The physiological reason behind this change from a decreased complexity at rest to an increased complexity during exercise is unknown and that direct interpretations of the measured indices are complicated.[39] With this information, we can see that it may be possible to detect early signs of an impaired autonomic nervous system with the use of an exercise test and HRV measurement.

Chapter 2: Statement of the Problem and Methods

2.1 Statement of the Problem

With an aging population, there is a need to better predict and identify frailty in the population. A limited number of studies have examined HRV in the context of frailty; however, each of those studies used the Fried frailty phenotype to identify the presence of frailty. A FI presents a more sensitive assessment tool that is able to detect many more increments of frailty on a continuous scale.[43] For example, in a study done by Blodgett et al.[7], it was identified that a FI better identified frailty at earlier stages of the frailty continuum when compared to the frailty phenotype. With a limited body of evidence regarding HRV across frailty status and assessment measures,[4] there is a novel opportunity to assess the association of frailty (as determined by a FI) and cardiac autonomic nervous system function assessed through a non-invasive HRV measurement.

There is no research examining HRV and its response to physical activity in individuals across the frailty continuum. Participating in low-moderate physical activity in those who appear to be robust may present as an opportunity to test HRV and further identify individuals at-risk of advancing along the frailty continuum. If a less complex HRV at rest or an increased HRV complexity during physical activity is a predictor for frailty, this non-invasive method may become a useful tool for identifying frailty risk, improving assessment and may serve as a tool for guiding prehab/rehab interventions.[4] HRV may also be useful for screening and monitoring clinical vulnerability.[31]

2.2 Hypotheses

I will test the following hypotheses:

1) HRV complexity will *differ* between frailty status identified by:
    a) FI
        i) In the resting cohort (hypothesis 1a)
        ii) In the exercise cohort (hypothesis 1c)

b) Standardized frailty phenotype

i) In the resting cohort (hypothesis 1b)

ii) In the exercise cohort (hypothesis 1d)

2) HRV complexity will be *associated* with frailty identified by:

a) FI

i) In the resting cohort (hypothesis 2a)

ii) In the exercise cohort (hypothesis 2c)

b) Standardized frailty phenotype

i) In the resting cohort (hypothesis 2b)

ii) In the exercise cohort (hypothesis 2d)

2.3 Methods

2.3.1 Study Design

This research study utilized a secondary, cross-sectional analysis of baseline data to examine the relationship between frailty status and HRV measured at rest and during a 6-minute walk test. The data was collected as part of the WARM Hearts research study (Women's Advanced Risk-Assessment in Manitoba), which is a prospective, observational study that is recruiting 1000 women to test a novel prognostic tool for identifying women at risk of cardiovascular disease (ClinicalTrials.gov Identifier: NCT03938155). The study protocol was reviewed by both the University of Manitoba Health Research Ethics Board (H2019:063) and the St. Boniface Hospital Research Review Committee (RRC/2019/1833). A detailed protocol paper describing the WARM Hearts cohort is being prepared for publication. The following sections will briefly describe the methodological approaches that are specific to my thesis research.

This thesis will follow Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines designed for cohort, case-control and cross-sectional studies[44] and the Sex and Gender Equity in Research (SAGER) reporting guidelines.[45] A checklist for each of these guidelines can be found in Appendix A and Appendix B respectively.

2.3.2 Participants

Recruitment for the WARM Hearts study began on October 10, 2019. To date, 480 individuals have been recruited to participate in this trial as of March 23, 2020. However, only 240 of these individuals have fully completed their data collection. This date is relevant due to the COVID-19 public health pandemic, as this is when the University of Manitoba and St. Boniface Hospital suspended research activities with human participants. Recruitment and data collection will continue when health officials in Manitoba along with those from both the University of Manitoba and the St. Boniface Hospital deem it safe to do so. For this thesis, a total of 125 females will be used for analysis. A total of 115 participants did not have complete HRV data so they have been excluded from use within this thesis. This was due to technological complications when measuring HRV resulting in data being dropped. An example of this is provided in Appendix E.

A convenience sampling method was implemented for the WARM Hearts trial using print, online and media advertisement methods. Individuals who were interested in participating contacted the WARM Hearts Study by email or telephone. Screening was done during this contact to determine eligibility. The inclusion criteria were:

1. Women aged 55 years and older; and,
2. Resident of Manitoba and must have a Manitoba Personal Health Information Number (PHIN).

Participants were excluded if:

1. They have a previous hospitalization or diagnosis of ischemic heart disease, acute myocardial infarction, stroke, percutaneous coronary intervention, coronary artery bypass surgery, congestive heart failure, or peripheral artery disease; and,
2. They were a previous participant from "The Assessment of Large and Small Artery Elasticity for the Early Detection of Cardiovascular Disease (NCT02863211)" research study.

If eligible, participants were invited to come to the Applied Research Centre at the University of Manitoba. At the initial appointment participants were informed about the study and provided a copy of the informed consent. Interested individuals were given enough time to consider their participation and were given time to ask questions where they were provided with adequate answers. Written informed consent was obtained before data collection occurred.

Women are often underrepresented in cardiovascular clinical trials which is a major reason we decided to recruit only women for the WARM Hearts research study. This underrepresentation is concerning as women often have a different clinical presentation and prognosis compared to men.[46] Additionally, traditional risk assessment tools, such as the Framingham Risk Score, underestimate the risk of experiencing a cardiovascular event in women.[47]

2.3.3 Data Collection

Data collection took place over the course of two days that were approximately 8-14 days apart. Day 1 of data collection took place at the Applied Research Centre at the University of Manitoba, whereas, day 2 of data collection occurred at the Albrechtsen St. Boniface General Hospital Research Centre. The participant was also sent home with a questionnaire pack between the two appointments.

Prior to the day 1 appointment participants were given fasting instructions including no food, smoking or caffeine for 2 hours and no alcohol or strenuous exercise within 6 hours of the appointment which was due to the body composition, fitness testing assessments. Prior to the day 2 appointment participants were also given fasting instructions including no food, alcohol or caffeine for 8 hours prior to the appointment due to the blood draw and fitness testing assessment. These instructions also minimized the influence that caffeine, nicotine or alcohol consumption and amount of exercise may have on study outcomes. Demographic information was collected both at the day 1 appointment and with the take-home questionnaire package. Variables for the FI were collected through both days and through the questionnaires; the phenotype and HRV data were collected during the day 1 appointment.

Demographics

Various demographic information was collected to characterize the study population. Height (cm) and weight (kg) were directly measured using a SECA brand stadiometer and an InBody 270 device, respectively. These values were then used to calculate body mass index (BMI) (kg/m^2). Questionnaires and self-report methods were used to collect age, living arrangement, level of education, tobacco use, and high blood pressure or diabetes diagnosis. Specific information regarding how these variables were collected can be found in Appendix C.

Frailty Index

A FI classifies frailty by counting a number of deficits, with the more deficits an individual has the frailer or more vulnerable they will be. A FI was constructed for the WARM Hearts research study using variables that were collected within this research study (WH-FI). This FI used 42 deficits from various systems including physical, weight, mood and depression, cognition, nutrition, exhaustion, quality of life, comorbidities and lab values. A detailed description of the FI created for the WARM Hearts research study is listed in Appendix D. This FI was created following a standard protocol

published by Searle et al.[2] This approach ensures that each deficit follows certain criteria including: 1) relation to health status, 2) associated with age, 3) must not saturate too early, 4) must cover a range of systems and 5) the same deficits must be used if measuring an individual more than once (Table 3).[2] Another criteria identified within the literature is that a variable must not be too common (present in over 80% of the sample) and also must not be too uncommon (have at least 1% prevalence) to be included in the index.[7] Each variable was coded as either dichotomous or continuous depending on the deficit and scoring options available. An overall FI score was then given, ranging from robust (0) to total frailty (1).[7] Participants were classified as robust ($\leq$ 0.1), pre-frail (>0.1 to 0.25) or frail (>0.25)[2]. For the purpose of this thesis, the WH-FI had to be modified for the smaller sample size of 125 as three deficits had <1% prevalence and needed to be removed. These variables included the Paffenbarger physical activity questionnaire, HDL cholesterol levels, and gait speed. This thesis-specific FI with the three deficits removed is referred to as the WH-HRV FI throughout this document.

Table 3. ***Criteria used for selecting deficits to include in the Frailty Index***[2,7]

1	Deficit must be associated with health status
2	Deficit prevalence must increase with age
3	The deficit must not saturate too early
4	The deficits must cover a wide range of body systems
5	If an index is to be used more than once on the same person, the same deficits must be used
6	Deficit must not be too common (>80% of the sample)
7	Deficit must not be too uncommon (<1% of the sample)

Frailty Phenotype

Frailty status was measured using both the frailty index and phenotype assessment methods. The phenotype method was assessed using the methods outlined by Fried et al.[3] This includes assessing the following five criteria: 1) Exhaustion; 2) Weight loss; 3) Diminished activity; 4) Slow walking speed; and 5) Weak grip strength. Participants that present with three or more criteria were identified as

frail, 1-2 criteria are pre-frail and those with no criteria present are robust.[3] Exhaustion was measured using the two-item Center for Epidemiological Studies Depression Scale (CES-D). Weight loss was assessed by self-report using the question "Have you unintentionally lost weight (i.e. not due to dieting or exercise)?" The Paffenbarger Physical Activity Scale (PPAS) was used to assess diminished activity, and a 5-meter gait speed walking test was used to assess slow walking speed.[3] Lastly a Jamar hand dynamometer was used to measure grip strength. Standardized cut-offs for these variables were published by Boreskie et al.[48] which measured frailty status using the same criteria in a similar group of community-dwelling older women recruited in Manitoba. The standardized cut off values that will be used in this thesis are listed on Table 4.

Table 4. ***Standardized Fried Phenotype Cut off values from Boreskie et al.***[48]

Criteria	**Standardized Cut Off Values**
Weight Loss	Have you unintentionally lost weight (i.e. not due to dieting or exercise)? *Score 1 if participant selects "yes"*
Exhaustion	Two questions from the CES-D: How often in the last week did you feel: (1) that everything was an effort? (2) that you could not 'get going'? 0= rarely or none of the time (<1 day) 1= some or a little of the time (1-2 days) 2= a moderate amount of time (3-4 days) 3= most of the time *Score 1 if answering 2 or 3 to either of these questions*
Diminished Activity	Kcal of leisure physical activity identified from the Paffenbarger Physical Activity Scale: <640.1 Kcal *Score 1 if Kcal of leisure physical activity is below this cut off*
Slow Walking Speed	Gait speed stratified by height: Height ≤ 161cm (≤ 1.10 m/s) Height > 161cm (≤ 1.15 m/s) *Score 1 if gait speed lower than this cut off*
Weak Grip Strength	Grip Strength stratified by BMI quartiles: BMI ≤ 23.3 (≤ 21 kg) BMI 23.4-26.1 (≤ 22 kg) BMI 26.2-29.6 (≤ 22 kg) BMI >29.6 (≤ 22 kg) *Score 1 if grip strength is lower than this cut off*

Heart Rate Variability (HRV)

The measurement of HRV was taken using a Polar Electro H7 heart rate monitor (Polar Electro Oy, Kempele, Finland) that was connected by Bluetooth to an accelerometer (ActiGraph wGT3X-BT, Actigraph Corporation, Pensacola, FL) at a sampling rate of 100 Hz.[9] The heart rate monitor was placed on an elastic chest strap and worn on the thorax in direct contact with skin, while the accelerometer was worn attached to a band on the participants non-dominant wrist. All data collection was taken between the hours of 8 AM and 12 PM to avoid the influence of circadian rhythm.[9]

Two separate HRV measurements were taken during both a static measurement and during a walking test. First, a static measurement of HRV was taken with the participant in a sitting position for 10 minutes, where they were informed to spontaneously breathe and to not speak or move. HRV was measured for the entire 10 minutes; however, only a 5-minute section of the data with the highest data quality was used for analysis for each participant. High quality data was assessed as data that did not include drops of HRV signal, and data that did not need more than 5% of ectopic beats corrected as identified by the Kubios software.[49]

Immediately following the static measurement, HRV was then recorded during a 6-minute walk test. Participants were asked to walk for six minutes trying to cover as much distance as possible around a 172-meter track. This track was located in a fitness centre that was open to the public, had music playing, bright lights and an average indoor temperature. The same accelerometer that was connected to the heart rate monitor during the static test was worn during the walking test. The participant was asked to score their Rate of Perceived Exertion (RPE) at the end of the walking test on a scale of 6-20 to determine self-reported intensity of exercise.[50] The last 2-minutes of this 6-minute walk test was used for the analysis to account for participants reaching a steady state heart rate. A stationary or stable signal is required as the statistics used to analyze each HRV variable assume a stable environment.[51] As previously mentioned, only high-quality data was used as assessed by the same conditions mentioned for the static measurements.[49]

Kubios software was used to calculate the HRV indices from the R-R intervals.[49] The time-domain variables include the mean RR interval, standard deviation of normal beat-beat intervals (SDNN), and the root mean square of successive R-R intervals (RMSSD). Frequency domain indices included low frequency (LF) and high Frequency (HF) measures. The low frequency (0.04-0.15 hertz) reflects sympathetic and parasympathetic modulation.[31] The high frequency (0.15-0.4 hertz) primarily reflects heart rate fluctuations due to parasympathetically mediated respiratory sinus arrhythmia, although non-respiratory sinus arrhythmia (erratic rhythm) can also influence it.[31] Total Power (TP) which is the overall heart rate variance across all frequency bands, and ratio LF/HF which is a marker of sympathetic/parasympathetic balance was also calculated.[31] The very low frequency (<0.04 hertz) measure was not included in the analysis as it is not recommended for short-term recordings. [22,52]

In addition to the time and frequency domain analysis, a non-linear variable, the approximate entropy for a heart rate time series (ApEn) was also analyzed using the Kubios software. This non-linear statistic quantifies the regularity of heart rate fluctuations over time.[8,31]

2.3.4 Statistical Analyses

Statistical analyses were performed using SPSS software, Version 26.0 (IBM Corporation, Armonk, NY, USA). Descriptive statistics are presented as mean ± standard deviation for continuous variables and as number (n) and percent of the population (%) for categorical variables. Data was analyzed using a one-way ANOVA for continuous variables and a Chi-Square for categorical variables. A Turkey's post hoc test was then used to identify differences between specific means when appropriate. The normality of the continuous data distribution was tested using the Shapiro-Wilk test.

For the resting data, an ANCOVA was first considered for the purpose of determining if there was a difference in HRV depending on frailty status, using age and BP medication as covariates (BP medication was considered to be any medication take for the purpose of hypertension including beta-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers and calcium channel

blockers and was coded as a yes/no variable). However, these two covariates did not meet the assumptions for this statistical test, as age was not linearly related to the dependent variable (HRV), and BP medication as a categorical variable was not appropriate for an ANCOVA. A two-way ANOVA (2 x 3) was then considered using frailty status and BP medication status as the two factors; however, after checking the sample sizes within the groups, it was determined that this was not an appropriate test as the sample size was too low for some groups, with only one person being frail and not being on BP medications. As a result, a one-way ANOVA was used to determine if there is a difference in HRV between frailty levels using both the FI and the standardized frailty phenotype at rest. In the exercise cohort, an ANCOVA was used to determine if there was a difference in HRV depending on frailty status, while controlling for exercise intensity (measured by RPE) as a covariate using both the FI and the standardized frailty phenotype.

Hierarchical multiple regression was used to determine the association between frailty and HRV while controlling for BP medication use, and exercise intensity (RPE) in the exercise cohort when using the FI and the standardized frailty phenotype as continuous variables. In the resting cohort, two models were used. The first was the unadjusted univariate model where the second added in BP medication status. In the exercise cohort three models were used. The first two are the same as the resting cohort, where model 3 added in exercise intensity (RPE) in order to control for this variable. A p value of ≤ 0.05 determined statistical significance.

The primary hypotheses of the association between the FI and the HRV indices ApEn was used to determine sample size for this thesis. To do this, a significance level of 0.05, with a power of 0.80 was used along with an effect size of 0.35 (calculated from the Takahashi et al. study[8]). This sample size calculation determined the minimum sample size to be 67.

Chapter 3: Results

3.1 Participant Characteristics/Demographics

The original plan was to examine data from one cohort of participants in this thesis. However, as I reviewed the resting and exercise HRV recordings from each participant and examined each file for data quality, it became apparent that technical challenges with the HRV recordings resulted in data being dropped (see Appendix E). As such, the original approach was not viable. Therefore, I created two cohorts for the resting and exercise data and only included data that met the required criteria reported in the methods section of this thesis to examine the original hypotheses. After completing this process, a total of 125 participants are included in the resting data cohort and 102 participants are included in the exercise data cohort. Participant characteristics did not differ between the two cohorts, as reported in Table 5. The age of the resting and the exercise cohorts were 64.0 ± 6.1 and 64.1 ± 6.2 respectively, with the youngest participant being 55 years of age and the oldest being 81 years of age. The average BMI of the resting cohort was 26.0 ± 5.0, and the exercise cohort a BMI of 25.4 ± 4.5. In the resting cohort, 21 (16.8%) participants live alone, and 15 (14.7%) participants lived alone in the exercise cohort. More than 80% of both cohorts had completed post-secondary education. There was no prevalence of type 1 Diabetes in either cohort; however, there were 2 participants in the resting cohort that reported a diagnosis of type 2 Diabetes.

Table 5. Participant Characteristics

Characteristic	Resting Cohort (n= 125)	Exercise Cohort (n=102)	P-Value
Age	64.0 ± 6.1	64.1 ± 6.2	0.982
Height (cm)	163.7 ± 5.3	163.9 ± 5.6	0.804
Weight (kg)	69.8 ± 14.2	68.5 ± 13.1	0.490
BMI (kg/m^2)	26.0 ± 5.0	25.4 ± 4.5	0.402
Living Alone	21 (16.8)	15 (14.7)	0.444
Post-Secondary Education	101 (80.8)	86 (84.3)	0.730
Ex/Current Tobacco Use	65 (52.0)	47 (46.1)	0.644
Type 2 Diabetic	2 (1.6)	0 (0.0)	0.503
High Blood Pressure	29 (23.2)	19 (18.6)	0.419

Continuous variables expressed as Mean ± standard deviation and compared using a T-Test; Categorical variables expressed as N (%) and compared using either a Chi-Square or a Fisher's Exact Test; ***BMI*** *= Body Mass Index.*

3.2 Frailty Prevalence Characteristics of the Resting Cohort

Two methods were used to assess frailty status in the overall sample. The prevalence of robust, pre-frail and frail individuals were different when using the WH-HRV FI compared to the Standardized Phenotype method. From the resting cohort, the WH-HRV Frailty Index identified that 19 participants were robust, 78 were pre-frail and 28 were identified as being frail. From the same resting cohort, a standardized frailty phenotype identified 69 as robust, 51 as pre-frail and 5 as frail. The comparison of these two tools is listed in Table 6.

Table 6. Comparison of Frailty Classification Tools in Resting Cohort

Standardized Phenotype Classification (n=125)	WH-HRV Frailty Index Classification (n=125)		
	Robust (n = 19)	Pre-Frail (n = 78)	Frail (n = 28)
Robust (n = 69)	13	48	8
Pre-Frail (n = 51)	6	29	16
Frail (n = 5)	0	1	4

3.3 Does HRV complexity of the resting cohort differ between groups based on FI frailty level?

Participant characteristics for those classified as robust, pre-frail and frail based on the WH-HRV FI in the resting cohort are listed in Table 7. No differences in age or height were detected between groups. Although the groups were not different in weight, there was a trend (p=0.054) that the

frail group was heavier than the other 2 groups. The frail group tended to weigh approximately 10.6 and 9.1 kg more than the robust and pre-frail groups, respectively. BMI was different between groups (p=0.008), where a Tukey's post hoc test identified that BMI was highest in the frail group compared to both the pre-frail group (p=0.001) and the robust group (p=0.002). In the resting cohort the prevalence of participants living alone and those that were ex or current tobacco users was not different between robust, pre-frail and frail groups. The prevalence of having a post-secondary education was different between groups (p=0.015), with the pre-frail group having the highest prevalence compared to the robust group. Both a type 2 diabetes diagnosis (p=0.030) and a diagnosis of high blood pressure (p<0.001) had the highest prevalence in the frail group compared to both the robust or pre-frail groups.

Table 7. Resting cohort participant characteristics by WH-HRV FI frailty level

Characteristic	Robust (n = 19)	Pre-Frail (n = 78)	Frail (n=28)	P-Value
Age	64.5 ± 6.2	64.2 ± 6.3	63.3 ± 5.4	0.731
Height (cm)	164.5 ± 3.9	163.9 ± 5.7	162.5 ± 5.1	0.365
Weight (kg)	66.5 ± 9.4	68.0 ± 12.1	77.1 ± 19.5	0.054
BMI (kg/m^2)	24.2 ± 3.2	25.3 ± 4.1	29.1 ± 6.8^{A,B}	0.008*
Living Alone	3 (15.8)	15 (19.0)	3 (10.7)	0.597
Post-Secondary Education	12 (63.2)	69 (88.5)^{A}	20 (71.4)	0.015*
Ex/Current Tobacco Use	8 (42.1)	38 (48.7)	19 (67.9)	0.142
Type 2 Diabetic	0 (0)	0 (0)	2 (7.1)^{A,B}	0.030*
Diagnosed High BP	3 (15.8)	11 (14.1)	15 (53.6)^{A,B}	<0.001*

Continuous variables expressed as Mean ± standard deviation and compared using a one-way ANOVA; Categorical variables expressed as N (%) and compared using a Chi-square test; ***BMI*** *= Body Mass Index,* ***BP*** *= Blood Pressure. * significantly differences between groups detected by ANOVA (P<0.05).* ^{A} *significantly different than Robust (P<0.05) based on a Tukey's post hoc test.* ^{B} *significantly different than Pre-Frail (P<0.05) based on a Tukey's post hoc test.*

A normal distribution of the dependent variable is needed to run a one-way ANOVA. A Shapiro-Wilk normality test was run on the eight HRV variables (Mean RR, SDNN, RMSSD, LF, HF, TP, LF/HF Ratio, and ApEn) to determine if these HRV variables had a normal distribution. Mean RR and ApEn were not statistically significant with the Shapiro-Wilk test and passed this assumption, allowing their raw data to be used for subsequent ANOVA testing. However, SDNN, RMSSD, LF, HF,

LF/HF Ratio and TP were statistically significant with the Shapiro-Wilk test and, therefore, failed this assumption. Log transformed data for LF, HF and TP were provided by the Kubios software when running the initial data analysis and were subsequently tested for normalcy. The Shapiro-Wilk test determined that there was a normal distribution for these log transformed variables. Therefore, the log transformed variables were used for the one-way ANOVA. SDNN, RMSSD and the LF/HF Ratio were log transformed in order to satisfy the normality assumption with the one-way ANOVA.

To determine if HRV differs between frailty levels identified by the WH-HRV FI during rest, a one-way ANOVA was run. A one-way ANOVA determined that there were no statistical differences between groups (Table 8). Although not significant, the LF_{log} variable was approaching significance (a trend; p=0.052). A Tukey's post hoc revealed that this difference was between the frail and pre-frail group, with a decreased value in the frail group (p=0.040). The LF/HF_{log} was also trending (p=0.085). A Tukey's post hoc also revealed that this difference was between the frail and pre-frail group, similarly with a decreased value in the frail group (p=0.090).

Table 8: Resting HRV parameters based on WH-HRV FI Frailty Group classifications

Characteristic	Robust (n = 19)	Pre-Frail (n = 78)	Frail (n=28)	P-Value
Mean RR	921 ± 141	899 ± 105	884 ± 140	0.594
$SDNN_{log}$	1.36 ± 0.19	1.38 ± 0.22	1.30 ± 0.24	0.262
$RMSSD_{log}$	1.30 ± 0.22	1.30 ± 0.24	1.25 ± 0.31	0.619
LF_{log}	5.42 ± 0.99	5.60 ± 1.09	5.02 ± 1.07^{B}	0.052
HF_{log}	4.96 ± 0.97	4.89 ± 1.22	4.70 ± 1.37	0.708
TP_{log}	6.12 ± 0.93	6.22 ± 1.05	5.81 ± 1.06	0.201
LF/HF_{log}	0.20 ± 0.26	0.32 ± 0.37	0.14 ± 0.45^{B}	0.085
ApEn	1.13 ± 0.07	1.10 ± 0.09	1.13 ± 0.09	0.113

Variables expressed as Mean ± standard deviation and compared using a one-way ANOVA; ***SDNN*** *= Standard Deviation of Normal beat-beat intervals,* ***RMSSD*** *= Root Mean Square of Successive RR intervals,* ***LF*** *= Low Frequency,* ***HF*** *= High Frequency,* ***TP*** *= Total Power,* ***LF/HF*** *= Low Frequency to High Frequency Ratio,* ***ApEn*** *= Approximate Entropy for a heart rate time series. * significant differences between groups detected by ANOVA (P<0.05).* ^{A} *significantly different than Robust (P<0.05) based on a Tukey's post hoc test.* ^{B} *significantly different than Pre-Frail (P<0.05) based on a Tukey's post hoc test.*

3.4 Does HRV complexity of the resting cohort differ between groups based on Standardized Phenotype frailty level?

Participant characteristics for those classified as robust, pre-frail and frail based on the standardized frailty phenotype in the resting cohort are listed in Table 9. No differences in age, weight, BMI were identified (Table 9). There was a significant difference in height (p=0.018). With a Tukey's post hoc test, it was identified that the frail group was shorter than the robust group (p=0.025). There was no significant difference between groups for living alone, having a post-secondary education, being an ex- or current tobacco user or having a type 2 diabetes diagnosis (Table 9). The frail group had a significantly higher prevalence of diagnosed high BP (p=0.003) when compared to both the robust and pre-frail groups with 80% of the frail population having diagnosed high BP, compared to 15.9% and 27.5% of the Robust and Pre-Frail populations, respectively.

Table 9. Resting cohort participant characteristics by Standardized Frailty Phenotype level

Characteristic	Robust (n = 69)	Pre-Frail (n = 51)	Frail (n=5)	P-Value
Age	63.6 ± 6.2	64.4 ± 6.0	67.0 ± 4.6	0.434
Height (cm)	164.6 ± 5.5	163.1 ± 4.9	158.2 ± 3.9[A]	0.018*
Weight (kg)	70.1 ± 14.0	69.9 ± 14.8	65.5 ± 12.6	0.787
BMI (kg/m^2)	25.7 ± 4.8	26.2 ± 5.3	26.0 ± 3.9	0.861
Living Alone	10 (14.5)	11 (21.6)	0 (0)	0.350
Post-Secondary Education	59 (85.5)	38 (74.5)	4 (80.0)	0.318
Ex/Current Tobacco Use	33 (47.8)	29 (56.9)	3 (60.0)	0.579
Type 2 Diabetic	1 (1.4)	1 (2.0)	0 (0)	0.935
Diagnosed High BP	11 (15.9)	14 (27.5)	4 (80.0)[A,B]	0.003*

Continuous variables expressed as Mean ± standard deviation and compared using a one-way ANOVA; Categorical variables expressed as N (%) and compared using a Chi-square test; ***BMI*** *= Body Mass Index,* ***BP*** *= Blood Pressure. * significant differences between groups detected by ANOVA (P<0.05). [A] significantly different than Robust (P<0.05) based on a Tukey's post hoc test. [B] significantly different than Pre-Frail (P<0.05) based on a Tukey's post hoc test.*

To determine if HRV differs between frailty groups identified by the Standardized Frailty Phenotype at rest a one-way ANOVA was run. The results from the one-way ANOVAs comparing frailty group and HRV are listed in Table 10. No significant differences were found in any of the HRV variables on frailty level.

Table 10: Resting HRV parameters based on the Standardized Frailty Phenotype Group classifications

Characteristic	Robust (n = 69)	Pre-Frail (n = 51)	Frail (n=5)	P-Value
Mean RR	903 ± 124	890 ± 110	931 ± 148	0.702
$SDNN_{log}$	1.37 ± 0.20	1.35 ± 0.24	1.29 ± 0.28	0.700
$RMSSD_{log}$	1.31 ± 0.23	1.27 ± 0.27	1.26 ± 0.38	0.777
LF_{log}	5.51 ± 1.04	5.42 ± 1.18	4.86 ± 0.66	0.434
HF_{log}	4.94 ± 1.16	4.78 ± 1.24	4.59 ± 1.82	0.668
TP_{log}	6.18 ± 0.98	6.06 ± 1.11	5.75 ± 1.15	0.588
LF/HF_{log}	0.25 ± 0.37	0.28 ± 0.38	0.12 ± 0.54	0.656
ApEn	1.11 ± 0.09	1.11 ± 0.09	1.15 ± 0.09	0.506

Variables expressed as Mean ± standard deviation and compared using a one-way ANOVA; ***SDNN*** *= Standard Deviation of Normal beat-beat intervals,* ***RMSSD*** *= Root Mean Square of Successive RR intervals,* ***LF*** *= Low Frequency,* ***HF*** *= High Frequency,* ***TP*** *= Total Power,* ***LF/HF*** *= Low Frequency to High Frequency Ratio,* ***ApEn*** *= Approximate Entropy for a heart rate time series. * significant differences between groups detected by ANOVA (P<0.05).* ^{A} *significantly different than Robust (P<0.05) based on a Tukey's post hoc test.* ^{B} *significantly different than Pre-Frail (P<0.05) based on a Tukey's post hoc test.*

3.5 Frailty Prevalence Characteristics of the exercise cohort

From the participants who were included in the exercise data cohort, the WH-HRV FI identified 22 as robust, 63 as pre-frail and 17 as frail; whereas, the Standardized Phenotype identified 61 as robust, 37 as pre-frail and 4 as frail (Table 8).

Table 11. Comparison of frailty classification tools in exercise cohort

Standardized Phenotype Classification (n=102)	Frailty Index Classification (n=102)		
	Robust (n = 22)	Pre-Frail (n = 63)	Frail (n = 17)
Robust (n = 61)	16	40	5
Pre-Frail (n = 37)	6	22	9
Frail (n = 4)	0	1	3

3.6 Does HRV complexity of the exercise cohort differ between groups based on FI frailty level?

Participant characteristics for those classified as robust, pre-frail and frail in the exercise cohort are listed in Table 12. Age was not different between frailty groups. However, there was a difference in height between the groups, where a Tukey's post hoc test determined the frail group was shorter than the robust group (p=0.019). There was no difference in weight between the groups, but there was a difference in BMI (p=0.007), where a Tukey post hoc test determined that the frail group had a higher BMI compared to the robust group (p=0.005). The prevalence of living alone was not different between groups. There was a significantly higher prevalence of participants who have a post-secondary education in the pre-frail group (90.5%) compared to the robust group (68.2%) (p=0.045). There was also a difference in tobacco use, with higher prevalence in the frail group compared to the robust group (p=0.009) and higher prevalence of high blood pressure in the frail group compared to the pre-frail group (p=0.003). There was no prevalence of type 2 diabetes in the exercise cohort.

Table 12. Exercise cohort participant characteristics by WH-HRV FI frailty level

Characteristic	Robust (n = 22)	Pre-Frail (n = 63)	Frail (n=17)	P-Value
Age	64.8 ± 6.2	64.1 ± 6.4	63.2 ± 5.8	0.733
Height (cm)	166.1 ± 5.2	163.9 ± 5.4	161.2 ± 6.0^{A}	0.026*
Weight (kg)	65.7 ± 9.4	68.2 ± 12.5	73.4 ± 17.9	0.183
BMI (kg/m^2)	23.6 ± 3.4	25.4 ± 4.2	28.0 ± 5.6^{A}	0.007*
Living Alone	3 (13.6)	9 (14.3)	3 (17.6)	0.930
Post-Secondary Education	15 (68.2)	57 (90.5)^{A}	14 (82.4)	0.045*
Ex/Current Tobacco User	6 (27.3)	28 (44.4)	13 (76.5)^{A}	0.009*
Type 2 Diabetic	0 (0)	0 (0)	0 (0)	---
High Blood Pressure	4 (18.2)	7 (11.1)	8 (47.1)^{B}	0.003*

Continuous variables expressed as Mean ± standard deviation and compared using a one-way ANOVA; Categorical variables expressed as N (%) and compared using a Chi-square test; ***BMI*** *= Body Mass Index. * significant differences between groups detected by ANOVA (P<0.05).* ^{A} *significantly different than Robust (P<0.05) based on a Tukey's post hoc test.* ^{B} *significantly different than Pre-Frail (P<0.05) based on a Tukey's post hoc test.*

A Shapiro-Wilk test was run on the eight HRV variables to determine if these variables had a normal distribution within the exercise cohort. The raw data for ApEn had a normal distribution as identified by the Shapiro-Wilk test, this raw data will be used to run the one-way ANCOVA. The remaining seven HRV variables (Mean R-R, SDNN, RMSSD, LF, HF, TP, and LF/HF) were statistically significant with the Shapiro-Wilk test, which indicated that they did not have a normal distribution. A normal distribution is needed for a one-way ANOVA. The eight variables were log transformed in order to satisfy the normality assumption for the ANOVA.

To determine if HRV differs between frailty groups identified by the WH-HRV FI during exercise a number of one-way ANCOVA's were run (Table 13). These ANCOVA's controlled for RPE. The robust group had an average RPE of 12±2, the pre-frail group averaged 11±2 and the frail group averaged 12±2. These values were not statistically significant between the groups (p=0.114). These ANCOVA's identified that there were no statistically significant differences in any of the HRV variables and frailty level as identified by the WH-HRV FI.

Table 13. Exercise HRV parameters based on WH-HRV FI Frailty Group Classifications

Characteristic	Robust (n = 22)	Pre-Frail (n = 63)	Frail (n=17)	ANCOVA P-Value
Mean RR_{log}	2.68 ± 0.06	2.70 ± 0.6	2.71 ± 0.08	0.238
$SDNN_{log}$	0.73 ± 0.27	0.75 ± 0.26	0.80 ± 0.22	0.549
$RMSSD_{log}$	0.21 ± 0.10	0.22 ± 0.12	0.20 ± 0.09	0.784
LF_{log}	1.87 ± 1.11	2.09 ± 1.29	2.04 ± 1.25	0.833
HF_{log}	1.52 ± 1.48	1.60 ± 1.32	1.48 ± 0.94	0.999
TP_{log}	2.66 ± 1.23	2.78 ± 1.29	2.74 ± 1.25	0.928
LF/HF_{log}	0.24 ± 0.41	0.24 ± 0.36	0.23 ± 0.40	0.979
ApEn	1.02 ± 0.09	1.01 ± 0.10	0.98 ± 0.09	0.374

Variables expressed as Mean ± standard deviation and compared using a one-way ANCOVA; ***SDNN*** *= Standard Deviation of Normal beat-beat intervals,* ***RMSSD*** *= Root Mean Square of Successive RR intervals,* ***HF*** *= High Frequency,* ***TP*** *= Total Power,* ***LF/HF*** *= Low Frequency to High Frequency Ratio,* ***ApEn*** *= Approximate Entropy for a heart rate time series. * significant differences between groups detected by ANOVA (P<0.05).* [A] *significantly different than Robust (P<0.05) based on a Tukey's post hoc test.* [B] *significantly different than Pre-Frail (P<0.05) based on a Tukey's post hoc test.*

3.7 Does HRV complexity of the exercise cohort differ between groups based on standardized phenotype level?

Participant characteristics for those classified as robust, pre-frail and frail when measured using the Standardized Frailty Phenotype in the exercise cohort are listed in Table 14. There was no difference in age, weight or BMI between frailty groups. However, there was a statistically significant difference in height between the groups (p=0.004). A Tukey's post hoc identified that there was a decrease in height between the frail group and the robust group (p=0.022) and the pre-frail and the robust group (p=0.042). The prevalence of living alone, having a post-secondary education and being an ex or current tobacco user were not different between groups. There was no prevalence of type 2 diabetes in the exercise cohort. There was a higher prevalence of diagnosed high blood pressure in the frail groups compared to both the pre-frail and robust groups (p=0.011).

Table 14. Exercise cohort participant characteristics by Standardized Frailty Phenotype level

Characteristic	Robust (n = 61)	Pre-Frail (n = 37)	Frail (n=4)	P-Value
Age	63.8 ± 6.4	64.2 ± 6.0	66.3 ± 7.6	0.748
Height (cm)	166.1 ± 5.2	163.9 ± 5.4[A]	161.2 ± 6.0[A]	0.004*
Weight (kg)	65.7 ± 9.4	68.2 ± 12.5	73.4 ± 17.9	0.673
BMI (kg/m^2)	23.6 ± 3.4	25.4 ± 4.2	28.0 ± 5.6	0.110
Living Alone	9 (14.8)	6 (16.2)	0 (0)	0.685
Post-Secondary Education	54 (88.5)	28 (75.7)	4 (100.00)	0.161
Ex/Current Tobacco User	24 (39.3)	20 (54.1)	3 (75.0)	0.182
Type 2 Diabetic	0 (0)	0 (0)	0 (0)	---
High Blood Pressure	9 (14.8)	7 (18.9)	3 (75.0)[A,B]	0.011*

Continuous variables expressed as Mean ± standard deviation and compared using a one-way ANOVA; Categorical variables expressed as N (%) and compared using a Chi-square test; ***BMI*** *= Body Mass Index,* ***BP*** *= Blood Pressure. * significant differences between groups detected by ANOVA (P<0.05). [A] significantly different than Robust (P<0.05) based on a Tukey's post hoc test. [B] significantly different than Pre-Frail (P<0.05) based on a Tukey's post hoc test.*

To determine if HRV differs between frailty groups identified by the standardized frailty phenotype during exercise, one-way ANCOVA's were run on each of the HRV variables (Table 15). Similarly, these ANCOVA's controlled for RPE. The robust group had an average RPE of 11±2, the pre-frail group averaged 11±2 and the frail group averaged 12±1. These values were not statistically significant between groups (p=0.579). These one-way ANCOVA's revealed significant differences of mean RR_{log} between the frailty levels (p=0.002). A Tukey's post hoc revealed that there was a significant difference between the frail group and both the robust (p=0.001) and pre-frail (p=0.002) group. There was also a significant difference in $SDNN_{log}$ between frail groups (p=0.046). A post hoc test determined that this was a non-significant difference between the pre-frail and robust groups (p=0.077). A third variable was also significant, specifically HF_{log}, between frailty groups (p=0.046) with a post hoc identifying that the difference was between the pre-frail and robust groups (p=0.044). Three of the other HRV variables were trending towards significance including TP_{log} (p=0.057), LF_{log} (0.072), and ApEn (p=0.085). The last two variables $RMSSD_{log}$ and LF/HF_{log} were not significantly different.

Table 15: Exercise HRV parameters based on the Standardized Frailty Phenotype Group classifications

Characteristic	Robust (n = 61)	Pre-Frail (n = 37)	Frail (n=4)	ANCOVA P-Value
Mean RR_{log}	2.69 ± 0.07	2.69 ± 0.05	2.79 ± 0.05[A,B]	0.002*
$SDNN_{log}$	0.79 ± 0.29	0.69 ± 0.18[A]	0.87 ± 0.22	0.046*
$RMSSD_{log}$	0.21 ± 0.12	0.23 ± 0.10	0.15 ± 0.07	0.197
LF_{log}	2.18 ± 1.26	1.75 ± 1.16	2.38 ± 1.45	0.072
HF_{log}	1.77 ± 1.45	1.20 ± 0.97[A]	1.74 ± 0.56	0.046*
TP_{log}	2.91 ± 1.32	2.43 ± 1.12	3.14 ± 1.20	0.057
LF/HF_{log}	0.21 ± 0.39	0.28 ± 0.32	0.28 ± 0.60	0.631
ApEn	1.00 ± 0.11	1.01 ± 0.07	0.91 ± 0.11	0.085

Variables expressed as Mean ± standard deviation and compared using a one-way ANCOVA; ***SDNN*** *= Standard Deviation of Normal beat-beat intervals,* ***RMSSD*** *= Root Mean Square of Successive RR intervals,* ***LF*** *= Low Frequency,* ***HF*** *= High Frequency,* ***TP*** *= Total Pow er,* ***LF/HF*** *= Low Frequency to High Frequency Ratio,* ***ApEn*** *= Approximate Entropy for a heart rate time series. * significant differences between groups detected by ANOVA (P<0.05). [A] significantly different than Robust (P<0.05) based on a Tukey's post hoc test. [B] significantly different than Pre-Frail (P<0.05) based on a Tukey's post hoc test.*

3.8 Is HRV complexity of the resting cohort associated with frailty based on the WH-HRV FI?

A hierarchical multiple regression was run to understand the effect of frailty as a continuous variable on HRV in the resting cohort with two separate models (Table 16). Model 1 was unadjusted where model 2 adjusted for the use of BP medications. In model 1, the HRV variable LF_{log} was trending towards, (p=0.063), but did not reach significance. No other HRV variables were significantly associated to frailty with model 1 (Mean RR, $SDNN_{log}$, $RMSSD_{log}$, HF_{log}, TP_{log}, LF/HF_{log} and ApEn).

In model 2, we adjusted for the use of BP medications. This model identified that frailty level (WH-HRV FI) and BP medication were significantly associated with LF_{log} (p=0.048) and accounted for 4.9% of the explained variability in LF. The variable TP_{log} was approaching but did not reach statistical significance (p=0.075). With model 2, frailty was not associated with any of the other HRV variables when measured at rest including Mean RR, SDNNlog, RMSSDlog, HF_{log}, LF/HF_{log} and ApEn.

Table 16: Multivariable linear regression for the WH-HRV FI and HRV variables in the resting cohort

	Model 1				**Model 2**			
Variable	**β**	**SE**	**R^2**	**p-value**	**β**	**SE**	**R^2**	**p-value**
Mean R-R	-0.07	119.55	0.004	0.471	-0.09	130.25	0.008	0.631
$SDNN_{log}$	-0.14	0.22	0.020	0.114	-0.08	0.24	0.039	0.091
$RMSSD_{log}$	-0.13	0.26	0.016	0.159	-0.09	0.28	0.025	0.218
LF_{log}	-0.17	1.09	0.028	0.063	-0.11	1.18	0.049	0.048*
HF_{log}	-0.13	1.22	0.016	0.163	-0.10	1.32	0.019	0.302
TP_{log}	-0.15	1.04	0.023	0.089	-0.10	1.12	0.042	0.075
LF/HF_{log}	-0.04	0.38	0.002	0.663	0.00	0.42	0.012	0.490
ApEn	0.04	0.09	0.001	0.681	0.02	0.10	0.002	0.871

SDNN *= Standard Deviation of Normal beat-beat intervals,* ***RMSSD*** *= Root Mean Square of Successive RR intervals,* ***LF*** *= Low Frequency,* ***HF*** *= High Frequency,* ***TP*** *= Total Power,* ***LF/HF*** *= Low Frequency to High Frequency Ratio,* ***ApEn*** *= Approximate Entropy for a heart rate time series,* ***β*** *= standardized coefficient; SE = standard error,* R^2 *= coefficient of determination;*
** significant differences. Model 1 = unadjusted, Model 2 = adjusted for BP medication use.*

3.9 Is HRV complexity of the resting cohort associated with frailty based on the Standardized Frailty Phenotype?

Next, a hierarchical regression was run to understand the effect of frailty on HRV complexity while using the standardize frailty phenotype at rest. For the purpose of this regression, the standardized frailty phenotype was used as a continuous variable. Similarly, two separate models were used with the model 1 being unadjusted and model 2 adjusted for the use of BP medications. Model 1 was not significantly associated with any of the eight HRV variables (Table 17) (Mean RR, $SDNN_{log}$, $RMSSD_{log}$, LF_{log}, HF_{log}, TP_{log}, LF/HF_{log}, or ApEn).

Model 2 was run to understand the effect of frailty and BP medication use on HRV complexity measured at rest. The variable LF_{log} was approaching (p=0.079) but did not reach significance. Frailty was not significantly associated any of the other HRV variables when using this model (Mean RR, $SDNN_{log}$, $RMSSD_{log}$, HF_{log}, TP_{log}, LF/HF_{log}, or ApEn).

Table 17: Multivariable linear regression for the standardized frailty phenotype and HRV variables in the resting cohort

	Model 1				**Model 2**			
Variable	**β**	**SE**	**R^2**	**p-value**	**β**	**SE**	**R^2**	**p-value**
Mean R-R	0.04	12.44	0.001	0.682	0.03	13.02	0.002	0.903
$SDNN_{log}$	-0.06	0.02	0.003	0.522	-0.01	0.02	0.033	0.133
$RMSSD_{log}$	-0.04	0.03	0.001	0.695	0.00	0.03	0.018	0.326
LF_{log}	-0.10	0.11	0.009	0.292	-0.04	0.12	0.041	0.079
HF_{log}	-0.06	0.13	0.003	0.523	-0.03	0.13	0.012	0.483
TP_{log}	-0.08	0.11	0.006	0.407	-0.03	0.11	0.034	0.117
LF/HF_{log}	-0.05	0.04	0.002	0.601	-0.02	0.04	0.012	0.481
ApEn	0.06	0.01	0.004	0.485	0.06	0.01	0.005	0.755

SDNN *= Standard Deviation of Normal beat-beat intervals,* ***RMSSD*** *= Root Mean Square of Successive RR intervals,* ***LF*** *= Low Frequency,* ***HF*** *= High Frequency,* ***TP*** *= Total Power,* ***LF/HF*** *= Low Frequency to High Frequency Ratio,* ***ApEn*** *= Approximate Entropy for a heart rate time series,* ***β*** *= standardized coefficient; SE = standard error,* R^2 *= coefficient of determination;*
** significant differences. Model 1 = unadjusted, Model 2 = adjusted for BP medication use.*

3.10 Is HRV complexity of the exercise cohort associated with frailty based on the WH-HRV FI?

A hierarchical regression was then run to understand the effect of frailty on HRV complexity measured with the WH HRV FI during exercise. With this regression, there were three models that were used. Model 1 was unadjusted, model 2 adjusted for BP medication use and model 3 adjusted for BP medication use and exercise intensity as measured by RPE (Table 18). With both the unadjusted model and model 2, frailty could not predict any of the eight HRV variables. With model 1, the HRV variable ApEn was trending towards, but did not reach significance (p=0.092).

With the addition of exercise intensity as a controlling factor in model 3, frailty was significantly associated to three HRV variables including Mean RR (p=0.006), LF_{log} (p=0.007), and TP_{log} (p=0.023). With each of these variables accounting for 12.3%, 11.7% and 9.4% of the explained variability in each variable respectively. $SDNN_{log}$ was trending towards (p=0.052) but did not reach significance. This model was not associated with the rest of the HRV variables ($RMSSD_{log}$, HF_{log}, LF/HF_{log}, and ApEn).

Table 18: Multivariable linear regression for the WH-HRV FI and HRV variables in the exercise cohort

	Model 1				Model 2				Model 3			
Variable	**β**	**SE**	**R^2**	**p-value**	**β**	**SE**	**R^2**	**p-value**	**β**	**SE**	**R^2**	**p-value**
Mean $R\text{-}R_{log}$	0.12	0.08	0.014	0.244	0.08	0.08	0.028	0.249	0.12	0.08	0.123	0.006*
$SDNN_{log}$	0.09	0.32	0.008	0.385	0.05	0.32	0.023	0.320	0.08	0.32	0.077	0.052
$RMSSD_{log}$	0.01	0.14	0.000	0.906	0.04	0.14	0.020	0.375	0.02	0.14	0.043	0.237
LF_{log}	0.01	1.49	0.000	0.925	0.03	1.57	0.013	0.539	0.01	1.50	0.117	0.007*
HF_{log}	0.01	1.57	0.000	0.909	0.01	1.66	0.004	0.829	0.02	1.64	0.051	0.167
TP_{log}	-0.00	1.52	0.000	0.980	-0.04	1.60	0.015	0.489	-0.00	1.55	0.094	0.023*
LF/HF_{log}	-0.06	0.46	0.004	0.528	-0.08	0.48	0.008	0.689	-0.07	0.49	0.013	0.727
ApEn	-0.17	0.11	0.029	0.092	-0.13	0.12	0.043	0.120	-0.14	0.12	0.056	0.135

SDNN *= Standard Deviation of Normal beat-beat intervals,* ***RMSSD*** *= Root Mean Square of Successive RR intervals,* ***LF*** *= Low Frequency,* ***HF*** *= High Frequency,* ***TP*** *= Total Power,* ***LF/HF*** *= Low Frequency to High Frequency Ratio,* ***ApEn*** *= Approximate Entropy for a heart rate time series,* ***β*** *= standardized coefficient; SE = standard error,* R^2 *= coefficient of determination;*
** significant differences. Model 1 = unadjusted, Model 2 = adjusted for BP medication use, Model 3 = adjusted for BP medication use and RPE.*

3.11 Is HRV complexity of the exercise cohort associated with frailty based on the Standardized Frailty Phenotype?

Lastly, a hierarchical regression was run to understand the effect of frailty on HRV complexity measured with the standardized frailty phenotype during exercise. With this regression the same three models that were used with the WH-HRV FI were also used. Model 1 was unadjusted, model 2 adjusted for BP medication use and model 3 adjusted for BP medication use and exercise intensity as measured by RPE (Table 19). All three models identified that frailty was significantly associated with mean RR_{log}. When accounting for both BP medication use and RPE (model 3) this accounted for 16.1% of explained variability (p=0.001). In model 1 and 2 frailty was not associated with any of the other HRV variables including, $SDNN_{log}$, $RMSSD_{log}$, LF_{log}, HF_{log}, TP_{log}, LF/HF_{log} and ApEn.

Model 3 (adjusting for BP medication use and exercise intensity) also identified that three other HRV variables were significantly associated with frailty including $SDNN_{log}$ (p=0.038), LF_{log} (p=0.004), and TP_{log} (p=0.013). Each of these variables accounted for 8.4%, 12.8% and 10.6% in the variability of each variable respectively. HF_{log} was trending towards (p=0.081) but did not reach a significant association.

Table 19: Multivariable linear regression for the standardized frailty phenotype and HRV variables in the exercise cohort

	Model 1				Model 2				Model 3			
Variable	**β**	**SE**	**R^2**	**p-value**	**β**	**SE**	**R^2**	**p-value**	**β**	**SE**	**R^2**	**p-value**
Mean $R\text{-}R_{log}$	0.26	0.01	0.065	0.010*	0.23	0.01	0.073	0.025*	0.23	0.01	0.161	0.001*
$SDNN_{log}$	-0.07	0.03	0.004	0.515	-0.11	0.03	0.033	0.194	-0.11	0.03	0.084	0.038*
$RMSSD_{log}$	0.00	0.01	0.000	0.970	0.04	0.01	0.020	0.376	0.04	0.01	0.044	0.228
LF_{log}	-0.07	0.14	0.005	0.476	-0.11	0.15	0.023	0.325	-0.11	0.14	0.128	0.004*
HF_{log}	-0.11	0.15	0.012	0.285	-0.13	0.16	0.020	0.368	-0.13	0.15	0.067	0.081
TP_{log}	-0.07	0.15	0.006	0.449	-0.12	0.15	0.026	0.283	-0.12	0.15	0.106	0.013*
LF/HF_{log}	0.05	0.04	0.003	0.616	0.04	0.05	0.003	0.859	0.05	0.05	0.010	0.799
ApEn	-0.10	0.01	0.009	0.342	-0.06	0.01	0.031	0.219	-0.06	0.01	0.041	0.260

SDNN *= Standard Deviation of Normal beat-beat intervals,* ***RMSSD*** *= Root Mean Square of Successive RR intervals,* ***LF*** *= Low Frequency,* ***HF*** *= High Frequency,* ***TP*** *= Total Power,* ***LF/HF*** *= Low Frequency to High Frequency Ratio,* ***ApEn*** *= Approximate Entropy for a heart rate time series,* ***β*** *= standardized coefficient; SE = standard error,* R^2 *= coefficient of determination;*
** significant differences. Model 1 = unadjusted, Model 2 = adjusted for BP medication use, Model 3 = adjusted for BP medication use and RPE.*

3.12 Summary of Results Section

A review table was created to provide a clear overview of the results for the relationship between frailty and HRV. The first table provides an overview of the results from the resting cohort including the one-way ANOVA, unadjusted regression (model 1) and the regression that controlled for BP medication (model 2; Table 20). Only one variable had significant results which was LF_{log} using the WH-HRV FI. Three other HRV variables were trending towards significance including $SDNN_{log}$, TP_{log}, and LF/HF_{log}. Most of these results, both significant and trending towards significance were found using the WH-HRV FI and not the standardized frailty phenotype.

The second table provides an overview of the results from the exercise cohort including the one-way ANCOVA that controlled for exercise intensity by RPE, the unadjusted regression (model 1), the regression that controlled for BP medication use (model 2) and lastly, the regression that controlled for both BP medication use and exercise intensity (model 3) (Table 21). Both mean RR_{log} and $SDNN_{log}$ were both significantly different between frailty groups and was also significantly association with frailty. LF_{log} and TP_{log} were significantly associated with frailty. The variable HF_{log} was significantly different between frailty groups when measured by the ANCOVA but was not associated with frailty. These results were primarily found using the standardized frailty phenotype and not the WH-HRV FI.

Table 20. Summary Table of Significant Results in Resting Cohort

Statistical Test	ANOVA		Model 1		Model 2	
FI Measure	**WH-HRV FI**	**Phenotype**	**WH-HRV FI**	**Phenotype**	**WH-HRV FI**	**Phenotype**
Mean RR	NS	NS	NS	NS	NS	NS
SDNN	NS	NS	NS	NS	TS	NS
RMSSD	NS	NS	NS	NS	NS	NS
LF	TS	NS	TS	NS	S	TS
HF	NS	NS	NS	NS	NS	NS
TP	NS	NS	TS	NS	TS	NS
LF/HF	TS	NS	NS	NS	NS	NS
ApEn	NS	NS	NS	NS	NS	NS

Model 1 = unadjusted, Model 2 = adjusted for BP medication use.
NS = not significant, TS = trending towards significance, S = significant.

Table 21. Summary Table of Significant Results in Exercise Cohort

Statistical Test	ANCOVA		Model 1		Model 2		Model 3	
FI Measure	**WH-HRV FI**	**Phenotype**	**WH-HRV FI**	**Phenotype**	**WH-HRV FI**	**Phenotype**	**WH-HRV FI**	**Phenotype**
Mean RR	NS	S	NS	S	NS	S	S	S
SDNN	NS	S	NS	NS	NS	NS	TS	S
RMSSD	NS	NS	NS	NS	NS	NS	NS	NS
LF	NS	TS	NS	NS	NS	NS	S	S
HF	NS	S	NS	NS	NS	NS	NS	TS
TP	NS	TS	NS	NS	NS	NS	S	S
LF/HF	NS	NS	NS	NS	NS	NS	NS	NS
ApEn	NS	TS	NS	NS	NS	NS	NS	NS

Model 1 = unadjusted, Model 2 = adjusted for BP medication use, Model 3 = adjusted for BP medication use and exercise intensity (RPE).
NS = not significant, TS = trending towards significance, S = significant.

Chapter 4: Discussion

4.1 Overview

Diseases of the heart along with high cardiovascular disease risk, have shown to be highly associated with frailty.[1] It may be reasonable to speculate that key components of the cardiovascular system, heart rate and HRV may be affected and may be associated with frailty. Previous research has attempted to provide evidence that the ANS, which controls the cardiovascular system and can be measured non-invasively through HRV, may impact or be impacted by frailty status.[4] A common hypothesis is that HRV will be less complex in those who are frail during resting conditions; however, there has been inconsistent results with some literature finding an increase in variability where others have reported a decrease in HRV variability or overall complexity.[8,9,31,34] Based on this literature, we saw an opportunity to add to this body of knowledge by using a frailty index where all other studies comparing frailty status and HRV have been done using the frailty phenotype method. I will also add to the knowledge of frailty and HRV by using HRV data during both rest and moderate self-paced exercise (6-minute walk test) as this is a novel area that has not previously been reported.

My thesis explored two key aspects of the relationship between frailty and HRV complexity. The first was to determine if there was a significant *difference* or *association* in HRV at rest depending on frailty status identified by the WH-HRV FI. I then utilized the standardized frailty phenotype in an attempt to duplicate the literature for HRV. The second aspect was to determine if there was a significant *difference* or *association* between frailty and HRV complexity measured by the WH-HRV FI or standardized frailty phenotype during exercise.

4.2 Findings from the Resting Cohort

The PNS branch of the ANS is responsible for our "rest and digest" functions and is responsible for the control of HR and BP when under resting conditions.[22] There are two common HRV measures

known throughout the literature that represent the activity of the PNS including RMSSD and HF. Four other measures represent activity of both the PNS and SNS including SDNN and TP (both measures of overall/global HRV), LF (measure of both PNS and SNS activity), and LF/HF (balance between SNS and PNS). With this information and knowledge of the actions of each branch of the ANS we can determine whether there are impairments in the ANS and where these may stem from.

At rest, our analysis determined that there was a trend towards a decrease in LF_{log} and LF/HF_{log} in those who were classified as frail compared to those who were identified as prefrail when measuring frailty level with the WH-HRV FI. There were no other trends or significant values found when using an ANOVA to detect differences in HRV among frailty levels in the resting cohort. These results tell a few key pieces of information. First, both LF_{log} and LF/HF_{log} (measures of both PNS and SNS modulation and the balance between the two branches) were trending lower in those who are frail and there were no differences in HF_{log} (indicator of PNS activity in resting conditions) among frailty levels. Because there were no differences in HF_{log} this tells us that there were no differences in PNS activity, but lower values of LF_{log} and LF/HF_{log} indicates that there may be an impairment in SNS modulation at rest in those who are frail.

To add to the knowledge of HRV at rest in frailty, we completed a multiple regression analysis. A regression allowed us to use the frailty measures as continuous variables instead of limiting frailty to three specific categories. It also allowed us to add BP medication use as a controlling factor. This regression found that as frailty level increased, there was significantly lower values of LF_{log} as measured by the WH-HRV FI. There was also a trend towards lower values of both $SDNN_{log}$ and TP_{log} with the WH-HRV FI. Similar trends were seen with the standardized frailty phenotype. This information reinforces that there are differences in LF_{log} in those with higher levels of frailty. Trending values of $SDNN_{log}$ and TP_{log} indicate that in those with higher levels of frailty there was less global variability in HRV meaning there was reduced autonomic modulation, regardless of the branch of the ANS (PNS or SNS).

The results from both the ANOVA and regression analysis indicate a reduction in the values of all variables that represent activity of both branches of the ANS in those who had a higher level of frailty (SDNN, TP, LF, LF/HF) but with no differences in those variables that represent only PNS activity (RMSSD, HF). This indicates that while there may be no difference in PNS, the SNS may be impaired in those who are frail. Previous studies have supported these findings including a study done by Chaves et al.[31] who also found a reduction in SDNN, TP, LF and LF/HF with no changes in HF in those who had higher levels of frailty. The authors noted that this indicated a higher sympathetic modulation at rest in participants who were frail compared to those who were robust or pre-frail.[4,31] A reduction in LF was also reported as an increase in SNS activity by Shaffer et al. While Katayama et al.[9] also found a trend towards a reduction in SDNN and LF in those who were frail and pre-frail compared to robust they also found a trend towards lower HF modulation indicating both an increase in SNS and a reduction of PNS activity in frail participants. These differences may be due to sample differences including sample size or an older age group in the Katayama study or other methodological differences including being supine instead of sitting upright as the participants were in our cohort.[9,22]

4.3 Findings from the Exercise Cohort

As we initiate activity, this triggers receptors that produce a rapid increase in HR. This process is largely mediated by a decrease in PNS activity with a increase in SNS activity as intensity increases, with a complete withdrawal of the PNS happening at peak intensity.[38] In a healthy population there tends to be a decrease in overall HRV during exercise as the SNS branch increases and PNS decreases in activity.[38] Similar to in the resting cohort, we can interpret exercise induced HRV by looking at the response of multiple HRV variables. During exercise an ANCOVA that adjusted for exercise intensity (RPE) identified longer mean RR_{log} intervals (inverse of HR) in those who were frail compared to those who were pre-frail or robust as measured by the standardized frailty phenotype. It also identified a reduction of $SDNN_{log}$ and HF_{log} in those who were pre-frail compared to those who were frail. Lastly,

LF_{log}, TP_{log} and ApEn were all approaching significance with the differences being between pre-frail and robust groups. This was all identified with the standardized frailty phenotype. There were no trends or significant differences in the ANCOVA results when frailty was measured using the WH-HRV FI in the exercise cohort.

Building on the ANCOVA, a regression was used to determine association between HRV and frailty while adjusting for both BP medication use and exercise intensity (RPE). This adjusted regression (Model 3) identified that similar HRV variables were associated with frailty regardless of which frailty tool was used (WH-HRV FI or standardized frailty phenotype). This included mean RR_{log}, TP_{log}, LF_{log} and $SDNN_{log}$. During moderate self-paced exercise mean RR_{log} (inverse of HR) was positively associated with frailty level, with the average RR_{log} interval increasing as frailty increased. This may mean that there is an impaired ANS response in those who are frail. This is supported in the literature as both Romero-Ortuno et al.[33] and Weiss et al.[32] found a significantly lower change in HR response to moderate exercise in those who were identified as frail. Model 3 (adjusted for BP medication use and exercise intensity) also identified an inverse relationship between TP_{log} (measure of global HRV) and frailty, also regardless of frailty tool being used. This means that as frailty level increases there is a decrease in global HRV. These two variables indicate that there is a longer average RR_{log} interval and reduced TP_{log} during exercise in those with higher levels of frailty showing that those who are frail may have a harder time adapting to external changes and they have a reduced global HRV.

However, the next two variables, LF_{log} and $SDNN_{log}$ (two variables that are often correlated in the literature), differ in the direction of their association depending on which frailty measure was being used. When measuring the association of HRV and frailty with the WH-HRV FI there was a significant positive association with LF_{log} and $SDNN_{log}$ but when frailty was measured with the standardized frailty phenotype there was a significant inverse association with LF_{log} and $SDNN_{log}$. The reason behind this difference is unknown; however, there are two key factors that may help explain these

contradictory results with both of them being directly related to the frailty measure being used. First, the percentage of participants that were frail were greatly different based on the tool being used. For example, the majority of participants (60%) were identified as robust with the frailty phenotype, where the majority of participants (62%) were identified as pre-frail with the WH-HRV FI. These differences in where the population may fall in the range of frailty may influence how the results are being interpreted. Associated with this explanation is the second key factor, which is the idea that different frailty measures may be appropriate depending on the needs and priorities of different settings.[53] This difference stems from the idea that different frailty scores measure different constructs or clinical presentations of frailty.[53] For example, an FI includes a much larger number of health deficits across a wider range of health systems where a phenotype method focuses more on the physical presentation of frailty. Knowing this, we can see that our WH-HRV FI includes only 20% of physical activity related measurements, whereas the phenotype has 60%. This divergence in what physical activity related deficits are included in the frailty tool may help explain why the two exercise measures identified different HRV responses to exercise as they may not be measuring the same clinical presentation of frailty. However, because this is the first study to use both frailty measurement approaches to look at the relationship with HRV further research is needed to determine if this divergence among tools is consistent among populations.

4.4 Strengths and Limitations

A key strength of this study was the inclusion of both a FI and a phenotype method of classifying frailty as these are the two most commonly used methods of measuring frailty within the literature. This is also a key strength as a FI was not previously used when looking at the connection between frailty and HRV.[4]

A second strength was that we were able to include both a rest and an exercise measure or HRV as we wanted to compare these two measures to see the exercise induced response. Being able to see how HRV may be affected by frailty level both at rest and during exercise many give an early indication of impairment if resting values were normal but did not respond as expected with an increase in exercise. However, this was also a limitation as we were not able to compare resting and exercise data. This was due to two main considerations. The first was that the resting data and the exercise data consisted of two separate populations with only a few participants being included in both groups due to technological complications. The second is that previous HRV literature has stated that you cannot compare different lengths of data collection for HRV.[22] This is not recommended as different lengths capture different cardiac fluctuations such as circadian rhythms or activity of the renin-angiotensin system, and that they may also capture different environmental stimuli influencing the variability in the results.[54] With this research study we had access to 5-minutes of resting HRV data and only 2-minutes of exercise HRV data. The decision was made to use only 2-minutes during exercise to ensure we had steady, high-quality data within this cohort as a stationary or steady signal is required to make an accurate HRV assessment.[51]

We realized during data analysis that there were technological complications when collecting HRV data due to drops in the Bluetooth connection of the HR monitor to the accelerometer. This included both short drops in measurement and/or completely missing data for participants. This drastically reduced the number of expected participants and impacted our opportunity to compare resting and exercise data as most participants had either high-quality resting data or high-quality exercise data and not both. To problem solve this, we started with turning off all technology that could connect to Bluetooth to limit the chance of the HR monitor trying to connect to a different device. These changes improved the data quality and will continue to be used in the WARM Hearts research project after recruitment can continue once the University of Manitoba and St. Boniface Research Centre deem it safe to do so due to COVID-19 restrictions. If this Bluetooth continues to be a

complication there is the option of incorporating an ECG to get a better connection as this is still the gold standard method of collecting RR interval data.[55]

Lastly, a limitation is the sample size within this thesis. The number of participants available for data analysis was limited for two main reasons. The first is due to COVID-19, as we were not able to continue data collection as long as expected, leaving us with a limited number of expected participants. Second, were the complications with collecting HRV data, which greatly reduced the number of available participants. This limited sample size meant that we may have been underpowered to detect HRV differences across frailty levels.

4.5 Future Research Directions

The relationship between HRV and frailty is an important area of frailty research that may help with the physiological understanding of frailty. For example HRV is a indirect measurement of the ANS which is a dynamic system that is consistently adjusting to maintain homeostasis within the human body.[13] HRV is also considered to be an indirect measure of the level of complexity with more complexity being related to higher resilience and a better ability to adapt to everyday changes.[4,13] More research on how this idea of resilience and complexity should be explored to gain a deeper understanding of how this may be presented with the concept of frailty. Further, the idea that different constructs of frailty may be measured using different tools should be explored within this setting to determine which frailty tool is the most appropriate to explore this relationship both at rest and during exercise.

Although we wanted to be able to compare the resting and exercise data in the same individuals to look at the response in HRV we were not able to use the same cohort of individuals for each measure (rest vs. exercise) due to the technological complications. However, the results that are presented in this thesis help to show that HRV is affected both at rest and during exercise, which shows that those who are frail may have an impairment in their ability to adapt to physical changes, reducing their

ability to maintain homeostasis. Further, if the exercise induced response is impaired in those who are frail, more research should be done to understand whether this can be improved with specific interventions, such as participating in physical activity, as it has been shown that exercise can improve variability.[30] Future research should continue to look at this exercise induced HRV response to further explain the impairment in those who have higher levels of frailty and whether or not this can be improved. With this, technological developments may need to be made to look at HRV during higher intensity activities. Currently, the most effective way to measure higher intensity activity is with the gold standard method of a Holter monitor or with a research grade ECG system.[55] However, a more accessible and affordable wearable device for daily living should be studied so the public can access and benefit from this technology.

Further, large population, longitudinal based studies that include multiple frailty and HRV measures in a more diverse population should be performed to better describe the relationship and covariates that may be influencing this relationship. For example, a large portion of the studies that have looked at the relationship between frailty and HRV have included female populations as women have largely been overlooked in cardiovascular research however this relationship should also be explored in other populations.[4]

Lastly, there has been some investigation into whether or not current statistical approaches are the best way to analyze HRV data. For example, it may be appropriate to utilize more complex mathematical approaches, such as a quadratic equation instead of a linear equation, to examine HRV data.[56] This was suggested by Goldberger et al.[56] as it appears that a quadratic fit was superior for four of the most common HRV measures including SDNN, RMSSD, LF, HF. It may also be appropriate to re-think how each HRV variable represents each division or function of the ANS, as there are some controversies throughout the literature surrounding which branch or activity each of the HRV variables represent.[57] This is because these variables have not always responded as expected when certain stimuli have been applied, but the cause of this is still unknown.[57] Overall, HRV is a valuable area of research;

however, methodological and analytical advancements may be needed to more optimally assess the functions of the different branches of the ANS.[58]

4.6 Conclusions

The results presented in this thesis support the hypothesis that there is an impairment in the activity of the ANS in those with increased levels of frailty. An ANOVA identified trending differences of LF_{log} and LF/HF_{log} ratio among frailty levels at rest and a multiple regression then confirmed these results with a significant association between lower levels of LF with higher levels of frailty along with trending values of $SDNN_{log}$ and TP_{log}. These results indicated an impairment in SNS activity at rest in those with higher levels of frailty. During exercise, an ANCOVA identified significant and trending values of mean RR_{log}, $SDNN_{log}$, HF_{log}, LF_{log}, TP_{log} and ApEn. A multiple regression identified a positive association between frailty and mean RR_{log} and an inverse association of TP_{log}. These results indicated an impairment in the ability to adapt and a reduced global HRV in those who were frail. There were inconsistent results regarding the direction of the association with the HRV variables $SDNN_{log}$ and LF_{log} between the two different frailty measures (WH-HRV FI and standardized frailty phenotype). Identifying that there is an impairment in the SNS at rest may be able to enhance tools to identify earlier stages of frailty, along with monitoring interventions to improve frailty status. Identifying the differences during exercise is also important as this may allow an even earlier detection of the ANS impairment in the pre-frail or frail population.

The FI was able to detect more differences at rest, where the standardized frailty phenotype was able to detect more differences during exercise. This difference may be directly related to the number of physical measures included in each frailty tool along with the distribution of frailty between the two tools; however, further research is required. The concept of different frailty measures being appropriate during different circumstances depending on priorities should continue to be explored and applied to the HRV and frailty relationship.

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Appendix A – STROBE Checklist

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	9-20
Objectives	3	State specific objectives, including any prespecified hypotheses	21-22
Methods			
Study design	4	Present key elements of study design early in the paper	22
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	24
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	23
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	24-29
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	24-29
Bias	9	Describe any efforts to address potential sources of bias	--
Study size	10	Explain how the study size was arrived at	27
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	--
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	27
		(*b*) Describe any methods used to examine subgroups and interactions	--
		(*c*) Explain how missing data were addressed	--
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	--
		(*e*) Describe any sensitivity analyses	--
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	23
		(b) Give reasons for non-participation at each stage	--
		(c) Consider use of a flow diagram	--
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	31-32
		(b) Indicate number of participants with missing data for each variable of interest	--
Outcome data	15*	Report numbers of outcome events or summary measures	--
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	34-45

1

		(*b*) Report category boundaries when continuous variables were categorized	--
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	--
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	--
Discussion			
Key results	18	Summarise key results with reference to study objectives	46-47
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	51-52
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	47-51
Generalisability	21	Discuss the generalisability (external validity) of the study results	53
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	--

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix B – SAGER Guidelines

General Principles		Completed
Authors should use the terms *sex* and *gender* carefully in order to avoid confusing both terms.		✓
Where the subjects of research comprise organisms capable of differentiation by sex, the research should be designed and conducted in a way that can reveal sex-related differences in the results, even if these were not initially expected.		✓
Where subjects can also be differentiated by gender (shaped by social and cultural circumstances), the research should be conducted similarly at this additional level of distinction.		✓
Title and abstract	If only one sex is included in the study, or if the results of the study are to be applied to only one sex or gender, the title and the abstract should specify the sex of animals or any cells, tissues and other material derived from these and the sex and gender of human participants	✓
Introduction	Authors should report, where relevant, whether sex and/or gender differences may be expected.	✓
Methods	Authors should report how sex and gender were taken into account in the design of the study, whether they ensured adequate representation of males and females, and justify the reasons for any exclusion of males or females.	✓
Results	Where appropriate, data should be routinely presented disaggregated by sex and gender. Sex- and gender-based analyses should be reported regardless of positive or negative outcome. In clinical trials, data on withdrawals and dropouts should also be reported disaggregated by sex.	✓
Discussion	The potential implications of sex and gender on the study results and analyses should be discussed. If a sex and gender analysis was not conducted, the rationale should be given. Authors should further discuss the implications of the lack of such analysis on the interpretation of the results.	✓

Appendix C – Demographic Information and Variables

Characteristic	Description/Collection Methods
Age	Calculated from date of birth that was reported during appointment 1 (dd/mm/yyyy)
Height	Measured using a stadiometer in centimeters (cm)
Weight	Measured using an InBody 270 scale in kilograms (kg)
BMI	Calculated using height and weight obtained from direct measurement (kg/m^2)
Living Arrangement	Collected through self-reported questionnaire by asking the question: *"Living Arrangement:"* Response options: • Alone • With spouse/partner • With relative
Level of Education	Collected through self-reported questionnaire by asking the question: *"What is your highest level of education completed?"* Response options included: • No Degree/certificate/diploma • High School • Post-Secondary
Tobacco Use	Collected through self-reported questionnaire by asking the question: *"Have you ever smoked tobacco?"* Response Options: • Never use tobacco • Currently use • Ex-tobacco user
Diabetes Diagnosis	Collected through self-reported questionnaire by asking the question: *"We are interested in conditions diagnosed by a health care professional. Have you been diagnosed with the following:* • *Type 1 Diabetes* • *Type 2 Diabetes*
High Blood Pressure Diagnosis	Collected through self-reported questionnaire by asking the question: *"We are interested in conditions diagnosed by a health care professional. Have you been diagnosed with the following:* • High Blood Pressure

Appendix D – WH 42 Item Frailty Index

As noted in the methods section, three variables from this list were removed for the purpose of this thesis as they had <1% prevalence in the 125 people being used in the sample. These three variables are 5 M Gait Speed Test, Paffenbarger PA Questionnaire, and HDL Cholesterol. After these three variables have been removed, the FI will be referred to as the WH-HRV FI.

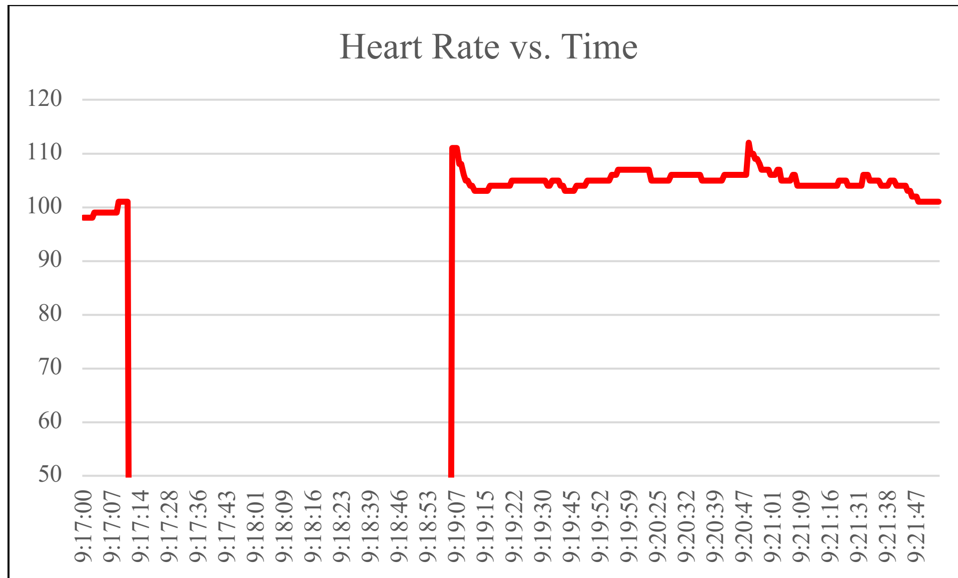
#	Domain	Tool used in WARM Hearts to Measure	Variable	Cut Offs Points	References
1	Physical	Hand Dynamometer	Grip Strength	[BMI ≤ 23, 1=≤ 17 kg, 0=>17 kg], [BMI 23.1-26, 1=≤ 17.3 kg, 0=>17.3 kg], [BMI 26.1-29, 1=≤ 18 kg, 0=>18 kg], BMI >29, 1=≤21 kg, 0=>21 kg]	Fried (2001)
2	Physical	Seniors Fitness Test	Chair Stand	[Age 50-64: 1=<12, 0=≥12], [Age 65-69: 1=<11, 0=≥11], [Age 70-74: 1=<10, 0=≥10], [Age 75-79: 1=<10, 0=≥10], [Age 80-84: 1=<9, 0=≥9], [Age 85-89: 1=<8, 0=≥8], [Age 90-94: 1=< 4, 0=≥ 4]	Rikli & Jones (2002)
3	Physical	5 M Gait Speed Test	Gait Speed	[Height ≤ 159 cm - 1=≤ 0.76 m/s, 0=>0.76 m/s], [Height >159 cm - 1=≤ 0.65 m/s, 0=>0.65 m/s]	Fried (2001)
4	Physical	Paffenbarger PA Questionnaire	Self Report PA	1= < 270 kcal/week, 0= ≥ 270 kcal/week	Fried (2001)
5	Physical	Seniors Fitness Test	Arm Curls	[Age 50-64: 1=<13, 0=≥13], [Age 65-69: 1=<12, 0=≥12], [Age 70-74: 1=<12, 0=≥12], [Age 75-79: 1=<11, 0=≥11], [Age 80-84: 1=<10, 0=≥10], [Age 85-89: 1=<10, 0=≥10], [Age 90-94: 1=<8, 0=≥8]	Rikli & Jones (2002)

6	Physical	Seniors Fitness Test	Back Scratch	[Age 50-64: 1=<-3, 0=≥-3], [Age 65-69: 1=<-3.5, 0=≥-3.5], [Age 70-74: 1=<-4.0, 0=≥-4.0], [Age 75-79: 1=<-5, 0=≥-5], [Age 80-84: 1=<-5.5, 0=≥-5.5], [Age 85-89: 1=<-7, 0=≥-7], [Age 90-94: 1=<-8, 0=≥-8]	Rikli & Jones (2002)
7	Physical	Seniors Fitness Test	Sit and Reach	[Age 50-64: 1=<-0.5, 0=≥-0.5], [Age 65-69: 1=<-0.5, 0=≥-0.5], [Age 70-74: 1=<-1, 0=≥-1], [Age 75-79: 1=<-1.5, 0=≥-1.5], [Age 80-84: 1=<-2, 0=≥-2], [Age 85-89: 1=<-2.5, 0=≥-2.5], [Age 90-94: 1=<-4.5, 0=≥-4.5]	Rikli & Jones (2002)
8	Physical	Seniors Fitness Test	8Ft Up and Go	[Age 50-64: 1=>6.0, 0=≤6.0], [Age 65-69: 1=>6.4, 0=≤6.4], [Age 70-74: 1=>7.1, 0=≤7.1], [Age 75-79: 1=>7.4, 0=≤7.4], [Age 80-84: 1=>8.7, 0=≤8.7], [Age 85-89: 1=>9.6, 0=≤9.6], [Age 90-94: 1=>11.5, 0=≤11.5]	Rikli & Jones (2002)
9	Physical	Seniors Fitness Test	6 Min Walk Test	[Age 50-64: 1=<498.35, 0=≥498.35], [Age 65-69: 1=<457.20, 0=≥457.20], [Age 70-74: 1=<438.91, 0=≥438.91], [Age 75-79: 1=<397.76, 0=≥397.76], [Age 80-84: 1=<352.04, 0=≥352.04], [Age 85-89: 1=<310.90, 0=≥310.90], [Age 90-94: 1=<251.46, 0=≥251.46]	Rikli & Jones (2002)
10	Weight	Tape Measure	Waist Circumference	1=>88 cm, 0=≤ 88 cm	Kehler (2020)
11	Body Comp	InBody	Fat Mass (kg)	1=<18.2 or ≥36.1, 0=18.2-36.099	Kehler (2020)
12	Body Comp	InBody	Fat-Free Mass (kg)	1=<40.9, 0.75=40,9-<43.3, 0.50=43.3-<45.8, 0.25=45.8-<49.3, 0=≥49.30	Kehler (2020)
13	Mood/Depression	PHQ-9	PHQ-9 Score	1=20-27 (severe), 0.80=15-19 (moderately severe), 0.60=10-14 (moderate), 0.40=5-9 (mild), 0.20=1-4 (minimal), 0=0 (none)	Kroenke (2001)
14	Mood/Depression	"Medical Conditions"	Clinical Diagnosis of Depression/Anxiety	1=yes, 0=no	

15	Cognition	MoCA	MoCA	1= ≤ 25, 0= ≥ 26	Nasreddine (2006)
16	Nutrition	Self Report Questionnaire	Unintentional Weight Loss	1=yes, 0=no	Fried (2001)
17	Nutrition	Self Report Questionnaire	Decline in Food intake	1=yes, 0=no	Reber (2019)
18	Nutrition/QOL	SCREEN II	Is biting or chewing food difficult for you?	1=Often or always, .6666=Sometimes, .3333=Rarely, 0=Never	
19	Nutrition/QOL	SCREEN II	Do you have any problems getting your groceries?	1=Always, .6666=Often, .3333=Sometimes, 0=Never or Rarely	
20	Exhaustion	CES-D	Feel everything was an effort	1=Most (3), .6666=Moderate (2), .3333=Sometimes (1), 0=Rarely (0)	Fried (2001)
21	Exhaustion	CES-D	Could not "get going"	1=Most (3), .6666=Moderate (2), .3333=Sometimes (1), 0=Rarely (0)	Fried (2001)
22	Quality of Life	EQ-5D-5L	Mobility	1=Extreme, 0.75=Severe, 0.50=Moderate, 0.25=Slight, 0=None	Janssen (2008)
23	Quality of Life	EQ-5D-5L	Self Care	1=Extreme, 0.75=Severe, 0.50=Moderate, 0.25=Slight, 0=None	Janssen (2008)
24	Quality of Life	EQ-5D-5L	Usual Activities	1=Extreme, 0.75=Severe, 0.50=Moderate, 0.25=Slight, 0=None	Janssen (2008)
25	Quality of Life	EQ-5D-5L	Pain/Discomfort	1=Extreme, 0.75=Severe, 0.50=Moderate, 0.25=Slight, 0=None	Janssen (2008)
26	Quality of Life	EQ VAS	Rate Health today	1-(EQ-VAS score/100)	Janssen (2008)
27	Comorbidities	"Medical History"	Diabetes	1=yes, 0=no	Kehler (2017)
28	Comorbidities	"Medical History"	Arthritis	1=yes, 0=no	Kehler (2017)
29	Comorbidities	"Medical History"	Sleep Apnea	1=yes, 0=no	Kehler (2017), Kehler (2020)
30	Comorbidities	"Medical History"	Cancer	1=yes, 0=no	Kehler (2017)

31	Comorbidities	"Medical History"	IBD	1=yes, 0=no	
32	Comorbidities	"Medical History"	Asthma	1=yes, 0=no	Kehler (2020)
33	Lab Values	Blood Pressure	Resting Systolic BP	1= <90 or >140 mmHg, 0= 90-140 mmHg	Kehler (2020)
34	Lab Values	Blood Pressure	Pulse Pressure	1= <30 or >60 mmHg, 0= 30-60 mmHg	Kehler (2020)
35	Lab Values	Heart Rate Monitor	Resting HR	1= <60 or >99 bpm, 0= 60-99 mmHg	Kehler (2017), Kehler (2020)
36	Lab Values	Blood Draw	Glucose (Fasting)	1=<3.9 or >6.1 mmol/L, 0=3.9-6.1 mmol/L	Kehler (2017), Kehler (2020)
37	Lab Values	Blood Draw	Total Cholesterol	1= > 6.2 mmol/L, 0= ≤ 6.2 mmol/L	Kehler (2020)
38	Lab Values	Blood Draw	Triglycerides	1= ≥ 1.67 mmol/L, 0= <1.67 mmol/L	Kehler (2020)
39	Lab Values	Blood Draw	HDL Cholesterol	1= < 1.03 mmol/L, 0= ≥ 1.03 mmol/L	Kehler (2020)
40	Lab Values	Blood Draw	LDL Cholesterol	1= <0.98 or >3.36 mmol/L, 0= 0.98-3.36 mmol/L	Kehler (2020)
41	Sleep Quality	PSQI	PSQI	1=5-21, 0=0-4	Tsai (2005)
42	Medications	# of Medications	# of Medications	1=≥5, 0=0-4	

Appendix E – HRV Data Collection Errors



This is a graph showing an example of the 5-minute resting segment and the technological complications that we had while collecting HRV data. This graph shows that there were periodical drops in Bluetooth signal during the 5-minute resting segment. This happened while collecting both resting and exercise data.