

Novel Biomarkers of Frailty in Middle-Aged and Older Females

by

Kevin Francis Boreskie

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Department of Applied Health Sciences

University of Manitoba

Winnipeg, Manitoba, Canada

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ABSTRACT

Generalized health declines and concomitant comorbidities will become an increasing concern for an aging population. Specifically, the burden of frailty and its associated health complications, including cardiovascular disease, is becoming a critical health concern. Frailty is a multifactorial physiological decline that increases vulnerability to health stressors. There are many different means of assessing frailty, but there is agreement on its ability to predict adverse health outcomes including hospitalization, the onset of comorbidities such as cardiovascular disease, and mortality.

Blood-based biomarkers have been suggested as a means of identifying frailty declines earlier, facilitating a better understanding of the pathophysiological decline seen in frailty, and offer a potential means of therapeutic intervention. Specifically, myokines, muscle-derived cytokines involved in inter-organ communication, have emerged as potential biomarkers of both frailty and cardiovascular disease given the deterioration in muscle health seen in these populations and the suspected associated physiological effects of myokines in relation to these declines. This dissertation investigates the association of myokines and bone metabolism analytes with frailty and associated cardiovascular disease risk through a series of studies utilizing both univariable and multivariable analyses.

Following an overview chapter, Chapter 2 of this thesis discusses the application of frailty assessment to the clinical setting. This context is important to explain the variety of frailty assessments currently available and their utility in a variety of settings to contextualize where biomarkers of frailty could fit into health promotion strategies for older adults.

Chapter 3 and 4 examine the association of univariable systemic myokine concentrations related to muscle and bone health to phenotypic frailty status and cardiovascular disease risk status using data from a cohort study examining novel cardiovascular disease risk factors. These works identified the immune modulating fractalkine/CX3CL1 as being associated with frailty, potentially indicating early immune dysregulation. IL-6, IL-15, TNF α , myokines associated with inflammation, and Leptin, which plays a role in metabolism, were associated with cardiovascular disease risk. These results support expert opinion and prior works examining myokine association with aging.

Chapter 5 utilizes a multivariable design to examine myokines associated with frailty in a larger data set analyzed from the WARM (Women's Advanced Risk Assessment in Manitoba)

Hearts cohort study based on current expert recommendation for both a phenotypic assessment of frailty and a frailty index. The stepwise multivariable regression analysis identified BDNF, FABP-3, OSM and osteonin in the model for the frailty index. These data support the association of myokines related to inflammation and metabolic function with frailty, as well as with cognition. In contrast, only SPARC, a myokine related largely to muscle function and mass, added to the model predicting Fried score.

These preliminary works support the notion of myokines acting as biomarkers of frailty with those involved in inflammation and metabolic function seeming to be most likely to add value to such a model. This work also supports the notion that the interplay of multiple myokines in a multivariable model did change results from univariable myokine associations, supporting expert opinion on the inclusion of a variety of biomarkers as opposed to searching for one unifying marker. These myokines could add value to a frailty index incorporating biomarkers for the detection of early frailty. Future work should further elucidate the physiological role myokines regulate in the human body, determine the optimal means of measuring myokines in a clinically feasible method, health interventions to modulate their expression and release, and clinical outcomes following this modulation.

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ABBREVIATIONS

ADL – Activities of daily living

ANOVA – Analysis of variance

aPWV – aortic pulse wave velocity

APLN – Apelin

ASCVD - Atherosclerotic Cardiovascular Disease

AVOID – Activity, vaccines, optimize medications, interaction, diet and nutrition

BDNF – Brain-derived neurotrophic factor

BMI – Body mass index

BP – Blood pressure

CFN – Canadian Frailty Network

CFS – Clinical Frailty Scale

CGA – Comprehensive Geriatric Assessment

CI – Confidence interval

CPAG – Canada’s Physical Activity Guidelines

CRP – C-reactive protein

CSEP – Canadian Society for Exercise Physiology

CVD – Cardiovascular disease

ED – Emergency Department

EDTA – Ethylenediaminetetraacetic acid

eFI – Electronic frailty index

EFS – Edmonton Frail Scale

EFT – Essential Frailty Toolset

EPO – Erythropoietin

EQ-5D-5L – EuroQol-5 Dimension-5 Level

EQ-VAS – EuroQol Visual-Analog Scale

FABP3 – Fatty acid-binding protein 3

FC – Fried Criteria

FFP – Fried frailty phenotype

FGF21 – fibroblast growth factor 21

FI – Frailty Index

FRAIL – Fatigue, Resistance, Ambulation, Illnesses & Loss of Weight

FRS – Framingham Risk Score

FSTL-1 – Follistatin-like 1 protein

GDF 8 – Growth differentiation factor 8

HDL – High density lipoprotein

HFRS – Hospital Frailty Risk Score

ICD-10 – International Statistical Classification of Diseases and Related Health Problems, Tenth Revision

ICU – Intensive Care Unit

IL-6 – Interleukin-6

IL-15 – Interleukin-15

IPAQ – International Physical Activity Questionnaire

ISAR – Identification of Seniors At Risk

LDL – Low density lipoprotein

LIF – Leukemia inhibitory factor

mFI – Modified Frailty Index

MI – Myocardial infarction

MoCA – Montreal Cognitive Assessment

MSTN – Myostatin/growth differentiation factor 8

MVPA – Moderate to vigorous physical activity

OR – Odds ratio

OSM – Oncostatin m

OSTN – Osteocrin/musclin

PA – Physical activity

PHIN – Personal health information number

PHQ-9 – Patient Health Questionnaire-9

PPAS - Paffenbarger Physical Activity Scale

PSQI – Pittsburgh Sleep Quality Index

RDS – Rasmussen Disease Score

SAVR – Surgical Aortic Valve Replacement

SCREEN II – Seniors in the Community: Risk Evaluation for Eating and Nutrition, V. II

SD – Standard deviation

SPARC – Secreted protein acidic and rich in cysteine

STROBE – STrengthening the Reporting of OBservational studies in Epidemiology

TAVI – Transcatheter Aortic Valve Implantation

TFI – Tilburg Frailty Indicator

TGF beta – Transforming growth factor beta

TNF- α – Tumor necrosis factor alpha

TRST – Triage Risk Screening Tool

WARM Hearts – Women’s Advanced Risk Assessment in Manitoba Hearts

6MWT – Six-minute walk test

CHAPTER 1: OVERVIEW

1.1 Aging

In 2015, the World Health Organization released the *World Report on Ageing and Health* to outline that increases in longevity across the world have also created a progressively aging global population.¹ The global population is living longer than ever before in a large part due to advances in medicine and healthcare. Unfortunately, this increase in lifespan has not always led to an increase in ‘health span’ with later years of life often lived in sickness and/or disability.¹⁻³

While ageing in and of itself is not a disease, it is associated with increased risk for a host of chronic diseases.¹ Over the past century, infectious and communicable diseases are no longer the leading causes of death worldwide, with chronic disease now taking the forefront. In fact, chronic diseases were the cause of 71% of deaths worldwide in 2016.⁴ The relative percentage of deaths due to noncommunicable disease is higher in high-income countries at 88% attributable; however, 78% of the global burden of noncommunicable disease is in low and middle-income countries. More specifically, ischemic heart disease, stroke, chronic obstructive pulmonary disease, dementias, lung cancers and diabetes are all amongst the top ten global causes of death.⁴

There are many proposed theories of aging that may explain this increased risk for chronic disease through systemic physiological dysfunction. These include theories of accumulated

oxidative stress,⁵ cellular senescence,⁶ epigenetic modification,⁷ mitochondrial dysfunction⁸ and telomere attrition,⁹ amongst others. These physiological deficits can accumulate over time, leading to a state of dysfunction, functional and quality of life impairments, chronic disease and, ultimately, mortality.^{10,11}

In Canada, it is estimated that the proportion of older adults ≥ 65 years of age in Canada will increase from 18.5% in 2021 to between 21.6-29.8% in the year 2068.¹² With this changing demographic, the prevalence of noncommunicable diseases will likely increase as will their associated precipitating risk factors. Thus, it is of importance to identify effective strategies for addressing risk factors that may lead to noncommunicable disease, specifically in older adult populations.

The leading modifiable, common risk factors for premature death due to noncommunicable disease include physical inactivity, obesity, hypertension and smoking.^{13,14} Modifiable risk factors such as these offer an opportunity to potentially reduce the prevalence of noncommunicable disease and improve health. With an aging population and healthcare systems already facing strains,¹ it is critical that effective methods of identifying individuals in the population who are at increased risk of health declines are developed to allow for targeted health initiatives. Strategies such as these have the potential to ‘compress’ periods of morbidity to the end of life, increasing the health span of the population.^{1,3}

1.2 Frailty

Frailty is now being discussed as a novel risk factor for noncommunicable disease and other adverse health outcomes.^{15–22} Frailty can be characterized by impairments in homeostatic maintenance that can lead to a reduced reserve when facing physiological health insults.²³ This occurs when the dysregulation of multiple physiological systems impair the ability of the body to respond to these health stressors as it would in someone who is robust.²⁴

Frailty can be thought of as a continuum ranging from robust to severe cases of frailty²² with increasing dysregulation and increasing risk for adverse outcomes across this spectrum. Considered an intermediary stage on this continuum, pre-frailty precedes frailty and increases the risk of further frailty progression by up to four-fold over a three to five year period.¹⁵ The corollary of individuals living with pre-frailty maintaining more homeostatic reserve compared

to their peers living with frailty is that they are thought to be more likely to recover from physiological insults they may face.²⁵

The prevalence of frailty in the population is dependent on the tool used to assess it and the population being assessed, but examination of the Canadian Health Measures Survey found prevalence ranging from 7.8% to 20.2% in those 65 years of age or older.²⁶ A systematic review and meta-analysis done on the prevalence of frailty in 62 countries in 1,755,497 subjects ≥ 50 years of age found a prevalence of 12% to 24% depending on the tool used. Despite frailty being distinct from chronological aging, its prevalence is higher in older adults.²⁶⁻²⁸ As well, there is a higher prevalence of frailty in females despite their greater longevity.²⁷⁻³⁰ While this paradox is not fully understood, it is thought to likely be multifactorial with both sociocultural and biological drivers.^{29,30} With an aging population, the prevalence of frailty is expected to rise.³¹

Frailty is an independent predictor of adverse outcomes such as hospitalizations,¹⁵ falls,¹⁵ a variety of comorbidities^{15,21,32,33} and mortality.^{21,27,33} Thus, frailty assessment has been suggested for use in care triage and planning.³⁴ In fact, in a cohort of 493,737 from the UK Biobank, pre-frailty and frailty were associated with odd ratios (OR) of 1.32 (1.30-1.34) and 2.27 (2.12-2.42), respectively, for having one comorbidity present.³³ These ORs were drastically increased to 3.31 (3.21-3.42) and 27.1 (25.3-29.1) for pre-frail and frail individuals, respectively, once looking at the presence of four or more comorbidities. Often described as an assessment of biological age, it has been suggested that frailty may provide more value in clinical assessment of risk for chronic disease, as compared to chronological age.³⁵ Aging is a heterogeneous process and, as such, frailty acts as a better measure of health and is more strongly associated with comorbidity.³⁶ With increasing recognition of the role that psychosocial factors can also play in health, psychosocial factors such as depression,³⁷ strained financial resources³⁸ and living alone^{39,40} have also been implicated in the health declines seen in frailty.⁴¹

1.2.1 Frailty in Primary Care

Primary prevention, defined as identifying risk for chronic disease in advance of its presentation, is the most cost-effective method of avoiding the health and economic costs of chronic disease.⁴² Focusing on secondary or tertiary prevention is less cost-effective and can lead to patients living with reduced quality of life for extended periods of time, reducing their healthspan.^{3,11} With an aging population presenting with increasingly complex health situations

it is difficult to adequately screen for a wide variety of factors that might place one at risk for specific adverse outcomes. A pragmatic approach might be instead to screen for frailty at the primary care level in order to identify individuals who may be at risk for a host of adverse biopsychosocial outcomes.^{43–45} Individuals with pre-frailty or frailty could then be identified for therapeutic intervention to reduce risk for these outcomes. The potential health burden this syndrome places on the population is becoming more understood, as it is a dynamic syndrome with many clinical and social consequences.⁴⁶ From a cost perspective, for example, it is estimated that frailty results in an estimated excess cost of 6 billion pounds per year due to increase healthcare utilization in the United Kingdom.⁴⁷ In Canada, frailty status is similarly associated with increased healthcare utilization and costs. In a cohort of over 150,000 home care recipients in Ontario, frailty was associated with average increased 1-year healthcare costs of \$10,845 (95% CI \$10,112–11,698) per patient in those with dementia and \$12,360 (95% CI \$11,849–12,981) per patient in those without dementia, as compared to their robust peers.⁴⁸ The range of consequences related to the public health burden of frailty again touches on its potential role in achieving successful aging.^{46,49}

1.2.2 Pathophysiology of Frailty

Frailty is associated with a wide range of adverse health outcomes, but the etiology and physiological declines that eventually lead to pathology in this population is not fully understood.²⁴ In order to better understand the homeostatic imbalances and reduced physiological reserve seen in frailty, research has examined associations with factors such as dyslipidemia and inflammation in what is commonly referred to as chronic “inflammaging”^{50–53} as well as factors related to immunosenescence.⁵⁴ The pathophysiology of frailty is a complex process, which makes sense because it represents generalized declines covering a variety of physiological systems.⁵⁵ It is important to also note that frailty is distinct from both comorbidity, the presence of medical conditions, and disability, difficulty in performing functions of daily living, although there is overlap between these (Figure 1).⁵⁶

Figure 1. Frailty, Comorbidity and Disability

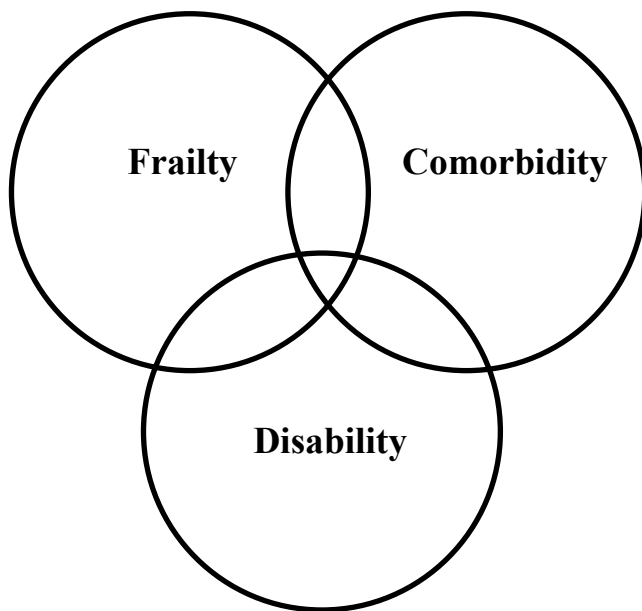


Figure 1 adapted from Fried et al.⁵⁶

Homeostatic imbalances paired with reduced physiological capacities as insulin resistance, chronic inflammation, and other aspects of metabolic syndrome can begin to exacerbate each other.⁵⁷ Wide ranging subclinical deficits that compile over the life course can be stressed beyond recovery in the context of a health stressor in an individual living with frailty who does not have the capacity to respond to such an event.²²

Physical activity and muscle health in frailty

Declines in muscle mass and/or quality are discussed as being a critical component of frailty progression regardless of the assessment methodology domain used.⁵⁴ Chronic inflammation, androgen deficiency and resulting insults to anabolic and catabolic balances can result in muscle wasting⁵⁷ – or what is known as sarcopenia. While muscle loss is a process that is thought to naturally occur with chronological aging,⁵⁸ it is often accelerated in frailty progression and can lead to a continually exacerbated cycle of physiological dysregulation and functional decline (Figure 2).⁵⁴

Playing a concurrent role with reduced muscle mass and quality in frailty is a reduction in physical activity.¹⁵ These factors often exacerbate each other, as reduced muscle mass/function

makes performing activity harder, leading to reductions in activity which lead to further reductions in muscle (Figure 2).¹⁵ Frailty is often associated with lower levels of moderate-to-vigorous physical activity and more sedentary behavior.^{59,60} The accumulation of deficits associated with physical inactivity develop into what has been described as the “diseasome of physical inactivity” – where the body is in a state of shared pathogenic mechanisms that can lead to adverse factors such as chronic inflammation through vicious cycles of physiological decline.⁶¹ Importantly, the presence of many of the adverse risk factors associated with physical inactivity are also associated with more sedentary behavior, further exacerbating the health declines seen.⁶² The recognition of disease states induced through the diseasome of physical inactivity, and its more generalized health decline, has led to the examination of factors that may mediate these declines. This includes intervention in frailty progression⁶³ and the newly understood action of muscle as an active endocrine organ.⁶⁴ This connection is further supported by the fact that the most effective frailty interventions to date are thought to be those that include aspects of physical activity.⁶⁵ Frailty interventions will be discussed further in section 1.3.4.

Figure 2. Physical activity, muscle health, and frailty

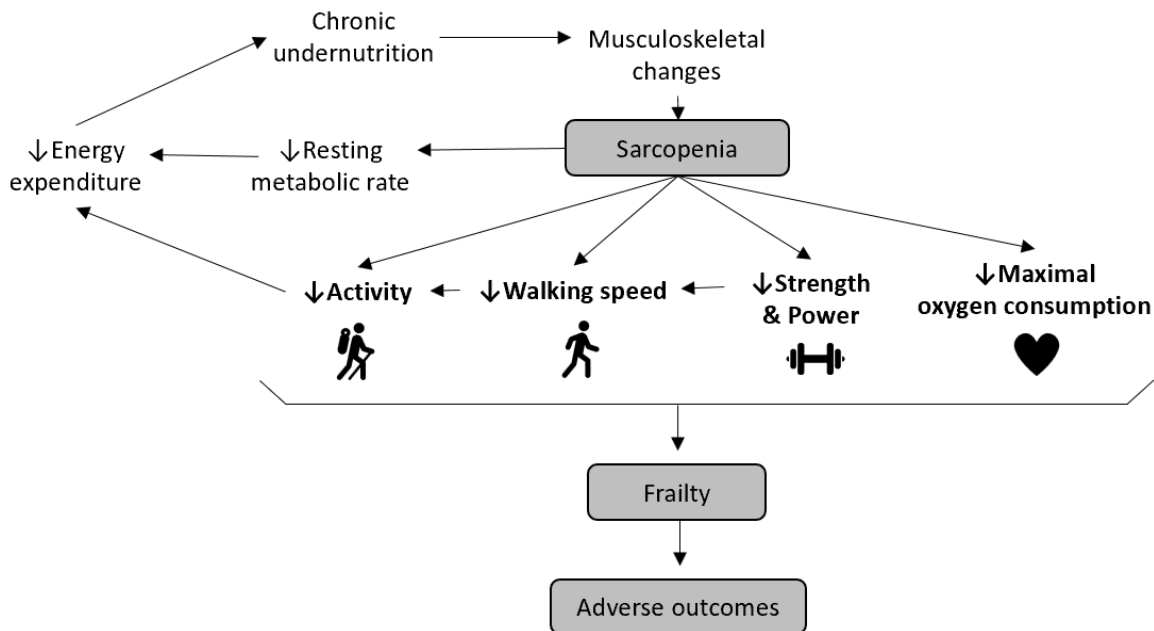


Figure 2 adapted from Fried et al.¹⁵

1.3 Detection of Frailty

There is no gold-standard frailty assessment method across all clinical contexts. In fact, over 50 assessments of frailty have been identified in the literature.^{66,67} The following section will identify major classifications of frailty assessment techniques, and then will discuss two of the most used frailty assessment approaches in detail.

1.3.1 Models of Frailty Detection

The Comprehensive Geriatric Assessment (CGA) may be the closest to filling the role as a gold-standard frailty assessment tool.^{68,69} A CGA is a interdisciplinary assessment to identify health limitations in older adults across a variety of domains such as social, psychological, environmental, physical, comorbidities, medications, as just a few examples.^{70–72} Assessment team composition can vary but are typically multidisciplinary with a geriatric specialized clinician. The limitation of the CGA is that it is often not feasible in many clinical settings because of the thoroughness required for such an assessment. Other models of frailty assessment

can be classified into one of three general categories: 1) phenotypic approaches; 2) multidimensional approaches; or 3) accumulation of deficits approaches, which are also multidimensional. Some of the more commonly used approaches in each of these domains are listed in Table 1. Each of these approaches have advantages and disadvantages with efficacy and feasibility being balanced to different extents based on the clinical context of their utilization.^{73,74} The detection of categorical frailty status has previously been compared between some of the most prominent tools in the literature.^{75,76} While the tools themselves do not agree well on categorical assignment of frailty status, likely as they are thought to examine different components of frailty, they do all act as predictors of adverse outcomes, and their use should be matched to the purpose and setting of assessment.^{67,73}

Phenotypic approaches focus on physical, clinically identifiable characteristics to identify frailty, such as weight loss, fatigue and low functional ability.¹⁵ These models of assessment have been critiqued for not being feasible in all settings and for focusing too heavily on solely phenotypic attributes of frailty while ignoring a large holistic picture of health with additional biopsychosocial aspects.⁷⁷ For example, many of these assessments rely on testing such as gait speed or grip strength assessments, which are not feasible to collect in all medical settings due to restrictions of the physical space, acuity of the patient presentation, and unfamiliarity with this type of testing.

As we gain knowledge on the potential importance of psychosocial domains in frailty,⁷⁸ it is thought that these factors should also be included into an assessment of frailty.⁷⁸ Frailty assessments built on multidimensional aspects focus on this wider variety of biopsychosocial health aspects in an attempt to look at frailty in a more holistic picture. The accumulation of deficits models, described as frailty indices, assess through examining an accumulation of deficits (i.e. sign, symptoms, disabilities, diseases and laboratory tests) that may be present in an individual.²² The main critiques of frailty indices are the sometimes lengthy questionnaires and/or plethora of information required to create these assessments that may not be feasible in some settings or with a lack of information depending on the components included.⁷⁹ Although, frailty index (FI) models have been created that rely on lab values,⁸⁰ or routinely collected electronic health records to ease this process,⁸¹ for example. Models of deficit accumulation have also received the critique that their creation could potentially include factors that are non-modifiable or may represent factors that represent disability, not frailty.

Table 1. Common Frailty Assessments

Assessment Name	Brief description	Citation
Phenotypic		
Fried Frailty Phenotype (FFP)	<i>Assesses five phenotypic domains: 1) muscle weakness; 2) slow walking speed; 3) low physical activity levels; 4) unintentional weight loss; 5) self-reported exhaustion</i>	Fried et al., 2001 ⁶³
FRAIL Scale	<i>Assesses five criteria via questionnaire: 1) fatigue; 2) resistance; 3) ambulation; 4) illness; 5) loss of weight</i>	Van Kan et al., 2008 ⁸²
Short Physical Performance Battery	<i>Assesses objective balance tests, a gait speed test and a chair stand test</i>	Guralnik et al., 1994 ⁸³
SHARE-FI	<i>Validated alternative to Fried that assesses similar criteria (weight loss is replaced by appetite and slowness is replaced by functional limitation)</i>	Romero-Ortuno et al., 2010 ⁸⁴
Multidimensional		
Comprehensive Geriatric Assessment (CGA)	<i>Detailed assessment of medical, psychosocial and functional independence</i>	Parker et al., 2008 ⁷⁰
Edmonton Frail Scale	<i>Brief screening interview that assesses cognition, general health status, functional independence, social supports, medication use, nutrition, mood and continence.</i>	Rolfson et al., 2006 ⁴⁴
Essential Frailty Toolset	<i>Recommended for use in cardiovascular surgery – assesses chair stand, cognitive impairment, hemoglobin and serum albumin</i>	Afilalo et al., 2017 ⁸⁵
Hospital Frailty Risk Score (HFRS)	<i>Uses ICD-10 codes to create a risk score through pre-existing routinely collected patient data.</i>	Gilbert et al., 2018 ⁸⁶
Accumulation of deficits		
Frailty Index (FI)	<i>Ratio between zero and one constructed by counting the number of existing deficits divided by the total number of deficits measured</i>	Searle et al., 2008 ⁸⁷
FI-Lab	<i>FI created using common clinical and laboratory tests</i>	Blodgett et al., 2017 ⁸⁰
Electronic FI (eFI)	<i>Uses routinely collected data in electronic health records to create an FI</i>	Clegg et al., 2016 ⁸¹

1.3.2 Fried Phenotype of Frailty

One of the most widely used models of frailty,⁸⁹ the Fried Frailty Phenotype⁶³ (FFP) was developed by Dr. Linda Fried using data collected from the Cardiovascular Health Study.⁹⁰ The FFP assesses five phenotypic aspects of frailty. Those being: 1) muscle weakness; 2) slow walking speed; 3) low physical activity levels; 4) unintentional weight loss; and 5) self-reported exhaustion. In her initial cohort, Fried found that creating cutoffs based on the lower quintile of the cohort in each of these aspects could create a scoring system that was able to identify individuals at risk for falls, hospitalization and mortality. Total scores for this assessment range from 0 to 5 (i.e. 0 = robust; 1-2 = pre-frail; ≥ 3 = frail) depending on the number of aspects present in the participant. Walking speed was originally assessed with the 15-ft walk test with lowest quintiles dependent on participant height, although a 5-meter gait speed test is now commonly used. Muscle weakness was assessed with a grip strength measure using hand dynamometer with cutoffs values stratified by body mass index (BMI) quartiles. Unintentional weight loss is assessed by using questionnaire asking, “In the last year, have you lost more than 10 pounds unintentionally (i.e., not due to dieting or exercise)?” If yes, then the participant was considered frail for this criterion. Exhaustion was assessed using a portion of the Center for Epidemiologic Studies – Depression Scale.⁹¹ Physical activity was characterized using the Paffenbarger Physical Activity Scale (PPAS).⁹² Based on this methodology, community prevalence has been found to be 5.3% in those 18-34 years of age, 5.7% in those 35-49 years of age, 6.9% in those 50-64 years of age, and 7.8% in 65+ years of age using data from the Canadian Health Measures Study.²⁶ International data from 62 countries in persons aged ≥ 50 years of age identified a frailty prevalence of 13% (95% CI 4-25%) using the FFP.²⁸

1.3.3 Rockwood Frailty Index

The Frailty Index (FI) model developed by Dr. Kenneth Rockwood is based on examining frailty as an accumulation of deficits.^{22,87} Deficits that have commonly been used in these models include clinical signs, symptoms, disabilities, disease presence and laboratory tests. An FI is a ratio presented by a value between zero and one that is calculated by counting the number of

existing deficits present divided by the total number of deficits assessed. A score of 0.25/1 or higher is used to classify an individual as being frail.⁹³ The following criteria are recommended when creating an FI:⁸⁷ (1) the deficit should be related to health status; (2) the prevalence of the selected deficits should increase with chronological age; (3) the prevalence of selected deficits should not saturate too early nor be collectively present at early ages or absent in older age; (4) the selected deficits should represent a broad range of physiological systems, and; (5) the same FI should be used serially in a cohort if multiple assessments are planned over time. Typically, the inclusion of around 30 variables is recommended for an FI.⁹⁴ Based on this methodology, community prevalence has been found to be 1.8% in those 18-34 years of age, 4.3% in those 35-49 years of age, 11.6% in those 50-64 years of age, and 20.2% in 65+ years of age using data from the Canadian Health Measures Study.²⁶ International data from 62 countries in persons aged ≥ 50 years of age identified a frailty prevalence of 24% (95% CI 22-26%) using the FI.²⁸ More specific uses of FIs have been introduced in the form of electronic FIs (eFI) using data that is commonly included in electronic health records to create a score to be used in primary care,⁸¹ FIs incorporating common clinical and laboratory tests (FI-Lab),^{80,95} and an FI-ED⁹⁶ using data that would be readily available in an emergency department presentation, as examples.

1.3.4 Frailty Interventions

Frailty is characterized by broad physiological declines, thus it is thought that multifaceted interventions likely would be the most efficacious approaches for intervention.^{97,98}

The lack of a consensus on the definition and components have frailty have made it difficult to design interventions,^{17,46} but this is further complicated by the need of different tools for different clinical settings to facilitate feasible and purposeful frailty assessment.^{73,74} Interventions have shown to benefit individuals living with frailty, but the ideal methodology for these interventions has not been identified – although, physical exercise provided in groups and nutritional supplementation both received a Level of Evidence of 1a for frailty intervention methodologies from a recent, comprehensive systematic review.⁹⁷ This indicates a strong recommendation with a high level of evidence supporting it. These methodologies will likely compose critical components of future frailty interventions and have also been supported in other subsequent reviews.⁶⁵ The Canadian Frailty Network recommends a general approach to

intervening in frailty trajectories through their AVOID acronym.⁹⁹ Specifically, they advocate for:

A) Activity: Multicomponent exercise including aerobic, strength, balance and flexibility training are recommended for frailty intervention.^{100,101} While they are not specific to frailty, Canada's Physical Activity Guidelines (CPAG) for Older Adults¹⁰² currently recommend 150 minutes of MVPA per week in bouts of ten minutes or more with at least two days per week of strength activities and balance training to prevent falls. Meeting the Canadian 24-hour movement guidelines¹⁰³ is associated with lower frailty prevalence.⁶⁰ Newer research points to the fact that physical activity in bouts of any length likely have a positive effect on health.¹⁰⁰ Multicomponent physical activity interventions are currently seen as being one of the most effective methods of intervening in frailty progression.⁹⁷ Reducing sedentary time should also be considered to prevent frailty progression as increased sedentary time is associated with higher frailty levels.^{59,104}

V) Vaccinate: Immunosenescence leads to immune dysregulation, which is often found in association with frailty.¹⁰⁵ Thus, frail individuals often present with an inability to fight off infection, placing increasing importance on receiving up to date vaccinations, such as the annual influenza vaccine, or receiving booster vaccinations, such as Tdap (tetanus, diphtheria and pertussis), in order to prevent complications and declines in health associated with infection.⁹⁹

O) Optimize medications: Polypharmacy has been associated with increased risk for developing frailty in a dose-response relationship.¹⁰⁶ Trying to optimize medication intake and examining potential interactions between drugs can help to prevent confusion, poor nutrient absorption and/or dizziness.⁹⁹

I) Interact: Holistic medicine through an additional focus on both psychosocial aspects of health, beyond just the biological, has received increasing recognition in the field of frailty.^{78,107} Cognitive and emotional impairments may play critical roles along with physical deficits as frailty is supposed to describe a syndrome effecting health in a broad, multidimensional fashion.⁷⁷

D) Diet and nutrition: Malnutrition, an unintentional nutrition intake imbalance,¹⁰⁸ is a risk factor associated with frailty¹⁰⁹ as it can lead to sarcopenia and dynapenia in older adults.^{110,111} Specifically, maintaining an adequate intake of protein¹¹² and vitamin D in those who are deficient¹⁷ is strongly recommended.

1.4 Cardiovascular Disease

Cardiovascular disease (CVD) can be used to describe disease or injury of the various parts of the cardiovascular system: the heart, the blood vessels of the heart and the vasculature throughout the rest of the body. Globally, CVD is the leading cause of death, representing 45% of all non-communicable disease deaths.¹¹³ The majority of this burden is faced by low and middle-income countries,¹¹⁴ but CVD is still the second-leading cause of death in Canada¹¹⁵ and the leading cause of hospitalization.¹¹⁶ Approximately 2.4 million Canadians 20 years of age or older live with diagnosed heart disease¹¹⁷ with an associated economic burden of \$20.9 billion.¹¹⁸ The incidence of CVD has decreased over the past decade, as has the death rate of those with CVD,¹¹⁷ but this will continue to carry a significant health burden with an aging population and increasing prevalence of obesity and diabetes – factors that are associated with CVD risk.¹¹⁹ While there are many distinct etiologies for CVD that can lead to pathological progression through a variety of routes, this thesis document will focus on chronic CVD that is typically developed through the development of atherosclerosis – vessel narrowing due to plaque build-up leading to ischemia.

1.4.1 Pathophysiology of Cardiovascular Disease

Chronic CVD develops over a long period of time due to accumulated damage and insults to the various components of the cardiovascular system.¹¹⁴ The most typical pathological development of chronic CVD is through the development of damage and plaque accumulation in arteries – also known as atherosclerosis.¹²⁰ Processes of oxidation and inflammation over time can cause damage to the arterial wall – composed of the intima, media and adventitia. This process can be aggravated through stressors like shear forces, toxins and excess systemic glucose.¹²¹ Damage to the endothelium leads to increased permeability and low-density lipoprotein (LDL) deposition which, in turn, causes further release of inflammatory markers. These markers signal monocyte infiltration leading to the development of macrophages that phagocytose the LDL creating foam cells. This is what is known as the development of “fatty streaks”. Later in the process, smooth muscle cells from the media migrate and proliferate in the subintima. The build-up of these plaques in this high inflammatory process can lead to partial occlusion of the vessels leading to ischemia downstream.¹²⁰ Vulnerable plaques with thin fibrous caps can also rupture leading to thrombosis and the potential for development of emboli that can cause infarction when arteries supplying vital organs like the heart or brain are occluded.¹²² The

process of atherosclerotic development is the leading cause of CVD¹²¹ as it can lead to clinical outcomes like acute coronary syndromes, peripheral artery disease, and stroke.¹²²

The development of the atherosclerotic process can be aggravated by the presence of biological and behavioral risk factors.¹¹⁴ The development of seminal longitudinal studies, like the Framingham Heart Study, has led to drastic improvements in our understanding of what precipitating factors lead to CVD pathological progression. Typically, these risk factors are divided into those that can be modified or managed with either lifestyle modification or pharmaceutical intervention and those that are non-modifiable. CVD risk factors commonly discussed in the literature that can be managed or modified include hypertension, diabetes, obesity, dyslipidemia, depression, physical inactivity, alcohol intake, smoking and poor diet.^{123–126} Non-modifiable risk factors include advanced age, genetic predisposition, gender and sex.^{120,121} The presence of or differences in these risk factors can lead to increased odds for the presence of inflammatory and oxidative processes that exacerbate the atherosclerotic process.¹²¹

There are a variety of different cardiovascular pathologies that derive from different physiological processes, although they share many of the same risk factors and same etiologies or can be the result of earlier infarction or ischemia.¹²⁷ These include clinical outcomes such as heart failure, valvular diseases or cardiac electrical issues. These clinical outcomes can either be a cause of or be a result of cardiac damage due to ischemia and infarction.¹²⁷ Congenital CVD would be a structural deficiency in the heart, the cardiac valves or the predominant vasculature that is present from birth.¹²⁸

1.4.2 Assessing Cardiovascular Disease Risk

An increased understanding of risk factors for CVD has led to the development of risk screening tools for use in the primary care setting. Currents efforts in Canada, as recommended by the Canadian Cardiovascular Society,¹²⁹ focus on the use of the Framingham Risk Score (FRS) which was developed through analysis of the data collected in the Framingham Heart Study.¹³⁰ Longitudinal data on the Framingham cohort was used to create a multivariate risk factor algorithm designed to assess 10-year risk for coronary artery disease, stroke, peripheral artery disease, heart failure and cardiovascular mortality.¹³¹ The variables used in the risk calculation are described in Table 3. The scoring system used for the FRS through the Canadian Cardiovascular Society is described in Appendix 1.

Table 2. Framingham Risk Score

FRS Variables	
Sex	Age
Systolic blood pressure	Treatment for hypertension
Smoking status	Presence of diabetes
High density lipoprotein	Total cholesterol
Risk classification (10-year risk %)	
Low risk (<10%)	
Intermediate risk ($\geq 10\%$ and $< 20\%$)	
High Risk ($\geq 20\%$)	

The American College of Cardiology and the American Heart Association have also developed the Atherosclerotic Cardiovascular Disease (ASCVD) risk score to assess risk for myocardial infarction or stroke.¹³² This score includes the same factors as the FRS but with the addition of race as a variable. The European Society of Cardiology has also developed its own risk score called the Systematic Coronary Risk Evaluation (SCORE).¹³³ This risk assessment tool uses the same variables as the FRS but examines total cholesterol and high-density lipoprotein (HDL) as a ratio and adds country of origin as an additional factor. This tool is only used to predict the percentage risk of cardiovascular mortality over a ten-year period.

Methods of improving the above global CVD risk scores have been suggested, such as the use of the modified FRS by the Canadian Cardiovascular Society.¹³⁴ This modification involves doubling the risk percentage of an individual from the original FRS if they are between the age of 30 and 59, if they do not have diabetes and if they have a history of premature CVD in a first-degree relative (before 55 years of age for men or 65 for women). Current global CVD risk scores have biomarkers in their composite scores, such as HDL and total cholesterol, but a variety of novel individual biomarkers of CVD risk have been identified in the literature.¹³⁵ These have included measures associated with inflammation, thrombosis or of the presence of abnormal lipids. The interest in identifying relevant biomarkers for CVD risk assessment also led to the American Heart Association releasing a statement on the topic to outline standards for novel

biomarker evaluation.¹³⁶ Specifically, this document emphasizes the importance of assessing biomarkers based on patient management and outcomes. Some of the novel biomarkers identified in the literature are outlined in Table 4. While some of these measures are used in private medical facilities, they have yet to receive regular use in the clinical setting due to some barriers to use that still exist – namely, cost, a lack of definite therapeutic options to address the abnormal biomarker and a lack of understanding of ideal target levels for these new biomarkers.

Table 3. Novel biomarkers of cardiovascular disease

Inflammation-related biomarkers	High sensitivity C-reactive protein (CRP); Fibrinogen; Interleukin-6 (IL-6); Tumor necrosis factor- α (TNF- α)
Thrombosis-related biomarkers	Homocysteine; Lipoprotein-associated phospholipase 2
Lipid-related biomarkers	Lipoprotein markers; Cholesteryl transfer protein; Coronary artery calcium
Arterial health biomarkers	Flow mediated dilation; Pulse wave velocity; Carotid intima-media thickness
Blood pressure-related biomarkers	Ankle-brachial index; Central blood pressure

1.4.3 Cardiovascular Disease and Women

Cardiovascular disease is the number one cause of premature death in women in Canada.¹³⁷ In fact, the top three causes of death for women in Canada are ischemic heart disease, stroke and heart failure.¹³⁸ Despite this, it is commonly perceived that CVD is a ‘man’s disease,’¹³⁹ although there is increasing recognition that women also have a high burden of CVD.¹³⁸

Men and women are known to have differences in the pathophysiological processes leading to CVD.¹⁴⁰ Women are typically at lower risk for developing CVD, as compared to men, until after menopause.¹⁴¹ As compared to the average onset of CVD in men, this occurs 10-15 years later in women as a result at approximately 60-65 years of age,¹⁴² thought potentially secondary to the protective effect of estrogen in pre-menopausal women.¹³⁹ Unfortunately, the

lack of focus on CVD as a significant cause of premature mortality in women has led to adverse systemic outcomes including underrepresentation in cardiovascular clinical trials.¹⁴³ As such, improvements in care related to new knowledge in the area has not translated into similar improvements in diagnosis, treatment or prevention of CVD in women.^{138,144} These deficits have led to women suffering from acute MI being more likely to die or have a subsequent cardiovascular event as compared to men.¹⁴⁵

Traditional risk factors do not affect men and women the same way and the implementation of clinical care practices incorporating these differences to benefit the cardiovascular care of women has been a slow process.¹⁴⁰ Women have been underrepresented in CVD research.¹³⁸ The disparity in women's cardiovascular health information is typically defined by a series of "unders": 1) under awareness; 2) under diagnosis; 3) under treated; 4) under support; and 5) under researched.¹⁴⁶ Research and health practice should work to improve awareness for both women and clinicians and strive to improve prognostic tools in this population through better understanding of the unique pathophysiological processes.¹³⁸ Thus, it is critical that research is done to gain a better understanding of the unique biopsychosocial factors that impact women's health and the pathophysiological differences in their development of CVD.

Current methods used to assess CVD risk in Canada, the FRS, underestimates the risk of women experiencing an acute MI^{144,147} with as much as 80% of women being deemed low risk but having subsequent acute MIs.¹⁴⁸ Thus, there is a need for refined tools to identify women at early risk for CVD. As a further example of differences in CVD progression in women, researchers have previously noted widespread differences in concentration of biomarkers of CVD between sexes. For example, biomarkers of inflammation, metabolic dysregulation and adiposity may contribute more to the development of pathophysiological mechanisms in disease progression in women.^{149,150}

1.4.2 Frailty and Cardiovascular Disease

The prevalence of both frailty and CVD increases with advancing age, but this relationship goes beyond merely an association – while still not fully elucidated, the progression of both clinical outcomes is thought to share many pathophysiological processes, such as mitochondrial dysfunction, impaired muscle homeostasis, and chronic inflammation.^{57,151} Given this, frailty assessment has been suggested as a means of improving CVD risk management by

incorporating it as an additional vital sign.^{35,152} Biological age may be a more important indicator of CVD risk than chronological age.³⁵

The commonality between the pathophysiological process of frailty and CVD does not come as a surprise as they share many common risk factors¹⁵³ - poor nutrition,¹⁵⁴ smoking,¹⁵⁵ poor weight control,¹⁵⁶ lack of physical activity,⁵⁹ sedentary behaviour,¹⁰⁴ and multicomorbidity.¹⁵¹ These associated conditions are thought to act bidirectionally with each increasing risk for the other, but there is still a poor understanding of the pathophysiology connecting frailty and CVD.¹⁵⁷ Components such as chronic inflammation^{158,159} and immunosenescence¹⁶⁰ likely play roles in this shared physiological decline.¹⁵¹

Our previous work has identified that pre-frail and frail women had odds ratios of 2.11 (95% CI, 1.60-2.78) and 4.55 (95% CI, 2.60-7.94), respectively, for having an intermediate to high risk FRS compared to their peers in a cross-sectional analysis.¹⁶¹ This finding is supported by longitudinal studies that have identified increased risk for CVD morbidity and mortality in pre-frail and frail men and women, as compared to their robust peers. In a cohort of 154,696 participants with a mean age of 70.8 years, Farooqi et al. found that individuals with frailty identified with an FI had a higher risk of CVD morbidity (IRR 1.97; 95% CI 1.85-2.08) and CVD mortality (HR 1.91; 95% CI 1.77-2.05) as compared to robust over a median 3.2 years.²⁰ Furthermore, the FI used was better able to discriminate those who had incident CVD events (C-statistic 0.60; 95% CI 0.60-0.61) compared to the FRS (C-statistic 0.58; 95% CI 0.57-0.58), a tool specifically designed to do so. When the FI score was combined with the FRS, the discriminatory ability of the tests improved (C-statistic 0.66; 95% CI 0.66-0.67). These findings are similarly supported by an analysis of over 3 million veterans in the United States that found frailty severity by FI was increasingly associated with CV mortality and CV major events (HR 7.9; 95% CI 6.2-10.3 for severe frailty) and that this was independent of the presence of CV risk factors.¹⁸ Data drawn from the English Longitudinal Study of Ageing on 5294 participants examined the ability of 35 different frailty scores to identify those at risk of CVD, and found that 23 of these scores were significantly associated with the incidence of CVD over a 7-year follow-up.²¹ Generally, the accumulation of deficits approach performed the best, even after controlling for a wide variety of CVD risk factors. These findings strongly emphasize the risk for CVD in frail individuals and speak to the concomitant processes of physiological decline that must be monitored to better identify morbidity risk for older adults. The opposite directionality has also

been previously found with individuals with a higher FRS being at increased risk of incident frailty over a four-year follow-up (OR 2.15; 95% CI 1.68-2.75)¹⁶² and patients with heart failure have also been found to be at increased risk (HR 2.7; 95% CI 1.5-5.1) for incident frailty over a median follow-up of 8.4 years.¹⁶³

As our population ages, the concomitant prevalence of frailty and CVD will become an increasing concern for the health of the population.^{46,57} The prevalence of frailty is high in those with CVD, specifically in women, and specifically in those with heart failure or valvular disease.¹⁶⁴ Regardless of the severity of existing CVD, frailty confers a 2.5-3.5 fold increased risk for mortality in these patients.¹⁶⁴ Traditional risk factors and prediction models for CVD only perform moderately well in those 60 years of age and older as compared to those who are middle-aged.¹⁶⁵ For example, an analysis of the Multimorbidity and Mental health Cohort Study in Frailty and Aging (MiMiCS-FRAIL) in Brazil (n=339) identified that the strength of associations observed between traditional CVD risk factors like hypertension, diabetes and dyslipidemia with incident CVD was reduced with increasing frailty status.¹⁹ Additionally, a meta-analysis (n=21,906) found that individuals with frailty and hypertension were not at increased risk of mortality as compared to individuals with frailty and normal blood pressure over a mean of six years.¹⁶⁶

The earlier pre-frailty stage is a critical point to intervene and slow or reverse the progression of frailty-related disease and decline.¹⁶⁷⁻¹⁶⁹ Pre-frail individuals are four times more likely to become frail over a four year period.⁶³ Further elucidating the physiological declines that precipitate frailty-related disease progression and discovering these changes early will allow for the identification and implementation of effective therapeutic strategies. Thus, it is imperative that we identify individuals early in frailty-related disease progressions so that we can intervene to change their health trajectory.⁸⁹ Moreover, early identification of frailty status can assist in evaluating patient risk-benefit ratio for CVD prevention strategies to guide best practice strategies for that individual using shared decision making.¹⁵²

1.5 Biomarkers of Frailty

Biomarkers are a means of identifying individuals living with frailty at increased risk for health declines.^{88,170-174} In fact, this promising area of research has led to its discussion and support of further work in this area by The International Conference on Frailty and Sarcopenia

Research (ICFSR) Task Force.¹⁷⁵ Biomarker approaches have the benefit of potentially identifying at-risk individuals earlier in frailty progression before established clinical signs saturate.^{174,176} Identification of biomarkers associated with frailty could also allow for the better understanding of the pathophysiological declines involved in frailty progression, which are not well understood.¹⁷⁵ This early identification could allow for resources to be put in place to improve health trajectory sooner while it is more amenable to change.^{167,168} These same biomarkers could also be used to assess the effectiveness of therapeutic approaches at both a population and individualized level through their change.^{173,174} Thus, the identification and characterization of biomarkers associated with frailty-related chronic disease declines could provide primary care value on several fronts.

Most biomarker research and discussion to date has focused on generalized biomarkers that are thought to be indicative of accelerated aging, or sarcopenia, as opposed to frailty specifically.¹⁷⁷ These works have typically focused on biomarkers associated with chronic inflammation, immunosenescence, changes related to the hypothalamic-pituitary-adrenal axis, or other subcellular changes^{173,175,178} many of which are similar to those previously described in the biomarkers of CVD section of this thesis (Section 1.4.2). Some of the biomarkers previously examined in relation to aging and/or frailty are described in Table 5. Typically, biomarkers that have been examined for frailty and aging have four different focuses for their application:^{179,180}

1. Biomarkers that estimate risk of developing frailty in advance of onset;
2. Biomarkers for identifying current frailty;
3. Biomarkers for describing the severity of frailty on a continuum; and,
4. Biomarkers for use as prognostic tools for adverse outcomes associated with frailty-related health declines.

Much of the work on biomarkers of aging and frailty thus far have focused on cross-sectional analyses.¹⁷⁵ Longitudinally, the identification that observable frailty deficits may be preceded by subclinical changes has led to research by Rockwood's research team into the use of the FI-Lab.^{80,181} In an exploratory analysis, they compared a 32-item FI-Lab to an FI involving self-report for their ability to predict mortality and found that performed similarly, with the conclusion that subcellular deficits may provide value in our understanding of aging.⁸⁰ A subsequent additional cohort of long-term care residents when comparing an FI-Lab to a clinical FI had similar findings.¹⁸¹ More recently, a systematic review and meta-analysis of studies using the FI-

Lab demonstrated its ability to predict mortality (HR 1.04; 1.03-1.03 95% CI per 0.01 FI increase) and a range of adverse outcomes in a range of populations.⁹⁵ The performance of the FI-Lab was similar to typically constructed FIs, but uses measures that may be less time and cost-consuming to collect, especially on a larger scale. Furthermore, Rockwood's research team has also looked at a biomarker-based frailty index (FI-B) using a series of biomarkers collected from blood sample.¹⁸² The clinical deficit FI and the FI-B performed similarly in discriminative ability (AUC 0.71 vs 0.66, respectively) for predicting mortality, but the combination of clinical deficits and biomarkers further improved the discriminative ability (AUC = 0.75) over a 7-year period in a cohort of 845 individuals with a mean age of 85.5 years. Namely, this FI-B used biomarkers of cellular senescence, inflammation, hematological values and markers of immunosenescence. The ability of this FI-B approach to discriminate mortality risk indicates that biomarker approaches may be able to give insight into subclinical deficits leading to frailty progression, thus provide an opportunity for therapeutic intervention.¹⁸²

Table 4. Biomarkers of frailty/aging

Biomarker	Description	Relation to frailty/aging
Inflammation		
IL-6	Pro-inflammatory cytokine. Influences immune response, energy metabolism in muscle and fat and osteoclast formation.	↑ frailty ¹⁷⁸ /↑ aging ¹⁸³
C-reactive protein	Acute inflammatory protein. Binds on cells to active the complement system.	↑ frailty ¹⁸⁴ /↑ aging ¹⁸⁵
TNF- α	Regulation of immune cells and systemic inflammation.	↑ aging ¹⁸³
Leptin	Regulates energy balance, role in neuroendocrine regulation and bone metabolism.	↑ frailty ¹⁸⁶ /↓ aging ¹⁸⁷
Platelet count	Play critical role in blood clot formation (primary hemostasis).	↓ frailty ¹⁸⁸ /↓ aging ¹⁸⁹
White blood cells	Cells involved in many functions of the immune response.	↑ frailty ¹⁹⁰ /↑ aging ¹⁹¹
Immunosenescence		
T-cell Phenotypes	Modulators of immune response.	↑ frailty ^{88,192}
Cellular aging		
Telomere length	Region of repetitive nucleotides that cap the ends of chromosomes.	↓ frailty/↓ aging ¹⁹³
Genetic/Epigenetic		
DNA methylation	Methyl groups can be added to DNA to repress transcription of segments.	↑ aging ¹⁹⁴
Other		
Vitamin D	Fat-soluble vitamin important in bone metabolism.	↓ frailty ^{195,196}
Gut Microbiota	Characterization of microflora living in the intestine.	↓ diversity in frailty ^{197,198}

IL-6, interleukin-6; TNF- α , tumor necrosis factor alpha

1.5.1 Myokines as biomarkers of frailty

In addition to producing physical movements through contraction, skeletal muscle acts as an autocrine, paracrine and endocrine organ, which is thought to drive the health promotion benefits seen from physical activity.^{199–201} First described as a potential exercise or muscle ‘factor’ by Goldstein,²⁰² the discovery of muscle’s additional actions in the body has helped to make sense of the diverse benefits achieved through participation in physical activity, as well as the detriments seen in the absence of physical activity.²⁰³ ‘Myokines’, small peptides and cytokines released independently from muscle received much of the earlier attention in research in this role.^{199–201}

Originally, myokines were thought to, namely, play a role in the immune system due to the related presence of these cytokines, such as IL-6, but myokines are now known to have wide-ranging effects throughout the body. As such, myokines are likely to play key roles in maintaining functional health status.²⁰⁴ Beyond many of their hypertrophic effects on muscle, the pleiotropic effects of myokines may explain some of the generalized health decline seen with physical inactivity and the concomitant increase in risk of a “diseasome state” as described by Pederson et al., who first coined the term ‘myokine’.^{61,205} Emerging evidence supports the fact that myokines likely play intimate roles in the positive anti-inflammatory, immune, and cell function adaptations that occur with exercise.⁶⁴ The mechanisms stimulating myokines are far from fully understood, but it is thought that only a subset of these myokines are regulated through muscle contraction.²⁰⁶

The relative release of cytokines during exercise from muscle as compared to other tissues is not well understood²⁰¹ but an even broader range of factors from a broader range of organ tissues are now thought to play a role in physical activity-mediated health benefits.²⁰⁷ ‘Exerkines’²⁰⁸ are signaling factors released in response to physical activity from tissues including, not only muscle, but cardiometabolic, immune, nervous and bone tissue as well.^{207,209} The broad benefits of physical activity are now thought to be achieved through these even broader moieties beyond just myokines in response to both acute and chronic exercise.²⁰⁷ The importance of myokines, and the broader exerkines, to healthy aging and disease prevention are of note when viewed in the context of only 11% of Canadians aged 60-79 years meeting Canada’s Physical Activity Guidelines.²¹⁰

Many myokines have been examined in association with age-related disease progression.^{211–213} Additionally, the role of various myokines and their association with

precipitating risk factors of noncommunicable disease are now being examined in the concomitant decline leading to chronic disease due to their association with adverse health states.^{172,204,212} This has included specific discussion and examination of the potential role that myokines play in the development of cardiovascular conditions such as hypertension²¹² more generalized CVD progression including endothelial dysfunction,²¹⁴⁻²¹⁶ cognitive declines,²⁰³ and metabolic changes.²⁰³

Chronic disease and frailty are both often accompanied by wasting of muscle and bone tissue, leading to further accelerated declines in health.²¹⁷ These detriments can lead to reduced quality of life and functional capacity, as well as reduced capacity for performing physical activity in order to prevent, mitigate, or slow advancing disease and frailty.²¹⁸ This can lead to a vicious cycle that characterizes the health decline seen in frailty-related disease states.¹⁵⁷ One of the possible mediators of these concomitant reductions in muscle, bone and more widespread health is that of myokines, which are understood to regulate communication between muscle and many wider spread targets throughout the body.^{201,217} Thus, myokines likely play a role in generalized deteriorating health seen in conditions such as frailty.^{172,175,219,220} With declines in muscle health often being a feature of frailty it is possible that deleterious changes in their expression from muscle or simply that reduced physical activity, which is associated with frailty status,⁶⁰ would prevent the necessary muscular stimulation to secrete myokines.²²¹ However, myokine response to acute and chronic exercise still requires further elucidation.²⁰⁷ Much of the prior research has focused primarily on aspects of physical frailty and/or sarcopenia given their direct relation to muscle health,¹⁷⁴ but the wide-ranging and diverse effects of myokines throughout the body may mean that they play a broader role in health, similar to that which multidimensional frailty describes.²²¹ Myokines may provide value as biomarkers as they may act as flags of subclinical changes in advance of the typically assessed clinically observable changes in phenotypic frailty assessments like walking speed and muscle strength. This could allow for earlier identification of individuals at risk for functional declines creating an opportunity for earlier intervention.¹⁷² The wide-ranging effects seen from myokines could help to explain the detriments seen across multiple physiological systems in frailty, their association with physical inactivity, as well as the pathophysiology connecting frailty to noncommunicable disease.^{17,63} Not only this, but they could also identify targets for therapeutic approaches that could have wide spread physiological benefits.^{217,221} The understood effects of some of these

myokines based on published literature are described in Table 6. An example of some of their possible associations with accumulated deficits seen in frailty are outlined in Figure 3.

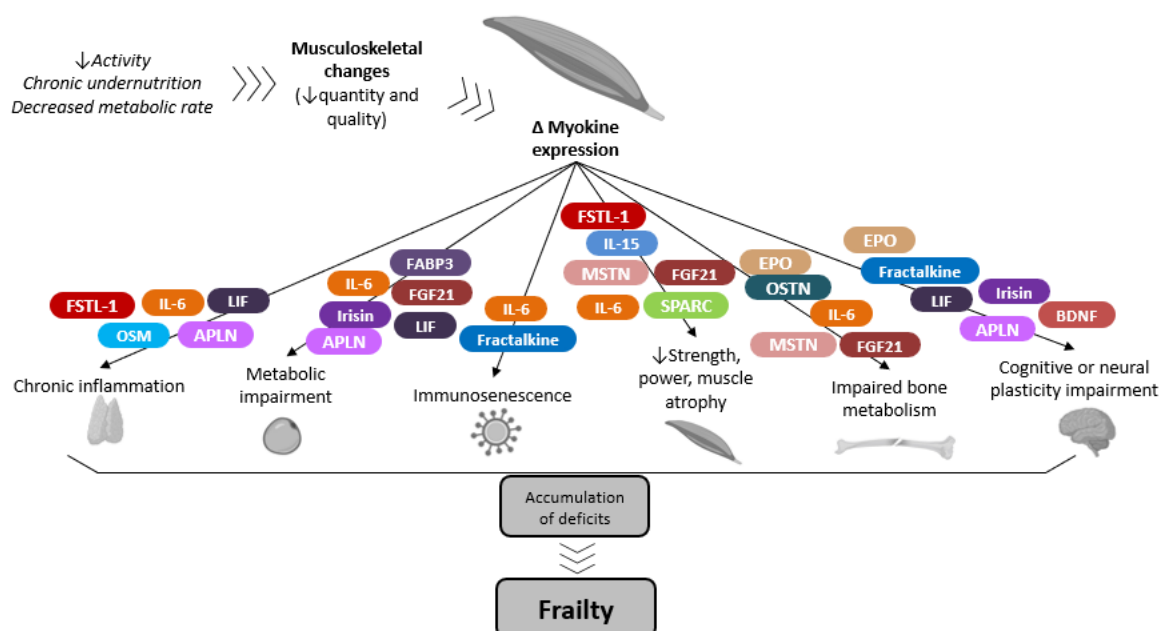
Table 5. Myokines potentially associated with frailty, aging and cardiovascular disease

Myokine	Description
IL-6	Pro-inflammatory cytokine and anti-inflammatory myokine. Influences immune response, energy metabolism in muscle and fat and osteoclast formation.
Apelin	Influences muscle metabolism, blood pressure and angiogenesis.
BDNF	Acts on neurons of the central and peripheral nervous systems to support and encourage growth of new neurons and synapses.
Erythropoietin	Controls red blood cell production.
FABP3	Involved in fatty acid metabolism.
FGF-21	Stimulates glucose uptake in adipocytes.
Fractalkine/CX3CL1	Role in leukocyte adhesion, attracts macrophages to skeletal muscle tissue.
FSTL1	Role in lung, central nervous system and skeletal development.
IL-15	Regulates activation of macrophages and insulin production.
IL-16	Regulates activation of macrophages and insulin production.
Irisin	Induces thermogin expression in fat cells and is associated with glucose tolerance in humans.
LIF	Influences cell growth by inhibiting differentiation.
Myostatin/GDF 8	Inhibits muscle cell growth and differentiation.
Oncostatin M	Involved in liver development, inflammatory process and central nervous system development.
Osteocrin/musclin	Likely plays a role in muscle metabolic adaptations to exercise.

Osteonectin/SPARC	May play a role in the repair of muscle satellite cells from damage. Also found to block tumor progression through induced apoptosis.
TNF- α	Regulation of immune cells and systemic inflammation.

***IL-6**, interleukin-6; **BDNF**, brain-derived neurotrophic factor; **FABP3**, fatty acid binding protein 3; **FGF-21**, fibroblast growth factor 21; **FSTL1**, follistatin-related protein 1; **IL-15**, interleukin 15; **IL-16**, interleukin 16; **LIF**, leukemia inhibitory factor; **GDF 8**, Growth differentiation factor 8; **TNF- α** , tumor necrosis factor alpha*

Figure 3. Myokines, accumulation of deficits, and frailty proposed pathophysiology



***BDNF**, brain-derived neurotrophic factor; **EPO**, erythropoietin; **FABP-3**, fatty acid-binding protein 3; **FGF-21**, fibroblast growth factor 21; **FSTL-1**, follistatin-like 1 protein; **IL-6**, interleukin-6; **IL-15**, interleukin-15; **LIF**, leukemia inhibitory factor; **OSM**, oncostatin M; **OSTN**, osteocrin/musclin; **SPARC**, osteonectin*

1.5.2 Muscle-Bone crosstalk and biomarkers of frailty

Much of the interaction between skeletal muscle and bone was originally thought to be only of a mechanical nature. Wolff's Law describes how mechanical forces acting through muscle contraction pulling on bone and gravitational forces on bone stimulate bone metabolism and anabolism.²²² As with muscle, it is now understood that bone is highly dynamic and capable of communicating with other organs.²²³ Similarly, bone also mediates communication between

itself and other organs through the release of extracellular vesicles.²²³ The effects of a variety of cytokines are related to both muscle and bone regulation in what is known as muscle-bone crosstalk.²²⁴ In fact, many of the cytokines described in Table 6 also regulate bone metabolism.²²³ This crosstalk is thought to play a critical role in health maintenance and impairments in this communication may be used to identify early dysregulation.^{203,217,224} Specifically, this dysregulation may lead to concomitant osteoporosis and sarcopenia.²²⁴ Myokines and exerkins are now thought to have even broader cross-talk beyond organ systems including immune, gut, nervous system and cardiometabolic tissues, thereby, playing a critical role in maintaining generalized health.²⁰⁷

Individuals with frailty, specifically phenotypic frailty, often present with sarcopenia and osteoporosis, although the directionality of this process is unclear.^{174,225} Both pre-frailty and frailty have been associated with increased risk for bone fracture^{226,227} and impaired remodeling.^{228,229} Frailty and CVD have aspects of shared pathophysiology and precipitants (as summarized in Section 1.4.2), such as decreased physical activity, which lead to bone health impairments that are often present in CVD progression as well.^{230,231} In fact, dysregulation of bone remodeling has also been discussed in association with CVD risk.^{230,231}

1.6 Purpose of thesis

Novel frailty assessment tools using biomarkers are showing promise in their ability to predict adverse health outcomes and a greater understanding of generalized frailty declines and concomitant chronic disease.^{88,170} Most of the existing research has focused on biomarkers associated with immunosenescence or inflammaging.^{5,6,8,160,178} Given that frailty is often characterized by deleterious changes in muscle and bone health,⁶³ myokines or bone metabolism analytes may play a role in early frailty-related disease progression in advance of clinical outcomes and, thus, act as biomarkers of frailty.²²¹ Furthermore, myokines have also been suggested to have a role in CVD development,²¹⁶ a clinical outcome that is known to be associated with frailty-related health declines.^{20,89} Despite this, little research has been done to examine the utility of myokine biomarkers to identify those at risk for frailty or CVD progression.¹⁷⁰ Tools for assessment of this nature could be of specific use to women as they have been underrepresented in CVD research and current assessment tools are not as good at identifying risk in this population.¹³⁸ Women are also at increased risk for frailty-related health declines.^{29,30}

My research will address this gap by characterizing concentrations of myokine and cytokines related to bone metabolism in a population of middle-aged to older women as well as performing an early exploratory analysis of their potential role as frailty biomarkers.

1.7 Specific aims

Frailty status reflects a quantifiable state of risk when faced with adverse health stressors, such as CVD, making its identification critical in characterizing older adults.^{36,182} Early identification of frailty remains a clinical challenge that could potentially benefit older adults living with frailty through earlier attempts to mitigate its progression.⁹⁷ This thesis aims to evaluate systemic myokines and bone metabolism analytes as candidate biomarkers of early frailty identification and associated CVD risk through the following objectives:

1. Contextualize frailty assessment, its utility in healthcare and identify opportunities for biomarker integration.
2. Examine univariable associations between individual myokines, frailty status and CVD risk.
3. Examine multivariable myokine biomarker approaches in association with frailty status using regression analyses.
4. Interpret the potential utility of myokines as biomarkers to early frailty identification and discuss their potential for identifying interventions in frailty trajectories.

1.8 Hypotheses

1. Systemic blood concentrations of myokines and bone metabolism analytes will differ by frailty status in middle-aged and older females.
2. Multivariable myokine analysis will identify a panel of myokines associated with frailty independent of age.
3. Biomarker profiles associated with frailty status will differ depending on frailty assessment approach (e.g., phenotypic vs index-based), reflecting the specific physiological domains each tool captures.

1.9 Organization of thesis and study objectives

Chapter 1 provides an overview of frailty and its proposed role in the clinical setting as well as the variety of assessment methods. Subsequently, the pathophysiology of frailty-related disease progression is discussed and the shared relationship with cardiovascular disease. Finally, new proposed methods of identifying frailty risk earlier through biomarkers are reviewed as well as the benefits this may provide in understanding and intervening in frailty progression.

Chapter 2 is a published literature review discussing frailty assessment modalities and their proposed roles in the clinical setting.⁷⁴ Given the wide variety of frailty assessment methods, it is important to understand specific clinical context as it can help direct tool selection. The review discusses the utility and strategies for implementing frailty assessment in a variety of clinical settings (e.g., primary care, acute care and surgical care). This discussion establishes the important role that frailty can play in the clinical setting, which in turn gives context to the examination of biomarkers of frailty.

Chapter 3 is a univariable analysis examining a select panel of myokines as biomarkers of both frailty and cardiovascular disease in community-dwelling middle-aged and older women (n = 104) using a case-control design. This work has been published.¹⁷⁰

Chapter 4 uses a similar case-control design to Study 1. Again, a univariable analysis was used to examine cytokines related to bone metabolism as biomarkers of frailty and cardiovascular disease in community-dwelling middle-aged and older women (n = 104). This work has also been published.²³²

Chapter 5 builds off the prior two studies by incorporating the latest expert recommendations to perform multivariable analyses when examining biomarkers of frailty, and specifically myokines. As such, this study uses a multivariable analysis of a select panel of myokines informed by our prior work to examine their utility as biomarkers of both the phenotypic FP and the accumulation of deficits-based FI in a cohort of community-dwelling middle-aged and older women (n = 329). This work is unpublished.

Chapter 6, the final chapter, discusses the overarching findings of this thesis research and contextualizes the novelty of the work by discussing how the research adds, supports, and differs from published literature. This chapter also identifies future directions for research in this area.

Chapter 2: Frailty-aware care: giving value to frailty assessment across different healthcare settings

2.1 Preface to the Manuscript written exclusively by Kevin Boreskie

The following chapter is a published literature review developed in response to discussion with committee members about the relevance of frailty to the clinical setting. Now part way through my residency training as an emergency medicine physician, this relevance is important for my future career as I plan on specializing in geriatric emergency medicine. The context of frailty in the clinical setting is critical as it also establishes the importance of biomarkers needed to identify, elucidate, and intervene in the progression of frailty. Despite continued expert opinion extolling the importance of frailty considerations in a variety of healthcare settings, frailty assessments are not commonly used in most medical practice. Slow uptake has partially been attributed to confusion by clinicians due to a wide variety of frailty assessments and the ways in which this information can be used differently depending on the clinical setting. Thus, this review paper was created as a primer for those looking to implement frailty assessment into practice by providing clarification on the utility of frailty assessment in clinical settings, which is an important consideration when examining biomarkers of frailty as in the rest of this thesis. This review paper compares frailty assessment in primary care, acute care and surgical care including recommended tools and main considerations.

2.2 Manuscript status

Published in *BMC Geriatrics*, January 3, 2022, volume 22.⁷⁴ Including the following manuscript in this thesis document does not require copyright permission as per the copyright permission officer of *BMC Geriatrics*.

2.3 Authors

Kevin F. Boreskie, Jacqueline L. Hay, Patrick E. Boreskie, Rakesh C. Arora, Todd A. Duhamel

2.4 Contributions of Authors

Dr. Duhamel, Dr. Arora and I conceptualized the review. As first author of the manuscript, I led the development of the manuscript and drafted the original manuscript. All authors

contributed to the literature search, reviewing, and editing the manuscript as well as assisted with the creation of the included tables and figures.

2.5 Manuscript

Abstract

Healthcare systems need to adapt to better serve an aging population with complex presentations. Frailty assessments are a potential means to address this heterogeneity in aging to identify individuals at increased risk for adverse health outcomes. Furthermore, frailty assessments offer an opportunity to optimize patient care in various healthcare settings. While the vast number of frailty assessment tools available can be a source of confusion for clinicians, each tool has features adaptable to the constraints and goals of different healthcare settings. This review discusses and compares barriers, facilitators, and the application of frailty assessments in primary care, the emergency department/intensive care unit and surgical care to cover a breadth of settings with different frailty assessment considerations. The implementation of frailty-aware care across healthcare settings potentiates better healthcare outcomes for older adults.

Introduction

People are living longer, however, this longevity often does not equate to years of good health-related quality of life.^{1,3} While aging itself is not a pathological process, biological aging is heterogenous, contributing to diverse patient presentation in healthcare and deviations from what has been traditionally considered the standard patient presentation.^{1,36} Health systems need to adapt to best serve our aging populations.¹ Best care practices may be improved using frailty assessments as tools to better identify complex patients at risk and by personalizing their care using insight beyond chronological age.^{66,67}

Frailty

While no singular definition of frailty has been universally accepted, frailty is generally characterized as a reduced physiologic reserve to adapt to health stressors.²⁴ Thus, frailty is a predictor of adverse outcomes such as falls,¹⁵ hospitalizations,¹⁵ morbidity^{15,21,32,33}, and mortality.^{21,27,33} Though frailty prevalence increases with age,²⁶ age and frailty are distinct concepts.³⁶ Biological age assessed through frailty may be more important than chronological

age when assessing risk for adverse health outcomes.^{33,46} A recent meta-analysis of 240 studies from 62 countries by O’Caoimh et al. identified the overall prevalence of frailty ranges from 12 to 24% in community-dwelling adults dependent on the tool used, with prevalence generally increasing with age.²³³ This analysis also found a higher prevalence of frailty in females (15-29%) as compared to males (11-20%).²³³ This finding supports prior discussions of the higher frailty prevalence in females despite their greater longevity.^{29,30} Importantly, the identification of frailty status offers a potential opportunity to implement targeted interventions to halt, slow, or reverse health declines, and provides information on the patient that can direct individualized care. The collection of chronological age alone does not offer these opportunities given the heterogeneity seen in aging.

Frailty Assessment

Increasing recognition of frailty assessment as a potential tool in healthcare has led to the development of over 50 frailty tools for a variety of settings.^{66,67} While there is low agreement between many frailty assessments, different tools likely identify different constructs of frailty.^{73,75} Frailty assessments are broadly classified by methodological approach: phenotypic, multidimensional, or an accumulation of deficits. Phenotypic approaches focus on physical clinical criteria including unintentional weight loss, low physical activity levels, and reduced muscle strength as a means of identifying frailty.^{15,66} Critics argue phenotypic approaches may be limited in scope and recommend the incorporation of a wider range of criteria, such as cognitive or psychological factors.^{66,78} To this end, multidimensional approaches have been developed to identify frailty beyond phenotypic presentation.⁶⁶ Furthermore, the accumulation of deficits approach seeks to identify frailty by examining a range of deficits (i.e. signs, symptoms, disabilities or disease presence) that would increase risk for adverse outcomes.⁹⁴

Frailty assessment is a recommended addition to the clinician toolkit to help identify individuals at risk for health decline aggravated by health stressors.^{66,67} The importance of these frailty assessments in an aging population is evident by the calls to action supporting their incorporation into clinical practice to better predict adverse health outcomes.^{17,46,234} In fact, the Canadian Frailty Network top ten research priorities focus on frailty assessment as a means for healthcare practitioners to inform treatment and care decisions, as well as to avoid unnecessary hospitalization and emergency department visits for older adults.²³⁵ Implementing frailty

assessments in clinical care has the potential to improve patient care.^{17,46,234} Despite the existence of obstacles to the incorporation of frailty into clinical practice such as uncertainty in tool selection,^{17,69} support for the incorporation of frailty across healthcare settings continues to evolve.

This review is intended to offer a current understanding of the value of frailty assessment in different settings to facilitate the implementation of frailty into practice by clinicians. MeSH terms such as “Frail Elderly”, “Geriatrics”, “Geriatric Assessment/methods” and “Frailty/diagnosis” were first used to identify relevant manuscripts using additional terms specific to the various clinical settings. Thereafter, a forward-and backward search strategy was implemented to identify additional relevant publications. We also used resources such as Connected Papers (www.connectedpapers.com) to identify related works. However, the search strategy was not formally developed or reviewed by a librarian and should not be considered a systematic methodology. Current opportunities and challenges for frailty-aware care will be compared between primary care, the emergency department (ED)/intensive care unit (ICU), as well as the perioperative context^{96,236,237}. Figure 4 summarizes the spectrum of considerations in each setting – human resource constraints, patient population, and outcomes of interest. While these settings do not cover the gamut of clinical practice, the competing interests for frailty assessment in each of these settings are widely applicable given the breadth of practice covered. As opposed to comparing frailty assessment methodologies within each context, this review paper seeks to compare overarching principles in frailty assessment between clinical contexts. Similarly, the frailty assessment tools discussed are not exhaustive, but we intend to highlight some of the most researched approaches related to each clinical setting.

Figure 4. Frailty assessment across clinical settings

	Primary Care	ED / ICU	Surgical
Patient population*	• General population • Less acute illness	• Higher acuity illness	• Mixed acuity illness
Goal of frailty identification	• Assess risk in advance of disease/disability • Identify targets for intervention, like cognition or mobility	• Gain better understanding of acute illness in context of pre-existing health status • Assist in patient triage and care management	• Assess iatrogenic stress tolerance • Refer at-risk patients to prehabilitation to optimize outcomes
Potential benefit	• Early intervention may halt or delay disease/disability onset • Medical management decisions will optimize patient-centered outcomes	• Guide disposition decisions • Medical or surgical management decisions will optimize patient-centered outcomes	• Assess likelihood of benefit from treatment • Medical or surgical management decisions will optimize patient-centered outcomes
Main barriers to implementation	• Risk of over-diagnosis • Further consensus needed on best care practices for patients with frailty	• Potentially limited time for assessment • Acute illness may limit assessment options • Further consensus needed on best care practices for patients with frailty	• Potentially limited time for assessment • Acute illness may limit assessment options • Further consensus needed on best care practices for patients with frailty
<i>*Patient presentations and healthcare constraints exist on a wide spectrum. This table is based on what is typical but may not be true for all readers' reality.</i>			

Frailty in Primary Care

As chronic diseases continue to be a higher attributable cause of death¹¹⁵ and as the population ages,²³⁸ frailty assessment approaches gain merit as a means of directing primary prevention efforts.²³⁹ Primary care serves a critical aspect of prevention by providing longitudinal care for patients. Since physiologic deficits accumulate over time, primary care offers an opportunity to intervene further upstream, potentially before the appearance of clinical symptoms.^{3,11} Healthcare systems are largely based on addressing single-system disease⁴⁴ and patient management challenges increase with higher prevalence of multisystem disease.²³⁸ Frailty assessment offers an opportunity to identify those at risk of multisystem downward health trajectories with more complex presentations and to direct therapeutic interventions at components of frailty earlier.^{240,241}

Applying Frailty Assessment in Primary Care

Early intervention is vital, as health trajectories typically accelerate downward with frailty progression and become increasingly difficult to reverse or slow.²³⁴ Thus, an effective frailty tool in the primary care setting should be one that can identify not only those who are already frail, as there is a high prevalence in the community,²³³ but also those in the early stages of frailty. Patient health trajectories are more amenable to change earlier and a greater number of therapeutic options may be available.^{97,242}

Frailty assessments in the primary care setting should also identify targets for intervention, such as health risk behaviours or domains of frailty, like cognition or mobility.²⁴³ Identifying targeted areas for health improvement allows for a personalized care approach and is more apt to pre-emptively improve the status of those who are at risk of frailty progression.^{97,243} For example, individuals with frailty and polypharmacy may benefit from a thorough prescription review,^{106,244} while those reporting challenges in activities of daily living may require a physical activity intervention to improve functional status.^{245,246} Currently, identifying optimal interventional strategies are challenging given varied frailty definitions and assessments, though multi-modal interventions involving physical activity and nutrition have demonstrated promise.⁹⁷

Validated frailty assessments for primary care vary in the ease of administration, with more intensive options likely requiring allied healthcare staff beyond the physician to save cost and time. In comparison, rapid options may require the referral of patients in need of intensive screening through more thorough methodologies with a multidisciplinary team.^{45,247,248} The International Conference on Frailty and Sarcopenia Research Consensus Guidelines recommend referral to a CGA after initial assessments identifying frailty given the low specificity often observed in frailty screening.^{45,79} A careful balance must be struck between the efficacy of the frailty approach used to identify interventional targets and the feasibility of utilizing the approach within resource constraints.

Importantly, frailty assessment in the primary care setting also potentiates improved clinical decision making for complex patients.²³⁹ For example, the pharmaceutical management of hypertension in adults with frailty is under reconsideration.^{249,250} Newer recommendations from experts suggest higher target blood pressure values for hypertensive adults with frailty than those who are robust, as aggressive treatment in those who are frail may lead to adverse outcomes.^{249,250} The direction of care and shared clinical decision making with regards to frailty

status requires communication between patient and physician. Communicating effectively on the topic of frailty with patients in the primary care setting requires a shared understanding of the concept with a focus on person-centered communication.²⁵¹

The incorporation of frailty assessment will require justifiable time, personnel, and technology to incorporate into primary care. In spite of the initial increased resource costs of frailty assessments, research indicates long-term cost-effectiveness and improved patient outcomes are possible and justified.^{97,252} As opposed to considering frailty a case of over-medicalization, leading geriatricians argue frailty assessments should be a part of general care and have likened the careful monitoring of frailty status to that of monitoring hypertension to reduce risk of myocardial infarction.²³⁴ Similarly, frailty status should be identified, treated, and monitored over time on a continuum as a means of reducing risk for disability and disease.²³⁴

Examples of Frailty Assessment in Primary Care

Frailty tools utilized in the primary care setting should have the ability to identify intervenable aspects of health and can identify those in need of increased healthcare attention. The following tools are far from an exhaustive list of recommended tools for this setting but can give insight on the variety of approaches available as well as some of their advantages and disadvantages.

The Fried physical frailty phenotype¹⁵ is one of the most commonly cited frailty assessments in the literature which focuses on five phenotypic aspects of frailty: 1) muscle weakness; 2) slow walking speed; 3) low physical activity levels; 4) unintentional weight loss; and 5) self-reported exhaustion. Frailty status is then assigned based on a total score ranging from zero to five (i.e. 0 = robust; 1-2 = pre-frail; ≥ 3 = frail) dependent on the number of criteria present as assessed through a combination of brief questionnaires and physical function testing, such as grip strength and gait speed. Phenotypic approaches, such as the PFP, are useful for identifying physical deficits and/or potential nutritional deficits in a patient but are potentially limited by only examining physical presentation.^{66,78} General signs of phenotypic decline do not provide direction for preventative or therapeutic strategies without then identifying root causes, but their collection has the benefit of not requiring preliminary clinical evaluation.²⁵³ Frailty assessments with physical components also have the limitation of requiring patients fit enough to complete the testing,²⁵⁴ although inability to complete functional testing can also sometimes be

informative. Additionally, physical assessments often take more time, lowering the feasibility of implementation in clinical practice.^{73,253}

Examining frailty as an accumulation of deficits, the FI⁸⁷ approach has been suggested as a means of frailty assessment applicable to a variety of clinical settings including primary care.^{17,44,255,256} An FI ratio between zero and one is determined by calculating the number of existing deficits presented, divided by the total number of deficits assessed in the patient.⁸⁷ Typically, deficits include clinical signs, symptoms, disabilities, morbidities, and/or laboratory tests that relate to health status and represent a broad range of physiological systems⁸⁷ – an approach that has the potential to identify specific intervenable domains of health.⁹⁴ The approach has been criticized for being cumbersome given an FI typically includes 35 or more deficits,⁸⁷ lowering its feasibility of implementation.⁷³ Creative uses of the FI approach have been introduced in the form of an electronic FI (eFI) that uses data already stored in electronic health records to create a score that could be automatically calculated for use in primary care^{81,256} as well as FIs that use less variables and focus on common clinical and laboratory tests (FI-Lab).⁸⁰

The FRAIL scale²⁵⁷ is also a phenotypic frailty assessment but it is administered entirely via a brief questionnaire that examines self-reported fatigue, resistance, ambulation, illness, and loss of weight.²⁵⁷ The FRAIL scale is easy to use⁷³ and has been validated in a variety of populations and has predictive ability for adverse health outcomes.^{247,257,258} Its use has been recommended as a tool to stratify those in need of a CGA to identify specific targets for intervention.²⁴⁷

The Kihon Checklist²⁵⁹ is a multidimensional frailty assessment widely used in Japan that consists of 25 yes/no questions covering categories like nutrition, activities of daily living, activity, socialization and cognition. The results of this rapid checklist correlate closely with other validated tools assessing frailty phenotypes²⁶⁰ and it has been shown to predict dependency and mortality.²⁶¹ Similarly, the validated Tilburg Frailty Indicator (TFI) uses a rapid self-report questionnaire to assess physical, psychological and social domains of frailty²⁶² to predict adverse outcomes like healthcare utilization and falls.²⁶³

In contrast to the FRAIL scale and the Kihon Checklist based solely on questionnaire, the multidimensional EFS uses a combination of questionnaire and assessment to assess functional, health and social factors while incorporating cognitive assessment through clock drawing and

functional performance with a timed up and go test.²⁶⁴ This tool has also been associated with adverse outcomes such as mortality.²⁶⁵

Frailty in the ED/ICU

Patient illness in the ED and ICU is typically acute in nature, making time per patient and patient flow necessary considerations. As such, patient triage and rapid decision-making tools validated in these environments are of importance. Using the best available clinical evidence, physicians must be able to assess the risk-benefit of various treatments, as well as have informed discussions regarding patient goals of care. Assessment and decision tools to assist staff in the ED/ICU setting to triage, care, and allocate resources are needed,⁸⁶ especially for an aging population presenting with increasing case complexity and comorbidities.²⁶⁶ Currently, triage models mistakenly under-triage older adults and provide interventions that may not yield benefit for the patient^{267,268} or simply cannot identify vulnerable older adults.²⁶⁹ Frailty assessment has been suggested to allow for a better understanding of patient presentation relative to their baseline health status.²⁴¹

Applying Frailty Assessment in the ED/ICU

Frailty assessment has been proposed as a potential means of improving triage methods and identifying appropriate care for older patients in the ED/ICU.^{34,96,270–272} Given that patients with frailty are more likely to experience adverse outcomes, either through presenting illness or iatrogenic stress, care has to be adapted to meet the patient needs.²⁷³ Recent systematic reviews have identified frailty as a predictor of in-hospital mortality, length of hospital stay, subsequent nursing home admission, and mortality in hospitalized older adults.^{274,275} Frailty assessment could assist with facilitating referrals to various health services and identifying optimal treatment options for that patient.²⁶⁸ In severe illness, the additional iatrogenic stress of intensive care interventions needs to be considered in patients with advanced frailty.²⁷⁶ Furthermore, frailty status affects the association between acuity of patient illness and mortality.^{277,278} For example, Pulok et al. found that high acuity illness was associated with significant mortality risk regardless of frailty status, but even those with low acuity illness were at risk for mortality when the degree of frailty was higher.²⁷⁷

The context of emergency and critical care necessitates considering different frailty tools than those best for primary care - validity must be carefully balanced with the feasibility of implementing an assessment in an acute care environment.^{275,279} A number of tools have been specifically developed and have demonstrated feasibility in urgent care, where time restraints on the clinical team and patient presentation limit the ability to perform certain tests.²⁸⁰ In the ED, many patients may be unable to perform common phenotypic frailty assessments, such as grip strength or gait speed, rendering brief assessments or clinical judgement more appealing.⁴⁵ Unfortunately, some rapid tools specifically designed for the ED, such as the Triage Risk Screening Tool²⁸¹ (TRST) and Identification of Seniors At Risk²⁸² (ISAR) tool have demonstrated poor prediction of adverse outcomes following ED encounters with older adults for outcomes like ED returns and functional decline.²⁶⁹

The selection of useful tools is dependent on choosing appropriate goals for frailty identification. In the emergency and critical care settings, frailty assessments namely identify optimal treatment and/or triage as well as inform shared decision making. Furthermore, frailty assessment in critical care may also lead to subsequent referrals to additional health services to better support the patient long-term.^{268,279} Theou *et al.* have recommended that frailty assessments in the ED/ICU could prevent premature case closure, facilitate the creation of multi-disciplinary care plans prior to discharge, and give an indication of cases involving more than a single pathology.²⁶⁸ Early assessment of frailty status at the ED can allow for the timelier initiation of a thorough CGA that can be used to inform patient discharge,²⁷² such as the implementation of homecare supports. Rapid frailty assessments are potentially efficacious for this purpose, as long as they are able to predict adverse patient outcomes, can assist in optimizing treatment, and result in better informed decisions regarding goals of care.

Examples of Frailty Assessment in the ED/ICU

Though more thorough assessments could potentially be conducted upon admission to a critical care ward in the hospital, initially, rapid frailty assessments are recommended in the context of the ED/ICU.^{279,280,283} Each rapid frailty assessments tool has inherent limitations but offers the flexibility and ease of measurement required for use in the ED/ICU.

The CFS has been suggested as one of the more studied approaches to assess frailty in the ED/ICU^{255,283,284} and has demonstrated association with mortality, length of stay, ICU admission,

and readmission.^{285–287} It is the only tool to date that has been found to be valid and reliable for these outcomes in the acute care setting.²⁸⁷ The CFS has convergent validity with the FI and Fried approaches as well as measures from the CGA.^{270,285,288} Frailty status is assessed on geriatric clinical judgement of mobility impairments, function, and cognition from two weeks prior making it a multidimensional approach. This information can also come from collateral sources, such as family members,²⁸⁴ which would be particularly important in the intubated or obtunded patient. The CFS is only recommended for use in adults over the age of 65 years.²⁸⁴

The HFRS was developed in an attempt to avoid any inter-rater or response bias errors of a rapid assessment tool.⁸⁶ International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) codes are used to calculate the HFRS. ICD-10 codes identify the illnesses and injuries that have been claimed on hospital billing,²⁸⁹ making it feasible to implement the HFRS into existing hospital information systems. Moreover, the HFRS has been validated in multiple cohorts and has moderate agreement with the Fried and FI approaches.⁸⁶

The FI model of frailty assessment²⁸⁵ has also been recommended in the acute care context, albeit the approach is modified slightly to better suit the ED/ICU.⁹⁶ The same conceptual creation of a FI using a ratio of existing to total deficits can still be used in this setting.^{87,96} For example, the FI-ED utilizes variables easily measured and/or accessible in the ED.⁹⁶ Despite having only 24 variables, the FI-ED was able to identify increased risk for admission, prolonged hospital stay, discharge to long-term care, and mortality at 28 days.⁹⁶ Although FI-Labs have been developed^{80,290} and used in the hospital setting,²⁹¹ the design of these approaches would have to take precaution not to use measures that may be saturated in the typical patient presenting to critical care.⁸⁷ FI-Labs used in acutely hospitalized older patients have been associated with mortality post discharge.^{292,293}

Future research to better support the increasing number of complex patients with frailty presenting to the ED/ICU is needed. Further research is required to facilitate a greater understanding of the feasibility, convergent validity, and predictive validity of varied frailty assessments.^{280,294} Considerations for frailty assessment in the ED/ICU setting are summarized in Figure 4.

Frailty in the Surgical Context

Advances in medicine have led to the surgical referral of older patients.²⁹⁵ However, some patients are less equipped to handle the iatrogenic stress associated with invasive surgery.^{295–297} Enhancing recovery protocols for various surgical procedures have been developed in recognition that changes to clinical practice are needed to improve surgical outcomes.²⁹⁸ These approaches have re-examined perioperative management to improve patient outcomes.

As in the ED/ICU setting, identifying patients at risk for adverse outcomes could be valuable in assessing the likelihood of benefit of surgical intervention and potentiates referrals to interventions aimed at increasing patient resilience prior to surgery.^{299–301} If a patient with frailty is known to be less able to tolerate health stressors such as an invasive surgery, less invasive options or even non-surgical supportive therapy may better fit the goals of patient care. For example, surgical aortic valve replacement (SAVR) is often the preferred intervention for patients requiring valve replacement. The less invasive transcatheter aortic valve implantation (TAVI) has been, in earlier use, in those at higher risk of surgical and post-surgical adverse events.³⁰² An assessment of frailty status could be used as a means of making the most informed choice for surgical intervention^{302,303} and could also guide perioperative care, such as tailoring the anaesthetic or other specific approaches to prevent delirium onset²⁷¹ of which frail individuals are at increased risk.³⁰⁴

Cardiac surgery research and care has strongly adopted frailty assessment as a means to improve patient care.³⁰⁵ Increasingly, older patients at risk for frailty are being referred for consideration of procedures like coronary artery bypass grafts and TAVI.³⁰⁶ Frailty status increases patient risk for adverse cardiac surgical outcomes,^{236,307–310} and this led to the recommendation of prehabilitation to improve functional status in patients awaiting surgery.³¹¹ The potential for prehabilitation approaches to improve patient outcomes in cardiac procedures is promising,^{312–314} but further randomized controlled trials are needed to assess feasibility, cost-effectiveness and efficacy in this and other surgical contexts.³⁰¹ Importantly, preoperative frailty status has also identified individuals who are less likely to attend cardiac rehabilitation after cardiac surgery³¹⁵ – a rehabilitation approach that is known to reduce adverse events postoperatively³¹⁶ and may further improve frailty status.³¹⁷

Applying Preoperative Frailty Assessment

Frailty is recommended as an assessment of patient resiliency in the preoperative period.^{271,296} Increasing complexity of surgical patients with comorbidities and functional limitations has meant that frailty assessment increasingly provides additional utility to current methods of risk stratification preoperatively.³¹⁸ In a recent systematic review, frailty status was associated with surgical complications, readmission, and mortality across a wide variety of surgical procedures.³¹⁹ While severity of illness of the surgical patient is quite dependent on the procedure, physical limitations and acuity of presentation may restrict possible frailty testing. Elective surgeries, for the most part, would be less urgent than the interventions for a patient in the ED/ICU, leaving more time for thorough frailty assessment. Concern has previously been raised, however, regarding the implementation of performance-based testing in the outpatient setting given the additional workload and the feasibility of performing such tests in all populations.²⁶⁵

Examples of Preoperative Frailty Assessment

Due to flexibility and thorough validation, the FI approach has been suggested for use in the preoperative setting.^{319,320} Simplified FIs with less variables and easily collected data have been recommended.^{296,299} For example, a modified FI (mFI) has been created using a simplified FI designed with only 11 variables and has been tested in surgical settings.^{321,322} A recent systematic review of preoperative frailty assessment identified FI assessments as being feasible while demonstrating strong predictive validity for surgical complications.³²⁰

The Modified PFP and the CFS have been identified as tools that could be used to identify those at risk of reduced quality of life and mortality.^{320,323} Interestingly, further analysis in one study used these scores to improve the ability of existing surgical risk tools, specifically the European System for Cardiac Operative Risk Evaluation II which discriminates patients at risk of poor functional survival.³²³ As in other clinical contexts, there is some concern about the ability of frail preoperative patients to perform some phenotypic frailty assessments.^{271,320} It has been recommended that protocols should utilize scoring systems for frailty assessment that do not exclude patients who are unable to perform the assessment.²³⁶

The Essential Frailty Toolset (EFT) has been specifically recommended by Afilalo et al. as the optimal tool for the preoperative cardiovascular surgery assessment of patients being considered for TAVI or SAVR.⁸⁵ The EFT can be rapidly assessed, using only a 4-item scale that

includes: 1) lower extremity weakness; 2) cognitive impairment; 3) anemia, and; 4) hypoalbuminemia. In the FRAILTY-AVR Study, the EFT was the strongest predictor of worsening disability and mortality at both 30 days and one year as compared to the PFP, FI, and Short Performance Physical Battery, to name a few.⁸⁵ However, the EFT has yet to be tested in other surgical contexts.

Despite the promising research advocating for frailty assessment in perioperative practice, frailty assessment has yet to become widely adopted. Frailty assessment should be strongly considered for standard preoperative care to identify those who are less likely to receive benefit from invasive procedures and to identify those who should receive prehabilitation prior to surgical intervention.^{296,324} Considerations for frailty assessment in the preoperative setting are summarized in Figure 4.

Moving Toward Clinical Implementation of Frailty

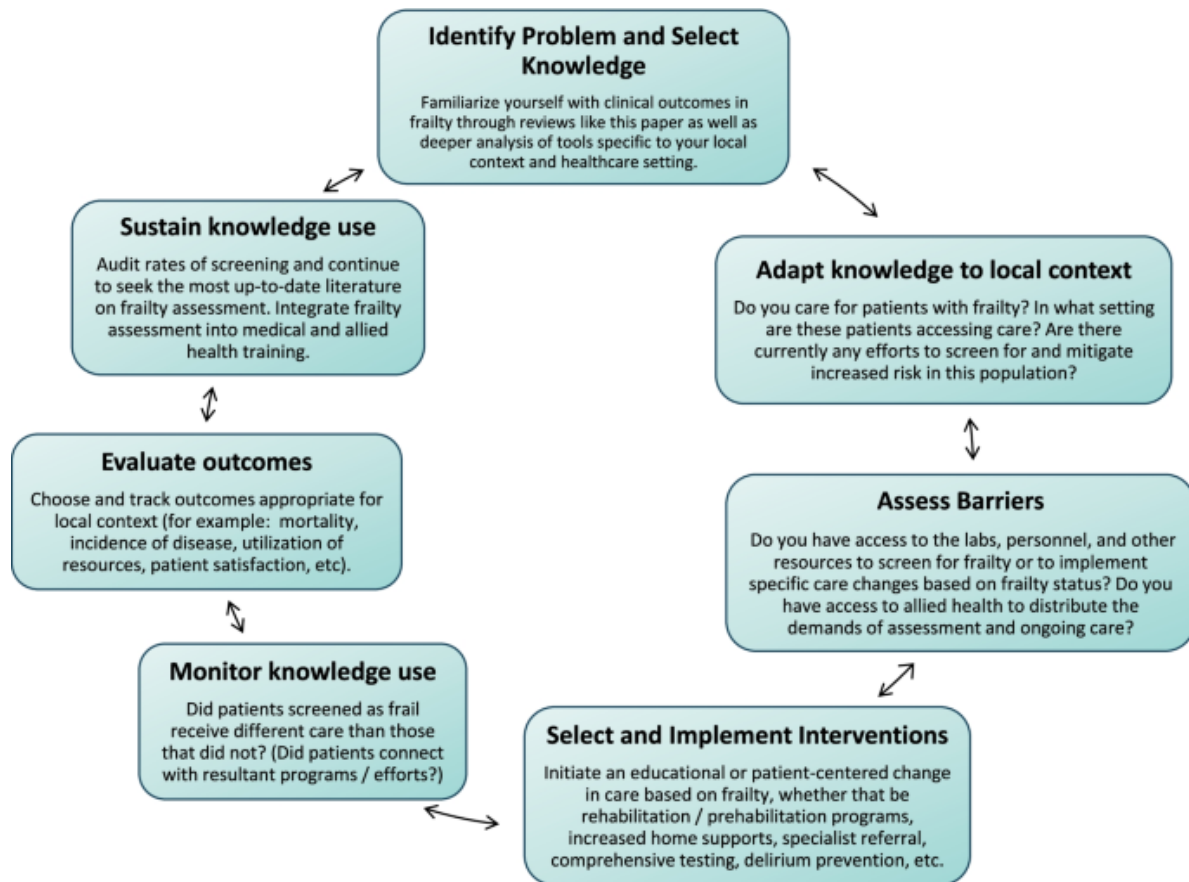
Successful adoption of frailty into clinical practice requires further consensus on both the definition and optimal assessment methodology for the proposed contexts.¹⁷ Optimal frailty assessment for various clinical settings, in turn, is dependent on the inherent constraints and the goals of use in each setting.⁷³ While there is no consensus of assessment for each individual setting, work such as that by Oviedo-Briones et al.⁷³ and Aguayo et al.⁷⁵ comparing the feasibility and application of various tools in a variety of settings is necessary to achieve this. These works have both found low agreement between many frailty assessments and conclude that different tools are likely assessing different aspects of frailty or that variable subtypes of frailty exist.^{73,75} Thus, tools should be selected based on feasibility and on their ability to identify the components of frailty important for that specific setting. Further work is needed to identify optimal approaches for acting on this information in those identified as being frail either through personalized care management, the identification of goals of care or interventional approaches. Clinical settings identify not only the need for intervention addressing frailty status, but also for frailty-informed care management.^{268,271,279,296}

Further consensus on tool selection in various healthcare settings will allow for the refinement of individualized care plans to best care for those identified as patients at increased risk for adverse outcomes based on their frailty status and to improve their resilience.^{17,234} The implementation of frailty assessment and intervention in the clinical setting has, in part, been

hampered by the incorrect use of presumed synonyms for frailty such as sarcopenia and disability, as well as the continual influx of new methodological approaches and tools¹⁷ that may be assessing different components of frailty.⁷³ Arguably, the most effective interventions are multi-dimensional, as the most successful interventions to date have included aspects of both physical activity and dietary intervention,^{97,100} as frailty itself is indicative of reduced physiologic reserve across a range of systems. Identifying an individual living with frailty could lead to subsequent CGA to provide more comprehensive information and to direct ongoing intervention.⁴⁵ Current interventions typically focus on components of frailty,⁹⁷ but a further understanding of the broad pathophysiology seen in frailty as well as clinically meaningful changes³²⁵ in frailty status are needed in order to address this syndrome of physiologic decline.

Initial analyses in the primary care setting have suggested that frailty-focused intervention, as opposed to usual care, provides better care and better patient outcomes without increasing costs.⁹⁷ Cost-analyses will have to be continually explored as new interventions and individualized care pathways are identified in expanding other clinical contexts, as in the ED/ICU^{272,279} and in preoperative care.³¹⁹ Further research is needed in order to identify the most efficacious approaches for therapeutic interventions in populations with frailty based on outcomes supported by the patient themselves, as well as to guide best care practices that take advantage of frailty identification in the clinical setting to support patient health.²⁷¹ Supporting patient health through the identification of frailty status requires a further understanding of effective patient-physician communication on the topic.²⁵¹ Future research on the use of frailty assessment in clinical settings should address this challenge and should use patient-oriented research designs to insure that the questions addressed are important to those living with frailty, such as the Top Ten Frailty Priorities developed by the Canadian Frailty Network.²³⁵ Advice regarding the specific implementation of frailty-aware care will be unique to local context and healthcare setting, so we advise using a Knowledge-to-Action (KTA) framework to determine steps forward (Figure 5). The KTA framework outlined by Graham *et al.* presents seven bi-directional steps to appropriately integrate knowledge while examining knowledge gaps and problems.³²⁶

Figure 5. Knowledge-to-action cycle for incorporating frailty into clinical settings



Conclusion

Barriers to implementing frailty assessments in clinical settings still exist - namely a lack of consensus on the assessment tool best suited in each context, uncertain cost-effectiveness, and few established interventions for frailty.^{17,44,234,239} What is known, however, is the significant health burden that frailty presents.^{46,252} Frailty costs our healthcare system^{241,252} in terms of increased hospital admissions, specialized consultations, longer hospital stays, and adverse outcomes.^{47,241} Adding frailty assessments into clinical practice could allow for further refinement of its effectiveness for directing individualized care pathways and practicality for implementation into a variety of clinical settings.^{234,239} Current healthcare systems are not designed to manage the complexity of patient presentation with multimorbidity that may become more common with an aging population.²⁴⁰ Though the integration of frailty assessment into clinical care is not yet widely accepted, the emerging body of literature is sufficient to guide

assessment implementation for a variety of medical settings as a step towards frailty-aware care that could improve acute and longitudinal patient treatment by guiding individualized care decisions and identifying the need for potential additional resources throughout care. Medicine has to shift with the aging demographic and changing presentation of the patient populations it is meant to help.¹ The implementation of frailty assessment across a variety of healthcare settings may get us closer to achieving better healthcare outcomes for older adults.

2.6 Brief Discussion of the Manuscript written exclusively by Kevin Boreskie

This published manuscript discusses the inevitable dilemma that healthcare systems will face across the globe, which is that of a globally aging population seeking care in healthcare systems that were not designed with their needs in mind.⁷⁴ It discusses how frailty screening can be used to better describe heterogeneous populations of older adults and, specifically, how chronological age does not capture this characterization adequately. Thus, frailty can be used to identify older adults who may require additional and different care needs from their robust peers. The more specific care goals related to frailty screening in each of primary care, acute care and surgical care differ but, generally, this identification can lead to the implementation of frailty-informed care pathways across all care settings to attempt to mitigate the adverse outcomes associated with frailty status. In the primary care setting, frailty identification can identify a patient requiring more connection to a broader clinical team for long-term primary prevention, connection to supports at home, and patient-centered outcomes regarding medical management choices over this longer term, such as with antihypertensives or blood thinners. In the acute care setting, more rapid frailty screening methods can identify an individual at risk of acute adverse outcomes, such as delirium, and can help guide informed goals of care discussions to guide patient disposition. In the surgical setting, frailty screening can be used to weigh benefits and risks of intervention and can identify a patient who requires further optimization prior to surgery to improve outcomes.

Since this manuscript was published in January 2022 it has been cited 81 times, is in the 93rd percentile of research outputs tracked on Altmetric and has been accessed just under 9,400 times. It has advanced the field as an impetus for further discussion regarding frailty-aware care pathways, largely in the acute care and cardiovascular disease space, as well as being used as rationale for studies examining frailty-associated outcomes in acute care settings and in

cardiovascular surgery settings. The manuscript has been cited in a number of different contexts but is most notably referenced in a policy document by the Health Promotion and Chronic Disease Prevention in Canada: Research, Policy and Practice document in September 2024 for a special issue on social prescribing in Canada.³²⁷ Specifically, it is cited in an article “Utilizing the determinants of healthy aging to guide the choice of social prescriptions for older adults” while discussing the lack of consensus on specific frailty tools in different settings. Other citations include studies in acute care settings examining outcomes of older adults with frailty, such as this notable one cited in *The Lancet Healthy Longevity* that showed worse outcomes for presenting trauma patients living with frailty compared to their robust peers in a sample of 193,156 patients.³²⁸ Additionally, other citations include studies discussing frailty in association with cardiovascular disease and cardiovascular surgery outcomes including in an update statement by the American Heart Association on acute coronary syndromes in older adults.³²⁹ My expertise in this topic area was also recognized by a guest editor of *Emergency Medicine Clinics* who invited me to write a manuscript for a special issue of this journal specifically on the use of frailty assessment in the emergency department, which is now published as an article in press.³³⁰

Despite evidence supporting the use of frailty screening in primary, acute care settings and in surgical settings, much work is still to be done to make this a reality in most areas. This manuscript outlined a summary of the supporting evidence for frailty screening across these different clinical domains and further research has been done supporting this use, but more work must be done explicitly outlining outcomes pre-post implementation of frailty-aware care pathways – not just studies looking at associated adverse outcomes of frailty and expert opinion stating that frailty-informed care pathways should improve outcomes for these patients. This conundrum of ongoing support for frailty informed care but few clinical areas fully implementing frailty specific care pathways was outlined in a recent article in the *Journal of the American Geriatrics Society* which stated the need for studies showing the effect of implementing frailty informed care on clinical outcomes for older adults living with frailty in a variety of care settings.³³¹

CHAPTER 3: Myokines as Biomarkers of Cardiovascular Disease Risk and Frailty in Females

3.1 Preface to the Manuscript written exclusively by Kevin Boreskie

This published case-control study was designed using initial data from the Duhamel lab's first cohort study examining novel screening tools of CVD in women. Using a propensity match of participants from this observational cohort of community-dwelling women aged 55 and older (NCT02863211) the hypothesis that myokines may act as biomarkers of both frailty and CVD risk was explored. To our knowledge, no prior works have examined the association of a panel of myokines with frailty and CVD risk status. As biomarker analyses are expensive, the case-control design with the propensity match allowed us to optimize the selection of our participants while still maintaining statistical power and reducing costs. The novelty of this research is evident as it was recognized with an invitation to compete as one of the four nationwide graduate student finalists at the 2018 Canadian Society for Exercise Physiology (CSEP) Annual Scientific Meeting in Niagara Falls, Ontario.¹⁷⁰ This data informed future research examining myokines as biomarkers of frailty described later in this thesis.

3.2 Manuscript status

Published in *Experimental Gerontology*, May 2020, Vol 133.¹⁷⁰ Used with permission from *Experimental Gerontology* with proof of this approval in Appendix E.

3.3 Authors

Kevin F. Boreskie, Christopher J. Oldfield, Jacqueline L. Hay, Teri L. Moffatt, Brett M. Hiebert, Rakesh C. Arora, Todd A. Duhamel

3.4 Contributions of Authors

Dr. Duhamel and I conceptualized the study and identified the methodology. I coordinated the cohort study. Data was collected by Jacqueline Hay, Chris Oldfield, Teri Moffatt and I. Brett Hiebert provided statistical support. As first author, I drafted the original manuscript with assistance from Chris Oldfield and Jacqueline Hay. All authors contributed academic expertise

and to reviewing and editing the manuscript. Dr. Duhamel and Dr. Arora acquired the funding for the project.

3.5 Manuscript

Abstract

Frailty is a risk factor for CVD. Biomarkers have the potential to detect the early stages of frailty, such as pre-frailty. Myokines may act as biomarkers of frailty-related disease progression, as a decline in muscle health is a hallmark of the frailty phenotype. Study 1 is a secondary analysis of 104 females, 55 years of age or older, with no previous history of CVD. Differences in systemic myokine concentrations based on frailty status and CVD risk profile were examined using a case-control design. Propensity matching identified two sets of 26 pairs with pre-frailty as the exposure variable in low or elevated CVD risk groups for a total 104 female participants. Frailty was assessed using the Fried Criteria and CVD risk was assessed using the Framingham Risk Score (FRS). Factorial ANOVA compared the main effects of frailty, CVD risk, and their interaction on the concentrations of 15 myokines. Differences were found when comparing elevated CVD risk status with low for the concentrations of EPO (384.76 ± 1046.07 vs. 206.63 ± 284.61 pg/mL, $p = 0.001$), FABP3 (2772.61 ± 3297.86 vs. 1693.31 ± 1019.34 pg/mL, $p = 0.017$), FGF21 (193.17 ± 521.09 vs. 70.18 ± 139.51 pg/mL, $p = 0.010$), IL-6 (1.73 ± 4.97 vs. 0.52 ± 0.89 pg/mL, $p = 0.023$), and IL-15 (2.62 ± 10.56 vs. 0.92 ± 1.25 pg/mL, $p = 0.022$). Pre-frail females had lower concentrations of fractalkine compared to robust (27.04 ± 20.60 vs. 103.62 ± 315.45 pg/mL, $p = 0.004$). Interaction effects between frailty status and CVD risk for FGF21 and OSM were identified. In elevated CVD risk, pre-frail females, concentrations of FGF21 and OSM were lower than that of elevated CVD risk, robust females (69.10 ± 62.86 vs. 317.24 ± 719.69 , $p = 0.011$; 1.73 ± 2.32 vs. 24.43 ± 69.21 , $p = 0.018$, respectively). These data identified specific biomarkers of CVD risk and biomarkers of frailty that are exacerbated with CVD risk.

Introduction

The population of Canada is rapidly aging, and by 2036, it is anticipated that older adults will compose 25% of the population.³³² This shifting demographic will present our healthcare system with new challenges, such as a higher prevalence of CVD³³³, and concomitant frailty.⁵⁷

Frailty is defined as a state of increased vulnerability to negative health outcomes^{63,334} that develops from cumulative declines across multiple physiological systems, and impairs one's ability to respond to stressors.^{57,335} Psychosocial factors also influence the development of frailty, as early life stress^{336,337} and low socioeconomic status³³⁸ have been implicated in the progression of frailty. Negative health outcomes associated with frailty include falls,³³⁹ disability,³⁴⁰ institutionalization,³⁴¹ hospitalization,³⁴² dementia,³⁴³ and mortality.³⁴⁴ Pre-frailty is an intermediate stage of frailty that increases risk for further frailty progression.⁶³ Although pre-frailty precedes frailty, it is still associated with negative health outcomes and is an independent predictor of disability, morbidity, and mortality.⁶³

CVD and frailty share common pathophysiologies such as chronic inflammation and insulin resistance, and as a result, can aggravate the presence of one another bidirectionally.^{345,346} A recent systematic review and meta-analysis identified that pre-frailty and frailty increased risk for CVD by 1.32 (95% CI 1.07-1.63) and 1.70 (95% CI 1.18-2.45) times, respectively, over a 4.4 year period.³⁴⁷ Similarly, those with a higher Framingham risk score (FRS) have an increased relative risk for incident frailty and pre-frailty of 2.15 (95% CI: 1.68-2.75) and 1.50 (95% CI: 1.29-1.74) times, respectively, over a 4-year follow-up, even after adjusting for cholesterol levels and smoking status.³⁴⁸ Our own work in this area has identified that pre-frail and frail females were at 1.5 and 2.8 higher odds of having a high FRS, as compared to their robust peers.³⁴⁹

The two most widely used models of frailty assessment are 1) the Fried Criteria (FC)⁶³ and 2) the Frailty Index developed by Rockwood et al.³⁵⁰ The FC measures the phenotypic aspects of frailty; whereas the Frailty Index assesses frailty based on the accumulation of health deficits present in an individual.³⁵¹ Health deficits can include signs, symptoms, laboratory values, disabilities, and diseases.³⁵² Given the phenotypic testing performed in frailty models such as the FC, skeletal muscle decline is strongly associated with frailty status.⁶³

Skeletal muscle acts as a paracrine and endocrine organ through the secretion of myokines - small peptides and cytokines released during muscular contraction.¹⁹⁹ Myokines have effects throughout the body beyond the immune system, and play a key role in maintaining functional health status.²⁰⁴ The concentration of various myokines have been associated with age-related disease progression.²¹¹ Specifically, a feature of frailty is a decline in muscle health; therefore, it is possible this characteristic decline effects the release of myokines from the muscle. Subclinical

changes in the concentration of these circulating myokines may occur in advance of clinically observable changes to phenotypic frailty assessments such as walking speed and grip strength, allowing for earlier identification of individuals at risk for functional declines. Additionally, the ranging effects of myokines may explain the decline seen across multiple physiological systems in frailty, as well as the shared pathophysiological connection between frailty and CVD.³⁵ Myokines may provide a snapshot in time of the health status for individual frailty status.²²¹

According to the FC, a decline in muscle health is characteristic of the frailty phenotype, however, little research has examined the value of muscle health biomarkers to screen for frailty and CVD risk.^{211,221} Females are at an increased risk of frailty, as compared to males,³⁵³ and have been underrepresented in CVD research.³⁵⁴ Therefore, the purpose of Study 1 was to investigate the association between myokines and frailty-related CVD progression in middle-aged and older females. Given that a decline in muscle health is a hallmark of the frailty phenotype, we hypothesized that myokines may act as biomarkers of frailty-related CVD progression.

Methods

Study Design

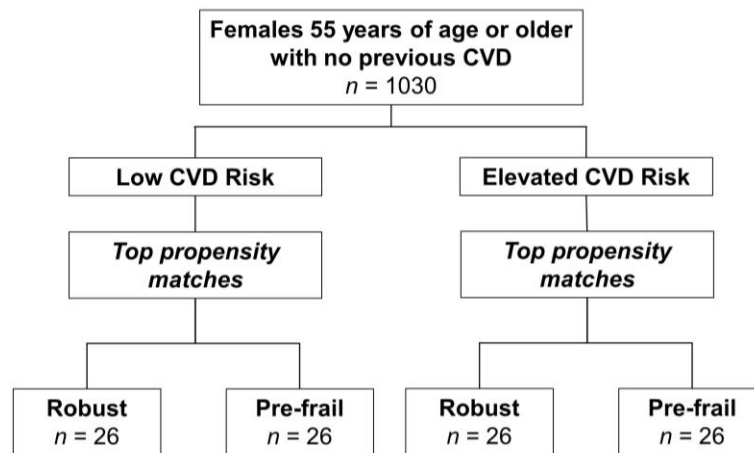
The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement guidelines for cross-sectional studies were followed in the development of this manuscript.³⁵⁵ This cross-sectional, secondary analysis used data from a previous observational cohort study³⁵⁶ that was approved by the University of Manitoba Health Research Ethics Board (H2014:224) and the St-Boniface Hospital Research Review Committee (RRC/2014/1417). The initial study used novel methods of CVD risk assessment, such as the standardized Fried Criteria (S-FC)^{63,357} and the Rasmussen Disease Score (RDS)³⁵⁸ to examine their sensitivity and specificity for predicting CVD events, as compared to traditional approaches, such as the Framingham Risk Score (FRS).³⁵⁹

Participants

The initial observational cohort study was conducted at the I. H. Asper Clinical Research Institute in Winnipeg, Manitoba, Canada. 1030 females were recruited through a convenience sampling approach using radio and media advertisements. Female participants aged 55 years or older with a Personal Health Information Number (PHIN) were included in the study. Participants

were excluded if they had previously been hospitalized for CVD, as described in the protocol.³⁵⁶ Next, propensity matching with pre-frailty as the exposure variable was used to identify the most similar two sets of 26 matched pairs in the low and elevated CVD risk groups for a total of 104 females (Figure 6).

Figure 6. Study flow diagram



Assessments

Frailty was measured using the FC as described by Fried et al.⁶³ Briefly, the Fried score examines the presence of five criteria: 1) low physical activity levels; 2) slow walking speed; 3) muscle weakness (grip strength); 4) self-reported exhaustion; and, 5) unintentional weight loss. Physical activity levels were assessed using the Paffenbarger Physical Activity Questionnaire³⁶⁰ and the International Physical Activity Questionnaire.³⁶¹ Muscle strength and walking speed were assessed using a grip strength dynamometer³⁶² and a 5 m gait speed test,³⁶³ respectively. Exhaustion was assessed using the Center for Epidemiological Studies – Depression Scale.³⁶⁴ Unintentional weight loss was determined using a participant questionnaire.³⁵⁶ Frailty status is determined based on the number of criteria present (i.e. 0 = robust, 1-2 = pre-frail, ≥ 3 = frail). Cutoffs for these criteria were based on those used in the original FC.⁶³

Participants were asked to fast for 12 hours prior to their appointment. Fasted blood samples were collected from all 1030 participants. Blood was immediately centrifuged at 2000 x

g for 10 minutes after collection. Plasma was then aliquoted into 500 uL samples and stored at -80° C.

FRS was determined based on the model described by D'Agostino et al.³⁵⁹ High-density lipoprotein (HDL) and total cholesterol were measured from the collected blood plasma. Resting blood pressure was measured in a supine position, following 10 minutes of rest in a calm and controlled, room temperature environment. Resting blood pressure and artery compliance were measured using the HD/PulseWave™ CR-2000 Research CardioVascular Profiling System. Height and weight measurements were obtained and used to calculate body mass index (BMI). Information on sex, age, smoking status, and prescribed medications were collected from participant questionnaires.

Plasma samples for the myokine analysis were collected at the same time as the plasma collected for the FRS analysis. Systemic myokine concentrations were analyzed with EMD Millipore's MILLIPLEX® Map Human Myokine Magnetic Bead Panel.³⁶⁵ This panel allows for the quantification of apelin (APLN), brain-derived neurotrophic factor (BDNF), erythropoietin (EPO), fatty acid-binding protein 3 (FABP3), fibroblast growth factor 21 (FGF21), fractalkine/CX3CL1, follistatin-like 1 protein (FSTL-1), interleukin-6 (IL-6), interleukin-15 (IL-15), irisin, leukemia inhibitory factor (LIF), myostatin/growth differentiation factor 8 (MSTN), oncostatin m (OSM), osteocrin/musclin (OSTN), and SPARC (osteonectin).

Statistical analysis

Participants within the cohort were classified as low CVD risk (as determined by a FRS <10%) or elevated (intermediate to high) CVD risk (FRS ≥10%). Propensity scores based on a greedy match with the exposure variable of pre-frailty status were calculated separately for the low and elevated CVD risk individuals to create a case-control design with four groups: i) Low FRS-Robust; ii) Elevated FRS-Robust; iii) Low FRS-Pre-frail, and; iv) Elevated FRS-Pre-frail. The propensity score matched females for age, BMI, continuous FRS, smoking status, and number of currently prescribed medications. Myokine concentrations were log transformed to adjust for skewness in the data distribution and to reduce variability in the data set. Factorial ANOVA was then used to compare the main and interaction effects of frailty and CVD risk on the log-transformed systemic concentration of 15 myokines. This study was powered using the concentration of the inflammatory cytokine IL-6, as this myokine has been implicated in the

frailty progression,³⁶⁶ from a similarly aged cohort³⁶⁷ (distribution of 9.5 ± 2.5 pg/mL [Mean $\pm$ SD]), which calculated a total sample size of 80 (20 in each group) to detect a 25% difference between groups (80% power; two-tailed alpha = 0.05). Only participants with data for that specific variable were included in the analysis.

Results

Descriptive data

The characteristics of the propensity matched elevated and low CVD risk groups were similar (Table 1). Robust participants in the low CVD risk group were younger, as compared to their pre-frail peers in the same group (62.92 ± 4.89 vs. 65.58 ± 7.10 years, $p = 0.02$). In the elevated CVD risk group, the prevalence of post-secondary education [22 (84.6%) vs 15 (57.7%), $p = 0.03$] and small artery compliance (3.80 ± 2.44 vs. 2.62 ± 1.13 mL/mmHg $\times$ 10, $p = 0.01$) were higher in robust females, as compared to pre-frail females. HDL cholesterol was higher in pre-frail females, as compared to robust females (1.63 ± 0.43 vs. 1.88 ± 0.61 mmol, $p = 0.04$), in the elevated CVD risk group.

Table 6. Participant Characteristics by Cardiovascular Disease Risk and Frailty Status

Low CVD Risk (n = 52)	Characteristics	Robust (n = 26)	Pre-frail (n = 26)	p-value
	Age (years)	62.92 ± 4.89	65.58 ± 7.10	0.02
	BMI (kg/m ²)	25.44 ± 4.14	24.92 ± 3.39	0.53
	Post-secondary education (% yes)	21 (80.8%)	18 (69.2%)	0.34
	Ex/Current smoker (% yes)	7 (26.9%)	10 (38.5%)	0.38
	FRS (%)	6.44 ± 2.12	6.92 ± 1.98	0.58
	Fried frailty score	0 ± 0	1.15 ± 0.37	<0.001
	Medication(s) (#)	0.42 ± 0.70	0.54 ± 0.81	0.50
	Hypertension medication(s)	1 (0.04%)	5 (19.2%)	0.08
	Diabetes medication(s)	0 (0%)	0 (0%)	-

	Lipid medication(s)	5 (19.2%)	4 (15.4%)	0.71
	HDL cholesterol (mmol/L)	1.92 ± 0.53	2.06 ± 0.44	0.36
	LDL cholesterol (mmol/L)	3.48 ± 0.67	3.62 ± 0.95	0.07
	Total cholesterol (mmol/L)	5.46 ± 0.75	5.74 ± 1.00	0.07
	Triglycerides (mmol/L)	1.13 ± 0.65	1.06 ± 0.40	0.10
	Blood glucose (mmol/L)	5.49 ± 0.72	5.28 ± 0.52	0.16
	Systolic blood pressure (mmHg)	124.15 ± 14.46	122.65 ± 10.93	0.64
	Diastolic blood pressure (mmHg)	70.35 ± 9.85	69.04 ± 8.05	0.39
	Small artery compliance (mL/mmHg $\times$ 10)	4.10 ± 3.17	4.52 ± 2.80	0.96
	Large artery compliance (mL/mmHg $\times$ 10)	12.41 ± 4.23	12.54 ± 4.48	0.58
	Grip strength (kg)	25.73 ± 2.74	23.27 ± 6.15	<0.001
	Walking speed (sec)	3.84 ± 0.53	4.15 ± 0.67	0.49
	6MWT (m)	580.11 ± 62.97	548.27 ± 73.09	0.19
	Total MVPA/week (mins)	613.20 ± 2156.80	313.18 ± 346.41	0.36

	Characteristics	Robust (n = 26)	Pre-frail (n = 26)	p-value
Elevated CVD Risk (n = 52)	Age (years)	67.92 ± 6.91	69.54 ± 5.23	0.09
	BMI (kg/m ²)	28.29 ± 3.67	27.01 ± 4.74	0.08
	Post-secondary education (% yes)	22 (84.6%)	15 (57.7%)	0.03
	Ex/Current smoker (% yes)	12 (46.2%)	13 (50.0%)	0.78
	FRS (%)	16.51 ± 5.88	16.29 ± 4.36	0.29
	Fried frailty score	0 ± 0	1.15 ± 0.37	<0.001

Medication(s) (#)	1.31 ± 1.38	1.54 ± 1.39	0.95
Hypertension medication(s)	15 (57.7%)	16 (61.5%)	0.78
Diabetes medication(s)	3 (11.5%)	4 (15.4%)	0.69
Lipid medication(s)	5 (19.2%)	7 (26.9%)	0.51
HDL cholesterol (mmol/L)	1.63 ± 0.43	1.88 ± 0.61	0.04
LDL cholesterol (mmol/L)	3.91 ± 0.80	3.64 ± 1.19	0.23
Total cholesterol (mmol/L)	5.84 ± 0.84	5.73 ± 1.26	0.26
Triglycerides (mmol/L)	1.60 ± 0.71	1.23 ± 0.56	0.16
Blood glucose (mmol/L)	6.29 ± 2.39	6.09 ± 1.74	0.61
Systolic blood pressure (mmHg)	143.65 ± 15.51	146.27 ± 10.12	0.15
Diastolic blood pressure (mmHg)	76.50 ± 7.75	77.73 ± 6.66	0.84
Small artery compliance (mL/mmHg $\times$ 10)	3.80 ± 2.44	2.62 ± 1.13	0.01
Large artery compliance (mL/mmHg $\times$ 10)	10.63 ± 3.04	9.85 ± 2.71	0.50
Grip strength (kg)	25.23 ± 3.54	21.62 ± 6.46	0.03
Walking speed (sec)	4.20 ± 0.58	4.48 ± 0.96	0.34
6MWT (m)	527.31 ± 59.20	509.79 ± 62.99	0.22
Total MVPA/week (mins)	339.96 ± 391.82	201.60 ± 266.50	0.87

*Continuous variables expressed as mean $\pm$ standard deviation and compared using T-test. **CVD**, cardiovascular disease; **BMI**, body mass index; **FRS**, Framingham Risk Score; **6MWT**, 6-minute walk test; **MVPA**, moderate-to-vigorous physical activity.*

Main results

The main effects for CVD risk and frailty as well as the interaction effects are described in Table 2. APLN and OSTN were removed from the analysis as the concentrations in the samples measured below detectable limits for the assay. Significantly higher concentrations were present in the elevated CVD risk, as compared to low CVD risk females for EPO (384.76 ± 1046.07 vs.

206.63 $\pm$ 284.61 pg/mL, $p = 0.001$), FABP3 (2772.61 $\pm$ 3297.86 vs. 1693.31 $\pm$ 1019.34 pg/mL, $p = 0.017$), FGF21 (193.17 $\pm$ 521.09 vs. 70.18 $\pm$ 139.51, $p = 0.010$), IL-6 (1.73 $\pm$ 4.97 vs. 0.52 $\pm$ 0.89 pg/mL, $p = 0.023$), and IL-15 (2.62 $\pm$ 10.56 vs. 0.92 $\pm$ 1.25 pg/mL, $p = 0.022$). Fractalkine/CX3CL1 concentrations were lower in pre-frail individuals, as compared to their robust peers (27.04 $\pm$ 20.60 vs. 103.62 $\pm$ 315.45 pg/mL, $p = 0.004$). Pre-frailty status and CVD risk had an interaction effect with FGF21 and OSM concentrations. In elevated CVD risk, pre-frail females, FGF21 concentration was lower than that of elevated CVD risk, robust females (69.10 $\pm$ 62.86 vs. 317.24 $\pm$ 719.69 pg/mL, $p = 0.011$). Similarly, concentrations of OSM were found to be lower in elevated CVD risk, pre-frail females when compared to elevated CVD risk, robust females (1.73 $\pm$ 2.32 vs. 24.43 $\pm$ 69.21 pg/mL, $p = 0.018$).

Table 7. Systemic Myokine Concentrations by Frailty and CVD Risk Status

Biomarker (pg/mL)	Robust (n = 52)	Pre-Frail (n = 52)	Main Effect Pre-Frailty (<i>p</i>-value)‡	Main Effect CVD Risk (<i>p</i>-value)‡	Interaction Effect (<i>p</i>- value)‡
BDNF					
Low CVD Risk	439.73 ± 169.54	487.91 ± 214.45	0.751	0.078	0.392
Elevated CVD Risk	597.13 ± 332.19	536.53 ± 246.52			
EPO					
Low CVD Risk	227.80 ± 308.58	185.47 ± 262.85	0.180	0.001	0.845
Elevated CVD Risk	547.55 ± 1474.17	221.98 ± 63.33			
FABP3					
Low CVD Risk	1557.96 ± 866.36	1828.66 ± 1153.68	0.746	0.017	0.800
Elevated CVD Risk	2773.68 ± 3489.89	2771.53 ± 3163.47			
FGF21					
Low CVD Risk	41.07 ± 48.01	99.29 ± 188.77	0.447	0.010	0.011
Elevated CVD Risk	317.24 ± 719.69	69.10 ± 62.86			
Fractalkine/CX3CL1					
Low CVD Risk	48.12 ± 71.46	26.14 ± 23.86	0.004	0.058	0.290
Elevated CVD Risk	159.12 ± 437.58	27.93 ± 17.16			
FSTL1					
Low CVD Risk	1439.62 ± 1860.57	887.03 ± 926.99	0.400	0.632	0.445
Elevated CVD Risk	1943.42 ± 4130.95	993.12 ± 885.88			
IL-6					
Low CVD Risk	0.56 ± 0.91	0.49 ± 0.88	0.814	0.023	0.501

Elevated CVD Risk	1.63 ± 5.27	1.83 ± 4.76			
IL-15					
Low CVD Risk	1.10 ± 1.72	0.74 ± 0.42	0.335	0.022	0.692
Elevated CVD Risk	4.14 ± 14.89	1.09 ± 1.02			
Irisin					
Low CVD Risk	242.20 ± 203.16	203.80 ± 159.38	0.068	0.070	0.254
Elevated CVD Risk	637.81 ± 1981.19	109.82 ± 118.77			
LIF					
Low CVD Risk	3.95 ± 5.51	3.34 ± 2.41	0.432	0.607	0.627
Elevated CVD Risk	12.60 ± 36.41	3.53 ± 3.13			
MSTN					
Low CVD Risk	420.69 ± 777.62	381.87 ± 636.50	0.097	0.150	0.085
Elevated CVD Risk	5378.21 ± 16261.62	351.25 ± 383.80			
OSM					
Low CVD Risk	1.51 ± 1.46	2.97 ± 5.87	0.232	0.248	0.018
Elevated CVD Risk	24.43 ± 69.21	1.73 ± 2.32			
SPARC					
Low CVD Risk	352.38 ± 1141.39	120.28 ± 47.04	0.173	0.301	0.989
Elevated CVD Risk	165.73 ± 71.97	164.19 ± 129.25			

Continuous variables expressed as mean pg/mL ± standard deviation and compared using two-way ANOVA. †P values presented are based on log transformed data. **BDNF**, brain-derived neurotrophic factor; **EPO**, erythropoietin; **FABP3**, fatty acid-binding protein 3; **FGF21**, fibroblast growth factor 21; **FSTL 1**, follistatin-like 1 protein; **IL-6**, interleukin-6; **IL-15**, interleukin-15; **LIF**, leukemia inhibitory factor; **MSTN**, myostatin/growth differentiation factor 8; **OSM**, oncostatin m; **SPARC**, secreted protein acidic and rich in cysteine/osteonectin.

Discussion

Higher concentrations of IL-6, IL-15, EPO, FABP3, and FGF23 were present in females of elevated CVD risk, as compared to those of low CVD risk. Fractalkine/CX3CL1 concentrations were lower in pre-frail females, as compared to their robust peers. Pre-frailty status and CVD risk had an interaction effect with FGF21 and OSM concentrations. In elevated CVD risk, pre-frail females, FGF21 concentrations were lower than that of elevated CVD risk, robust females. Similarly, OSM concentrations were lower in elevated CVD risk, pre-frail females when compared to elevated CVD risk, robust females.

Only the concentration of fractalkine/CX3CL1 was lower in pre-frail, as compared to robust females. Contrary to these findings, some previous literature suggests frail individuals may actually possess increased concentrations of fractalkine/CX3CL1 due to chronic inflammation, as fractalkine/CX3CL1 concentration is elevated during inflammatory conditions.³⁶⁸ Negative correlations between fractalkine/CX3CL1 and some chronic cognitive conditions have been observed,³⁶⁹ so it is possible that lower levels of fractalkine/CX3CL1 in these pre-frail females may be impairing their necessary immune response to health stressors. Impaired immune response is a key characteristic of the progression of frailty; therefore, this is a plausible rationale.³⁷⁰

This data supports the use of myokines to identify females at risk for physiological decline and CVD. IL-6 and IL-15 concentrations were higher in the elevated CVD risk group, as compared to the low CVD risk group. IL-6 and IL-15 are cytokines involved in the immune response³⁷¹ and compromised immune function and inflammation are thought to play a role in the development of both frailty⁵⁴ and CVD.³⁷² Frailty is associated with dysfunction of the immune system and increased inflammation,³⁷³ and increased inflammation is also an etiologic factor in CVD.³⁷⁴ IL-6 has been previously identified as a biomarker of future cardiovascular events³⁷⁵ and a polymorphism of the IL-6 gene is associated with the development and progression of CVD.³⁷⁶ IL-15 has already been identified as biomarker of CVD, as plasma concentrations of IL-15 were reported to be significantly higher in patients with coronary artery disease³⁷⁷ and peripheral artery disease,³⁷⁸ when compared to healthy subjects.

Concentrations of EPO, FABP3, and FGF21 were higher in the elevated CVD risk cohort, as compared to the low CVD risk females. EPO is the primary regulator of red blood cell production.³⁷⁹ EPO has also been reported to be produced, and locally released, by many tissues in response to stress.³⁸⁰ Higher levels of EPO may suggest a state of increased inflammation in the

elevated CVD risk cohort. FABP3 is a small, cytosolic protein abundantly expressed in cardiac and skeletal muscle.³⁸¹ Following myocardial injury, FABP3 is often released into the circulation^{382–384}; therefore, it may be a potential biomarker of myocardial injury. FGF21 functions in glucose homeostasis and metabolic regulation.³⁸⁵

Concentrations of IL-6, IL-15, EPO, FABP3, and FGF21 were all higher in the elevated CVD risk cohort, as compared to the low CVD risk cohort. This data supports our hypothesis that myokines can be used as biomarkers of CVD risk and previous studies have suggested the potential of myokines to act as diagnostic indicators of CVD risk.²¹³ Although, the propensity matched pairs were similar, the small difference in age between the low and elevated CVD risk females may explain some of these results.

An interaction effect of frailty and CVD risk status was present for both FGF21 and OSM. Seen as an adaptation to nutritional stress,³⁸⁶ higher levels of FGF21 were found in elevated CVD risk and females in the low CVD risk, pre-frail group had concentrations similar to those with elevated CVD risk. Some literature suggests higher levels of FGF21 can be beneficial for those with cardiometabolic diseases.³⁸⁷ Further, the systemic administration of FGF21 has been used as a treatment for obesity in rat models.³⁸⁸ However, recent research in humans has reported associations between increased concentrations of FGF21 and CVD.³⁸⁹ The higher concentrations seen in both elevated CVD risk and low CVD risk, pre-frail females may be due to a resistance to FGF21 that has developed over time.³⁹⁰ Concentration of OSM was higher in robust, elevated CVD risk females compared all other groups, including those who were pre-frail, elevated CVD risk. Interestingly, pre-frail, low CVD risk females had higher concentrations of OSM compared to their robust, low CVD risk peers. OSM is an inflammatory cytokine of the IL-6 family³⁹¹ and has been suggested to have a role in the progression of diseases involving chronic inflammation such as vascular diseases and cancers.³⁹² As such, the presence of only an interaction effect between frailty status and CVD risk status for OSM is difficult to explain. It may be that the females in our cohort had not progressed to stages of CVD risk or frailty that were advanced enough to present with changes in these myokines.

It is possible that simultaneous examination of complementary biomarkers may work better as biomarkers of risk for conditions involving complex and broad pathophysiological processes, such as frailty.¹⁷⁴ Due to ease of collection in a clinical setting, systemic myokine concentrations were examined, as opposed to directly measuring myokine release from muscle. Thus, it is possible

that some of the cytokines measured in the systemic circulation were released not just from skeletal muscle, but from other tissues.²²¹ The systemic myokine concentrations had large ranges, even within groups based on CVD and frailty status. Despite powering our study based on IL-6, the variability seen in our data for many myokines suggests that larger sample sizes may be needed to detect differences. The cause of such variation is unclear, but it is possible some participants performed more, or less, physical activity before their appointment, compared to their peers, despite being asked to avoid this, which could alter myokine release. Additionally, females may have to be further in the progression of frailty and CVD risk to present with more noticeable differences in systemic myokine concentrations. Future research should perform analyses such as these in larger cohorts. Another limitation of Study 1 was a lack of control for muscle mass. The measurement of muscle mass could have provided additional information on the muscular health of the participants, which may have assisted in explaining differences in the myokine concentrations between groups. This is a limitation we will be addressing in our future research in which we are assessing body composition.

Further refinement of novel biomarker-based diagnostic tools for frailty are needed. The vast pleiotropic effects of myokines throughout the body are not fully elucidated. Additional research identifying the effects of these signaling molecules, the optimal method for their measurement in a clinical setting, and defined physiological ranges are needed to better understand the roles of myokines in the body. Additionally, concentrations of these biomarkers need to be evaluated in different states, such as at rest versus during different exercise modalities. Not only will this provide a better understanding of the functions of myokines in the body, but it will also provide the information needed to use this knowledge in the development of therapeutic interventions.

These findings will inform the refinement of novel biomarker-based diagnostic tools to better detect early stages of CVD risk and frailty. This could allow for earlier identification of physiological decline and interventions that may be initiated at an early stage.¹⁷⁴ Additionally, this research is relevant as the use of frailty to inform treatment and the examination of the effects of physical activity on frailty is one of the top ten research priorities of the Canadian Frailty Network.²³⁵ The identification of myokines associated with frailty and CVD may elucidate the role that physical activity could play in preventing, halting or reversing frailty progression. The tracking of biomarkers related to frailty and CVD could allow for the response to therapeutic

interventions, such as physical activity, to be assessed.¹⁷⁴ These data also enhance our understanding of the potential importance of muscle health in linking the pathological processes of frailty and CVD progression. Exploration of the sensitivity and specificity of these biomarkers for predicting CVD events will be completed in 2022 after a 5-year follow-up period based on health data tracking using the Manitoba Health Data Repository.³⁵⁶

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3.6 Brief Discussion of the Manuscript written exclusively by Kevin Boreskie

This published manuscript was the first examination by our lab group examining blood-based biomarkers of frailty.³⁹³ Experts recommended research in this space as biomarkers of frailty were thought to potentially identify earlier frailty progression prior to notable clinical changes while simultaneously elucidating the generalized pathophysiological decline seen in frailty.^{175,180,211,221,394} Specifically, myokines were recommended as biomarkers of frailty given their release from muscle tissue and association with physical activity as well as the associated reduction in muscle quality and physical activity seen in frailty progression.¹⁷⁷ Despite this recommendation, only a few studies had examined the association of myokines with frailty. Myokines have also been associated with progression of CVD pathophysiology.^{214-216,395} Given the shared aspects of pathophysiology between frailty and CVD risk, as well as the thought that myokines likely play a role in part of this progression, we looked at the association of a panel of myokines in association with both frailty status and cardiovascular disease risk status. In order to efficiently do this as an initial exploratory analysis, we used a propensity match strategy as described in the manuscript. This analysis showed that EPO, FABP3, FGF21, IL-6 and IL-15

systemic concentrations were different in high CVD risk groups, as determined by Framingham Risk Score, as compared to their low-risk peers. This supports the notion that immunosenescence and chronic inflammation may be associated with CVD disease progression.¹⁵⁹ Pre-frail individuals had lower systemic concentrations of fractalkine/CX3CL1 as compared to their robust peers, another myokine that has been associated with either higher or lower concentrations in the context of chronic inflammation in chronic conditions.^{368,369}

Since publication in *Experimental Gerontology*, this manuscript has been cited 15 times. Of note, the manuscript was cited by a European Myeloma Network frailty position paper in *Leukemia*, a high impact factor journal (impact factor of 12.8).³⁹⁶ Part of this article was describing different modalities for frailty assessment/screening and a discussion on the potential for biomarkers of frailty. My manuscript was listed as an example of a study looking at such a thing. It has also been cited in documents examining myokines or cytokines as biomarkers in states other than frailty, such as in peripheral artery disease³⁹⁷ and reduced physical capacity.³⁹⁸ Additionally, it has been cited in general discussion in a manuscript on concomitant frailty and sarcopenia,³⁹⁹ as some examples.

This study added to the field as one of the few papers examining the association of systemic myokines with frailty, as opposed to general aging or sarcopenia, which has been researched more relatively.²²¹ Despite this, significant further research is needed before myokines can be implemented in any clinical capacity as biomarkers of frailty and cardiovascular disease. This includes works to identify ideal methodology for measuring myokines with good efficacy while being clinically feasible. Significant further work is also needed to continue the pleiotropic effects of myokines and exerkinases more generally.^{199,203,207} Myokine concentrations were assessed at rest in this study, but it may be more clinically relevant to measure their pre-post relative change after stimulus, such as different physical activity modalities, while also better characterizing ‘normal’ concentrations in different populations. Finally, interventions to alter expression of myokines associated with frailty and cardiovascular disease will have to be explored and subsequent research demonstrating that altering this expression, in turn, slows or reverses frailty and CVD progression.^{207,400}

CHAPTER 4: Bone Metabolism Analytes as Biomarkers of Pre-Frailty and Cardiovascular Disease Risk in Females

4.1 Preface to the Manuscript written exclusively by Kevin Boreskie

The following manuscript is a published case-control study using a similar approach to the study described in Chapter 3. Again, a propensity match was used to identify the most appropriate participants from the Duhamel lab's initial CVD cohort sample (NCT02863211) for an initial examination of the hypothesis that analytes related to bone metabolism may act as biomarkers of frailty and CVD risk. This complements the work in Chapter 3 looking at myokines as biomarkers of frailty and CVD due to the increased recognition of muscle-bone crosstalk as an important process in health regulation, specifically in aging.²²³ The case-control design with propensity match again allows us to optimally select participants to maintain statistical power and more efficiently performing the required assays.

4.2 Manuscript status

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4.3 Authors

Kevin F. Boreskie, Christopher J. Oldfield, Liam A. Thompson, Teri L. Moffatt, Brett M. Hiebert, Rakesh C. Arora, and Todd A. Duhamel

4.4 Contributions of Authors

Dr. Duhamel and I conceptualized the study and identified the methodology. I coordinated the data collection from the cohort study with Jacqueline Hay, Chris Oldfield and Teri Moffatt. Brett Hiebert provided statistical expertise. As first author, I drafted the original manuscript. All authors contributed to reviewing and editing. Dr. Duhamel and Dr. Arora acquired the funding for the project.

4.5 Manuscript

Abstract

Background: Frailty and cardiovascular disease (CVD) share common pathophysiology in their progression. Declines in bone health often occur concomitantly with frailty and CVD. Therefore,

analytes of bone metabolism may be useful biomarkers of pre-frailty and CVD risk. The aim of this study was to identify the effects of pre-frailty and CVD risk on the systemic concentrations of bone metabolism and inflammatory analytes in middle-aged and older females.

Method: This case-control study is a secondary analysis of data from 1030 females with no self-reported history of CVD. Frailty was measured using the Fried Criteria, and females were stratified into low and elevated CVD risk using the Framingham risk score. Greedy matching with pre-frailty as the exposure variable identified 26 matched pairs in the low and elevated CVD risk groups for a total of 104 females. Factorial ANOVA compared differences in the log transformed concentrations of 15 bone metabolism analytes based on pre-frailty status, CVD risk, and the interaction.

Results: Differences in the systemic concentrations of IL-6 (5.25 ± 14.30 vs. 1.35 ± 1.74 pg/mL, $p = 0.001$), TNF α (1.41 ± 1.83 vs. 0.89 ± 0.40 pg/mL, $p = 0.06$), and leptin (12628.48 ± 10472.90 vs. 7562.96 ± 4972.25 pg/mL, $p = 0.023$) were found in elevated CVD risk status compared to low. No differences in the concentrations of bone metabolism analytes were found based on pre-frailty status, nor were any interaction effects.

Conclusion: Differences were found in the concentrations of cytokines involved in bone metabolism based on CVD risk; however, no differences were found based on pre-frailty status. IL-6, TNF α , and leptin may act as biomarkers of CVD risk, but this study does not support the use of the examined analytes involved in bone metabolism as biomarkers of pre-frailty.

Introduction

The population of Canada is aging rapidly. Currently, 17% of the population is 65 and older,⁴⁰¹ and this proportion is expected to reach 25% by 2036.⁴⁰² From a public health perspective, this is likely to result in an increasing prevalence of both frailty²⁶ and cardiovascular disease (CVD)⁴⁰³ as the presence of these conditions is associated with age.

Frailty is characterized by a state of increased vulnerability to health stressors resulting from the dysregulation of multiple physiological systems.⁴⁰⁴ This leads to a reduction in physiological reserve and an impaired ability to respond to adverse health events.¹⁵ Pre-frailty is an intermediary stage preceding frailty that increases risk of frailty progression.¹⁵ Individuals who

are pre-frail are able to respond to health stressors,²⁵ but are still at increased risk for disability, mortality, and morbidities, including CVD.^{15,33}

Frailty and CVD share similar risk factors and pathophysiological progression.⁵⁷ Sarcopenia and osteoporosis, as well as chronic inflammation¹⁵⁸ are often associated with both conditions. As a result, the presence of frailty and CVD are thought to exacerbate each other.³⁴⁶ Recently, large pooled data sets have identified that frail individuals are at increased risk for CVD^{20,347} and this increased risk is apparent in the earlier pre-frail stage.^{347,405,406} This relationship is also bidirectional as individuals with a high CVD risk had an increased risk for incident frailty over a 4 year period (OR 2.15; 95% CI 1.68-2.75).¹⁶²

Biomarkers of both frailty and CVD are beginning to be examined with the goal of accurately identifying at-risk individuals earlier in downward health trajectory.^{88,149} For example, blood-based biomarkers of inflammation have been studied as biomarkers of frailty in the younger-old and the very-old.⁴⁰⁷ Given the related pathophysiology of frailty and CVD^{408,409}, these conditions may share common biomarkers.

Bone and muscle are both highly dynamic organs which secrete cytokines that act throughout the body.^{203,410-412} The cross-talk between bone and muscle is receiving increased attention as it may have a role in health maintenance and decline.^{203,217,224} Dysregulation in bone-muscle cross-talk can influence the progression of osteoporosis and sarcopenia²²⁴ – conditions often seen in frailty.^{174,225} Pre-frail and frail individuals are at increased risk for osteoporotic fracture^{226,227} and have been shown to present with reduced bone health.^{228,229} Bone and cardiovascular health are also suggested to be linked, since reduced bone health is associated with increased risk for CVD, and vice versa,^{230,231} potentially implicating this same dysregulated cross-talk in CVD progression. Specifically, females who are post-menopause may be at increased risk for frailty-related reductions in bone and cardiovascular health, as hormonal changes post-menopause increase risk for both osteoporosis and CVD.^{138,413} Bone metabolism analytes have previously been measured in the systemic circulation as a means of assessing bone health.^{412,414} Given the relevance of bone metabolism and bone-muscle cross-talk to frailty characteristics and cardiovascular health, it is possible that these analytes may act as biomarkers of frailty and CVD.

The objective of this study was to identify the main and interaction effects of pre-frailty and CVD risk on the systemic concentrations of bone metabolism and inflammatory analytes in middle-aged and older females. Reduced bone health can occur concomitantly with frailty and

CVD; therefore, bone metabolism analytes may be useful biomarkers of pre-frailty and CVD risk. The identification of biomarkers in the early pre-frailty stage could allow for easier detection and tracking of frailty progression.¹⁷⁴

Materials and Methods

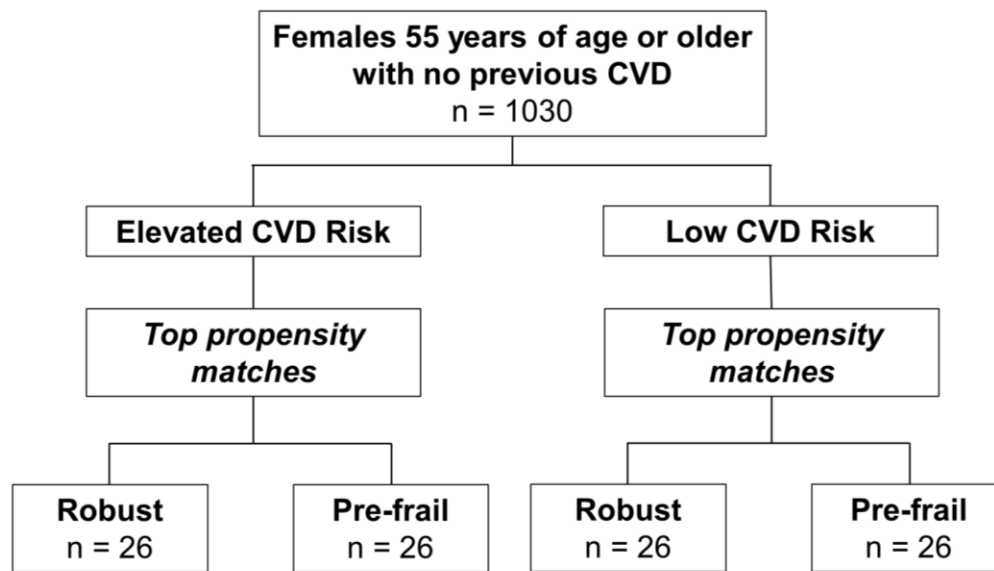
Study Design

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement guidelines for case-control studies were adhered to in the development of this manuscript.⁴¹⁵ This case-control study was a secondary analysis of data previously collected from an observational cohort study⁴¹⁶ approved by the University of Manitoba Health Research Ethics Board (H2014:224) and the St-Boniface Hospital Research Review Committee (RRC/2014/1417). The initial study examined the sensitivity and specificity of novel CVD risk assessment methods for predicting CVD events, such as the standardized Fried Criteria (FC)⁴¹⁷ and the Rasmussen Disease Score,⁴¹⁸ as compared to established approaches, such as the Framingham Risk Score (FRS).⁴¹⁹

Participants

The initial observational cohort study was conducted in Winnipeg, Manitoba, Canada and recruited 1030 females using radio and media ads. Participants were included if they were a female aged 55 years or older with no previous self-reported CVD, had a Personal Health Information Number (PHIN), and if informed consent was obtained. Participants were excluded if they had been previously hospitalized for CVD, as described in the protocol paper.⁴¹⁶ Propensity matching with pre-frailty as the exposure variable was then used to identify a top set of 26 matched pairs in the low and elevated CVD risk groups for a total of 104 females to better control for potential confounding variables (Figure 7).

Figure 7. Study flow diagram



Assessments

The FC was used to assess the frailty phenotype¹⁵. The FC assesses the presence of five criteria: 1) low physical activity levels; 2) slow walking speed; 3) muscle weakness (grip strength); 4) self-reported exhaustion; and 5) unintentional weight loss. Frailty status is determined using the number of criteria present (i.e. 0 = robust, 1-2 = pre-frail, ≥ 3 = frail). The cut-offs demarcating the presence or absence of the criteria were based on those of Fried et al.¹⁵

Participants were instructed to fast for 12 hours pre-appointment. Fasted blood samples were collected from all 1030 females. Immediately following sample collection, blood was centrifuged at 2000 x g for 10 minutes at 4° C. Plasma was then aliquoted into microcentrifuge tubes and stored at -80° C for future analyses.

The FRS was determined based on the model of D'Agostino et al.⁴¹⁹ High-density lipoprotein (HDL) and total cholesterol were measured using the collected plasma. Resting blood pressure was measured in a supine position, following 10 minutes of rest, in a calm environment at room temperature. Resting blood pressure was measured using the HD/PulseWaveTM CR-2000 Research CardioVascular Profiling System (Hypertension Diagnostics, Minneapolis, MN, USA). Participant sex, age, smoking status, diabetes status, and blood pressure treatment status were collected from participant questionnaires.

The systemic concentrations of bone metabolism analytes were measured with a EMD Millipore MILLIPLEX® Map Human Bone Magnetic Bead Panel - Bone Metabolism Multiplex Assay (EMD Millipore, Burlington, MA, USA).⁴²⁰ This multiplex assay allows for the concurrent quantification of adrenocorticotrophic hormone (ACTH), Dickkopf WNT signaling pathway inhibitor 1 (DKK1), interleukin-6 (IL-6), insulin, tumor necrosis factor α (TNF α), leptin, osteoprotegrin (OPG), osteocalcin (OC), osteopontin (OPN), interleukin-1 β (IL-1 β), parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF23). These analytes were selected due to their roles in bone metabolism, amongst others. Plasma samples used in the analysis of the analytes were collected simultaneously as the plasma samples used in the FRS analysis.

Statistical Analysis

Participants were classified as low CVD risk (FRS <10%) or elevated CVD risk (FRS $\geq$ 10%). Propensity scores based on a greedy match were calculated for the low and elevated CVD risk participants with the exposure variable of pre-frailty status, creating a case-control design with four groups: 1) Low FRS-Robust; 2) Elevated FRS-Robust; 3) Low FRS-Pre-frail, and; 4) Elevated FRS-Pre-frail. Propensity scores matched females for age, body mass index, continuous FRS, smoking status, and the number of currently prescribed medications. The analyte concentrations were log-transformed to adjust for skewness and reduce variability in the dataset. Factorial ANOVA compared the main and interaction effects of pre-frailty and CVD risk on the log-transformed systemic plasma concentration of 12 bone metabolism analytes. A Tukey post-hoc test was used to compare means where significant difference (p -value = <0.05) was found. The study was powered using the concentration of the inflammatory cytokine IL-6 from a similarly aged cohort⁴²¹ (distribution of 9.5 ± 2.5 pg/mL [Mean $\pm$ SD]), a total sample size of 80 (20 in each group) was calculated to detect a 25% difference between groups (80% power; two-tailed alpha = 0.05). All statistical calculations were made using SPSS version 26 (IBM Corporation, Armonk, NY, USA).

Results

Descriptive Data

Characteristics of the low and elevated CVD risk propensity matched groups were similar

(Table 1). Mean age was lower in the robust group as compared to the pre-frail group in females of low CVD risk (62.92 ± 4.89 vs. 65.58 ± 7.10 years, $p = 0.02$). Differences were found in the prevalence of post-secondary education [22 (84.6%) vs. 15 (57.7%), $p = 0.03$], HDL cholesterol (1.63 ± 0.43 vs. 1.88 ± 0.61 mmol, $p = 0.04$) and small artery compliance (3.80 ± 2.44 vs. 2.62 ± 1.13 mL/mmHg $\times$ 10, $p = 0.01$) between females who were robust and females who were pre-frail in the elevated CVD risk group. All females were post-menopause.

Table 8. Characteristics of participants by CVD risk and frailty status

	Characteristics	Robust	Pre-frail	<i>p</i> -value
		(n = 26)	(n = 26)	
Low CVD Risk (n = 52)	Age (years)	62.92 ± 4.89	65.58 ± 7.10	0.02
	BMI (kg/m ²)	25.44 ± 4.14	24.92 ± 3.39	0.53
	Post-secondary education (% yes)	21 (80.8%)	18 (69.2%)	0.34
	Ex/Current smoker (% yes)	7 (26.9%)	10 (38.5%)	0.38
	FRS (%)	6.44 ± 2.12	6.92 ± 1.98	0.58
	Fried frailty score	0 ± 0	1.15 ± 0.37	<0.001
	Medication(s) #	0.42 ± 0.70	0.54 ± 0.81	0.50
	Hypertension medication(s) (n)	1 (0.04%)	5 (19.2%)	0.08
	Diabetes medication(s) (n)	0 (0%)	0 (0%)	-
	Lipid medication(s) (n)	5 (19.2%)	4 (15.4%)	0.71
	HDL cholesterol (mmol/L)	1.92 ± 0.53	2.06 ± 0.44	0.36
	LDL cholesterol (mmol/L)	3.48 ± 0.67	3.62 ± 0.95	0.07
	Total cholesterol (mmol/L)	5.46 ± 0.75	5.74 ± 1.00	0.07
	Triglycerides (mmol/L)	1.13 ± 0.65	1.06 ± 0.40	0.10
	Blood glucose (mmol/L)	5.49 ± 0.72	5.28 ± 0.52	0.16
	Systolic blood pressure (mmHg)	124.15 ± 14.46	122.65 ± 10.93	0.64
	Diastolic blood pressure (mmHg)	70.35 ± 9.85	69.04 ± 8.05	0.39

Elevated CVD Risk (n = 52)	Small artery compliance (mL/mmHg $\times$ 10)	4.10 $\pm$ 3.17	4.52 $\pm$ 2.80	0.96
	Large artery compliance (mL/mmHg $\times$ 10)	12.41 $\pm$ 4.23	12.54 $\pm$ 4.48	0.58
	Grip strength (kg)	25.73 $\pm$ 2.74	23.27 $\pm$ 6.15	<0.001
	Walking speed (sec)	3.84 $\pm$ 0.53	4.15 $\pm$ 0.67	0.49
	6MWT (m)	580.11 $\pm$ 62.97	548.27 $\pm$ 73.09	0.19
	Total MVPA/week (mins)	613.20 $\pm$ 2156.80	313.18 $\pm$ 346.41	0.36
	Characteristics	Robust (n = 26)	Pre-frail (n = 26)	p-value
	Age (years)	67.92 $\pm$ 6.91	69.54 $\pm$ 5.23	0.09
	BMI (kg/m ²)	28.29 $\pm$ 3.67	27.01 $\pm$ 4.74	0.08
	Post-secondary education (% yes)	22 (84.6%)	15 (57.7%)	0.03
	Ex/Current smoker (% yes)	12 (46.2%)	13 (50.0%)	0.78
	FRS (%)	16.51 $\pm$ 5.88	16.29 $\pm$ 4.36	0.29
	Fried frailty score	0 $\pm$ 0	1.15 $\pm$ 0.37	<0.001
	Medication(s) #	1.31 $\pm$ 1.38	1.54 $\pm$ 1.39	0.95
	Hypertension medication(s) (n)	15 (57.7%)	16 (61.5%)	0.78
	Diabetes medication(s) (n)	3 (11.5%)	4 (15.4%)	0.69
	Lipid medication(s) (n)	5 (19.2%)	7 (26.9%)	0.51
	HDL cholesterol (mmol/L)	1.63 $\pm$ 0.43	1.88 $\pm$ 0.61	0.04
	LDL cholesterol (mmol/L)	3.91 $\pm$ 0.80	3.64 $\pm$ 1.19	0.23
	Total cholesterol (mmol/L)	5.84 $\pm$ 0.84	5.73 $\pm$ 1.26	0.26
	Triglycerides (mmol/L)	1.60 $\pm$ 0.71	1.23 $\pm$ 0.56	0.16
	Blood glucose (mmol/L)	6.29 $\pm$ 2.39	6.09 $\pm$ 1.74	0.61
	Systolic blood pressure (mmHg)	143.65 $\pm$ 15.51	146.27 $\pm$ 10.12	0.15

Diastolic blood pressure (mmHg)	76.50 ± 7.75	77.73 ± 6.66	0.84
Small artery compliance (mL/mmHg×10)	3.80 ± 2.44	2.62 ± 1.13	0.01
Large artery compliance (mL/mmHg×10)	10.63 ± 3.04	9.85 ± 2.71	0.50
Grip strength (kg)	25.23 ± 3.54	21.62 ± 6.46	0.03
Walking speed (sec)	4.20 ± 0.58	4.48 ± 0.96	0.34
6MWT (m)	527.31 ± 59.20	509.79 ± 62.99	0.22
Total MVPA/week (mins)	339.96 ± 391.82	201.60 ± 266.50	0.87

Continuous variables expressed as mean ± standard deviation and compared using T-test. CVD, cardiovascular disease; BMI, body mass index; FRS, Framingham Risk Score; 6MWT, 6-minute walk test; MVPA, moderate-to-vigorous physical activity.

Main Results

The main effects for pre-frailty and CVD risk status and their interaction effects are found in Table 2. ACTH and PTH were not included in the final analysis as concentrations in the majority of the samples measured below detectable limits for the assay. Significantly higher concentrations were present in females of elevated CVD risk, as compared to those of low CVD risk for IL-6 (5.25 ± 14.30 vs. 1.35 ± 1.74 pg/mL, $p = 0.001$), TNF α (1.41 ± 1.83 vs. 0.89 ± 0.40 pg/mL, $p = 0.006$), and leptin (12628.48 ± 10472.90 vs. 7562.96 ± 4972.25 pg/mL, $p = 0.023$). No differences in the concentrations of bone metabolism analytes were found based on pre-frailty status. No interaction effects between pre-frailty status and CVD risk were found.

Table 9. Concentrations of Systemic Bone Metabolism Analytes by Frailty and CVD Risk

Biomarker (pg/mL)	Robust (n = 52)	Pre-frail (n = 52)	Main Effect of Pre-frailty (<i>p</i>-value) ‡	Main Effect of CVD Risk (<i>p</i>-value) ‡	Interaction Effect (<i>p</i>-value) ‡
DKK1					
Low CVD Risk	156.34 (40.27)	164.00 (69.01)	0.778	0.138	0.944
Elevated CVD Risk	145.61 (53.28)	147.97 (52.20)			
IL-6					
Low CVD Risk	1.27 (1.71)	1.43 (1.80)	0.183	0.001	0.476
Elevated CVD Risk	5.34 (17.99)	5.17 (9.66)			
Insulin					
Low CVD Risk	346.39 (281.18)	274.54 (227.39)	0.173	0.228	0.942
Elevated CVD Risk	457.90 (581.96)	358.42 (324.19)			
TNFα					
Low CVD Risk	0.83 (0.42)	0.95 (.38)	0.546	0.006	0.320
Elevated CVD Risk	1.55 (2.45)	1.27 (.88)			
Leptin					
Low CVD Risk	7179.27 (4087.90)	7946.64 (5780.87)	0.177	0.023	0.138

Elevated CVD Risk	13730.65 (9171.93)	11526.31 (11708.94)			
OPG					
Low CVD Risk	203.15 (72.24)	216.11 (66.55)	0.069	0.151	0.311
Elevated CVD Risk	220.54 (109.24)	366.81 (570.78)			
OC					
Low CVD Risk	9215.50 (3723.66)	9805 (4801.35)	0.985	0.153	0.900
Elevated CVD Risk	8688.65 (4758.53)	8791.65 (5299.55)			
OPN					
Low CVD Risk	5373.76 (4408.36)	6339.46 (2996.10)	0.361	0.755	0.218
Elevated CVD Risk	6521.58 (4391.42)	6956.11 (6994.28)			
IL-1β					
Low CVD Risk	862.38 (448.86)	767.30 (443.73)	0.669	0.132	0.812
Elevated CVD Risk	714.70 (559.30)	745.80 (616.32)			
FGF23					
Low CVD Risk	28.74 (9.83)	29.54 (9.18)	0.786	0.167	0.530
Elevated CVD Risk	41.50 (43.10)	35.38 (30.73)			

Continuous variables expressed as mean pg/mL $\pm$ standard deviation and compared using two-way ANOVA. [‡]p values presented are based on log transformed data. **DKK1**, Dickkopf WNT signaling pathway inhibitor 1; **IL-6**, interleukin-6; **TNF α** , tumor necrosis factor α ; **OPG**, osteoprotegerin; **OC**, osteocalcin; **OPN**, osteopontin; **IL-1 β** , interleukin-1 β ; **FGF23**, fibroblast growth factor 2

Discussion

No differences in the concentrations of the assessed bone metabolism analytes were found in females who were pre-frail, compared to those who were robust, and no interaction effects were found between pre-frailty and CVD risk status. Based on these findings, analytes involved in bone metabolism may not be useful biomarkers of pre-frailty.

Reductions in bone health may occur concomitantly with frailty⁴²² as frailty has been predictive of osteoporotic fractures.⁴²³ There is some evidence to suggest changes to bone health in the earlier, pre-frail stage as reduced bone mineral density has been identified in pre-frail females,⁴²⁴ but this research was on a slightly older cohort (mean age = 70 years). Pre-frail males have been found to have reduced bone health compared to frail males, as assessed by quantitative ultrasound.⁴²² Though, the bone mineral density scans of these pre-frail males were not significantly different from the robust group, indicating only early changes to bone health.⁴²² We found no differences in the concentrations of bone metabolism analytes between pre-frail and robust females. This may be because pre-frailty is too early in frailty progression for changes in bone health to be detected with biomarkers associated with bone metabolism. As such, differences in the concentrations of bone metabolism analytes between these two groups may not be identifiable until later frailty stages. Markers of bone metabolism have been reported to be higher in females with established frailty.⁴²⁵ Therefore, had this study utilized females who were frail instead of pre-frail and who were older, differences in the concentrations of bone metabolism analytes between groups might have been found. This finding could warrant the use of alternative biomarkers allowing for early identification and intervention prior to the onset of associated adverse health outcomes. Thus far, evidence suggests that interventions implemented in the earlier pre-frail stage are more likely to successfully slow or reverse frailty progression.⁴²⁶

Although no differences were found in the concentrations of bone metabolism analytes based on pre-frailty, differences were found based on CVD risk status. IL-6, TNF α , and leptin were higher in females at elevated CVD risk, as compared to females at low CVD risk.

IL-6 is an inflammatory cytokine typically involved in the body's immune response.³⁷¹ IL-6 has a wide range of inflammatory effects throughout the body⁴²⁷ and it is involved in bone metabolism.⁴²⁸ IL-6 has been reported to stimulate the genesis of osteoclasts, multinucleated giant cells with the capacity to breakdown bone tissue.⁴²⁹ Thus, increased inflammation is associated with reduced bone mass.⁴³⁰ Increased inflammation is also implicated in the development of

CVD³⁷² and frailty.⁵⁴ We identified higher levels of IL-6 in females at elevated CVD risk, however, there was no difference in IL-6 concentration between robust and pre-frail females. Despite this finding, increases in IL-6 have often been identified in pre-frail and frail individuals.⁴³¹ This discrepancy may have resulted from the large variations in IL-6 concentrations between groups which might have impacted our ability to identify higher levels of IL-6 in pre-frail females. In relation to CVD, the higher levels of IL-6 found in females at elevated CVD risk status is supported by previous evidence identifying IL-6 as a biomarker of CVD.³⁷⁵

TNF α is also an inflammatory cytokine with an important role in the immune response and has pleiotropic effects throughout the body.⁴³² Like IL-6, TNF α is able to induce osteoclastogenesis, leading to the destruction of bone tissue.⁴³³ Additionally, TNF α is able to suppress the activity of osteoblasts, cells responsible for forming new bone,⁴³⁴ further reducing bone health. As such, this inflammatory cytokine is thought to be associated with altered bone metabolism.⁴³⁵ TNF α has already been identified as a biomarker of CVD⁴³⁶ and because of its link with inflammation, evidence also supports its use as a potential biomarker of frailty with higher levels often being present in pre-frail and frail individuals.⁴³¹ Higher levels of TNF α were identified in females at elevated CVD risk but frailty status did not influence TNF α concentration in our study participants. Again, this discrepancy may be due to large variations in the concentration of this cytokine between groups. Past research supports the finding of higher levels of TNF α found in females at elevated CVD risk,⁴³⁷ indicating the efficacy of this cytokine in CVD risk assessment.

Leptin is a hormone predominately synthesized and secreted by adipocytes.⁴³⁸ Leptin regulates bodyweight and energy balance,⁴³⁹ yet also has a complex role in bone metabolism.^{440,441} The specific effects of leptin on bone metabolism are difficult to summarize,⁴⁴² though research suggests leptin is necessary for both the inhibition and stimulation of bone formation.^{443,444} A relationship between leptin and CVD has been established⁴⁴⁵ and the use of leptin as a biomarker of CVD is supported by clinical studies demonstrating increased concentration of leptin is predictive of cardiovascular events.⁴⁴⁶ Additionally, leptin concentrations are higher in obesity and other metabolic disorders which predispose for the

development of CVD.⁴⁴⁷ Thus, the increase in leptin concentration present in females at elevated risk for CVD might result from the presence of metabolic risk factors for CVD in this group.

Limitations

Despite using results from a similarly aged cohort to calculate sample size for this study,⁴²¹ large variations in the concentrations of bone metabolism analytes in the systemic concentration were found within groups. This variation may have impacted our ability to identify differences between groups. The specific cause of this variation is unclear, and improved methodological approaches are needed to address variability in the future.⁴⁴⁸ The measured analytes, while selected for their involvement in bone metabolism, also play a variety of pleiotropic roles throughout the body, such as the well-known roles of IL-6 and TNF α in inflammation.^{371,432} This makes conclusively stating which pathophysiological processes connect the studied analytes to outcomes like pre-frailty or elevated CVD risk difficult to ascertain. Further, the collection of bone mineral density measures and information on potential hormone replacement therapies in the females of this cohort could have provided additional information on the bone health of the participants, which may have assisted in explaining why the bone metabolism analytes were not found to be effective biomarkers of pre-frailty.

Future Directions

There is an increasing need to identify and validate biomarkers of pre-frailty and frailty as current identification is based on specific criteria which can sometimes be inconsistent³⁹⁴. While the measurement of bone metabolism analytes is effective in the monitoring of conditions like osteoporosis⁴⁴⁹, bone metabolism analytes may not be useful biomarkers of pre-frailty as reductions in bone health may only be evident later in the frailty progression of most females. Additional research is necessary to (1) identify biomarkers of pre-frailty; (2) define the physiological ranges of these biomarkers; (3) identify the optimal method of assessing established biomarkers; and (4) examine the underlying pathophysiology connecting said biomarkers to frailty progression in more detail.

These findings will support the development of novel biomarker-based methods of pre-frailty and CVD risk assessment. The identification of effective biomarkers of pre-frailty may be used to identify potential physiological dysfunction, facilitate early intervention, and lead to the

development of therapeutic strategies.²²¹ Promising biomarkers of pre-frailty and CVD may be identified in our cohort in 2022, following the completion of a 5-year follow up period.⁴¹⁶

Conclusions

No differences were found for the concentrations of any bone metabolism analytes based on pre-frailty status, nor were any interaction effects between pre-frailty and CVD risk. It is possible these females were too early in the progression of frailty to have developed reductions in bone health that were detectable using biomarkers of bone metabolism. Concentrations of IL-6, TNF α , and leptin were found to be higher in females who were at elevated risk of developing CVD, as compared to females at low risk for developing CVD. This research suggests these cytokines involved in bone metabolism may act as potential biomarkers of CVD risk; however, it did not identify potential biomarkers of pre-frailty in middle-aged and older females.

4.6 Brief Discussion of the Manuscript written exclusively by Kevin Boreskie

Our initial analysis examining systemic myokines associated with CVD and frailty discussed in the prior chapter proved the feasibility of our cytokine assays and of the propensity match methodology that we used to efficiently examine samples from our larger cohort study. We wanted to expand our panel of cytokines being measured, as it is likely that any biomarker approach would require a range of markers, given the complexity of their function and the pathophysiology of frailty.^{175,221,450,451} Frailty progression is associated with reduced physical activity and decreased muscle quality; thus, our interest is in the association of myokines.²²¹ Bone metabolism also has a complex interplay with muscle health, physical activity with newer discovered wide effects physiologically,²²³ so we also wanted to examine the association of cytokines related to bone metabolism with frailty and CVD progression. Using a similar cytokine assay, specifically focused on cytokines related to bone metabolism, we examined their association with phenotypic frailty in our cohort, employing the same propensity match design.²³² This data found that differences in concentration of IL-6, TNF α and leptin between low and high CVD risk individuals, but no differences in cytokine concentration between robust and individuals with pre-frailty. No individuals in the cohort were living with frailty. It is possible that the earlier pre-frailty stage was too early in frailty progression for most of the study sample to be exhibiting markers of poorer bone health relative to their robust peers.

This publication was included as part of a special issue in *Advances in Geriatric Medicine and Research* on frailty. Tracking its outputs is difficult, as unfortunately, it is not listed in Web of Science, despite being expected to be included in this journal. This has likely led to less interactions with this work by others as well. It has been viewed 888 times and has been cited in an article in *Nutrients* (impact factor of 5.717).⁴⁵² The *Nutrients* article was a sub-analysis of data from the Frailty Syndrome in Daily Practice of Interventional Cardiology Ward (FRAPICA) study, a prospective cohort study examining the prognostic ability of phenotypic frailty for older adults admitted to hospital on interventional cardiology wards.⁴⁵² The analysis looked at initial data from the first 78 cohort patients recruited to examine the association of systemic myokine concentrations, not necessarily associated with bone metabolism, with frailty status and fat-free mass. They then used data from our study²³² as part of their discussion to compare results.⁴⁵³

The data from this chapter found that cytokines related to bone metabolism were not associated with pre-frailty status but were associated with CVD risk status. Specifically, IL-6, TNF α and leptin also play roles in inflammation and energy balance. The association between chronic inflammation and CVD is well supported, but more recognition of the interplay between aging, frailty and chronic disease progression, especially CVD, continues to be explored, specifically in the context of how exerkines might play a role in this.^{209,454} Significant further work is needed to identify clinically feasible biomarkers associated with frailty progression, allowing for potential earlier identification. Validation studies are also required to confirm these findings and provide a signal to help elucidate personalized intervention modalities. Similar to the discussion in Chapter 3, in order to clinically implement work like this, the roles of associated myokines will have to be elucidated, their ideal/feasible methodology for measurement, and interventions that alter their expression which, in turn, slow or reverse frailty and CVD progression.^{207,400}

CHAPTER 5: Multivariable Analysis of Myokines as Biomarkers of Frailty in Females

5.1 Preface to the Manuscript written exclusively by Kevin Boreskie

Chapter 3 employed a case-control design with a propensity match to identify the most suitable participants from the Duhamel lab's initial CVD cohort sample (NCT02863211) for an initial examination of the hypothesis that myokines may serve as biomarkers of frailty-related and CVD risk. This data informed the subsequent study (Chapter 5), which built further on this concept in a larger sample size (n = 329) from the WARM Hearts study (NCT03938155). This work further advanced our past study by implementing multivariable analysis approaches to better understand the complex interactions of myokines in relation to frailty status as supported by the latest expert opinion. This research also incorporated the use of a multidimensional FI, in addition to the phenotypic Fried approach, for frailty assessment. While we had originally planned to also examine myokine association with CVD, as with the prior research described in Chapter 3 and 4, the proposed statistical approaches were not feasible with such an assessment, and it would have led to a more convoluted analysis/interpretation of the data. Additionally, while chronic disease and frailty are different, they are invariably interlinked bidirectionally. Therefore, we no longer thought it made sense to explicitly separate frailty and CVD risk, as previously done. This chapter is not yet submitted for peer review/publication.

5.2 Manuscript status

This is an original chapter that has not yet been submitted for publication.

5.3 Authors

Kevin F. Boreskie, Jacqueline L. Hay, Daniel Schwade, Kaitlin M. Reilly, Ayesha Saleem, Rakesh C. Arora, and Todd A. Duhamel

5.4 Contributions of Authors

I conceptualized the study and identified the methodology. Along with Jacqueline Hay and Dr. Duhamel, I contributed to the development of the cohort study (WARM Hearts) that the data for this analysis was collected. Daniel Schwade and Kaitlin M. Reilly coordinated study sites and managed the trial data. As first author, I drafted the original manuscript. All authors

contributed to reviewing and editing the document. Dr. Duhamel acquired the funding for the project.

5.5 Manuscript

Abstract

Background: Individuals living with frailty have reduced physiological reserve for tolerating health stressors compared to robust peers. Myokines are cytokines released from skeletal muscle that have pleiotropic effects throughout the body. Although suggested as a means of facilitating earlier identification and elucidating the pathophysiological decline seen in frailty, little research has explored the association of myokines with frailty status.

Methods: This secondary, cross-sectional analysis of the Women's Advanced Risk Assessment in Manitoba (WARM) Hearts observational cohort comprised of females aged 55 years or older with no previous history of cardiovascular disease (NCT03938155). All participants were assessed using a frailty index (FI) and the Fried frailty phenotype (FP). Systemic myokine blood concentrations were quantified using the MILLIPLEX® Map Human Myokine Magnetic Bead Panel and log10 transformed for all analyses. Cohort characteristics and myokine concentrations differences by frailty status were assessed using ANOVAs. Linear regressions were used to examine associations between individual myokine concentrations and the FI and FP, respectively. A stepwise multivariable regression was used to examine the value of adding each myokine to a model identifying the FI and then FP score after adjusting for age.

Results: The mean ($\pm$ SD) age of the cohort was 64 ± 6 years ($n = 329$). The FI identified 28 robust females, 248 living with pre-frailty and 53 living with frailty. The stepwise multivariable regression adjusting for age included brain-derived neurotrophic factor (BDNF) (β 0.143, SE 0.013), fatty acid binding protein 3 (FABP-3) (β 0.149, SE 0.020), oncostatin M (OSM) (β 0.144, SE 0.005) and osteocrin (OSTN) (β -0.197, SE 0.010) in the model for the FI. Comparatively, the FP identified 270 females as robust, 58 as pre-frail and none as frail (one participant had missing FP criteria). Only secreted protein acidic and rich in cysteine (SPARC) (β 0.121, SE 0.107) was included in the final stepwise multivariable regression model for FP adjusted for age.

Conclusion: Myokines BDNF, FABP-3, OSM and osteocrin contributed to a stepwise regression model predicting the FI after adjusting for age, whereas only SPARC remained in a model predicting FP score. Future studies validating these and emerging myokines of interest for frailty

biomarker tools are needed.

Introduction

An aging global population means that individuals will present with increasing clinical complexity due to comorbidities and other concomitant health stressors.¹ Associated with, but separate from chronological age, frailty is defined as a susceptibility to health stressors due to dysregulated physiological reserve that increases risk for adverse health outcomes.^{15–17} Frailty is associated with risk for a range of adverse outcomes, including hospitalization,¹⁵ comorbidities,^{15,21,32,33} and mortality.^{21,27,33} Many frailty assessment tools have been described in the literature^{66,67} with their use depending on the clinical setting and purpose of assessment.⁷⁴ Methodologies can generally be grouped into frailty assessments based on an accumulation of deficits or as a phenotypic expression. More recently, blood and urine-based biomarkers have been incorporated into approaches as a means to potentially enhance the prediction of adverse health outcomes earlier in the pre-frail stage of frailty.^{80,95,451,455} For example, the Frailty Index Lab (FI-Lab) demonstrated that even seemingly minor abnormalities from 32 common blood, urine, and hemodynamic (pulse and blood pressure) assessment were associated with mortality.⁸⁰ A recent systematic review and meta-analysis supported the FI-Lab approach predicting mortality (per 0.01 increase in FI HR = 1.04; 95% CI 1.03-1.05) regardless of the number of items included in the FI-Lab (< or >25).⁹⁵ Therefore, lab-based biomarkers may provide unique insights into the complex physiological decline and facilitate identifying new therapies to prevent or slow frailty progression.^{173–175,455}

The pleiotropic beneficial effects of physical activity have more recently been explained through the role of ‘exerkines’.^{207,208} These signaling factors are released in response to acute or chronic exercise from a variety of cardiometabolic, immune, nervous and bone tissues, having endocrine, paracrine and autocrine effects throughout the body.²⁰⁸ While this broad definition encompasses a variety of moieties, the term ‘myokines’ has been used to describe cytokines that are released from skeletal muscle in response to physical activity.^{199,209} Since deteriorating muscle health and reduced physical activity are often part of frailty progression,¹⁸⁰ biomarkers that are upregulated or downregulated in response to physical activity and/or muscle mass may act as markers of early frailty progression. As with the broader exerkines, myokines have a variety of autocrine, paracrine, and endocrine functions, which produce pleiotropic disease-modifying

effects.^{209,456} As such, it is thought that changes in myokine production and release may explain part of the complexity in pathogenesis toward multimorbidity and frailty.^{177,180,219,220}

Multiple reviews have endorsed further research into the role of myokines as promising potential biomarkers for frailty,^{173,175,177,180} but few works have examined this association. Reviews have identified myokines such as myostatin/growth differentiation factor 8 (MSTN), irisin, brain-derived neurotrophic factor (BDNF), and myokines associated with inflammatory processes like interleukin 6 (IL-6) as being potentially associated with frailty in middle and older age adults.^{177,180} In a sample of 53 undernourished, inpatients 65 years of age and older, Liu et al. found that patients with frailty had higher leptin, adenosine 5'-monophosphate-activated protein kinase (AMPK) and myostatin (MSTN) serum concentrations, while pre-frail and frail participants had lower irisin concentrations, as compared to their robust peers.⁴⁵⁷ In a subsequent multiple linear regression for predicting frailty including other biomarkers and clinical scoring tools, leptin and MSTN concentrations remained as some of the most influential components of the regression model. A cross-sectional univariable analysis from our lab identified that females with pre-frailty had lower fractalkine/CX3CL1 concentrations as compared to their robust peers.¹⁷⁰ Our group has also previously examined the univariable association of cytokines related to bone metabolism and frailty, many of which were also related to muscle function,²³² and none were found different in pre-frail participants compared to robust.

While our previous approaches assessed individual myokines, expert recommendations now suggest that examining biomarkers in isolation, as opposed to using multivariable approaches, may result in a loss of information, given the complex pathophysiology present in frailty.^{174,180,455} Therefore, we are building off our past work examining univariable associations between myokines and frailty¹⁷⁰ by incorporating a multivariable approach using a larger sample to better examine the potential complex interaction between myokines and frailty. This new research is also strengthened as we examined frailty scores as continuous variables, as opposed to categorical, and incorporates both a multidimensional approach to frailty (a Frailty Index - FI)⁴⁵⁸ and a phenotypic approach (the Fried Phenotype - FP).¹⁵ Identifying myokines of interest in association with frailty is an important first step to help direct future work examining their use as frailty predictors and therapeutic targets.

This study aims to examine associations between frailty status and systemic myokine blood concentrations at rest. We hypothesize that a multivariable myokine analysis will identify

a panel of myokines associated with frailty, even after adjusting for age, given the shared pleiotropic effects of both frailty and myokines in the body, as well as their shared connection to physical function, muscle health, and activity.

Materials and Methods

Study design

This secondary, cross-sectional analysis of data was collected from the Women's Advanced Risk Assessment in Manitoba (WARM) Hearts study,⁴⁵⁹ an observational cohort study recruiting Manitoban females 55 years of age and older with no previous history of CVD to examine novel CVD risk factors in this population (ClinicalTrials.gov: NCT03938155). The study was approved by the University of Manitoba Health Research Ethics Board and the St-Boniface Hospital Research Review Committee (Ethics Number HS22576 (H2019:063)). The cohort study used novel methods to assess CVD risk, such as arterial elasticity assessment, as compared to traditional approaches, such as the Framingham Risk Score (FRS).⁴⁵⁹ The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement guidelines³⁵⁵ for cross-sectional studies were followed in the development of this manuscript as well as the Sex and Gender Equity in Research (SAGER) guidelines.⁴⁶⁰ Females were specifically recruited given their higher prevalence of frailty compared to males,²⁸ differing presentations of same,³⁰ suspected differences in the relationship between cytokines and frailty by sex,⁴⁶¹ as well as differences in the expression of myokines by sex.⁴⁶²

Setting and recruitment

The WARM Hearts study was conducted in Winnipeg, Manitoba at two locations – the University of Manitoba Active Living Centre and the Albrechtsen St. Boniface General Hospital Research Centre. One-thousand and eighteen (1018) women aged 55 years or older were recruited into the WARM Hearts cohort through a convenience sample via newspaper articles, an online webpage describing the project, media interviews, and presentations at community events. Table 10 identifies the inclusion and exclusion criteria of the study. Data collection for the WARM Hearts study took place between November 2019 and January 2022 at the University of Manitoba and the Asper Research Centre in Winnipeg, Manitoba, Canada. Recruitment for the study was notably impacted by the COVID-19 pandemic. The recruitment process began on October 10,

2019, but was temporarily paused on March 23, 2020, after enrolling 480 participants, 240 of whom completed data collection. Data collection resumed on September 16, 2020, but was again halted on November 10, 2020, due to public health restrictions in the province. Recruitment restarted on October 2, 2021, and continued until December 20, 2021, with 522 participants enrolled, 330 of whom completed data collection. Following a pause due to the Omicron variant, recruitment resumed on February 1, 2022, and concluded on December 22, 2022, with 1,018 participants completing data collection. This analysis is based on the first 329 participants with complete data.

Table 10. Inclusion and exclusion criteria⁴⁵⁹

Inclusion criteria <i>(both need to be fulfilled for eligibility)</i>	Exclusion criteria <i>(any of the following result in ineligibility)</i>
1) Women aged 55 and older 2) Possess a Manitoba Personal Health Information Number	Pre-hospitalisation or treatment for: 1) Ischemic heart disease 2) Acute myocardial infarction 3) Stroke/transient ischemic attack 4) Percutaneous coronary intervention 5) Coronary artery bypass surgery 6) Congestive heart failure 7) Peripheral artery disease 8) Congenital heart defects 9) Arrhythmia Additional exclusion: 10) Medical advice against physical activity 11) Previous participant in the Assessment of Large and Small Artery Elasticity for the Early Detection of Cardiovascular Disease (NCT02863211)

Study procedure

Eligible participants attended two research appointments 8-14 days apart to complete data collection. Take-home assessment packages were provided at the first appointment and completed forms were returned at the second data collection appointment. Participants were given pre-appointment instructions to fast and not to smoke or consume caffeine two hours prior, and to avoid strenuous exercise and alcohol within 6 hours of the appointment. Participants were given the same instructions prior to their second appointment; however, fasting protocols were 12 hours before data collection. Blood samples were collected at this second appointment. This methods section describes measures relevant to this analysis from WARM Hearts. A complete list of data collected in WARM Hearts can be found in the published protocol paper.⁴⁵⁹

Anthropometry and body composition

Height was measured on a portable Seca stadiometer to the nearest 0.5 cm. In accordance with the Canadian Society for Exercise Physiology (CSEP) guidelines, waist circumference was measured at the superior iliac crest to the nearest 0.1 cm on the skin at the end of exhalation.^{463,464} The InBody 270 was used to estimate body composition, including weight, skeletal muscle mass, body fat percentage and total body fat mass using bioelectrical impedance analysis following a two hour fasting period.^{465,466}

Blood sampling

Plasma samples were collected to examine traditional CVD risk biomarkers for use in the FRS as well as novel biomarkers of cardiovascular and muscle health. Participants were asked to fast 12h prior to their appointment. Fifteen (15) mL of blood was drawn and separated into 4 tubes: a serum silica tube (1x4mL), a lithium heparin tube (1x4mL), and two EDTA tubes (2x3mL). Two aliquots of whole blood from the serum silica tube were snap frozen and stored at -80 °C.. Blood samples in the EDTA and lithium heparin tubes were centrifuged at 2000 x g for 10 minutes and then the plasma was snap frozen and eight aliquots were stored at -80 °C. Cholesterol, triglycerides, and blood glucose were measured using one of the lithium heparin plasma samples.

Myokine Analysis

The EDTA plasma samples were used for the targeted myokine analysis. The analysis was performed by Eve Technologies in Calgary, Alberta, an experienced biomarker laboratory

using a EMD Millipore's MILLIPLEX® Map Human Myokine Magnetic Bead Panel. Briefly, this Millipore Sigma kit uses Luminex xMAP technology to identify fluorescent-coded magnetic beads coated with a specific capture antibody and allows for the simultaneous quantification of protein biomarkers. Our lab has used this same myokine panel previously as well as a similar panel examining cytokines associated with bone metabolism.^{170,232} This panel quantifies apelin (APLN), BDNF, erythropoietin (EPO), fatty acid-binding protein 3 (FABP3), fibroblast growth factor 21 (FGF21), fractalkine/CX3CL1, follistatin-like 1 protein (FSTL-1), IL-6, IL-15, irisin, leukemia inhibitory factor (LIF), myostatin (MSTN), oncostatin m (OSM), osteocrin/musclin (OSTN), and osteonectin (SPARC). While there are hundreds of myokines discussed in the literature,²⁰³ these myokines were identified as ones of specific interest available in a compatible panel. All assays were run in duplicate with the average value being used for each participant. Values below the range of the standard curve were designated as the lowest quantifiable value obtained for that analyte.

Non-invasive measure of peripheral and central hemodynamics

Seated blood pressure (BP) measurements were taken at the left upper arm using the validated Mobil-O-Graph(IEM, Stolberg, Germany) in accordance with Canadian Cardiovascular Society guidelines.⁴⁶⁷ The Mobil-O-Graph ambulatory blood pressure (BP) monitor is validated for the non-invasive assessment of brachial blood pressure and estimates of aortic pulse wave velocity (aPWV), augmentation index, and central blood pressure.⁴⁶⁸⁻⁴⁷⁰ All of these assessments were quantified simultaneously in triplicate through oscillometric pulse wave form analysis. The first measurement was discarded and the second and third measurements were averaged.

For adults, a resting BP of $\leq 120/80$ mmHg is considered normal, 120-139/80-89 mmHg is considered pre-hypertensive, and $\geq 140/90$ mmHg is classified as hypertension, as defined by the Canadian Cardiovascular Society guidelines.⁴⁶⁷ A resting BP of 160 mmHg systolic or 90 mm Hg diastolic, and a heart rate of 100 beats per minute were used as screening cut-offs for proceeding with the data collection appointments as recommended by the CSEP guidelines.⁴⁶⁴ Participants who exceeded BP or heart rate cut-offs were given five minutes to rest before the measurements were retaken. If a participant was above either of the cut-offs on two consecutive

measurements, the appointment was terminated, and the participant was recommended to speak with their primary care provider.

Framingham Risk Score

A multivariable risk factor algorithm described by D'Agostino *et al*¹³¹ was used to calculate the FRS for each participant. This tool is recommended for use in clinical settings in Canada by the Canadian Cardiovascular Society.⁴⁶⁷ Age, sex, and smoking status were collected by questionnaire; whereas, fasting glucose, HDL, and total cholesterol measured from blood samples. As previously described, BP was recorded using the Mobil-O-Graph.

Cognitive assessment

The Montreal Cognitive Assessment (MoCA) was used to assess cognitive impairment in the cohort.^{471,472} Research assistants all completed the MoCA training. The MoCA is scored out of 30 with lower scores being indicative of increased cognitive impairment. Mild cognitive impairment is considered present with scores below 26/30.⁴⁷²

Questionnaires

Participants completed a series of questionnaires:

1. Demographic questionnaire including age, menopausal status, as well as sex and gender identity using the two question method.⁴⁷³
2. The International Physical Activity Questionnaire (IPAQ) short version⁴⁷⁴ collected self-reported duration and intensity of physical activity completed in the last 7 days.
3. The EuroQol Five Dimensions Five Level (EQ-5D-5L) evaluates the dimensions of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.⁴⁷⁵ The EuroQol Visual-Analog Scale (EQ-VAS) provided complementary information of the patients' views of their health.⁴⁷⁶
4. Sleep quality over a 1-month time interval was assessed using the Pittsburgh Sleep Quality index (PSQI).⁴⁷⁷
5. The Seniors in the Community: Risk Evaluation for Eating and Nutrition Version II (SCREEN II) was used for the identification of risk for impaired nutritional states in community-living older adults.⁴⁷⁸

6. The Patient Health Questionnaire-9 (PHQ-9) was used to screen for depressive symptoms.⁴⁷⁹ PHQ-9 scores of 5, 10, 15, and 20 represent mild, moderate, moderately severe and severe depression respectively. Question 9 on the PHQ-9 screens for the presence of and duration of suicidal ideation.

Frailty status

Frailty was assessed using two approaches, a FI⁴⁵⁸ and the FP.¹⁵ No single frailty assessment tool has established itself as a gold standard as there are more than fifty tools that exist⁶⁶ serving different purposes for different clinical contexts.⁷⁴ Both of these frailty assessment approaches were used in this study as they are the two most regularly used in the literature and there is no clear consensus supporting the use of one over the other.⁶⁷

The FI was created using variables collected in WARM Hearts study (Appendix F) following the general guidelines as described by Searle et al.⁴⁵⁸ Variable selection is based on the follow criteria: (1) deficits must have an association with health status; (2) selected deficit prevalence should increase with chronological age; (3) selected health deficits should not saturate too early; (4) selected deficits should cover a broad range of physiological systems; (5) frailty indices should be kept the same if planned to be used on the same cohort at multiple time points.⁴⁵⁸ Proposed cut points for FI scores have included ≤ 0.08 as robust, ≥ 0.25 as frail and everything in between as pre-frail.⁴⁸⁰

The FP uses measures previously described by Fried et al.¹⁵ This phenotypic to frailty assessment classifies participants as frail if three or more of the following criteria were met: (1) unintentional weight loss, (2) self-reported exhaustion, (3) weakness, (4) slow walking speed, (5) low physical activity. Exhaustion was assessed by the Center for Epidemiologic Studies – Depression Scale.⁴⁸¹ Self-reported physical activity levels were assessed by the Paffenbarger Physical Activity Scale⁴⁸² and unintentional weight loss was assessed via questionnaire. Gait speed was assessed by the 5-meter walk test.⁴⁸³ Weakness was assessed by grip strength using a hand held dynamometer.⁴⁸³ Participants are considered robust if they have 0 criteria present, pre-frail if 1-2 criteria are present or frail with 3 or more criteria are present.

Statistical analysis

Statistical support was provided by Dr. Robert Balshaw, a biostatistician from the George & Fay Centre for Healthcare Innovation, University of Manitoba. One-way between group ANOVAs were used to compare characteristics variables of the cohort based on five bins designated by FI score ranges with the lowest score bin (≤ 0.08) representing robust and the highest score bin (≥ 0.25) representing those living with frailty based on previously used cut points.⁴⁸⁰ This binning approach was used as opposed to using an approach that categorizes as robust versus pre-frail versus frail to better describe the continuum of frailty in our cohort

One-way between group ANOVAs were used on the log10 transformation of the systemic concentration of the myokines to assess differences by FI and FP score. Bonferroni post-hoc analysis was used to explore between-group differences when the ANOVA was statistically significant. Chi-Square tests were used to assess difference in categorical variables. GraphPad Prism was used to generate included graphs (Figure 8, 9 and 10).

Univariable linear regression was used to examine associations between individual log10 myokine concentrations and with frailty using both the FI and FP approach. An additional linear regression model was then run adjusting for age. Homoscedasticity was assessed by visual inspection of a plot of standardized residuals versus standardized predicted values.

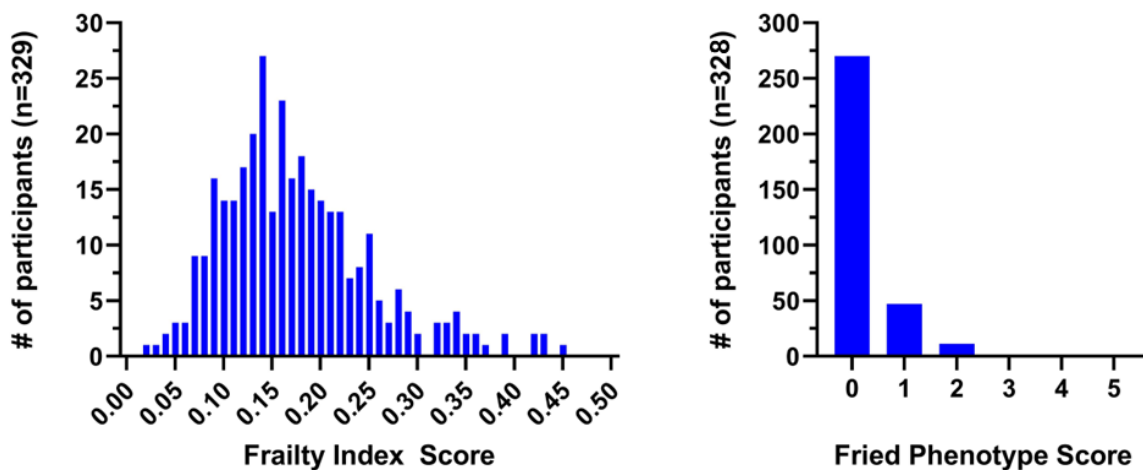
Maintaining age in the model, a stepwise, forward multivariable regression was used to identify myokines as possible predictors of FI and then FP score. Each step involved including the myokine with the highest correlation with the outcome if it met criterion ($p < 0.05$) until no myokines met this criterion. The inclusion of myokines in these models suggest that they should be of interest for further examination in future studies exploring the pathophysiology of frailty, biomarker-based approaches for identifying frailty, and potential therapeutic targets. Multivariable regressions conservatively require 10-15 participants per variable examined,⁴⁸⁴ meaning that a sample size of 240 was required to power this analysis for the 16 analytes evaluated. P-values are not demonstrated for these models as they are biased toward fitting a single model. All statistical analyses were conducted using IBM SPSS Statistics, version 31 (IBM Corp., Armonk, NY, USA).

Results

Descriptive data

This cohort was composed of 329 females. The entirety of the cohort identified as women, 99% of the cohort was post-menopausal, and 97% of the cohort were White. The average ($\pm$ SD) age of the cohort was 64 ± 6 years. The FI identified 28 (8.5%) of the participants as robust, 248 (75.4%) participants with pre-frailty and 53 (16.1%) of the participants with frailty. Comparatively, the FP identified 270 participants (81.6%) as robust, 58 participants (17.5%) as pre-frail and none as frail. Distribution of FI and FP scores in the cohort are presented in Figure 8. The characteristics of the cohort as organized into bins by FI score are summarized in Table 11. There was no difference in age between the five FI categories. Higher FI scores were generally associated with higher BMIs, greater waist circumference, more prescribed medications, and higher systolic blood pressure.

Figure 8. Distribution of frailty index scores in the cohort



FI and FP presented as continuous score distributions for the cohort.

Table 11. Characteristics of cohort by FI score

Characteristic	Cohort <i>n</i> = 329	Group 1 ≤0.08 <i>n</i> = 28	Group 2 0.09-0.13 <i>n</i> = 82	Group 3 0.14-0.19 <i>n</i> = 111	Group 4 0.20-0.24 <i>n</i> = 55	Group 5 ≥0.25 <i>n</i> = 53
Age (years)	63.96 ±5.97	63.32 ±5.85	63.44 ±6.03	64.31 ±6.06	64.27 ±6.37	64.08 ±5.48
Frailty index	0.17 ±0.08	0.06 ±0.02	0.11 ±0.01*	0.16 ±0.02*†	0.22 ±0.01*†‡	0.31 ±0.06*†‡§
Post-secondary education (%)	257 (78.1%)	22 (78.6%)	69 (84.1%)	86 (77.5%)	41 (74.5%)	39 (73.6%)
Living alone (%)	64 (19.5%)	4 (14.3%)	12 (14.6%)	26 (23.4%)	10 (18.2%)	12 (22.6%)
BMI (kg/m ²)	26.50 ±5.03	24.20 ±3.62	24.56 ±3.30	25.89 ±3.82	28.30 ±5.51*†‡	30.12 ±6.97*†‡
Waist circumference (cm)	91.47 ±13.00	84.77 ±10.91	85.81 ±10.52	90.51 ±10.62	96.83 ±12.53*†‡	100.23 ±15.65*†‡
Fat-free mass (kg)	45.07 ±6.16	44.38 ±4.23	45.08 ±5.63	44.31 ±5.81	45.87 ±6.58	46.18 ±7.79
Skeletal muscle mass (kg)	24.64 ±3.60	24.35 ±2.58	24.59 ±3.37	24.12 ±3.07	25.25 ±4.16	25.31 ±4.59
Percent body fat (%)	35.39 ±7.49	32.32 ±6.80	32.04 ±6.81	34.76 ±6.17	38.49 ±6.86*†‡	40.28 ±8.36*†‡
Ex or current smoker (%)	158 (48%)	8 (28.6%)	37 (45.1%)	54 (48.6%)	28 (50.9%)	31 (58.5%)
Prescribed medication(s) (#)	1.63 ±1.67	0.85 ±0.91	0.96 ±1.14	1.48 ±1.34	1.88 ±1.84*†	3.12 ±2.12*†‡§
MoCA score	26.22 ±2.38	27.86 ±1.27	26.63 ±2.11	26.06 ±2.16*	26.02 ±2.51*	25.25 ±2.94*†
PHQ-9 score	2.37 ±2.36	1.36 ±1.57	1.68 ±1.81	2.12 ±2.02	3.27 ±2.38*†‡	3.57 ±3.25*†‡
HDL cholesterol (mmol/L)	1.70 ±0.43	1.76 ±0.38	1.79 ±0.42	1.72 ±0.44	1.59 ±0.38	1.63 ±0.49
LDL cholesterol (mmol/L)	3.48 ±0.92	2.86 ±0.61	3.30 ±0.79	3.64 ±0.99*	3.64 ±0.84*	3.61 ±0.98*

Total cholesterol (mmol/L)	5.29 ±0.95	4.69 ±0.42	5.13 ±0.82	5.46 ±1.06*	5.41 ±0.91*	5.38 ±1.03*
Triglycerides (mmol/L)	1.14 ±0.53	1.00 ±0.42	0.92 ±0.42	1.13 ±0.52†	1.34 ±0.58*†	1.39 ±0.55*†‡
Blood glucose (mmol/L)	5.83 ±0.66	5.63 ±0.54	5.60 ±0.38	5.77 ±0.54	6.00 ±0.76†	6.22 ±0.94*†‡
Systolic blood pressure (mmHg)	120.32 ±14.28	115.04 ±13.22	115.83 ±12.05	119.41 ±13.17	122.73 ±13.12†	129.49 ±16.80*†‡
Diastolic blood pressure (mmHg)	75.49 ±10.13	74.14 ±10.62	73.61 ±8.73	75.27 ±10.20	76.49 ±8.74	78.53 ±12.38
Pulse wave velocity (m/s)	8.98 ±1.14	8.58 ±1.14	8.77 ±1.13	9.02 ±1.10	9.09 ±1.21	9.33 ±1.08*†
Framingham Risk Score	7.13 ±4.26	4.53 ±2.51	5.36 ±2.73	6.89 ±3.70*	8.20 ±4.46*†	10.55 ±5.27*†‡§
Hypertension (%)	71 (21.6%)	5 (17.9%)	4 (4.9%)	21 (18.9%)	17 (30.9%)	24 (45.3%)
Type 2 diabetes (%)	6 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.8%)	5 (9.45%)
Past cancer (%)	45 (13.7%)	2 (7.1%)	6 (7.3%)	13 (11.7%)	11 (20.0%)	13 (24.5%)
5m gait time (s)	4.16 ±0.64	4.01 ±0.47	3.94 ±0.46	4.12 ±0.61	4.23 ±0.56	4.60 ±0.87*†‡§
Grip strength (kg)	25.62 ±5.721	26.64 ±3.68	27.91 ±4.80	24.91 ±5.24†	24.02 ±6.26†	24.66 ±7.16†
6MWT (m)	592.51 ±79.38	650.77 ±74.94	613.27 ±73.10	602.50 ±65.88*	571.28 ±64.83*†	530.33 ±88.29*†‡§
Total MVPA/week (mins)	273.64 ±287.27	311.70 ±301.31	283.92 ±264.83	309.66 ±316.71	242.12 ±267.97	191.10 ±254.33

Continuous variables expressed as mean ± standard deviation and compared using ANOVA. Categorical variables expressed as N (%) compared using Chi-square test. **BMI**, body mass index; **MoCA**, Montreal Cognitive Assessment; **PHQ-9**, Patient Health Questionnaire-9; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein; **IBD**, inflammatory bowel disease; **6MWT**, 6-minute walk test; **MVPA**, moderate-to-vigorous physical activity.

* indicates statistically significant difference from Group 1. † indicates statistically significant difference from Group 2. ‡ indicates statistically significant difference from Group 3. § indicates statistically significant difference from Group 4.

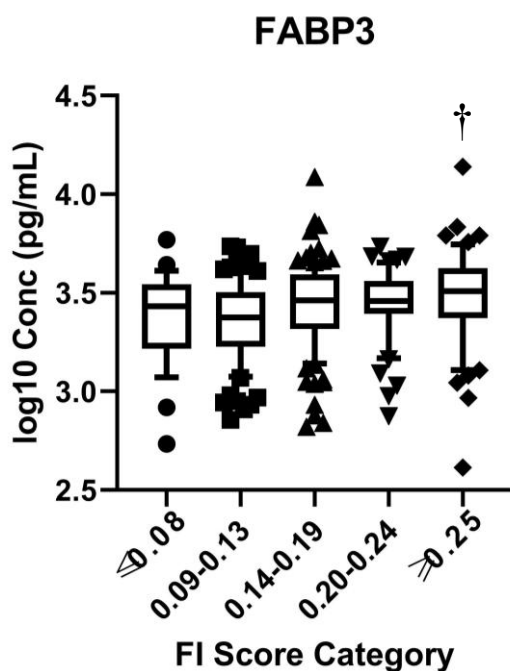
Frailty biomarkers designated as the lowest quantifiable value

The following describes the percentage of samples in the cohort that had myokine concentration values below the standard curve range for each respective myokine: Apelin 18%; BDNF 0%; EPO 33%; FABP-3 0%; FGF-21 80%; FSTL-1 18%; Fractalkine 30%; IL-6 50%; IL-15 61%; Irisin 81%; LIF 34%; Myostatin 77%; OSM 11%; OSTN 7%; SPARC<1%. These values were designated as the lowest quantifiable value obtained for that analyte.

Results based on FI score bins

Myokine concentration values in pg/mL by FI score bins are presented in Supplementary Table 14 with the log10 values assessed by ANOVA. Between-group differences were assessed with a Bonferroni post-hoc analysis. Group 5 (FI ≥ 0.25) had higher FABP3 concentrations than Group 2 (FI 0.09-0.13)(Figure 9).

Figure 9. Box plot of significant log10 myokine concentrations by FI score



*FI scores are expressed as continuous values. FABP3 values are presented as pg/mL log10 concentrations. The boxes represent lower quartile, median and upper quartile while the whiskers represent minimum and maximum values with outliers displayed. **FABP-3**, fatty acid-binding protein 3.*

† indicates statistically significant difference from FI Score 0.09-0.13 Group.

Univariable regressions for each of the log10 myokine concentrations by FI are presented in Table 12. When a stepwise regression including age was performed, only BDNF, FABP-3, OSM and osteocrin added value to the model. The strength of the effect of independent variables (myokine concentration) on the dependent variable (FI) are presented as standardized β weights. BDNF (Standardized β 0.143, SE 0.013), FABP-3 (Standardized β 0.149, SE 0.020) and OSM (Standardized β 0.144, SE 0.005) all had positive standardized beta weights, while OSTN (Standardized β -0.197, SE 0.010) had a negative beta weight, in the final stepwise model.

Table 12. Linear regression models of log10 myokine concentrations by FI score

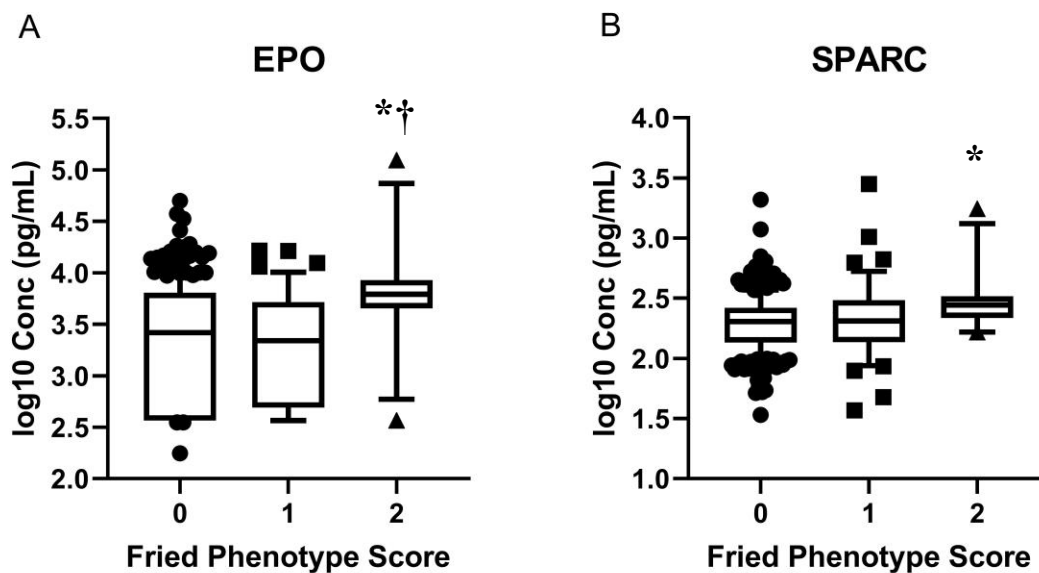
Variable	Univariable regression Standardized β (SE)	Age-adjusted regression Standardized β (SE)	Stepwise regression Standardized β (SE)
Age	0.043 (0.001)	-	-
Apelin	-0.038 (0.004)	-0.038 (0.004)	-
BDNF	0.124 (0.012)	0.126 (0.012)	0.143 (0.013)
EPO	0.022 (0.007)	0.024 (0.007)	-
FABP-3	0.159 (0.019)	0.156 (0.020)	0.149 (0.020)
FGF-21	0.066 (0.007)	0.063 (0.007)	-
FSTL-1	0.000 (0.005)	-0.006 (0.005)	-
Fractalkine	0.020 (0.006)	0.019 (0.006)	-
IL-6	-0.003 (0.005)	-0.007 (0.005)	-
IL-15	-0.014 (0.004)	-0.014 (0.004)	-
Irisin	0.052 (0.005)	0.051 (0.005)	-
LIF	-0.035 (0.003)	-0.039 (0.003)	-
Myostatin	0.032 (0.007)	0.030 (0.007)	-
OSM	0.116 (0.005)	0.115 (0.005)	0.144 (0.005)
OSTN	-0.033 (0.008)	-0.033 (0.008)	-0.197 (0.010)
SPARC	0.060 (0.017)	0.056 (0.017)	-

All variables expressed as standardized β (standard error). **BDNF**, brain-derived neurotrophic factor; **EPO**, erythropoietin; **FABP-3**, fatty acid-binding protein 3; **FGF-21**, fibroblast growth factor 21; **FSTL-1**, follistatin-like 1 protein; **IL-6**, interleukin-6; **IL-15**, interleukin-15; **LIF**, leukemia inhibitory factor; **OSM**, oncostatin M; **OSTN**, osteocrin/musclin; **SPARC**, osteonectin

Results by FP score

Myokine concentration values in pg/mL by FP score are presented in Supplementary Table 15 with log10 values assessed by ANOVA. Both EPO and SPARC concentrations were higher in higher FP scores (Figure 10). The FP group with a score of 2 had higher EPO concentrations than both groups with FP scores of 0 or 1 ($p < 0.05$). The FP group with a score of 2 had higher SPARC concentrations than the FP group with a score of 0 ($p < 0.05$).

Figure 10. Box plots of significant log10 myokine concentrations by FP score



FP scores are expressed as continuous values. Myokine values are presented as pg/mL log10 concentrations. The boxes represent lower quartile, median and upper quartile while the whiskers represent minimum and maximum values with outliers displayed. **EPO**, erythropoietin; **SPARC**, osteonectin.

* indicates statistically significant difference from Group 1. † indicates statistically significant difference from Group 2.

Univariable regressions for each of the log10 myokine concentrations by FP score are presented in Table 13. Fifteen (15) myokines were identified in the univariable regression and age-adjusted regression; whereas, only SPARC (0.121, SE 0.107) added value to the final stepwise model with age for the FP assessment.

Table 13. Linear regression models of log10 myokine concentrations by FP score

Variable	Univariable regression <i>Standardized β (SE)</i>	Age-adjusted regression <i>Standardized β (SE)</i>	Stepwise regression <i>Standardized β (SE)</i>
Age	0.146 (0.004)	-	-
Apelin	0.025 (0.023)	0.026 (0.022)	-
BDNF	0.114 (0.074)	0.119 (0.073)	-
EPO	0.086 (0.045)	0.091 (0.044)	-
FABP-3	0.110 (0.122)	0.090 (0.123)	-
FGF-21	0.045 (0.043)	0.032 (0.043)	-
FSTL-1	0.073 (0.032)	0.056 (0.032)	-
Fractalkine	0.053 (0.039)	0.051 (0.039)	-
IL-6	0.051 (0.031)	0.037 (0.031)	-
IL-15	0.023 (0.026)	0.022 (0.026)	-
Irisin	-0.012 (0.032)	-0.016 (0.032)	-
LIF	0.065 (0.020)	0.076 (0.020)	-
Myostatin	0.038 (0.041)	0.030 (0.041)	-
OSM	0.072 (0.029)	0.069 (0.029)	-
OSTN	0.048 (0.051)	0.050 (0.050)	-
SPARC	0.135 (0.107)	0.121 (0.107)	0.121 (0.107)

*All variables expressed as standardized β (standard error). **BDNF**, brain-derived neurotrophic factor; **EPO**, erythropoietin; **FABP-3**, fatty acid-binding protein 3; **FGF-21**, fibroblast growth factor 21; **FSTL-1**, follistatin-like 1 protein; **IL-6**, interleukin-6; **IL-15**, interleukin-15; **LIF**, leukemia inhibitory factor; **OSM**, oncostatin M; **OSTN**, osteocrin/musclin; **SPARC**, osteonectin*

Discussion

This study identified systemic myokine blood concentrations associated with frailty status, as measured by both the FI and the FP. Specifically, while 15 myokines were associated in both the univariable and age-adjusted regression analyses, only 4 (i.e.; BDNF, FABP-3, OSM and OSTN) were included in the FI stepwise regression model with age; whereas only SPARC was included in the FP stepwise model with age included. This approach was used, as the latest expert recommendation suggests that biomarkers such as these should be examined in multivariable approaches to avoid simplifying what are likely complex relationships into univariable assessments.^{174,180,455} Based on our findings, the specific myokines BDNF, FABP-3, OSM and OSTN should be considered of interest for future work examining myokines as biomarkers of frailty and for their potential contribution to frailty-related health declines.

The FI and FP are two of the most established approaches for assessing frailty. Importantly, they identify frailty differently.²⁵³ While the FI examines frailty from a multidimensional aspect as an accumulation of deficits,²² the FP is a phenotypic-focused assessment.¹⁵ As such, these assessments do not have strong agreement between them and the prevalence of frailty in the same population can vary depending on the assessment tool used.⁴⁸⁵ In this study, for example, the FI identified 53 (16.1%) of the participants as living with frailty, whereas the FP did not identify any individuals living with frailty. This is supported by past work that has similarly identified higher rates of frailty identification in middle and older ages with the FI as compared to the FP.^{26,28}

The identification of myokines associated with pre-frailty and frailty through multivariable approaches has the potential to assist with the creation of tools to identify individuals requiring intervention to reduce risk for adverse outcomes earlier in their frailty progression.^{173,177} Past work has shown that creating FIs focusing on lab-based measures can identify individuals at risk for further physiological decline and identify subclinical deficit accumulation before identification with common clinical assessments.¹⁸¹ This study expands the type of lab-based measures by specifically identifying myokine biomarkers that could potentially add value to such an approach.

BDNF, FABP-3, OSM and OSTN all remained statistically significant contributors to the FI stepwise regression. Increased BDNF is thought to play a key role in the cognitive improvements seen with physical activity through neuronal growth^{211,486} and it is also thought to

play a role in modulating inflammation.^{211,487} This work showed a positive association between BDNF and FI score in the stepwise model. While increased BDNF has been associated with positive aging prior,⁴⁸⁸ other works have also identified higher BDNF in older adults with inflammatory conditions, such as osteoarthritis,⁴⁸⁷ as well as in type 2 diabetes mellitus.⁴⁸⁹ In our cohort, individuals living with further advanced frailty (i.e.; higher FI score) had higher diabetes prevalence as well as slightly lower MoCA scores, representing worse cognition.

FABP3 is involved in fatty acid metabolism and has previously been found to be released in association with myocardial injury.^{382–384} Additionally, FABP3 knockdown has been shown to have positive effects on aged skeletal muscle, with improved protein synthesis and reduced endoplasmic reticulum stress through changes in lipid composition,⁴⁹⁰ which may explain its positive association with FI scores in the stepwise regression analysis in our cohort.

OSM is increased with chronic inflammation³⁹¹ in pathologies like vascular disease or cancer,³⁹² and it is thought to play a role in driving muscle atrophy associated with these comorbidities.⁴⁹² These roles may be attributable to its positive association with FI in our cohort as chronic inflammation and atrophy are thought to play key components in the pathogenesis of frailty, including its relation to CVD.¹⁵⁹

OSTN is stimulated with physical activity and may play a preventative role in pathologic cardiac remodeling.⁴⁹³ Low OSTN has previously been associated with frailty and adverse outcomes in patients undergoing transcatheter aortic valve implantation (TAVI).⁴⁹⁴ Similarly, lower serum concentrations of OSTN were associated with higher FI score in our final stepwise model.

Although 15 myokines were included in the univariable regression and age-adjusted regression, only SPARC remained in the FP stepwise regression model. This finding contrasts our prior work that identified only fractalkine as being associated with pre-frailty; granted, that approach used categorical frailty assessment as opposed to the continuous evaluation of FP implemented in the current work.¹⁷⁰ BDNF and FABP3 were statistically significant in the linear regression for FP score but were also strongly associated with age so they were no longer significant in the age-adjusted models. SPARC has garnered some interest as a myokine due to its potential role in sarcopenia with age-related decreases in expression,^{495,496} as well as in tumor suppression with increased expression.⁴⁹⁷ SPARC knockout mice have been found to develop smaller myofiber diameters compared to wild-type mice⁴⁹⁵ and show signs of accelerated

aging.⁴⁹⁸ In contrast to these prior works, our sample identified SPARC as being positively associated with increasing FP score. However, this does align with findings that increased SPARC may impair adipogenesis and insulin sensitivity⁴⁹⁹ as well as its association with hypertension and inflammation.⁵⁰⁰ Further work will have to elucidate this relationship given these various findings as well as compare both tissue-specific regulation in relation to circulating levels of SPARC.

The measurement of novel blood-based biomarkers, specifically myokines, is challenging due to the wide heterogeneity in systemic concentrations and the lack of established guidelines for measurement in the clinical setting. EPO, FGF-21, IL-6, IL-15, irisin, LIF and myostatin all had more than a third of the values below the standard curve range, meaning that many of the values for these myokines were estimated as the lowest quantifiable values. Description of the number of samples below the standard curve range is provided in Supplementary Table 16. None of these myokines appeared in the final stepwise models, which may have been due to a reduction in statistical power when assigning the lowest quantifiable value to all values below the standard range. Without established normal concentrations for these analytes in this population, it is difficult to determine what the cause of this may have been. The mechanical and metabolic stress of physical activity alters the release of myokines,⁵⁰¹ so it may be that this stimulus is required to better assess biosynthetic capacity of the muscle in response to acute activity and, in turn, their association with frailty.¹⁷⁷ This could be done based on either absolute concentration release or the change in concentration from rest to post-exercise.

The quantifiable myokine values covered a wide range for the included participants, with some outlier values. While data was log transformed to address this, further work is needed to better understand typical concentrations of systemic myokine concentrations in different populations both at rest from chronic physical activity and in response to acute stimuli. This information will give better context to future study results, thus the inclusion of Supplementary Tables 5 and 6. Additional variables may also play a role in resting concentrations of the various myokines captured in this panel. For example, hormonal changes associated with menstrual cycle can potentially lead to variation in some myokine concentrations,⁹¹ as can potential circadian influences.⁹² This was likely mitigated by the fact that 99% of the cohort was post-menopausal.

The FP is a phenotypic assessment of frailty,¹⁵ so we anticipated that more myokines would be associated with FP score as their stimulus is dependent on physical activity and/or muscle mass.²²⁰ It is possible that the FI was associated with more myokines in this multivariable

analysis as the FI is designed to cover a wider range of physiological systems that may have cross over with the known pleiotropic effects of the myokines included in this panel.

Beyond the limitation of assessing systemic myokine concentrations at rest, as previously described, this study has some additional limitations to consider. While the analytes selected were specifically chosen due to their potential association with frailty and their known release from muscle tissue as myokines, these cytokines are also released from additional sources, including immune and other cardiometabolic tissues.^{199,207} Thus, the cytokines quantified in our assay may have originated from tissue other than skeletal muscle, but the cytokines selected for analysis were those that are thought to have altered expression with muscle stimulation. Many additional exerkines have also been identified beyond myokines specifically²⁰⁷ and it may be that the exclusion of some of these moieties would best identify early changes associated with frailty progress. This analysis was limited to the 15 pre-identified myokines selected. The EMD Millipore assay selected also was not validated using additional biochemical methodology in this analysis. The cross-sectional nature of this analysis associating myokine concentrations with frailty scores means that a potential causative role of these myokines in frailty-related health declines cannot be explored.

Notwithstanding these limitations, the study offers insight into the potential role that myokines may play as biomarkers of frailty in White females 55 years of age and older. These myokines may be used to identify early physiological dysfunction, facilitate early intervention, and lead to the development of therapeutic strategies to slow or reverse frailty progression.^{173,221} The identification of myokines associated with frailty could also inform future interventional trials aimed at altering frailty-related myokine expression using exercise interventions, simulated exercise interventions such as with neuromuscular electrical stimulation,⁵⁰² or using recombinant myokine injection in mouse models of frailty. Given the pleiotropic effects of myokines systemically, interventions targeting these may show success intervening with the broad deleterious effects of frailty – and may help to explain part of the success of physical activity interventions in this area already.⁹⁷

Conclusion

This study identified BDNF, FABP-3, OSM and OSTN as myokines that contributed to a stepwise regression model predicting FI score after adjusting for age, whereas only SPARC

remained in a stepwise model predicting FP score. Future work looking at creating and validated frailty biomarker tools should include these probable myokines of interest. The pleiotropic effects of myokines, the relevance of different serum concentrations, and the causes of acute/chronic fluctuations in myokine expression in people living with frailty requires further elucidation. Future work should look at the role these myokines play in frailty progression and aging, as well as their therapeutic potential through targeted therapies.

Table 14. Supplementary - Differences in myokine concentration by frailty index score

Myokine	Cohort <i>n</i> = 329	Group 1 ≤0.08 <i>n</i> = 28	Group 2 0.09-0.13 <i>n</i> = 82	Group 3 0.14-0.19 <i>n</i> = 111	Group 4 0.20-0.24 <i>n</i> = 55	Group 5 ≥0.25 <i>n</i> = 53
Apelin	87.79 (228.57)	109.29 (417.17)	90.47 (186.40)	86.00 (256.43)	64.60 (204.00)	111.04 (239.42)
BDNF	2039.78 (2570.42)	2054.79 (2951.75)	1628.64 (2707.21)	1829.77 (2566.98)	2102.35 (2206.45)	2355.30 (3112.01)
EPO	2795.80 (6029.97)	3365.70 (6295.04)	2188.90 (5969.33)	2795.80 (5694.81)	3317.17 (6179.55)	3317.17 (6178.76)
FABP-3	2791.23 (1635.47)	2705.10 (1862.12)	2372.22 (1504.98)	2900.97 (1852.40)	2876.39 (1163.87)	3226.88 (1877.26) [†]
FGF-21 [¶]	4.94 (0.00)	4.94 (0.00)	4.94 (0.00)	4.94 (0.00)	4.94 (0.00)	4.94 (5.32)
FSTL-1	4513.17 (9007.53)	5719.97 (9773.14)	4648.63 (9261.89)	4211.05 (8918.28)	3597.36 (7488.07)	5010.64 (9445.16)
Fractalkine	107.24 (280.19)	97.49 (304.43)	86.11 (260.17)	145.11 (307.51)	88.12 (241.45)	95.96 (328.86)
IL-6 [¶]	0.33 (8.61)	3.26 (14.65)	0.26 (6.54)	0.90 (10.32)	0.26 (6.37)	0.26 (9.68)
IL-15 [¶]	0.07 (3.74)	0.29 (5.83)	0.07 (2.00)	0.37 (4.16)	0.07 (1.22)	0.07 (4.35)
Irisin [¶]	30.27 (0.00)	30.27 (0.00)	30.27 (0.00)	30.27 (0.00)	30.27 (0.00)	30.27 (0.00)
LIF	8.20 (43.46)	16.39 (84.68)	7.00 (49.27)	9.40 (45.13)	10.20 (49.27)	5.40 (32.67)
Myostatin [¶]	255.76 (0.00)	255.76 (1331.81)	255.76 (53.98)	255.76 (0.00)	255.76 (0.00)	255.76 (573.34)
OSM	1.38 (2.91)	1.83 (5.34)	0.97 (2.47)	1.40 (2.37)	1.40 (3.24)	1.67 (3.96)
OSTN	35.62 (42.60)	40.85 (58.82)	28.94 (39.46)	40.47 (37.65)	31.71 (44.48)	36.45 (49.74)
SPARC	204.65 (138.01)	217.64 (140.38)	201.65 (122.17)	196.78 (129.90)	204.81 (139.02)	224.30 (111.21)

*All values are presented in pg/mL. Continuous variables expressed as median (IQR) and compared using ANOVA on the log10 transformed values. ¶ indicates analytes with more than 35% of the total samples outside of range. **BDNF**, brain-derived neurotrophic factor; **EPO**, erythropoietin; **FABP-3**, fatty acid-binding protein 3; **FGF-21**, fibroblast growth factor 21; **FSTL-1**, follistatin-like 1 protein; **IL-6**, interleukin-6; **IL-15**, interleukin-15; **LIF**, leukemia inhibitory factor; **OSM**, oncostatin M; **OSTN**, osteocrin; **SPARC**, osteonectin.*

** indicates statistically significant difference from Group 1. † indicates statistically significant difference from Group 2. ‡ indicates statistically significant difference from Group 3. § indicates statistically significant difference from Group 4.*

Table 15. Supplementary - Differences in myokine concentration by Fried Phenotype

Myokine	Overall Cohort <i>n</i> = 328	0 <i>n</i> = 270	1 <i>n</i> = 47	2 <i>n</i> = 11
Apelin	87.79 (228.57)	92.26 (228.71)	48.56 (148.86)	194.84 (469.99)
BDNF	2039.78 (2570.42)	1924.09 (2546.85)	2257.83 (2307.31)	4229.86 (6645.48)
EPO	2795.80 (6029.97)	2624.33 (6060.13)	2188.90 (4707.23)	6182.37 (3945.60)*†
FABP-3	2791.23 (1635.47)	2713.02 (1779.70)	3033.45 (1483.00)	3452.79 (1631.26)
FGF-21 [¶]	4.94 (0.00)	4.94 (0.00)	4.94 (0.00)	4.94 (78.56)
FSTL-1	4513.17 (9007.53)	4185.64 (9029.49)	4663.18 (10080.61)	6082.30 (12103.37)
Fractalkine	107.24 (280.19)	96.45 (298.36)	96.00 (210.39)	261.46 (176.37)
IL-6 [¶]	0.33 (8.61)	0.26 (8.60)	0.33 (6.53)	1.81 (27.83)
IL-15 [¶]	0.07 (3.74)	0.07 (3.89)	0.07 (3.37)	0.07 (11.29)
Irisin [¶]	30.27 (0.00)	30.27 (0.00)	30.27 (0.00)	30.27 (1522.47)
LIF	8.20 (43.46)	7.75 (47.26)	13.39 (31.27)	31.35 (33.94)
Myostatin [¶]	255.76 (0.00)	255.76 (0.06)	255.76 (0.00)	255.76 (4680.76)
OSM	1.38 (2.91)	1.33 (2.85)	1.38 (2.54)	2.35 (5.23)
OSTN	35.62 (42.60)	34.35 (43.32)	30.34 (30.61)	62.18 (41.54)
SPARC	204.65 (138.01)	202.30 (127.95)	204.50 (168.32)	278.24 (110.88)*

*All values are presented in pg/mL. Continuous variables expressed as median (IQR) and compared using ANOVA on the log10 transformed values. [¶]indicates analytes with more than 35% of the total samples outside of range. **BDNF**, brain-derived neurotrophic factor; **EPO**, erythropoietin; **FABP-3**, fatty acid-binding protein 3; **FGF-21**, fibroblast growth factor 21; **FSTL-1**, follistatin-like 1*

protein; **IL-6**, interleukin-6; **IL-15**, interleukin-15; **LIF**, leukemia inhibitory factor; **OSM**, oncostatin M; **OSTN**, osteocrin; **SPARC**, osteonectin.

* indicates statistically significant difference from Group 1. † indicates statistically significant difference from Group 2.

Table 16. Supplementary - Myokine concentration data ranges for duplicate samples

Myokine	Out of range (n)	Out of range (%)	Minimum value (pg/mL)	Maximum value (pg/mL)
Apelin	121	18%	0.25	20804.75
BDNF	0	0%	46.00	15136.00
EPO	218	33%	366.13	127769.41
FABP-3	0	0%	410.00	14238.00
FGF-21 [¶]	527	80%	4.94	2866.06
FSTL-1	117	18%	104.00	211415.00
Fractalkine	197	30%	8.78	7149.94
IL-6 [¶]	327	50%	0.00	226.00
IL-15 [¶]	403	61%	0.07	1160.48
Irisin	531	81%	30.00	213892.00
LIF	227	34%	0.08	3708.21
Myostatin [¶]	507	77%	256.00	624662.00
OSM	71	11%	0.01	319.61
OSTN	48	7%	1.00	1105.00
SPARC	2	0%	33.86	3134.29

*Samples were all analysed in duplicate. With n = 329 participants, this resulted in 658 samples run per myokine. [¶]indicates analytes with more than 35% of the total samples outside of range. **BDNF**, brain-derived neurotrophic factor; **EPO**, erythropoietin; **FABP-3**,*

*fatty acid-binding protein 3; **FGF-21**, fibroblast growth factor 21; **FSTL-1**, follistatin-like 1 protein; **IL-6**, interleukin-6; **IL-15**, interleukin-15; **LIF**, leukemia inhibitory factor; **OSM**, oncostatin M; **OSTN**, osteocrin; **SPARC**, osteonectin*

5.6 Brief Discussion of the Manuscript written exclusively by Kevin Boreskie

This unpublished work built on the studies in the prior chapters, examining the association of myokines with frailty status in a number of different ways. Expert opinion in this space began discussing the importance of looking at biomarkers using multivariable analyses given the presumed complexity of their interactions in pathophysiological declines.^{175,221} Our previous research had only done univariable analyses in a relatively small sample size. Additionally, we had examined only the phenotype of frailty in association with myokines. Therefore, we recruited a larger sample size (n = 329) from the initially recruited participants for the WARM Hearts cohort study (NCT03938155), a cohort study examining unique CVD risk in middle-aged and older women.

This larger sample enabled a cross-sectional, multivariable analysis examining the association between a panel of selected myokines and frailty status. Frailty was assessed with both the Fried phenotype of frailty as well as with a Frailty Index (FI), as both are supported for use in different clinical contexts.⁷⁴ Originally, we had planned on again examining the association of the myokines with CVD risk status, but the proposed statistical approaches were not feasible with this design and it took away from the important story of their association with frailty, our main outcome of interest. The progression of CVD and frailty are also interlinked bidirectionally, making it difficult to separate these concepts in their earlier stages.^{35,159,163} Therefore, we decided to focus exclusively on frailty in the research design. Systemic myokine blood concentrations were determined using the same MILLIPLEX® Map Human Myokine Magnetic Bead Panel as Chapter 3. A stepwise, multivariable regression was used to assess the value of adding each of the selected 15 myokines to a model predicting both FI and FP after adjusting for age. This methodology would allow us to hopefully capture a more intricate interplay between myokines when examined in association with frailty status. It also allowed us to examine associations with both the FP and the multidimensional FI. This allows for a broader understanding of myokine associations with various frailty assessments; however, the FI is also measured in a more continuous fashion than the FP, enabling a more sensitive analysis of these associations.

BDNF, FABP-3, OSM and osteonin were all included in a stepwise multivariable regression predicting FI score while only SPARC added value to the model for FP in addition to age. The difference in myokines associated with the two frailty assessment modalities is likely

because they examine frailty differently, with the FI being more of a multidimensional assessment, and the FI is also more sensitive in identifying much more of our cohort living with frailty. These myokines should be considered of interest in future research examining biomarkers of frailty and their association with frailty-related health declines, although much work would still have to be done prior to their clinical implementation. For one, their effects throughout the body will have to be further elucidated, given their vast, pleiotropic effects. The identification of myokines associated with frailty-related health declines could also allow for the creation of a tool, such as an FI-Lab, incorporating these data points.^{80,173,177,182} FIs incorporating biomarkers, such as myokines, offer the opportunity to potentially identify frailty-related changes earlier before overt clinical outcomes like reduced grip strength, for example.^{80,181} It also identifies possible therapeutic targets. For example, if a panel of myokines was found to be associated with frailty, one could attempt to alter their expression through interventions, such as various physical activity modalities. Such an approach would then require further research into how their expression can be altered, and whether their altered expression would slow or mitigate frailty-related progression.

Chapter 6: Thesis synthesis and conclusions

6.1 Thesis synthesis

An aging population and longitudinal data suggesting an increase in the proportion of individuals living with a high degree of frailty mean that healthcare systems will have to adapt to meet this challenge.^{1,31,44,238} As outlined in this thesis work, frailty assessment can assist in a transition to healthcare provision that is more personalized and better serves a complex patient population.^{36,67,74,271,284} Frailty assessment provides a better description of this population, assists with more accurate direction of patient-centered care, and allows for opportunities for intervention, as compared to using chronological age to guide care pathways.⁷⁴ Given the important role that frailty can play in health as well as in directing better care, clinicians must be able to identify it early in its progression to improve health, and as it is likely that frailty becomes increasingly difficult to intervene with after further progression.^{97,169,243} Not only this, but its pathophysiological progression must be better understood so that targeted therapeutic interventions can be implemented to improve patient trajectories. The work of this thesis has demonstrated the early potential of blood-based biomarkers, specifically myokines, as a means of doing this, as well as their relevance to the larger clinical picture of care provision for healthy aging.

The Canadian Frailty Network (CFN) created a list of top ten priorities for research on patients with frailty following the methods of the James Lind Alliance by consulting those living with frailty and their caregivers (Table 17).²³⁵ Research should be patient-focused and thus work toward patient-identified needs. The works covered in this thesis document touch on at least six of these ten outlined priorities. First, to contextualize the importance of biomarkers in frailty assessment and progression, the importance of frailty was reviewed in thesis Chapter 2. Regardless of the acuity or setting of the clinical care being provided, frailty serves as an opportunity to better understand personalized prognosis, management, and disposition for that specific patient.⁷⁴ Chapter 2 summarizes important existing literature and aligns with the first, second, fifth, and ninth frailty priorities in the CFN top ten. Chapter 2 also serves to provide clinicians and researchers alike with knowledge to continue incorporating geriatric principles into their work through understanding how frailty assessment can be used in their own practice, directly addressing the sixth research priority of the CFN. This application will only become increasingly relevant with

an aging population¹ that will require better characterization than chronological age can provide in order to navigate presentations with multi-comorbidity and social complexity.⁷⁴

Table 17. Canadian Frailty Network top ten frailty priorities²³⁵

Top Ten Frailty Priorities	Associated Thesis Chapter
1. How can health systems be organized to provide integrated/coordinated care that would better meet the health and social care needs of older adults living with frailty, and their family/caregivers?	Chapter 2
2. How can care, services and treatments be tailored to meet the needs of older adults living with frailty who are isolated and/or without family/caregiver support or advocates?	Chapter 2
3. What is the impact of community- and home-based services, programs, and resources in preventing and managing frailty (including slowing progression and/or minimizing the impact of frailty)?	
4. What are the costs and benefits of alternative models of housing, including multigenerational or shared living, for older adults living with frailty?	
5. What would help avoid unnecessary hospitalizations and emergency department visits for older adults living with frailty?	Chapter 2
6. What frailty-related attitudes, skills and knowledge should health and social care providers have? What are effective ways of improving attitudes and providing skills and knowledge about frailty for health and social care providers?	
7. What would help older adults living with frailty and their family/caregivers recognize when living at home is no longer viable?	
8. What are effective ways of supporting family/caregivers of older adults living with frailty to maintain their own health and well-being and/or that of older adults living with frailty?	
9. How can frailty measures be used by health care practitioners, older adults and family/caregivers to inform treatment and care decisions?	Chapter 2, 3, 4, 5

10. What is the impact of exercise and physical activity (including type, duration and intensity) in preventing and managing frailty (including slowing progression and/or minimizing the impact of frailty)?	Chapter 3, 4, 5
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Measurement of myokine concentration in individuals to assist in an FI-Lab may also allow for further identification of specific pathophysiological changes that could be addressed through intervention and assessing for appropriate changes in concentration with said intervention. Interventions could be personalized based on the tracked response of these biomarkers to said intervention. Newer data continues to show that traditional risk markers of disease are not as effective in older adults, specifically in CVD. For example, in a systematic review of 12 studies including adults 60 years of age and older ranging from 302 to 40,825 participants, the authors found that current risk prediction modeling only performed moderately and the value of traditional risk factors were reduced.¹⁶⁵ Specifically, age, sex and diabetes maintained their predictive value, but factors like cholesterol, BMI and left ventricular hypertrophy added no value. A more recent analysis of 339 older adults (mean age 75.2±9.1 years) demonstrated that the strength of associated traditional CVD risk factors such as smoking, hypertension and dyslipidemia was reduced with increasing frailty status.¹⁹ Furthermore, a large meta-analysis of 21,906 older adults found that individuals with frailty and hypertension were not at increased risk for mortality relative to their normotensive peers with frailty.¹⁶⁶ These data, along with large datasets identifying frailty as outperforming traditional CVD risk detection tools²⁰ indicate that a shift from traditional CVD risk factors to factors focusing more on function and broader health declines may be warranted.

Chapter 2 introduces the contextual clinical utility of frailty assessment and, more specifically, the application of biomarker-based approaches to assessing frailty, such as the FI-Lab.¹⁸¹ Using such an approach offers the availability of frailty assessment when there is a paucity of other information that may be needed to complete a typical FI, it is not feasible to perform extensive questionnaires or clinical testing, or an inability to complete testing that is often done as part of a phenotypic assessment, such as grip strength or gait speed. Past work has identified that FI-Labs using such an approach are predictors of mortality per unit increase in FI score as well as a variety of other adverse outcomes, supporting such an approach.⁹⁵ It is unlikely that any single biomarker will be able to perform well compared to a more comprehensive FI, but their inclusion in an FI type model could help improve prognostic accuracy.⁴⁵¹ FIs comprised entirely of lab

values have performed similarly to typical FIs in predicting adverse outcomes,^{80,181} but combining blood-based markers with clinical deficits has been shown to improve prognostication.¹⁸² Importantly, one of the proposed benefits of a blood-based marker approach for frailty assessment is that it may be able to identify frailty progression earlier than clinical deficits typically assessed in FI-based approaches or phenotypic approaches to frailty.^{173–175} While many different pathophysiological processes have been suggested as contributing to frailty-related declines, this process is still not well understood. Identifying blood-based markers related to frailty may also help further elucidate this process.¹⁷⁵

The studies described in Chapters 3 and 4 started to address these knowledge gaps by performing an exploratory analysis into the association of biomarkers, specifically cytokines associated with physical activity and bone metabolism, with frailty status. Through a panel of pre-identified myokines thought to be associated with frailty and CVD-related health declines, Chapter 3 further supported the notion that immune modulating cytokines may play a role in the progression of the phenotype of frailty and thus, serve as biomarkers of same – specifically, fractalkine/CX3CL1.¹⁷⁰ Cytokines associated with inflammation and the immune response, IL-6 and IL-15 were also associated with CVD risk in this same analysis. With further support toward the notion of exerkines communicating with a variety of tissues across the body,²⁰⁷ Chapter 4 explored a panel of cytokines associated with bone metabolism in association with phenotypic frailty status and CVD risk, as bone health can also be impaired with aging, and bone-muscle cross talk plays a role in the physiological benefits of physical activity with aging.²²³ None of these ‘bonekines’ were associated with frailty in this analysis, but it was thought that potentially this was only the case as our cohort population only consisted of robust and pre-frail females – none were frail based on the FP criteria.²³² However, similar with our prior analysis, cytokines associated with immune response again were associated with CVD risk – specifically, IL-6 and TNF α , as well as Leptin, which is thought to play a role in metabolism.⁴³⁹

The findings from Chapters 3 and 4 provided some insight supporting, largely, the association of myokines playing a role in inflammation and immune function to frailty and CVD-related health declines. This preliminary examination demonstrated the potential of these biomarkers and allowed us to work with the assay kits in a smaller initial sample. Chapter 5 built on this work further by incorporating current expert opinion that investigations into biomarkers of

frailty should likely look at the interplay of multiple biomarkers given the likely complex pathophysiology of frailty.^{174,180,455} The multivariable statistical approach used with the myokine assay panel allowed us to do this, as well as allowed us to incorporate both assessments of FP and an FI. The FI is a more multidimensional assessment of frailty, and is potentially more sensitive to frailty at less significant degrees, which better reflected our cohort as shown by the higher prevalence of frailty identified with this tool.²³³ The scoring of frailty on a continuous scale with more steps of discernment by increments of 0.01 with the FI also potentially allowed for a more sensitive assessment of associations with myokines, although clinically meaningful change in the FI is thought to likely be 0.03.³²⁵

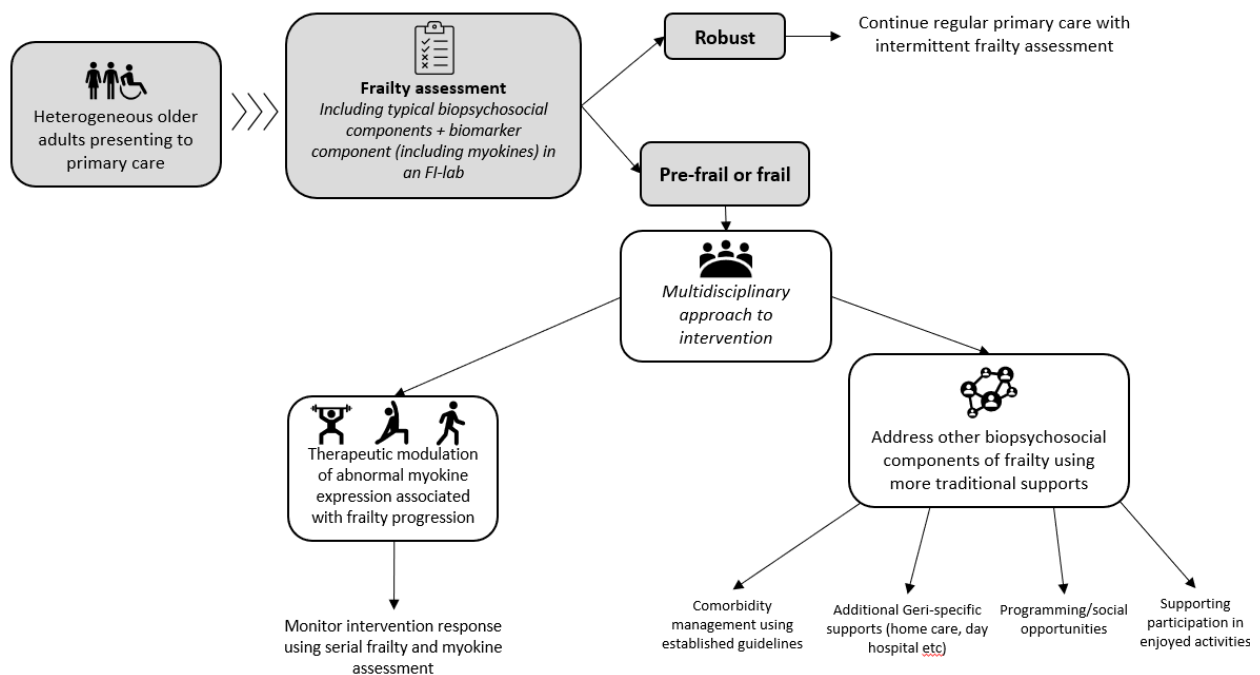
This investigation identified that BDNF, FABP-3, OSM and osteocrin all contributed to a stepwise regression model predicting FI score. These data again support the association of myokines related to inflammation and metabolic function with frailty, as well as with cognition. In contrast, only SPARC, a myokine related largely to muscle function and mass, added to the model predicting Fried score. The difference in myokines identified between the two models likely reflects the differences conceptually between these frailty models with a myokine related to muscle function/mass being associated with the phenotypic model. Additionally, as previously mentioned, the FI is assessed on more of a continuous scale and is more sensitive to lesser degrees of frailty, which may be why more myokines fit in the model. Interestingly, the multivariable models identified different myokines (BDNF, osteocrin, OSM, FABP-3 for FI; SPARC for FP) as being related to frailty than our prior univariable analyses (fractalkine). Our prior analyses were likely less sensitive given the categorization of variables as opposed to a continuous analysis, as well as the hope that the multivariable analyses would capture the interplay of multiple myokines, which aligns with expert opinion.^{175,455}

Overall, this thesis research contributes novel insights into the intricate relationship between myokines and frailty status. The identification of biomarkers, specifically myokines (Chapter 3 and Chapter 5), associated with frailty progression could also help address the ninth and tenth research priorities as established by the CFN.¹³⁷ These include examining how frailty measures can inform treatment/care decisions and examining the relationship between physical activity and frailty. The ninth priority is also addressed in Chapter 2, but the identification of biomarkers associated with frailty progression could allow for personalized intervention should modification of these biomarkers also be elucidated through physical activity or through mimetics,

such as bioactive compounds or pharmaceuticals.^{207,400} These findings hold implications to support further research in this space and, eventually, the potential for clinical application which will be discussed further in Section 6.1 Future Directions.

Research has increasingly focused on personalizing risk prediction and intervention for disease in older adults as our population ages. Past work has shown that creating FIs focusing on both clinical deficits and lab-based measures can identify individuals at risk for further physiological decline and may provide additional value in identifying subclinical deficit accumulation.⁹⁵ The data in this thesis work has identified biomarkers that could add value to such an approach. Figure 11 describes such an approach with frailty assessment at the primary care level followed by multidisciplinary interventions, part of which could be personalized through the use of myokine assessment and modulation.

Figure 11. Frailty, biomarkers, and the clinical context



6.2 Thesis strengths and limitations

With an aging population, frailty prevalence is expected to continue to rise.²³³ This will make its identification, pathophysiological understanding and intervention increasingly relevant in order to support the health of an aging population. A strength of this thesis document is its

alignment with the Canadian Frailty Network top ten frailty priorities as established by those living with frailty and their caregivers.²³⁵ Biomarkers of frailty have specifically been identified as an area of interest within this research space because of their potential to identify frailty earlier and provide a better understanding of the underlying mechanisms of frailty progression.^{175,450} This work not only aligns with these calls but, more specifically, with the calls for research examining myokines as biomarkers of frailty.^{175,221} Beyond these expert statements, prior to the research included in this thesis document, previous works had either examined biomarker panels only including a few myokines univariable myokine associations with frailty, or myokine associations with related, but distinct, conditions such as sarcopenia.^{451,455,457,503,504} This makes the multivariable analyses in this document novel.

The findings of this thesis are then bridged into clinical context by situating this biomarker work within practical frailty assessment frameworks across healthcare settings and through the identification of future potential translational studies, such as the proposal for the findings to be incorporated into an FI-Lab tool,^{80,181} charting a potential roadmap from discovery to intervention which will be discussed more in the subsequent section.

The results presented herein are limited by several factors and should be understood with the limits of the approaches used. All studies included in this work are of a cross-sectional design, precluding determination of whether altered myokine concentrations levels drive frailty progression or merely reflect changes that have already occurred. This could be addressed with future longitudinal works and intervention studies as discussed in section 6.3. These included analyses are also all on data collected from middle aged to older adult females collected in a convenience sample method, limiting generalizability to this population only. The analysis of myokine concentrations in this thesis also has limitations because of the work being novel and my decision that the tools used have an emphasis on being clinically feasible. Normal ranges of systemic myokine concentration are not well understood in different populations and several of the analytes used in the analyses either fell below standard curve limits and/or had wide inter-individual variability, complicating their interpretation. This was a limitation of my thesis work and the field more broadly. Without analyses specifically measuring myokines as they are released from muscle (which can be done by measuring venous-arteriole differences in specific myokine concentrations across contracting muscle), it also remains unclear how systemic cytokine concentrations reflect release from muscle tissue versus contribution from immune or

other tissues. Lastly, while the association of myokines with frailty status was examined, these analytes have many pleiotropic effects, which makes it difficult to state the pathophysiological pathways linking them without future mechanistic studies.

6.3 Future research directions

This thesis supports the use of myokines as biomarkers of frailty and, in turn, identifies possible pathophysiological connections to elucidate the pathophysiological declines seen in frailty. This work is only preliminary - a significant amount of further research is needed to better characterize myokine concentrations in older adults living with frailty. Additional research is needed to more fully elucidate the role of the identified myokines in human physiology. Other research is needed to determine the optimal means of measuring myokines from blood samples collected that is clinically feasible. The results of this thesis require further validation in different cohorts as well as in samples that include both males and females to determine if these results differ by cohort and/or sex.

In 2018, the CFN brought together a panel of experts to discuss the development of research on biomarkers for frailty.⁴⁵⁰ Aligned with this thesis document, CFN experts outlined the importance of biomarkers reflecting a pathophysiological pathway that is likely to play a role in frailty progression/development. The data and discussion of the physiological basis for cytokines and frailty in this document indicate what may be a role in this progression, meaning that impairments in this biomarker may be actionable.⁴⁵⁰ On that note, they also discuss that an ideal biomarker would be one that is able to help assess the efficacy of an intervention. Myokines may fit this ideal as their change in expression could be assessed after different modalities, such as physical activity.⁵⁰⁵ Finally, the expert discussion also concluded that personalized frailty assessment and care will likely require both biomarkers and existing assessment instruments in unison, something that will be possible to examine with this data set and cohort in the future. These recommendations are also reflected by those of the ICFSR task force previously discussed in this document.¹⁷⁵

The identification of myokines associated with frailty and CVD progression gets us closer to understanding the role physical activity may have in this trajectory, but the vast effects of even the few myokines used in the assays in this thesis are far from fully understood - not to mention the pleiotropic effects of the hundreds of other myokines identified thus far and the broader

exerkines.^{199,203,207} Future research will have to continue to explore the autocrine, paracrine and endocrine effects of these signaling molecules while also better defining expected norms for physiological ranges in a variety of populations. Expected concentration ranges and a better understanding of myokine effects will allow us to further refine their use as biomarkers, especially given the increased variability between subjects that has been identified in the works that have examined cytokines associated with frailty as well as with these thesis works.^{170,232,461} Furthermore, longitudinal changes in myokine concentration and expression in relation to frailty and CVD trajectory has not been studied. While our data does indicate more substantial changes in systemic myokine concentration from pre-frail to frail individuals as compared to robust, longitudinal assessments are needed to determine directionality of the relationship between these changes in myokines and health status.

While both myokine studies in this thesis were assessed from systemic blood samples at rest,¹⁷⁰ there is an urgent need to identify the optimal timing and conditions under which to draw blood samples from participants. Myokine concentrations are altered by muscle contractions or after certain physiological stressors. Physical activity is thought to alter exerkine expression both acutely and chronically.^{207,209} For example, differences in systemic myokine concentrations between rest and post-exercise may provide more value as frailty biomarkers by identifying altered expression in the context of an acute stimulus. This alteration may be either due to an overall reduction in physical activity associated with frailty progression or a preceding change in muscle health that could subsequently also lead to reduced physical activity, creating a cycle as outlined by Fried in Figure 2. Examining the timeline of systemic myokine release following different exercise modalities, such as following resistance training versus following various aerobic training modalities, may provide a better understanding of how these signal proteins function and how we can best alter their release therapeutically. For those who may be unable to perform physical activity, identifying alternative means of altering myokine and exerkine expression will also be important for this work.^{207,400}

Research is needed to more fully understand how stimuli (e.g. health behaviours) modulate myokine expression and their influence on clinical outcomes after such modulation. Research is only just starting to identify the critical and vast influence of exerkines on a variety of aging and disease states.²⁰⁹ Further work examining the alteration of myokine systemic concentration pre- and post-both acute and chronic physical activity interventions in populations living with frailty

should be conducted to continue moving this research space forward, as this is still not well elucidated.^{207,505} These studies should also examine varying physical activity modalities, such as aerobic training versus strength training, although expert opinion suggests that focusing on myokines stimulated by resistance training, as opposed to aerobic training, should be the focus of future research in this space given age-related muscle declines seen in strength that may be related to part of frailty progression.²²⁰ Given the high heterogeneity seen in the resting systemic myokine concentrations seen in our work, it is possible that changes, or lack of, systemic myokine concentrations in response to a stimulus could be a better biomarker for frailty. This information could also inform future interventions for mitigating frailty progression by targeting myokine stimuli in myokines associated with frailty progression through different exercise modalities. A recent systematic review and meta-analysis found highly variable myokine response to acute resistance and acute aerobic training in commonly examined myokines (IL-15, irisin, SPARC, OSM and decorin) at varying time points.⁵⁰⁵ Of note, no significant difference in response was found between the resistance and aerobic training pooled data. This analysis was conducted in a grouped population of 1,193 participants with a median age of approximately 30 years and numerous sources of heterogeneity between interventions. Thus, much further work is needed to better inform this data.

Beyond systemic blood concentrations of various myokines, measurement of direct release of myokines from the muscle via novel myotube models is necessary to get more precise measurement of the cytokines only being released from muscle. There is crossover in release of some cytokines from immune cells, amongst other tissues, although myotube assessment is less feasible from a clinical standpoint.^{506,507} Nederveen et al. discuss the challenges associated with trying to track skeletal-muscle specific extracellular vesicles once they enter systemic circulation.²⁰¹ While such an approach would potentially help solidify the role that cytokines released specifically from muscle play in frailty and assessing potential for exercise interventions subsequently, it would not be a feasible approach for identifying frailty in the clinical setting given the increased complexity of a measurement like this. Furthermore, it may be more important to examine the systemic release of these cytokines from various tissues in response to physical activity. Approaches could also be used to examine associations between various chronic movement patterns, such as physical activity or sedentary behaviour, and their association with systemic myokine concentrations, as well as their impact on frailty status.

The identification of myokines associated with frailty and CVD risk may get us closer to the creation of an optimized FI-Lab^{80,95,181} or an FI-B,¹⁸² or other blood-based frailty assessment that is better at identifying early subclinical changes before the appearance of observable frailty deficits. Past research from Dr. Rockwood's group has identified that adding biomarkers to an FI composed of clinical deficits improved its ability to predict mortality.¹⁸² Even if an FI-Lab performs equivalently to clinical-based FIs in predicting adverse outcomes, as has been demonstrated with multiple datasets, lab markers potentially allow for easier and/or less expensive collection, especially in large datasets.⁹⁵ Biomarkers could also be added to a quantile frailty index (FI-Q) that does not binarize deficits included in the FI, instead creating an average risk quantile for the included biomarkers.⁵⁰⁸ Such an approach may allow for richer prognostic information without the dichotomization of included deficits.⁹⁵

The data collected from this thesis may also be used to inform a randomized controlled trial in mouse models of frailty. Using data from the regression model, we have identified some specific myokines that may be of interest in the pathophysiological process of frailty progression, but it is important to establish their effects in an experimental study. While it is unethical to perform such an experiment in human participants without first completing phase 1 and 2 experimental trials in model systems, the PolG mouse exhibits premature aging and frailty due to accumulated mitochondrial DNA (mtDNA) mutations,⁵⁰⁹ and can be used to test therapeutic approaches (such as pharmacological intervention) for the treatment of frailty and CVD risk using myokine treatment through tail intravenous injections. Frailty status could be assessed using specific frailty assessments for mice^{510,511} at baseline and then at various time points following recombinant protein injection to compare frailty progression between treatment and control arms. Similar experiments could be conducted to determine if health behaviour modification interventions targeting physical activity, sleep, or sedentary behaviours can also be conducted using the PolG mouse model. Further work still needs to be done in order to ensure that circulating cytokines in mice models respond similarly in relation to frailty status; however, as some suspected difference have already been identified.⁴⁶¹ Building from these works, a randomized controlled trial could identify therapeutic approaches for slowing, or reversing, the progression of frailty using recombinant myokines. This would also provide further evidence for the myokines of importance in frailty progression so that their expression can be modified therapeutically to prevent progression, either through exogenous or endogenous means.

6.4 Summary and impact

This thesis adds data to address the calls from expert opinion asking for research to examine the potential association between myokines and frailty status.^{175,177} Given how few data sets have examined the association of a panel of myokines with frailty status, a data set this large, using a multivariable analyses examining both FP and FI is a step forward in this specific research area. Specifically, much of the prior work in related research areas have either looked at univariable markers or markers in association with aging more generally or sarcopenia. Significant further work on various fronts is needed to bring this data closer to clinical application. However, this data suggests that this may be an area worth continuing to pursue, providing some direction for future research. More generally, the work in this thesis also highlights the importance of frailty in aging, chronic disease progression, and quality of life. This will become increasingly relevant in an aging population. Incorporating frailty-aware care into clinical practice through research and its implementation in various settings will be crucial to better understand and, in turn, care for our older patients. The published works from this thesis, especially the review paper, have been cited by other experts with an interest in the field of frailty. While this work is still far from clinical implementation, it hopefully guides future research toward some of the steps outlined in the Future Directions section and allows others to build upon it.

REFERENCES

1. WHO | World report on ageing and health 2015. <http://www.who.int/ageing/events/world-report-2015-launch/en/>.
2. National Center for Health Statistics (US). *Health, United States, 2007: With Chartbook on Trends in the Health of Americans*. (National Center for Health Statistics (US), Hyattsville (MD), 2007).
3. Seals, D. R., Justice, J. N. & LaRocca, T. J. Physiological geroscience: targeting function to increase healthspan and achieve optimal longevity. *J. Physiol.* **594**, 2001–2024 (2016).
4. The top 10 causes of death. <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>.
5. Liguori, I. *et al.* Oxidative stress, aging, and diseases. *Clin. Interv. Aging* **13**, 757–772 (2018).
6. Wei, W. & Ji, S. Cellular senescence: Molecular mechanisms and pathogenicity. *J. Cell. Physiol.* **233**, 9121–9135 (2018).
7. Sen, P., Shah, P. P., Nativio, R. & Berger, S. L. Epigenetic mechanisms regulating longevity and aging. *Cell* **166**, 822–839 (2016).
8. Jang, J. Y., Blum, A., Liu, J. & Finkel, T. The role of mitochondria in aging. *J. Clin. Invest.* **128**, 3662–3670 (2018).
9. Zhu, Y., Liu, X., Ding, X., Wang, F. & Geng, X. Telomere and its role in the aging pathways: telomere shortening, cell senescence and mitochondria dysfunction. *Biogerontology* **20**, 1–16 (2019).
10. Fried, L. P. *et al.* Nonlinear multisystem physiological dysregulation associated with frailty in older women: implications for etiology and treatment. *J. Gerontol. A. Biol. Sci. Med. Sci.* **64**, 1049–1057 (2009).
11. Seals, D. R. & Melov, S. Translational geroscience: emphasizing function to achieve optimal longevity. *Aging* **6**, 718–730 (2014).

12. Government of Canada, S. C. Population Projections for Canada (2021 to 2068), Provinces and Territories (2021 to 2043). <https://www150.statcan.gc.ca/n1/pub/91-520-x/91-520-x2022001-eng.htm> (2022).
13. Non communicable diseases. <https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases>.
14. Blair, S. N., Sallis, R. E., Hutber, A. & Archer, E. Exercise therapy - the public health message. *Scand. J. Med. Sci. Sports* **22**, e24-28 (2012).
15. Fried, L. P. *et al.* Frailty in older adults: evidence for a phenotype. *J. Gerontol. A. Biol. Sci. Med. Sci.* **56**, M146-156 (2001).
16. Peng, Y., Zhong, G.-C., Zhou, X., Guan, L. & Zhou, L. Frailty and risks of all-cause and cause-specific death in community-dwelling adults: a systematic review and meta-analysis. *BMC Geriatr.* **22**, 725 (2022).
17. Morley, J. E. *et al.* Frailty consensus: a call to action. *J. Am. Med. Dir. Assoc.* **14**, 392–397 (2013).
18. Shrauner, W. *et al.* Frailty and cardiovascular mortality in more than 3 million US Veterans. *Eur. Heart J.* **43**, 818–826 (2022).
19. Aprahamian, I. *et al.* The association between cardiovascular risk factors and major cardiovascular diseases decreases with increasing frailty levels in geriatric outpatients. *Exp. Gerontol.* 111475 (2021) doi:10.1016/j.exger.2021.111475.
20. Farooqi, M. A. M., Gerstein, H., Yusuf, S. & Leong, D. P. Accumulation of Deficits as a Key Risk Factor for Cardiovascular Morbidity and Mortality: A Pooled Analysis of 154 000 Individuals. *J. Am. Heart Assoc.* **9**, e014686 (2020).
21. Aguayo, G. A. *et al.* Comparative analysis of the association between 35 frailty scores and cardiovascular events, cancer, and total mortality in an elderly general population in England: An observational study. *PLOS Med.* **15**, e1002543 (2018).

22. Mitnitski, A. B., Mogilner, A. J. & Rockwood, K. Accumulation of deficits as a proxy measure of aging. *ScientificWorldJournal* **1**, 323–336 (2001).
23. Bergman, H. *et al.* Frailty: an emerging research and clinical paradigm--issues and controversies. *J. Gerontol. A. Biol. Sci. Med. Sci.* **62**, 731–737 (2007).
24. Fulop, T. *et al.* Aging, frailty and age-related diseases. *Biogerontology* **11**, 547–563 (2010).
25. Lang, P.-O., Michel, J.-P. & Zekry, D. Frailty syndrome: a transitional state in a dynamic process. *Gerontology* **55**, 539–549 (2009).
26. Kehler, D. S. *et al.* Prevalence of frailty in Canadians 18-79 years old in the Canadian Health Measures Survey. *BMC Geriatr.* **17**, 28 (2017).
27. Shamliyan, T., Talley, K. M. C., Ramakrishnan, R. & Kane, R. L. Association of frailty with survival: A systematic literature review. *Ageing Res. Rev.* **12**, 719–736 (2013).
28. O’Caoimh, R. *et al.* Prevalence of frailty in 62 countries across the world: a systematic review and meta-analysis of population-level studies. *Age Ageing* **50**, 96–104 (2021).
29. Hubbard, R. E. & Rockwood, K. Frailty in older women. *Maturitas* **69**, 203–207 (2011).
30. Gordon, E. H. & Hubbard, R. E. Differences in frailty in older men and women. *Med. J. Aust.* **212**, 183–188 (2020).
31. Blodgett, J. M., Rockwood, K. & Theou, O. Changes in the severity and lethality of age-related health deficit accumulation in the USA between 1999 and 2018: a population-based cohort study. *Lancet Healthy Longev.* **2**, e96–e104 (2021).
32. Veronese, N. *et al.* Risk of cardiovascular disease morbidity and mortality in frail and pre-frail older adults: Results from a meta-analysis and exploratory meta-regression analysis. *Ageing Res. Rev.* **35**, 63–73 (2017).
33. Hanlon, P. *et al.* Frailty and pre-frailty in middle-aged and older adults and its association with multimorbidity and mortality: a prospective analysis of 493 737 UK Biobank participants. *Lancet Public Health* **3**, e323–e332 (2018).

34. Boreskie, K. F., Boreskie, P. E. & Melady, D. Age is just a number - and so is frailty: Strategies to inform resource allocation during the COVID-19 pandemic. *CJEM* 1–5 (2020) doi:10.1017/cem.2020.358.
35. Forman, D. E. & Alexander, K. P. Frailty: A Vital Sign for Older Adults With Cardiovascular Disease. *Can. J. Cardiol.* (2016) doi:10.1016/j.cjca.2016.05.015.
36. Mitnitski, A., Howlett, S. E. & Rockwood, K. Heterogeneity of Human Aging and Its Assessment. *J. Gerontol. A. Biol. Sci. Med. Sci.* **72**, 877–884 (2017).
37. Soysal, P. *et al.* Relationship between depression and frailty in older adults: A systematic review and meta-analysis. *Ageing Res. Rev.* **36**, 78–87 (2017).
38. Franse, C. B. *et al.* Socioeconomic inequalities in frailty and frailty components among community-dwelling older citizens. *PLoS ONE* **12**, (2017).
39. Kelly, S., O’Brien, I., Smuts, K., O’Sullivan, M. & Warters, A. Prevalence of frailty among community dwelling older adults in receipt of low level home support: a cross-sectional analysis of the North Dublin Cohort. *BMC Geriatr.* **17**, (2017).
40. Thompson, M. Q. *et al.* Frailty prevalence and factors associated with the Frailty Phenotype and Frailty Index: Findings from the North West Adelaide Health Study. *Australas. J. Ageing* (2017) doi:10.1111/ajag.12487.
41. Hoogendijk, E. O. *et al.* Frailty Combined with Loneliness or Social Isolation: An Elevated Risk for Mortality in Later Life. *J. Am. Geriatr. Soc.* **68**, 2587–2593 (2020).
42. Schwappach, D. L., Boluarte, T. A. & Suhrcke, M. The economics of primary prevention of cardiovascular disease - a systematic review of economic evaluations. *Cost Eff. Resour. Alloc. CE* **5**, 5 (2007).
43. Lacas, A. & Rockwood, K. Frailty in primary care: a review of its conceptualization and implications for practice. *BMC Med.* **10**, 4 (2012).

44. Muscedere, J. *et al.* Screening for Frailty in Canada's Health Care System: A Time for Action. *Can. J. Aging Rev. Can. Vieil.* **35**, 281–297 (2016).
45. Ruiz, J. G. *et al.* Screening for and Managing the Person with Frailty in Primary Care: ICFSR Consensus Guidelines. *J. Nutr. Health Aging* (2020) doi:10.1007/s12603-020-1492-3.
46. Buckinx, F. *et al.* Burden of frailty in the elderly population: perspectives for a public health challenge. *Arch. Public Health* **73**, (2015).
47. Han, L., Clegg, A., Doran, T. & Fraser, L. The impact of frailty on healthcare resource use: a longitudinal analysis using the Clinical Practice Research Datalink in England. *Age Ageing* **48**, 665–671 (2019).
48. Mondor, L. *et al.* The Incremental Health Care Costs of Frailty Among Home Care Recipients With and Without Dementia in Ontario, Canada: A Cohort Study. *Med. Care* **57**, 512 (2019).
49. Lowry, K. A., Vallejo, A. N. & Studenski, S. A. Successful Aging as a Continuum of Functional Independence: Lessons from Physical Disability Models of Aging. *Aging Dis.* **3**, 5–15 (2011).
50. Schalk, B. W. M., Visser, M., Deeg, D. J. H. & Bouter, L. M. Lower levels of serum albumin and total cholesterol and future decline in functional performance in older persons: the Longitudinal Aging Study Amsterdam. *Age Ageing* **33**, 266–272 (2004).
51. Morley, J. E. Developing novel therapeutic approaches to frailty. *Curr. Pharm. Des.* **15**, 3384–3395 (2009).
52. Landi, F. *et al.* Serum high-density lipoprotein cholesterol levels and mortality in frail, community-living elderly. *Gerontology* **54**, 71–78 (2008).
53. Hazzard, W. R. Depressed albumin and high-density lipoprotein cholesterol: signposts along the final common pathway of frailty. *J. Am. Geriatr. Soc.* **49**, 1253–1254 (2001).
54. Wilson, D., Jackson, T., Sapey, E. & Lord, J. M. Frailty and sarcopenia: The potential role of an aged immune system. *Ageing Res. Rev.* **36**, 1–10 (2017).

55. Walston, J. *et al.* Research agenda for frailty in older adults: toward a better understanding of physiology and etiology: summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults. *J. Am. Geriatr. Soc.* **54**, 991–1001 (2006).
56. Fried, L. P., Ferrucci, L., Darer, J., Williamson, J. D. & Anderson, G. Untangling the Concepts of Disability, Frailty, and Comorbidity: Implications for Improved Targeting and Care. *J. Gerontol. Ser. A* **59**, M255–M263 (2004).
57. Afilalo, J., Karunanathan, S., Eisenberg, M. J., Alexander, K. P. & Bergman, H. Role of frailty in patients with cardiovascular disease. *Am. J. Cardiol.* **103**, 1616–1621 (2009).
58. Larsson, L. *et al.* Sarcopenia: Aging-Related Loss of Muscle Mass and Function. *Physiol. Rev.* **99**, 427–511 (2019).
59. Blodgett, J., Theou, O., Kirkland, S., Andreou, P. & Rockwood, K. The association between sedentary behaviour, moderate-vigorous physical activity and frailty in NHANES cohorts. *Maturitas* **80**, 187–191 (2015).
60. Liu, Y., Gao, S., Dou, Z., Chen, Z. & Tang, J. Is meeting 24-hour movement guidelines associated with a lower risk of frailty among adults? *Int. J. Behav. Nutr. Phys. Act.* **22**, 21 (2025).
61. Pedersen, B. K. The Physiology of Optimizing Health with a Focus on Exercise as Medicine. *Annu. Rev. Physiol.* **81**, 607–627 (2019).
62. da Silva, V. D. *et al.* Association between frailty and the combination of physical activity level and sedentary behavior in older adults. *BMC Public Health* **19**, 709 (2019).
63. Fried, L. P. *et al.* Frailty in older adults: evidence for a phenotype. *J. Gerontol. A. Biol. Sci. Med. Sci.* **56**, M146-156 (2001).
64. Pedersen, B. K. Muscles and their myokines. *J. Exp. Biol.* **214**, 337–346 (2011).
65. Sun, X. *et al.* Comparative effectiveness of non-pharmacological interventions for frailty: a systematic review and network meta-analysis. *Age Ageing* **52**, afad004 (2023).

66. Faller, J. W. *et al.* Instruments for the detection of frailty syndrome in older adults: A systematic review. *PloS One* **14**, e0216166 (2019).
67. Buta, B. J. *et al.* Frailty assessment instruments: Systematic characterization of the uses and contexts of highly-cited instruments. *Ageing Res. Rev.* **26**, 53–61 (2016).
68. Lee, H., Lee, E. & Jang, I. Y. Frailty and Comprehensive Geriatric Assessment. *J. Korean Med. Sci.* **35**, e16 (2020).
69. de Vries, N. M. *et al.* Outcome instruments to measure frailty: a systematic review. *Ageing Res. Rev.* **10**, 104–114 (2011).
70. Parker, S. G. *et al.* What is Comprehensive Geriatric Assessment (CGA)? An umbrella review. *Age Ageing* **47**, 149–155 (2018).
71. Veronese, N. *et al.* Comprehensive geriatric assessment in older people: an umbrella review of health outcomes. *Age Ageing* **51**, afac104 (2022).
72. Comprehensive geriatric assessment - UpToDate. <https://www.uptodate-com.uml.idm.oclc.org/contents/comprehensive-geriatric-assessment>.
73. Oviedo-Briones, M. *et al.* A Comparison of Frailty Assessment Instruments in Different Clinical and Social Care Settings: The Frailtools Project. *J. Am. Med. Dir. Assoc.* (2020) doi:10.1016/j.jamda.2020.09.024.
74. Boreskie, K. F., Hay, J. L., Boreskie, P. E., Arora, R. C. & Duhamel, T. A. Frailty-aware care: giving value to frailty assessment across different healthcare settings. *BMC Geriatr.* **22**, 13 (2022).
75. Aguayo, G. A. *et al.* Agreement Between 35 Published Frailty Scores in the General Population. *Am. J. Epidemiol.* **186**, 420–434 (2017).
76. Sison, S. D. M. *et al.* A crosswalk of commonly used frailty scales. *J. Am. Geriatr. Soc.* **71**, 3189–3198 (2023).
77. Khezrian, M., Myint, P. K., McNeil, C. & Murray, A. D. A Review of Frailty Syndrome and Its Physical, Cognitive and Emotional Domains in the Elderly. *Geriatrics* **2**, (2017).

78. Mulasso, A., Roppolo, M., Giannotta, F. & Rabaglietti, E. Associations of frailty and psychosocial factors with autonomy in daily activities: a cross-sectional study in Italian community-dwelling older adults. *Clin. Interv. Aging* **11**, 37–45 (2016).
79. Clegg, A., Rogers, L. & Young, J. Diagnostic test accuracy of simple instruments for identifying frailty in community-dwelling older people: a systematic review. *Age Ageing* **44**, 148–152 (2015).
80. Blodgett, J. M., Theou, O., Howlett, S. E. & Rockwood, K. A frailty index from common clinical and laboratory tests predicts increased risk of death across the life course. *GeroScience* **39**, 447–455 (2017).
81. Clegg, A. *et al.* Development and validation of an electronic frailty index using routine primary care electronic health record data. *Age Ageing* **45**, 353–360 (2016).
82. Kan, G. A. van, Rolland, Y. M., Morley, J. E. & Vellas, B. Frailty: Toward a Clinical Definition. *J. Am. Med. Dir. Assoc.* **9**, 71–72 (2008).
83. Guralnik, J. M. *et al.* A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J. Gerontol.* **49**, M85-94 (1994).
84. Romero-Ortuno, R., Walsh, C. D., Lawlor, B. A. & Kenny, R. A. A Frailty Instrument for primary care: findings from the Survey of Health, Ageing and Retirement in Europe (SHARE). *BMC Geriatr.* **10**, 57 (2010).
85. Afilalo, J. *et al.* Frailty in Older Adults Undergoing Aortic Valve Replacement: The FRAILTY-AVR Study. *J. Am. Coll. Cardiol.* **70**, 689–700 (2017).
86. Gilbert, T. *et al.* Development and validation of a Hospital Frailty Risk Score focusing on older people in acute care settings using electronic hospital records: an observational study. *The Lancet* **391**, 1775–1782 (2018).
87. Searle, S. D., Mitnitski, A., Gahbauer, E. A., Gill, T. M. & Rockwood, K. A standard procedure for creating a frailty index. *BMC Geriatr.* **8**, 24 (2008).

88. Mitnitski, A. *et al.* Age-related frailty and its association with biological markers of ageing. *BMC Med.* **13**, 161 (2015).
89. Afilalo, J. *et al.* Frailty Assessment in the Cardiovascular Care of Older Adults. *J. Am. Coll. Cardiol.* **63**, 747–762 (2014).
90. Fried, L. P. *et al.* The Cardiovascular Health Study: design and rationale. *Ann. Epidemiol.* **1**, 263–276 (1991).
91. Radloff, L. S. The CES-D Scale A Self-Report Depression Scale for Research in the General Population. *Appl. Psychol. Meas.* **1**, 385–401 (1977).
92. Nowak, Z. *et al.* Paffenbarger Physical Activity Questionnaire as an additional tool in clinical assessment of patients with coronary artery disease treated with angioplasty. *Kardiol. Pol.* **68**, 32–39 (2010).
93. Rockwood, K., Andrew, M. & Mitnitski, A. A comparison of two approaches to measuring frailty in elderly people. *J. Gerontol. A. Biol. Sci. Med. Sci.* **62**, 738–743 (2007).
94. Rockwood, K. & Mitnitski, A. Frailty in Relation to the Accumulation of Deficits. *J. Gerontol. Ser. A* **62**, 722–727 (2007).
95. Sapp, D. G., Cormier, B. M., Rockwood, K., Howlett, S. E. & Heinze, S. S. The frailty index based on laboratory test data as a tool to investigate the impact of frailty on health outcomes: a systematic review and meta-analysis. *Age Ageing* **52**, afac309 (2023).
96. Brousseau, A.-A. *et al.* Identification of older adults with frailty in the Emergency Department using a frailty index: results from a multinational study. *Age Ageing* **47**, 242–248 (2018).
97. Apóstolo, J. *et al.* Effectiveness of interventions to prevent pre-frailty and frailty progression in older adults: a systematic review. *JBI Database Syst. Rev. Implement. Rep.* **16**, 140 (2018).
98. Brennan, T. H., Lewis, L. K., Gordon, S. J. & Prichard, I. Effectiveness of interventions to prevent or reverse pre-frailty and frailty in middle-aged community dwelling adults: A systematic review. *Prev. Med.* **185**, 108008 (2024).

99. AVOID Frailty. *Canadian Frailty Network* <https://www.cfn-nce.ca/frailty-matters/avoid-frailty/>.
100. Jadcak, A. D., Makwana, N., Luscombe-Marsh, N., Visvanathan, R. & Schultz, T. J. Effectiveness of exercise interventions on physical function in community-dwelling frail older people: an umbrella review of systematic reviews. *JBIM Database Syst. Rev. Implement. Rep.* **16**, 752–775 (2018).
101. Izquierdo, M. *et al.* Global consensus on optimal exercise recommendations for enhancing healthy longevity in older adults (ICFSR). *J. Nutr. Health Aging* 100401 (2024) doi:10.1016/j.jnha.2024.100401.
102. CSEP | SCPE. *CSEP | SCPE* <https://csepguidelines.ca>.
103. Ross, R. *et al.* Canadian 24-Hour Movement Guidelines for Adults aged 18-64 years and Adults aged 65 years or older: an integration of physical activity, sedentary behaviour, and sleep. *Appl. Physiol. Nutr. Metab. Physiol. Appl. Nutr. Metab.* **45**, S57–S102 (2020).
104. Kehler, D. S. *et al.* A systematic review of the association between sedentary behaviors with frailty. *Exp. Gerontol.* **114**, 1–12 (2018).
105. Fulop, T. *et al.* Frailty, Inflammation and Immunosenescence. *Interdiscip. Top. Gerontol. Geriatr.* **41**, 26–40 (2015).
106. Veronese, N. *et al.* Polypharmacy Is Associated With Higher Frailty Risk in Older People: An 8-Year Longitudinal Cohort Study. *J. Am. Med. Dir. Assoc.* (2017) doi:10.1016/j.jamda.2017.02.009.
107. Evans, I. E. M., Martyr, A., Collins, R., Brayne, C. & Clare, L. Social Isolation and Cognitive Function in Later Life: A Systematic Review and Meta-Analysis. *J. Alzheimers Dis. JAD* **70**, S119–S144 (2019).
108. Cederholm, T. *et al.* Diagnostic criteria for malnutrition - An ESPEN Consensus Statement. *Clin. Nutr. Edinb. Scotl.* **34**, 335–340 (2015).

109. Ligthart-Melis, G. C. *et al.* Frailty, Sarcopenia, and Malnutrition Frequently (Co-)occur in Hospitalized Older Adults: A Systematic Review and Meta-analysis. *J. Am. Med. Dir. Assoc.* (2020) doi:10.1016/j.jamda.2020.03.006.
110. Genton, L. *et al.* Body composition changes over 9 years in healthy elderly subjects and impact of physical activity. *Clin. Nutr. Edinb. Scotl.* **30**, 436–442 (2011).
111. Broadwin, J., Goodman-Gruen, D. & Slymen, D. Ability of fat and fat-free mass percentages to predict functional disability in older men and women. *J. Am. Geriatr. Soc.* **49**, 1641–1645 (2001).
112. Bauer, J. *et al.* Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J. Am. Med. Dir. Assoc.* **14**, 542–559 (2013).
113. Wang, H. *et al.* Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet* **388**, 1459–1544 (2016).
114. WHO | Cardiovascular diseases (CVDs). *WHO*
<http://www.who.int/mediacentre/factsheets/fs317/en/>.
115. Government of Canada, S. C. Leading causes of death, total population, by age group.
<https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310039401> (2018).
116. Inpatient Hospitalizations, Surgeries and Newborn Indicators, 2015–2016 | CIHI.
<https://www.cihi.ca/en/inpatient-hospitalizations-surgeries-and-newborn-indicators-2015-2016> (2018).
117. Canada, P. H. A. of. Heart Disease in Canada. *aem* <https://www.canada.ca/en/public-health/services/publications/diseases-conditions/heart-disease-canada.html> (2017).
118. Statistics - Heart and Stroke Foundation of Canada. *heartandstroke.ca*
<http://www.heartandstroke.com/site/c.ikIQLcMWJtE/b.3483991/k.34A8/Statistics.htm>.

119. Government of Canada, P. H. A. of C. Executive Summary - 2009 Tracking Heart Disease and Stroke in Canada - Public Health Agency of Canada. <http://www.phac-aspc.gc.ca/publicat/2009/cvd-avc/summary-resume-eng.php> (2009).
120. Scott, J. Pathophysiology and biochemistry of cardiovascular disease. *Curr. Opin. Genet. Dev.* **14**, 271–279 (2004).
121. Lusis, A. J. Atherosclerosis. *Nature* **407**, 233–241 (2000).
122. Bentzon, J. F., Otsuka, F., Virmani, R. & Falk, E. Mechanisms of Plaque Formation and Rupture. *Circ. Res.* **114**, 1852–1866 (2014).
123. Lusis, A. J. Atherosclerosis. *Nature* **407**, 233–241 (2000).
124. Scott, J. Pathophysiology and biochemistry of cardiovascular disease. *Curr. Opin. Genet. Dev.* **14**, 271–279 (2004).
125. Yusuf, S. *et al.* Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet Lond. Engl.* **364**, 937–952 (2004).
126. Bentzon, J. F., Otsuka, F., Virmani, R. & Falk, E. Mechanisms of Plaque Formation and Rupture. *Circ. Res.* **114**, 1852–1866 (2014).
127. Government of Canada, P. H. A. of C. Six types of cardiovascular disease - Public Health Agency Canada. <http://www.phac-aspc.gc.ca/cd-mc/cvd-mcv/cvd-mcv-eng.php> (2008).
128. pmhdev. Congenital Heart Defects - National Library of Medicine. *PubMed Health* <https://www.ncbi.nlm.nih.gov/pubmedhealth/PMHT0023239/>.
129. 3. Risk Assessment and Stratification.
<http://www.ccs.ca/eguidelines/Content/Topics/Dyslipidemia/3.%20Risk%20Assessment%20and%20Stratification.htm>.

130. Mahmood, S. S., Levy, D., Vasan, R. S. & Wang, T. J. The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. *Lancet Lond. Engl.* **383**, 999–1008 (2014).
131. D’Agostino, R. B. *et al.* General cardiovascular risk profile for use in primary care: The Framingham heart study. *Circulation* **117**, 743–753 (2008).
132. Goff David C. *et al.* 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk. *Circulation* **129**, S49–S73 (2014).
133. Conroy, R. M. *et al.* Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur. Heart J.* **24**, 987–1003 (2003).
134. Anderson, T. J. *et al.* 2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. *Can. J. Cardiol.* **29**, 151–167 (2013).
135. Vasan Ramachandran S. Biomarkers of Cardiovascular Disease. *Circulation* **113**, 2335–2362 (2006).
136. Hlatky, M. A. *et al.* Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. *Circulation* **119**, 2408–2416 (2009).
137. Naghavi, M. *et al.* Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet* **390**, 1151–1210 (2017).
138. Norris Colleen M. *et al.* State of the Science in Women’s Cardiovascular Disease: A Canadian Perspective on the Influence of Sex and Gender. *J. Am. Heart Assoc.* **9**, e015634 (2020).
139. Möller-Leimkühler, A. M. Gender differences in cardiovascular disease and comorbid depression. *Dialogues Clin. Neurosci.* **9**, 71–83 (2007).

140. Norris, C. M. *et al.* Systematic Incorporation of Sex-Specific Information Into Clinical Practice Guidelines for the Management of ST -Segment-Elevation Myocardial Infarction: Feasibility and Outcomes. *J. Am. Heart Assoc.* **8**, e011597 (2019).
141. Barrett-Connor, E. Menopause, atherosclerosis, and coronary artery disease. *Curr. Opin. Pharmacol.* **13**, 186–191 (2013).
142. Kettner, J. Chief Provincial Public Health Officer’s Report on the Health Status of Manitobans 2010 Priorities for Prevention: Everyone, Every Place, Every Day. 272 (2010).
143. Duprez, D. A. *et al.* Vascular and cardiac functional and structural screening to identify risk of future morbid events: preliminary observations. *J. Am. Soc. Hypertens. JASH* **5**, 401–409 (2011).
144. O’Callaghan, K. M. Solutions for disparities for women with heart disease. *J Cardiovasc. Transl. Res.* **2**, 518–525 (2009).
145. Go, A. S. *et al.* Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation* **127**, e6–e245 (2013).
146. Heart Report. *Heart and Stroke Foundation of Canada* <https://www.heartandstroke.ca/en/what-we-do/media-centre/heart-report/>.
147. Rodondi, N. *et al.* Framingham Risk Score and Alternatives for Prediction of Coronary Heart Disease in Older Adults. *PLoS ONE* **7**, (2012).
148. Dib, J. G., Alameddine, Y., Geitany, R. & Afiouni, F. National Cholesterol Education Panel III performance in preventing myocardial infarction in young adults. *Ann. Saudi Med.* **28**, 22–27 (2008).
149. Lau, E. S. *et al.* Sex Differences in Circulating Biomarkers of Cardiovascular Disease. *J. Am. Coll. Cardiol.* **74**, 1543–1553 (2019).
150. Lam Carolyn S.P. *et al.* Sex Differences in Clinical Characteristics and Outcomes in Elderly Patients With Heart Failure and Preserved Ejection Fraction. *Circ. Heart Fail.* **5**, 571–578 (2012).
151. Barbalho, S. M. *et al.* Benchside to the bedside of frailty and cardiovascular aging: Main shared cellular and molecular mechanisms. *Exp. Gerontol.* 111302 (2021) doi:10.1016/j.exger.2021.111302.

152. Chung, K. J. N. C., Wilkinson, C., Veerasamy, M. & Kunadian, V. Frailty Scores and Their Utility in Older Patients with Cardiovascular Disease. *Interv. Cardiol. Rev.* **16**, (2021).
153. Graciani, A., García-Esquinas, E., López-García, E., Banegas, J. R. & Rodríguez-Artalejo, F. Ideal Cardiovascular Health and Risk of Frailty in Older Adults. *Circ. Cardiovasc. Qual. Outcomes* **9**, 239–245 (2016).
154. León-Muñoz, L. M., García-Esquinas, E., López-García, E., Banegas, J. R. & Rodríguez-Artalejo, F. Major dietary patterns and risk of frailty in older adults: a prospective cohort study. *BMC Med.* **13**, 11 (2015).
155. Woods, N. F. *et al.* Frailty: emergence and consequences in women aged 65 and older in the Women's Health Initiative Observational Study. *J. Am. Geriatr. Soc.* **53**, 1321–1330 (2005).
156. García-Esquinas, E. *et al.* Obesity, fat distribution, and risk of frailty in two population-based cohorts of older adults in Spain. *Obes. Silver Spring Md* **23**, 847–855 (2015).
157. Flint, K. Which Came First, the Frailty or the Heart Disease?: Exploring the Vicious Cycle*. *J. Am. Coll. Cardiol.* **65**, 984–986 (2015).
158. Ferrucci, L. & Fabbri, E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat. Rev. Cardiol.* **15**, 505–522 (2018).
159. Soysal, P., Arik, F., Smith, L., Jackson, S. E. & Isik, A. T. Inflammation, Frailty and Cardiovascular Disease. *Adv. Exp. Med. Biol.* **1216**, 55–64 (2020).
160. Fülöp, T., Dupuis, G., Witkowski, J. M. & Larbi, A. The Role of Immunosenesence in the Development of Age-Related Diseases. *Rev. Investig. Clin. Organo Hosp. Enfermedades Nutr.* **68**, 84–91 (2016).
161. Duhamel, T. A. *et al.* Pre-Frailty and Cardiovascular Disease Risk in Women Age 55 Years and Older. *Can. J. Cardiol.* **34**, e19 (2018).
162. Gale, C. R., Cooper, C. & Sayer, A. A. Framingham cardiovascular disease risk scores and incident frailty: the English longitudinal study of ageing. *Age Dordr. Neth.* **36**, 9692 (2014).

163. Kleipool, E. E. *et al.* Frailty in Older Adults with Cardiovascular Disease: Cause, Effect or Both? *Aging Dis.* **9**, 489–497 (2018).
164. Marinus, N. *et al.* Frailty is highly prevalent in specific cardiovascular diseases and females, but significantly worsens prognosis in all affected patients: A systematic review. *Ageing Res. Rev.* **66**, 101233 (2020).
165. van Bussel, E. F. *et al.* Predictive value of traditional risk factors for cardiovascular disease in older people: A systematic review. *Prev. Med.* **132**, 105986 (2020).
166. Todd, O. M. *et al.* Is the association between blood pressure and mortality in older adults different with frailty? A systematic review and meta-analysis. *Age Ageing* **48**, 627–635 (2019).
167. Gary, R. Evaluation of frailty in older adults with cardiovascular disease: incorporating physical performance measures. *J. Cardiovasc. Nurs.* **27**, 120–131 (2012).
168. von Haehling, S., Anker, S. D., Doehner, W., Morley, J. E. & Vellas, B. Frailty and heart disease. *Int. J. Cardiol.* **168**, 1745–1747 (2013).
169. Xue, Q.-L., Bandeen-Roche, K., Tian, J., Kasper, J. D. & Fried, L. P. Progression of Physical Frailty and the Risk of All-Cause Mortality: Is There a Point of No Return? *J. Am. Geriatr. Soc.* **69**, 908–915 (2021).
170. Boreskie, K. F. *et al.* Myokines as biomarkers of frailty and cardiovascular disease risk in females. *Exp. Gerontol.* **133**, 110859 (2020).
171. Fernández-Garrido, J., Ruiz-Ros, V., Buigues, C., Navarro-Martinez, R. & Cauli, O. Clinical features of prefrail older individuals and emerging peripheral biomarkers: a systematic review. *Arch. Gerontol. Geriatr.* **59**, 7–17 (2014).
172. Coelho-Junior, H. J., Picca, A., Calvani, R., Uchida, M. C. & Marzetti, E. If my muscle could talk: Myokines as a biomarker of frailty. *Exp. Gerontol.* **127**, 110715 (2019).

173. Wang, J., Maxwell, C. A. & Yu, F. Biological Processes and Biomarkers Related to Frailty in Older Adults: A State-of-the-Science Literature Review: *Biol. Res. Nurs.* (2018) doi:10.1177/1099800418798047.
174. Calvani, R. *et al.* Biomarkers for physical frailty and sarcopenia. *Aging Clin. Exp. Res.* **29**, 29–34 (2017).
175. Rodriguez-Mañas, L. *et al.* ICFSR Task Force Perspective on Biomarkers for Sarcopenia and Frailty. *J. Frailty Aging* **9**, 4–8 (2020).
176. Sebastiani, P. *et al.* Biomarker signatures of aging. *Aging Cell* **16**, 329–338 (2017).
177. Coelho-Junior, H. J., Picca, A., Calvani, R., Uchida, M. C. & Marzetti, E. If my muscle could talk: Myokines as a biomarker of frailty. *Exp. Gerontol.* **127**, 110715 (2019).
178. Michaud, M. *et al.* Proinflammatory cytokines, aging, and age-related diseases. *J. Am. Med. Dir. Assoc.* **14**, 877–882 (2013).
179. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* **69**, 89–95 (2001).
180. Calvani, R. *et al.* Biomarkers for physical frailty and sarcopenia: state of the science and future developments. *J. Cachexia Sarcopenia Muscle* **6**, 278–286 (2015).
181. Rockwood, K., McMillan, M., Mitnitski, A. & Howlett, S. E. A Frailty Index Based on Common Laboratory Tests in Comparison With a Clinical Frailty Index for Older Adults in Long-Term Care Facilities. *J. Am. Med. Dir. Assoc.* **16**, 842–847 (2015).
182. Mitnitski, A. *et al.* Age-related frailty and its association with biological markers of ageing. *BMC Med.* **13**, (2015).
183. Soysal, P. *et al.* Inflammation and frailty in the elderly: A systematic review and meta-analysis. *Ageing Res. Rev.* **31**, 1–8 (2016).
184. Puts, M. T. E., Visser, M., Twisk, J. W. R., Deeg, D. J. H. & Lips, P. Endocrine and inflammatory markers as predictors of frailty. *Clin. Endocrinol. (Oxf.)* **63**, 403–411 (2005).

185. Tang, Y., Fung, E., Xu, A. & Lan, H.-Y. C-reactive protein and ageing. *Clin. Exp. Pharmacol. Physiol.* **44 Suppl 1**, 9–14 (2017).
186. Lana, A., Valdés-Bécares, A., Buño, A., Rodríguez-Artalejo, F. & Lopez-Garcia, E. Serum Leptin Concentration is Associated with Incident Frailty in Older Adults. *Aging Dis.* **8**, 240–249 (2017).
187. Isidori, A. M. *et al.* Leptin and aging: correlation with endocrine changes in male and female healthy adult populations of different body weights. *J. Clin. Endocrinol. Metab.* **85**, 1954–1962 (2000).
188. Hernández, B., Fuentes, E., Palomo, I. & Alarcón, M. Increased platelet function during frailty. *Exp. Hematol.* **77**, 12-25.e2 (2019).
189. Balduino, C. L. & Noris, P. Platelet count and aging. *Haematologica* **99**, 953–955 (2014).
190. Leng, S. X., Xue, Q.-L., Tian, J., Walston, J. D. & Fried, L. P. Inflammation and frailty in older women. *J. Am. Geriatr. Soc.* **55**, 864–871 (2007).
191. Ruggiero, C. *et al.* White blood cell count and mortality in the Baltimore Longitudinal Study of Aging. *J. Am. Coll. Cardiol.* **49**, 1841–1850 (2007).
192. Johnstone, J. *et al.* T-Cell Phenotypes Predictive of Frailty and Mortality in Elderly Nursing Home Residents. *J. Am. Geriatr. Soc.* **65**, 153–159 (2017).
193. Araújo Carvalho, A. C. *et al.* Telomere length and frailty in older adults-A systematic review and meta-analysis. *Ageing Res. Rev.* **54**, 100914 (2019).
194. Ciccarone, F., Tagliatesta, S., Caiafa, P. & Zampieri, M. DNA methylation dynamics in aging: how far are we from understanding the mechanisms? *Mech. Ageing Dev.* **174**, 3–17 (2018).
195. Buta, B. *et al.* The Association of Vitamin D Deficiency and Incident Frailty in Older Women: The Role of Cardiometabolic Diseases. *J. Am. Geriatr. Soc.* **65**, 619–624 (2017).
196. Zhou, J. *et al.* Association of vitamin D deficiency and frailty: A systematic review and meta-analysis. *Maturitas* **94**, 70–76 (2016).

197. Maffei, V. J. *et al.* Biological Aging and the Human Gut Microbiota. *J. Gerontol. A. Biol. Sci. Med. Sci.* **72**, 1474–1482 (2017).
198. Jackson, M. *et al.* Signatures of early frailty in the gut microbiota. *Genome Med.* **8**, (2016).
199. Pedersen, B. K. & Febbraio, M. A. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat. Rev. Endocrinol.* **8**, 457–465 (2012).
200. Trovato, E., Di Felice, V. & Barone, R. Extracellular Vesicles: Delivery Vehicles of Myokines. *Front. Physiol.* **10**, 522 (2019).
201. Nederveen, J. P., Warnier, G., Di Carlo, A., Nilsson, M. I. & Tarnopolsky, M. A. Extracellular Vesicles and Exosomes: Insights From Exercise Science. *Front. Physiol.* **11**, (2021).
202. Goldstein, M. S. Humoral nature of hypoglycemia in muscular exercise. *Am. J. Physiol.-Leg. Content* **200**, 67–70 (1961).
203. Severinsen, M. C. K. & Pedersen, B. K. Muscle-organ crosstalk: the emerging roles of myokines. *Endocr. Rev.* (2020) doi:10.1210/endrev/bnaa016.
204. Pedersen, B. K. Muscles and their myokines. *J. Exp. Biol.* **214**, 337–346 (2011).
205. Pedersen, B. K. *et al.* Searching for the exercise factor: is IL-6 a candidate? *J. Muscle Res. Cell Motil.* **24**, 113–119 (2003).
206. Laurens, C., Bergouignan, A. & Moro, C. Exercise-Released Myokines in the Control of Energy Metabolism. *Front. Physiol.* **11**, 91 (2020).
207. Chow, L. S. *et al.* Exerkines in health, resilience and disease. *Nat. Rev. Endocrinol.* **18**, 273–289 (2022).
208. Safdar, A., Saleem, A. & Tarnopolsky, M. A. The potential of endurance exercise-derived exosomes to treat metabolic diseases. *Nat. Rev. Endocrinol.* **12**, 504–517 (2016).
209. Lu, X. *et al.* Exercise and exerkines: Mechanisms and roles in anti-aging and disease prevention. *Exp. Gerontol.* **200**, 112685 (2025).

210. Government of Canada, S. C. Directly measured physical activity of Canadian adults, 2007 to 2011. <https://www150.statcan.gc.ca/n1/pub/82-625-x/2013001/article/11807-eng.htm> (2013).
211. Cardoso, A. L. *et al.* Towards frailty biomarkers: Candidates from genes and pathways regulated in aging and age-related diseases. *Ageing Res. Rev.* **47**, 214–277 (2018).
212. Chen, K., Zhou, M., Wang, X., Li, S. & Yang, D. The Role of Myokines and Adipokines in Hypertension and Hypertension-related Complications. *Hypertens. Res.* 1–8 (2019) doi:10.1038/s41440-019-0266-y.
213. Walsh, K. Adipokines, myokines and cardiovascular disease. *Circ. J. Off. J. Jpn. Circ. Soc.* **73**, 13–18 (2009).
214. Chung, H. S. & Choi, K. M. Adipokines and Myokines: A Pivotal Role in Metabolic and Cardiovascular Disorders. *Curr. Med. Chem.* **25**, 2401–2415 (2018).
215. Ouchi, N., Ohashi, K., Shibata, R. & Murohara, T. Protective Roles of Adipocytokines and Myokines in Cardiovascular Disease. *Circ. J.* **80**, 2073–2080 (2016).
216. Barbalho, S. M. *et al.* Physical Exercise and Myokines: Relationships with Sarcopenia and Cardiovascular Complications. *Int. J. Mol. Sci.* **21**, (2020).
217. Colaianni, G., Storlino, G., Sanesi, L., Colucci, S. & Grano, M. Myokines and Osteokines in the Pathogenesis of Muscle and Bone Diseases. *Curr. Osteoporos. Rep.* (2020) doi:10.1007/s11914-020-00600-8.
218. Fried, L. P. *et al.* Frailty in older adults: evidence for a phenotype. *J. Gerontol. A. Biol. Sci. Med. Sci.* **56**, M146-156 (2001).
219. Barros, D., Marques, E. A., Magalhães, J. & Carvalho, J. Energy metabolism and frailty: The potential role of exercise-induced myokines - A narrative review. *Ageing Res. Rev.* **82**, 101780 (2022).
220. Kwon, J. H., Moon, K. M. & Min, K.-W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. *Healthc. Basel Switz.* **8**, (2020).

221. Coelho-Junior, H. J., Picca, A., Calvani, R., Uchida, M. C. & Marzetti, E. If my muscle could talk: Myokines as a biomarker of frailty. *Exp. Gerontol.* **127**, 110715 (2019).
222. Chen, J.-H., Liu, C., You, L. & Simmons, C. A. Boning up on Wolff's Law: mechanical regulation of the cells that make and maintain bone. *J. Biomech.* **43**, 108–118 (2010).
223. He, C. *et al.* Bone and Muscle Crosstalk in Aging. *Front. Cell Dev. Biol.* **8**, 585644 (2020).
224. Li, G. *et al.* Muscle-bone crosstalk and potential therapies for sarco-osteoporosis. *J. Cell. Biochem.* (2019) doi:10.1002/jcb.28946.
225. Greco, E. A., Pietschmann, P. & Migliaccio, S. Osteoporosis and Sarcopenia Increase Frailty Syndrome in the Elderly. *Front. Endocrinol.* **10**, 255 (2019).
226. van den Bergh, J. P., van Geel, T. A. & Geusens, P. P. Osteoporosis, frailty and fracture: implications for case finding and therapy. *Nat. Rev. Rheumatol.* **8**, 163–172 (2012).
227. Li, G. *et al.* An overview of osteoporosis and frailty in the elderly. *BMC Musculoskelet. Disord.* **18**, 46 (2017).
228. Ecss, A. & Ecss, A. Association of Bone Mineral Density with Frailty, Pre-Frailty, and Osteoporosis in Community-Dwelling Elderly: A Prospective Study.
229. Cook, M. J. *et al.* Frailty and bone health in European men. *Age Ageing* (2017) doi:10.1093/ageing/afw205.
230. Warburton, D. E., Nicol, C. W., Gatto, S. N. & Bredin, S. S. Cardiovascular disease and osteoporosis: Balancing risk management. *Vasc. Health Risk Manag.* **3**, 673–689 (2007).
231. McFarlane, S. I., Muniyappa, R., Shin, J. J., Bahtiyar, G. & Sowers, J. R. Osteoporosis and cardiovascular disease: brittle bones and boned arteries, is there a link? *Endocrine* **23**, 1–10 (2004).
232. Boreskie, K. F. *et al.* Bone Metabolism Analytes as Biomarkers of Pre-Frailty and Cardiovascular Disease Risk in Females. *Adv. Geriatr. Med. Res.* **4**, (2020).
233. O'Caoimh, R. *et al.* Prevalence of frailty in 62 countries across the world: a systematic review and meta-analysis of population-level studies. *Age Ageing* doi:10.1093/ageing/afaa219.

234. Cesari, M. & Vellas, B. Frailty in Clinical Practice. *Frailty Pathophysiol. Phenotype Patient Care* **83**, 93–98 (2015).
235. Top Ten Frailty Priorities. *Canadian Frailty Network* <https://www.cfn-nce.ca/about-us/engaging-canadians/helping-to-set-frailty-priorities/top-ten-frailty-priorities-identified-by-citizens/>.
236. Sepehri, A. *et al.* The impact of frailty on outcomes after cardiac surgery: A systematic review. *J. Thorac. Cardiovasc. Surg.* **148**, 3110–3117 (2014).
237. Romero-Ortuno, R. Frailty in Primary Care. *Interdiscip. Top. Gerontol. Geriatr.* **41**, 85–94 (2015).
238. Canada, E. and S. D. Government of Canada — Action for Seniors report. *aem* <https://www.canada.ca/en/employment-social-development/programs/seniors-action-report.html> (2016).
239. Abbasi, M. *et al.* Identification and management of frailty in the primary care setting. *CMAJ* **190**, E1134–E1140 (2018).
240. Walston, J. *et al.* Integrating Frailty Research into the Medical Specialties – Report from a U13 Conference. *J. Am. Geriatr. Soc.* **65**, 2134–2139 (2017).
241. Hoogendijk, E. O. *et al.* Frailty: implications for clinical practice and public health. *The Lancet* **394**, 1365–1375 (2019).
242. Travers, J., Romero-Ortuno, R., Bailey, J. & Cooney, M.-T. Delaying and reversing frailty: a systematic review of primary care interventions. *Br. J. Gen. Pract. J. R. Coll. Gen. Pract.* (2018) doi:10.3399/bjgp18X700241.
243. Looman, W. *et al.* Managing frailty: exploring the effectiveness of integrated primary care interventions by distinguishing subpopulations of frail older people. *Int. J. Integr. Care* **17**, (2017).
244. Gutiérrez-Valencia, M. *et al.* The relationship between frailty and polypharmacy in older people: A systematic review. *Br. J. Clin. Pharmacol.* **84**, 1432–1444 (2018).

245. Cesari, M. *et al.* A physical activity intervention to treat the frailty syndrome in older persons- results from the LIFE-P study. *J. Gerontol. A. Biol. Sci. Med. Sci.* **70**, 216–222 (2015).
246. Marzetti, E. *et al.* Physical activity and exercise as countermeasures to physical frailty and sarcopenia. *Aging Clin. Exp. Res.* (2017) doi:10.1007/s40520-016-0705-4.
247. Woo, J. *et al.* Frailty Screening in the Community Using the FRAIL Scale. *J. Am. Med. Dir. Assoc.* **16**, 412–419 (2015).
248. Merchant, R. A. *et al.* Rapid Geriatric Assessment Using Mobile App in Primary Care: Prevalence of Geriatric Syndromes and Review of Its Feasibility. *Front. Med.* **7**, (2020).
249. Liu, P. *et al.* Frailty and hypertension in older adults: current understanding and future perspectives. *Hypertens. Res.* 1–9 (2020) doi:10.1038/s41440-020-0510-5.
250. Aprahamian, I. *et al.* Hypertension and frailty in older adults. *J. Clin. Hypertens. Greenwich Conn* (2017) doi:10.1111/jch.13135.
251. Lawless, M. T., Archibald, M. M., Ambagtsheer, R. C. & Kitson, A. L. Factors influencing communication about frailty in primary care: A scoping review. *Patient Educ. Couns.* **103**, 436–450 (2020).
252. Hajek, A. *et al.* Frailty and healthcare costs—longitudinal results of a prospective cohort study. *Age Ageing* **47**, 233–241 (2018).
253. Cesari, M., Gambassi, G., Abellan van Kan, G. & Vellas, B. The frailty phenotype and the frailty index: different instruments for different purposes. *Age Ageing* **43**, 10–12 (2014).
254. Bieniek, J., Wilczyński, K. & Szewieczek, J. Fried frailty phenotype assessment components as applied to geriatric inpatients. *Clin. Interv. Aging* **11**, 453–459 (2016).
255. Rockwood, K. *et al.* A global clinical measure of fitness and frailty in elderly people. *CMAJ* **173**, 489–495 (2005).
256. Lansbury, L. N. *et al.* Use of the electronic Frailty Index to identify vulnerable patients: a pilot study in primary care. *Br. J. Gen. Pract. J. R. Coll. Gen. Pract.* **67**, e751–e756 (2017).

257. MORLEY, J. E., MALMSTROM, T. K. & MILLER, D. K. A SIMPLE FRAILTY QUESTIONNAIRE (FRAIL) PREDICTS OUTCOMES IN MIDDLE AGED AFRICAN AMERICANS. *J. Nutr. Health Aging* **16**, 601–608 (2012).
258. Malmstrom, T. K., Miller, D. K. & Morley, J. E. A Comparison of Four Frailty Models. *J. Am. Geriatr. Soc.* **62**, 721–726 (2014).
259. Tomata, Y. *et al.* [Validation of the Kihon Checklist for predicting the risk of 1-year incident long-term care insurance certification: the Ohsaki Cohort 2006 Study]. *Nihon Koshu Eisei Zasshi Jpn. J. Public Health* **58**, 3–13 (2011).
260. Sampaio, P. Y. S., Sampaio, R. A. C., Yamada, M. & Arai, H. Systematic review of the Kihon Checklist: Is it a reliable assessment of frailty? *Geriatr. Gerontol. Int.* **16**, 893–902 (2016).
261. Satake, S., Shimokata, H., Senda, K., Kondo, I. & Toba, K. Validity of Total Kihon Checklist Score for Predicting the Incidence of 3-Year Dependency and Mortality in a Community-Dwelling Older Population. *J. Am. Med. Dir. Assoc.* **18**, 552.e1-552.e6 (2017).
262. Gobbens, R. J. J., van Assen, M. A. L. M., Luijkx, K. G., Wijnen-Sponselee, M. T. & Schols, J. M. G. A. The Tilburg Frailty Indicator: psychometric properties. *J. Am. Med. Dir. Assoc.* **11**, 344–355 (2010).
263. Gobbens, R. J., Boersma, P., Uchmanowicz, I. & Santiago, L. M. The Tilburg Frailty Indicator (TFI): New Evidence for Its Validity. *Clin. Interv. Aging* **15**, 265–274 (2020).
264. Rolfson, D. B., Majumdar, S. R., Tsuyuki, R. T., Tahir, A. & Rockwood, K. Validity and reliability of the Edmonton Frail Scale. *Age Ageing* **35**, 526–529 (2006).
265. Theou, O., Brothers, T. D., Mitnitski, A. & Rockwood, K. Operationalization of frailty using eight commonly used scales and comparison of their ability to predict all-cause mortality. *J. Am. Geriatr. Soc.* **61**, 1537–1551 (2013).
266. Gray, L. C. *et al.* Profiles of older patients in the emergency department: findings from the interRAI Multinational Emergency Department Study. *Ann. Emerg. Med.* **62**, 467–474 (2013).

267. Platts-Mills, T. F. *et al.* Accuracy of the Emergency Severity Index triage instrument for identifying elder emergency department patients receiving an immediate life-saving intervention. *Acad. Emerg. Med. Off. J. Soc. Acad. Emerg. Med.* **17**, 238–243 (2010).
268. Theou, O., Campbell, S., Malone, M. L. & Rockwood, K. Older Adults in the Emergency Department with Frailty. *Clin. Geriatr. Med.* **34**, 369–386 (2018).
269. Carpenter, C. R. *et al.* Risk Factors and Screening Instruments to Predict Adverse Outcomes for Undifferentiated Older Emergency Department Patients: A Systematic Review and Meta-analysis. *Acad. Emerg. Med.* **22**, 1–21 (2015).
270. Lewis, E. T. *et al.* Which frailty scale for patients admitted via Emergency Department? A cohort study. *Arch. Gerontol. Geriatr.* **80**, 104–114 (2019).
271. Hogan, D. B. *et al.* A Scoping Review of Frailty and Acute Care in Middle-Aged and Older Individuals with Recommendations for Future Research. *Can. Geriatr. J.* **20**, 22–37 (2017).
272. Hogervorst, V. M. *et al.* Emergency department management of older people living with frailty: a guide for emergency practitioners. *Emerg. Med. J.* (2021) doi:10.1136/emmermed-2020-210014.
273. Theou, O. & Rockwood, K. Should frailty status always be considered when treating the elderly patient? *Aging Health* **8**, 261–271 (2012).
274. Cunha, A. I. L., Veronese, N., de Melo Borges, S. & Ricci, N. A. Frailty as a predictor of adverse outcomes in hospitalized older adults: A systematic review and meta-analysis. *Ageing Res. Rev.* **56**, 100960 (2019).
275. Jørgensen, R. & Brabrand, M. Screening of the frail patient in the emergency department: A systematic review. *Eur. J. Intern. Med.* **45**, 71–73 (2017).
276. Muscedere, J. *et al.* The impact of frailty on intensive care unit outcomes: a systematic review and meta-analysis. *Intensive Care Med.* **43**, 1105–1122 (2017).

277. Pulok, M. H., Theou, O., van der Valk, A. M. & Rockwood, K. The role of illness acuity on the association between frailty and mortality in emergency department patients referred to internal medicine. *Age Ageing* **49**, 1071–1079 (2020).
278. Aliberti, M. J. R. *et al.* COVID-19 is not over and age is not enough: Using frailty for prognostication in hospitalized patients. *J. Am. Geriatr. Soc.* **69**, 1116–1127 (2021).
279. Theou, O. *et al.* What do we know about frailty in the acute care setting? A scoping review. *BMC Geriatr.* **18**, 139 (2018).
280. Elliott, A., Hull, L. & Conroy, S. P. Frailty identification in the emergency department-a systematic review focussing on feasibility. *Age Ageing* **46**, 509–513 (2017).
281. Meldon, S. W. *et al.* A brief risk-stratification tool to predict repeat emergency department visits and hospitalizations in older patients discharged from the emergency department. *Acad. Emerg. Med. Off. J. Soc. Acad. Emerg. Med.* **10**, 224–232 (2003).
282. McCusker, J. *et al.* Detection of older people at increased risk of adverse health outcomes after an emergency visit: the ISAR screening tool. *J. Am. Geriatr. Soc.* **47**, 1229–1237 (1999).
283. Pugh, R. J. *et al.* Feasibility and reliability of frailty assessment in the critically ill: a systematic review. *Crit. Care* **22**, 49 (2018).
284. Rockwood, K. & Theou, O. Using the Clinical Frailty Scale in Allocating Scarce Health Care Resources. *Can. Geriatr. J.* **23**, 254–259 (2020).
285. Chong, E. *et al.* Frailty in Hospitalized Older Adults: Comparing Different Frailty Measures in Predicting Short- and Long-term Patient Outcomes. *J. Am. Med. Dir. Assoc.* **19**, 450-457.e3 (2018).
286. Wallis, S. J., Wall, J., Biram, R. W. S. & Romero-Ortuno, R. Association of the clinical frailty scale with hospital outcomes. *QJM Mon. J. Assoc. Physicians* **108**, 943–949 (2015).
287. Kaeppli, T. *et al.* Validation of the Clinical Frailty Scale for Prediction of Thirty-Day Mortality in the Emergency Department. *Ann. Emerg. Med.* **76**, 291–300 (2020).

288. O’Caoimh, R. *et al.* Comparison of Frailty Screening Instruments in the Emergency Department. *Int. J. Environ. Res. Public. Health* **16**, (2019).
289. WHO | ICD-10 online versions. *WHO*
<http://www.who.int/classifications/icd/icdonlineversions/en/>.
290. Howlett, S. E., Rockwood, M. R. H., Mitnitski, A. & Rockwood, K. Standard laboratory tests to identify older adults at increased risk of death. *BMC Med.* **12**, 171 (2014).
291. Ellis, H. L. *et al.* Complementing chronic frailty assessment at hospital admission with an electronic frailty index (FI-Laboratory) comprising routine blood test results. *CMAJ Can. Med. Assoc. J. J. Assoc. Medicale Can.* **192**, E3–E8 (2020).
292. Jäger, J., Sieber, C. C., Gaßmann, K.-G. & Ritt, M. Changes of a frailty index based on common blood and urine tests during a hospital stay on geriatric wards predict 6-month and 1-year mortality in older people. *Clin. Interv. Aging* **14**, 473–484 (2019).
293. Klausen, H. H. *et al.* Association between routine laboratory tests and long-term mortality among acutely admitted older medical patients: a cohort study. *BMC Geriatr.* **17**, 62 (2017).
294. Flaatten, H. & Clegg, A. Frailty: we need valid and reliable tools in critical care. *Intensive Care Med.* **44**, 1973–1975 (2018).
295. Fowler, A. J., Abbott, T. E. F., Prowle, J. & Pearse, R. M. Age of patients undergoing surgery. *Br. J. Surg.* **106**, 1012–1018 (2019).
296. Nidadavolu, L. S., Ehrlich, A. L., Sieber, F. E. & Oh, E. S. Preoperative Evaluation of the Frail Patient. *Anesth. Analg.* **130**, 1493–1503 (2020).
297. Santhirapala, R., Partridge, J. & MacEwen, C. J. The older surgical patient – to operate or not? A state of the art review. *Anaesthesia* **75**, e46–e53 (2020).
298. Melnyk, M., Casey, R. G., Black, P. & Koupparis, A. J. Enhanced recovery after surgery (ERAS) protocols: Time to change practice? *Can. Urol. Assoc. J.* **5**, 342–348 (2011).

299. Amrock, L. G. & Deiner, S. The Implication of Frailty on Preoperative Risk Assessment. *Curr. Opin. Anaesthesiol.* **27**, 330–335 (2014).
300. Sacks, G. *et al.* Surgeon Perception of Risk and Benefit in the Decision to Operate. *Ann. Surg.* **264**, 896–903 (2016).
301. Boreskie, K. F. *et al.* Prehabilitation: The Right Medicine for Older Frail Adults Anticipating Transcatheter Aortic Valve Replacement, Coronary Artery Bypass Graft, and Other Cardiovascular Care. *Clin. Geriatr. Med.* **35**, 571–585 (2019).
302. Hinterbuchner, L. *et al.* Frailty scoring in transcatheter aortic valve replacement patients. *Eur. J. Cardiovasc. Nurs. J. Work. Group Cardiovasc. Nurs. Eur. Soc. Cardiol.* **15**, 384–397 (2016).
303. Thongprayoon, C., Cheungpasitporn, W. & Kashani, K. The impact of frailty on mortality after transcatheter aortic valve replacement. *Ann. Transl. Med.* **5**, (2017).
304. Brown, C. H. *et al.* The Association Between Preoperative Frailty and Postoperative Delirium After Cardiac Surgery. *Anesth. Analg.* **123**, 430–435 (2016).
305. Yanagawa, B., Graham, M. M., Afilalo, J., Hassan, A. & Arora, R. C. Frailty as a risk predictor in cardiac surgery: Beyond the eyeball test. *J. Thorac. Cardiovasc. Surg.* **156**, 172-176.e2 (2018).
306. Brown, N. A. & Zenilman, M. E. The Impact of Frailty in the Elderly on the Outcome of Surgery in the Aged. *Adv. Surg.* **44**, 229–249 (2010).
307. Green, P. *et al.* The impact of frailty status on survival after transcatheter aortic valve replacement in older adults with severe aortic stenosis: a single-center experience. *JACC Cardiovasc. Interv.* **5**, 974–981 (2012).
308. Lee, D. H., Buth, K. J., Martin, B.-J., Yip, A. M. & Hirsch, G. M. Frail patients are at increased risk for mortality and prolonged institutional care after cardiac surgery. *Circulation* **121**, 973–978 (2010).

309. Singh, M. *et al.* Influence of frailty and health status on outcomes in patients with coronary disease undergoing percutaneous revascularization. *Circ. Cardiovasc. Qual. Outcomes* **4**, 496–502 (2011).
310. Sündermann, S. *et al.* One-year follow-up of patients undergoing elective cardiac surgery assessed with the Comprehensive Assessment of Frailty test and its simplified form. *Interact. Cardiovasc. Thorac. Surg.* **13**, 119–123; discussion 123 (2011).
311. Expert recommendations - ERAS. <https://www.erascardiac.org/recommendations/expert-recommendations>.
312. Hulzebos, E. H. J., Smit, Y., Helders, P. P. J. M. & van Meeteren, N. L. U. Preoperative physical therapy for elective cardiac surgery patients. *Cochrane Database Syst. Rev.* **11**, CD010118 (2012).
313. Marmelo, F., Rocha, V. & Gonçalves, D. The impact of prehabilitation on post-surgical complications in patients undergoing non-urgent cardiovascular surgical intervention: Systematic review and meta-analysis. *Eur. J. Prev. Cardiol.* **25**, 404–417 (2018).
314. Snowdon, D., Haines, T. P. & Skinner, E. H. Preoperative intervention reduces postoperative pulmonary complications but not length of stay in cardiac surgical patients: a systematic review. *J. Physiother.* **60**, 66–77 (2014).
315. Kimber, D. E. *et al.* Pre-Operative Frailty Status Is Associated with Cardiac Rehabilitation Completion: A Retrospective Cohort Study. *J. Clin. Med.* **7**, (2018).
316. Dalal, H. M., Doherty, P. & Taylor, R. S. Cardiac rehabilitation. *The BMJ* **351**, (2015).
317. Kehler, D. S. *et al.* Association Between Cardiac Rehabilitation and Frailty. *Can. J. Cardiol.* **36**, 482–489 (2020).
318. Makary, M. A. *et al.* Frailty as a predictor of surgical outcomes in older patients. *J. Am. Coll. Surg.* **210**, 901–908 (2010).
319. Panayi, A. C. *et al.* Impact of frailty on outcomes in surgical patients: A systematic review and meta-analysis. *Am. J. Surg.* **218**, 393–400 (2019).

320. Aucoin, S. D. *et al.* Accuracy and Feasibility of Clinically Applied Frailty Instruments before Surgery A Systematic Review and Meta-analysis. *Anesthesiol. J. Am. Soc. Anesthesiol.* **133**, 78–95 (2020).
321. Ali, T. Z., Lehman, E. B. & Aziz, F. Modified Frailty Index Can Be Used to Predict Adverse Outcomes and Mortality after Lower Extremity Bypass Surgery. *Ann. Vasc. Surg.* **46**, 168–177 (2018).
322. Wahl, T. S. *et al.* Association of the Modified Frailty Index With 30-Day Surgical Readmission. *JAMA Surg.* **152**, 749–757 (2017).
323. Lytwyn, J. *et al.* The impact of frailty on functional survival in patients 1 year after cardiac surgery. *J. Thorac. Cardiovasc. Surg.* **154**, 1990–1999 (2017).
324. Ko, F. C. Preoperative Frailty Evaluation: A Promising Risk-stratification Tool in Older Adults Undergoing General Surgery. *Clin. Ther.* **41**, 387–399 (2019).
325. Theou, O. *et al.* Exploring Clinically Meaningful Changes for the Frailty Index in a Longitudinal Cohort of Hospitalized Older Patients. *J. Gerontol. Ser. A* **75**, 1928–1934 (2020).
326. Graham, I. D. *et al.* Lost in knowledge translation: time for a map? *J. Contin. Educ. Health Prof.* **26**, 13–24 (2006).
327. Canada, P. H. A. of. Health Promotion and Chronic Disease Prevention in Canada, Vol 44, No 9, September 2024. <https://www.canada.ca/en/public-health/services/reports-publications/health-promotion-chronic-disease-prevention-canada-research-policy-practice/vol-44-no-9-2024.html> (2024).
328. Carter, B. *et al.* A national study of 23 major trauma centres to investigate the effect of frailty on clinical outcomes in older people admitted with serious injury in England (FiTR 1): a multicentre observational study. *Lancet Healthy Longev.* **3**, e540–e548 (2022).

329. Cacciatore, S., Martone, A. M., Landi, F. & Tosato, M. Acute Coronary Syndrome in Older Adults: an Update from the 2022 Scientific Statement by the American Heart Association. *Heart Vessels Transplant.* **7**, 7–10 (2023).
330. Boreskie, P. E. & Boreskie, K. F. Frailty-aware Care in the Emergency Department. *Emerg. Med. Clin.* **0**, (2024).
331. Aliberti, M. J. R., Arteaga-Vargas, D. F. & Avelino-Silva, T. J. Frailty Matters—Why Isn't It Guiding Clinical Decisions? *J. Am. Geriatr. Soc.* **n/a**,.
332. Seniors. <https://www150.statcan.gc.ca/n1/pub/11-402-x/2011000/chap/seniors-aines/seniors-aines-eng.htm>.
333. Canada, P. H. A. of & Canada, P. H. A. of. Executive summary: Tracking Heart Disease and Stroke in Canada 2009. *aem* <https://www.canada.ca/en/public-health/services/reports-publications/2009-tracking-heart-disease-stroke-canada/executive-summary.html> (2009).
334. Vermeiren, S. *et al.* Frailty and the Prediction of Negative Health Outcomes: A Meta-Analysis. *J. Am. Med. Dir. Assoc.* **17**, 1163.e1-1163.e17 (2016).
335. Clegg, A., Young, J., Iliffe, S., Rikkert, M. O. & Rockwood, K. Frailty in elderly people. *Lancet Lond. Engl.* **381**, 752–762 (2013).
336. Haapanen, M. J. *et al.* Early life stress and frailty in old age: the Helsinki birth cohort study. *BMC Geriatr.* **18**, 179 (2018).
337. van der Linden, B. W. A. *et al.* Life-course circumstances and frailty in old age within different European welfare regimes: a longitudinal study with SHARE. *J. Gerontol. B. Psychol. Sci. Soc. Sci.* (2019) doi:10.1093/geronb/gbz140.
338. Szanton, S. L., Seplaki, C. L., Thorpe, R. J., Allen, J. K. & Fried, L. P. Socioeconomic status is associated with frailty: the Women's Health and Aging Studies. *J. Epidemiol. Community Health* **64**, 63–67 (2010).

339. Cheng, M.-H. & Chang, S.-F. Frailty as a Risk Factor for Falls Among Community Dwelling People: Evidence From a Meta-Analysis. *J. Nurs. Scholarsh. Off. Publ. Sigma Theta Tau Int. Honor Soc. Nurs.* **49**, 529–536 (2017).
340. Kojima, G. Frailty as a predictor of disabilities among community-dwelling older people: a systematic review and meta-analysis. *Disabil. Rehabil.* **39**, 1897–1908 (2017).
341. Kojima, G. Frailty as a Predictor of Nursing Home Placement Among Community-Dwelling Older Adults: A Systematic Review and Meta-analysis. *J. Geriatr. Phys. Ther.* **2001** **41**, 42–48 (2018).
342. Kojima, G. Frailty as a predictor of hospitalisation among community-dwelling older people: a systematic review and meta-analysis. *J. Epidemiol. Community Health* **70**, 722–729 (2016).
343. Kojima, G., Taniguchi, Y., Iliffe, S. & Walters, K. Frailty as a Predictor of Alzheimer Disease, Vascular Dementia, and All Dementia Among Community-Dwelling Older People: A Systematic Review and Meta-Analysis. *J. Am. Med. Dir. Assoc.* **17**, 881–888 (2016).
344. Kojima, G., Iliffe, S. & Walters, K. Frailty index as a predictor of mortality: a systematic review and meta-analysis. *Age Ageing* **47**, 193–200 (2018).
345. Afilalo, J. *et al.* Frailty assessment in the cardiovascular care of older adults. *J. Am. Coll. Cardiol.* **63**, 747–762 (2014).
346. Flint, K. Which came first, the frailty or the heart disease?: exploring the vicious cycle. *J. Am. Coll. Cardiol.* **65**, 984–986 (2015).
347. Veronese, N. *et al.* Risk of cardiovascular disease morbidity and mortality in frail and pre-frail older adults: Results from a meta-analysis and exploratory meta-regression analysis. *Ageing Res. Rev.* **35**, 63–73 (2017).
348. Gale, C. R., Cooper, C. & Sayer, A. A. Framingham cardiovascular disease risk scores and incident frailty: the English longitudinal study of ageing. *Age* **36**, (2014).

349. Duhamel, T. A. *et al.* Pre-Frailty and Cardiovascular Disease Risk in Women Age 55 Years and Older. *Can. J. Cardiol.* **34**, e19 (2018).
350. Rockwood, K. *et al.* A global clinical measure of fitness and frailty in elderly people. *CMAJ Can. Med. Assoc. J.* **173**, 489–495 (2005).
351. Rockwood, K., Mitnitski, A. B. & MacKnight, C. Some mathematical models of frailty and their clinical implications. *Rev. Clin. Gerontol.* **12**, 109–117 (2002).
352. Kehler, D. S. *et al.* Prevalence of frailty in Canadians 18–79 years old in the Canadian Health Measures Survey. *BMC Geriatr.* **17**, (2017).
353. Shamliyan, T., Talley, K. M. C., Ramakrishnan, R. & Kane, R. L. Association of frailty with survival: a systematic literature review. *Ageing Res. Rev.* **12**, 719–736 (2013).
354. Vitale, C., Rosano, G. & Fini, M. Are elderly and women under-represented in cardiovascular clinical trials? Implication for treatment. *Wien. Klin. Wochenschr.* **128**, 433–438 (2016).
355. STROBE Statement: Available checklists. <https://www.strobe-statement.org/index.php?id=available-checklists>.
356. Boreskie, K. F. *et al.* Protocol for the HAPPY Hearts study: cardiovascular screening for the early detection of future adverse cardiovascular outcomes in middle-aged and older women: a prospective, observational cohort study. *BMJ Open* **7**, e018249 (2017).
357. Boreskie, K. F. *et al.* Standardization of the Fried frailty phenotype improves cardiovascular disease risk discrimination. *Exp. Gerontol.* **119**, 40–44 (2019).
358. Duprez, D. A. & Cohn, J. N. Identifying early cardiovascular disease to target candidates for treatment. *J. Clin. Hypertens. Greenwich Conn* **10**, 226–231 (2008).
359. D’Agostino, R. B. *et al.* General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* **117**, 743–753 (2008).

360. Nowak, Z. *et al.* Paffenbarger Physical Activity Questionnaire as an additional tool in clinical assessment of patients with coronary artery disease treated with angioplasty. *Kardiol. Pol.* **68**, 32–39 (2010).
361. Lee, P. H., Macfarlane, D. J., Lam, T. H. & Stewart, S. M. Validity of the International Physical Activity Questionnaire Short Form (IPAQ-SF): a systematic review. *Int. J. Behav. Nutr. Phys. Act.* **8**, 115 (2011).
362. Leong, D. P. & Teo, K. K. Predicting cardiovascular disease from handgrip strength: the potential clinical implications. *Expert Rev. Cardiovasc. Ther.* **13**, 1277–1279 (2015).
363. Schoon, Y., Bongers, K., Van Kempen, J., Melis, R. & Olde Rikkert, M. Gait speed as a test for monitoring frailty in community-dwelling older people has the highest diagnostic value compared to step length and chair rise time. *Eur. J. Phys. Rehabil. Med.* **50**, 693–701 (2014).
364. Carleton, R. N. *et al.* The Center for Epidemiologic Studies Depression Scale: A Review with a Theoretical and Empirical Examination of Item Content and Factor Structure. *PLoS ONE* **8**, (2013).
365. MILLIPLEX MAP Human Myokine Magnetic Bead Panel | HMYOMAG-56K.
http://www.emdmillipore.com/CA/en/product/MILLIPLEX-MAP-Human-Myokine-Magnetic-Bead-Panel,MM_NF-HMYOMAG-56K.
366. Soysal, P. *et al.* Inflammation and frailty in the elderly: A systematic review and meta-analysis. *Ageing Res. Rev.* **31**, 1–8 (2016).
367. Cornish, S. M. & Chilibeck, P. D. Alpha-linolenic acid supplementation and resistance training in older adults. *Appl. Physiol. Nutr. Metab. Physiol. Appl. Nutr. Metab.* **34**, 49–59 (2009).
368. Ferretti, E., Pistoia, V. & Corcione, A. Role of Fractalkine/CX3CL1 and Its Receptor in the Pathogenesis of Inflammatory and Malignant Diseases with Emphasis on B Cell Malignancies. *Mediators Inflamm.* **2014**, (2014).
369. Chen, P., Zhao, W., Guo, Y., Xu, J. & Yin, M. CX3CL1/CX3CR1 in Alzheimer's Disease: A Target for Neuroprotection. *BioMed Res. Int.* **2016**, (2016).

370. Li, H., Manwani, B. & Leng, S. X. Frailty, Inflammation, and Immunity. *Aging Dis.* **2**, 466–473 (2011).
371. Akdis, M. *et al.* Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β , and TNF- α : Receptors, functions, and roles in diseases. *J. Allergy Clin. Immunol.* **138**, 984–1010 (2016).
372. Fernández-Ruiz, I. Immune system and cardiovascular disease. *Nat. Rev. Cardiol.* **13**, 503 (2016).
373. Yao, X., Li, H. & Leng, S. X. Inflammation and Immune System Alterations in Frailty. *Clin. Geriatr. Med.* **27**, 79–87 (2011).
374. Kaplan, R. C. & Frishman, W. H. Systemic inflammation as a cardiovascular disease risk factor and as a potential target for drug therapy. *Heart Dis. Hagerstown Md* **3**, 326–332 (2001).
375. Nishida, H. *et al.* Interleukin-6 as an independent predictor of future cardiovascular events in high-risk Japanese patients: comparison with C-reactive protein. *Cytokine* **53**, 342–346 (2011).
376. Aker, S. *et al.* Influence of interleukin-6 G-174C gene polymorphism on coronary artery disease, cardiovascular complications and mortality in dialysis patients. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc.* **24**, 2847–2851 (2009).
377. Gokkusu, C. *et al.* Influences of genetic variants in interleukin-15 gene and serum interleukin-15 levels on coronary heart disease. *Cytokine* **49**, 58–63 (2010).
378. Kaibe, M. *et al.* Serum interleukin-15 concentration in patients with essential hypertension. *Am. J. Hypertens.* **18**, 1019–1025 (2005).
379. Jelkmann, W. Erythropoietin. *Front. Horm. Res.* **47**, 115–127 (2016).
380. Nekoui, A. & Blaise, G. Erythropoietin and Nonhematopoietic Effects. *Am. J. Med. Sci.* **353**, 76–81 (2017).
381. Thumser, A. E., Moore, J. B. & Plant, N. J. Fatty acid binding proteins: tissue-specific functions in health and disease. *Curr. Opin. Clin. Nutr. Metab. Care* **17**, 124–129 (2014).

382. Kleine, A. H., Glatz, J. F. C., Van Nieuwenhoven, F. A. & Van der Vusse, G. J. Release of heart fatty acid-binding protein into plasma after acute myocardial infarction in man. *Mol. Cell. Biochem.* **116**, 155–162 (1992).
383. Kochansky, C. J., Lyman, M. J., Fauty, S. E., Vlasakova, K. & D'mello, A. P. Administration of Fenofibrate Markedly Elevates *Fabp3* in Rat Liver and Plasma and Confounds Its Use as a Preclinical Biomarker of Cardiac and Muscle Toxicity. *Lipids* **53**, 947–960 (2018).
384. Pelsers, M. M. A. L., Hermens, W. T. & Glatz, J. F. C. Fatty acid-binding proteins as plasma markers of tissue injury. *Clin. Chim. Acta* **352**, 15–35 (2005).
385. Salminen, A., Kaarniranta, K. & Kauppinen, A. Regulation of longevity by FGF21: Interaction between energy metabolism and stress responses. *Ageing Res. Rev.* **37**, 79–93 (2017).
386. Lundsgaard, A.-M. *et al.* Circulating FGF21 in humans is potently induced by short term overfeeding of carbohydrates. *Mol. Metab.* **6**, 22–29 (2017).
387. Kharitonov, A. *et al.* FGF-21 as a novel metabolic regulator. *J. Clin. Invest.* **115**, 1627–1635 (2005).
388. Coskun, T. *et al.* Fibroblast Growth Factor 21 Corrects Obesity in Mice. *Endocrinology* **149**, 6018–6027 (2008).
389. Shen, Y. *et al.* Serum FGF21 Is Associated with Future Cardiovascular Events in Patients with Coronary Artery Disease. *Cardiology* **139**, 212–218 (2018).
390. Villarroya, J. *et al.* Aging is associated with increased FGF21 levels but unaltered FGF21 responsiveness in adipose tissue. *Aging Cell* **17**, (2018).
391. Xie, J. *et al.* Oncostatin M was associated with thrombosis in patients with atrial fibrillation. *Medicine (Baltimore)* **96**, (2017).
392. Richards, C. D. The Enigmatic Cytokine Oncostatin M and Roles in Disease. *ISRN Inflamm.* **2013**, (2013).

393. Boreskie, K. F. *et al.* Myokines as biomarkers of frailty and cardiovascular disease risk in females. *Exp. Gerontol.* **133**, 110859 (2020).
394. Saedi, A. A., Feehan, J., Phu, S. & Duque, G. Current and emerging biomarkers of frailty in the elderly. *Clin. Interv. Aging* **14**, 389–398 (2019).
395. Fiuza-Luces, C. *et al.* Exercise benefits in cardiovascular disease: beyond attenuation of traditional risk factors. *Nat. Rev. Cardiol.* **15**, 731–743 (2018).
396. Cook, G., Larocca, A., Facon, T., Zweegman, S. & Engelhardt, M. Defining the vulnerable patient with myeloma—a frailty position paper of the European Myeloma Network. *Leukemia* **34**, 2285–2294 (2020).
397. Li, B. *et al.* The Identification and Evaluation of Interleukin-7 as a Myokine Biomarker for Peripheral Artery Disease Prognosis. *J. Clin. Med.* **13**, 3583 (2024).
398. Isanejad, M. *et al.* Dietary omega-3 polyunsaturated fatty acid and alpha-linolenic acid are associated with physical capacity measure but not muscle mass in older women 65–72 years. *Eur. J. Nutr.* **61**, 1813–1821 (2022).
399. Davies, B. *et al.* Differential Association of Frailty and Sarcopenia With Mortality and Disability: Insight Supporting Clinical Subtypes of Frailty. *J. Am. Med. Dir. Assoc.* **23**, 1712-1716.e3 (2022).
400. Samant, V. & Prabhu, A. Exercise, exerkins and exercise mimetic drugs: Molecular mechanisms and therapeutics. *Life Sci.* **359**, 123225 (2024).
401. Statistics Canada. Canada’s population estimates: Age and sex, July 1, 2018. Preprint at <https://www150.statcan.gc.ca/n1/en/daily-quotidien/190125/dq190125a-eng.pdf?st=7PJeGKWj>.
402. Seniors. Preprint at <https://www150.statcan.gc.ca/n1/pub/11-402-x/2011000/chap/seniors-aines/seniors-aines-eng.htm> (2018).
403. North, B. J. & Sinclair, D. A. The Intersection Between Aging and Cardiovascular Disease. *Circ. Res.* **110**, 1097–1108 (2012).

404. Bergman, H. *et al.* Frailty: An Emerging Research and Clinical Paradigm—Issues and Controversies. *J. Gerontol. A. Biol. Sci. Med. Sci.* **62**, 731–737 (2007).
405. Sergi, G. *et al.* Pre-frailty and risk of cardiovascular disease in elderly men and women: the Pro.V.A. study. *J. Am. Coll. Cardiol.* **65**, 976–983 (2015).
406. Boreskie, K. F. *et al.* Frailty status and cardiovascular disease risk profile in middle-aged and older females. *Exp. Gerontol.* 111061 (2020) doi:10.1016/j.exger.2020.111061.
407. Collerton, J. *et al.* Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: Cross-sectional findings from the Newcastle 85+ Study. *Mech. Ageing Dev.* **133**, 456–466 (2012).
408. Afilalo, J., Karunanathan, S., Eisenberg, M. J., Alexander, K. P. & Bergman, H. Role of frailty in patients with cardiovascular disease. *Am. J. Cardiol.* **103**, 1616–1621 (2009).
409. Soysal, P., Arik, F., Smith, L., Jackson, S. E. & Isik, A. T. Inflammation, Frailty and Cardiovascular Disease. *Adv. Exp. Med. Biol.* **1216**, 55–64 (2020).
410. Streeten, E. A. Bone as a classic endocrine organ: Interactions with non-bone tissues. *Rev. Endocr. Metab. Disord.* **16**, 77–78 (2015).
411. Datta, H. K., Ng, W. F., Walker, J. A., Tuck, S. P. & Varanasi, S. S. The cell biology of bone metabolism. *J. Clin. Pathol.* **61**, 577–587 (2008).
412. Zaidi, M., Yuen, T., Sun, L. & Rosen, C. J. Regulation of Skeletal Homeostasis. *Endocr. Rev.* **39**, 701–718 (2018).
413. Jackson, R. D. & Mysiw, W. J. Insights into the epidemiology of postmenopausal osteoporosis: the Women’s Health Initiative. *Semin. Reprod. Med.* **32**, 454–462 (2014).
414. Eastell, R. & Szulc, P. Use of bone turnover markers in postmenopausal osteoporosis. *Lancet Diabetes Endocrinol.* **5**, 908–923 (2017).
415. STROBE Statement: Home. <https://www.strobe-statement.org/index.php?id=strobe-home>.

416. Boreskie, K. F. *et al.* Protocol for the HAPPY Hearts study: cardiovascular screening for the early detection of future adverse cardiovascular outcomes in middle-aged and older women: a prospective, observational cohort study. *BMJ Open* **7**, e018249 (2017).
417. Boreskie, K. F. *et al.* Standardization of the Fried frailty phenotype improves cardiovascular disease risk discrimination. *Exp. Gerontol.* **119**, 40–44 (2019).
418. Duprez, D. A. & Cohn, J. N. Identifying early cardiovascular disease to target candidates for treatment. *J. Clin. Hypertens. Greenwich Conn* **10**, 226–231 (2008).
419. D’Agostino, R. B. *et al.* General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* **117**, 743–753 (2008).
420. MILLIPLEX MAP Human Bone Magnetic Bead Panel - Bone Metabolism Multiplex Assay | HBNMAG-51K. http://www.emdmillipore.com/CA/en/product/MILLIPLEX-MAP-Human-Bone-Magnetic-Bead-Panel-Bone-Metabolism-Multiplex-Assay,MM_NF-HBNMAG-51K.
421. Cornish, S. M. & Chilibeck, P. D. Alpha-linolenic acid supplementation and resistance training in older adults. *Appl. Physiol. Nutr. Metab. Physiol. Appl. Nutr. Metab.* **34**, 49–59 (2009).
422. Cook, M. J. *et al.* Frailty and bone health in European men. *Age Ageing* (2017) doi:10.1093/ageing/afw205.
423. Ensrud, K. E. *et al.* Frailty and risk of falls, fracture, and mortality in older women: the study of osteoporotic fractures. *J. Gerontol. A. Biol. Sci. Med. Sci.* **62**, 744–751 (2007).
424. Ecss, A. & Ecss, A. Association of Bone Mineral Density with Frailty, Pre-Frailty, and Osteoporosis in Community-Dwelling Elderly: A Prospective Study.
425. Álvarez-Sánchez, N. *et al.* Homocysteine levels are associated with bone resorption in pre-frail and frail Spanish women: The Toledo Study for Healthy Aging. *Exp. Gerontol.* **108**, 201–208 (2018).
426. Gené Huguet, L. *et al.* Pre Frail 80: Multifactorial Intervention to Prevent Progression of Pre-Frailty to Frailty in the Elderly. *J. Nutr. Health Aging* **22**, 1266–1274 (2018).

427. Wolf, J., Rose-John, S. & Garbers, C. Interleukin-6 and its receptors: a highly regulated and dynamic system. *Cytokine* **70**, 11–20 (2014).
428. Yoshitake, F., Itoh, S., Narita, H., Ishihara, K. & Ebisu, S. Interleukin-6 Directly Inhibits Osteoclast Differentiation by Suppressing Receptor Activator of NF- κ B Signaling Pathways. *J. Biol. Chem.* **283**, 11535–11540 (2008).
429. Lee, S.-H., Kim, T.-S., Choi, Y. & Lorenzo, J. Osteoimmunology: cytokines and the skeletal system. *BMB Rep.* **41**, 495–510 (2008).
430. Loi, F. *et al.* Inflammation, Fracture and Bone Repair. *Bone* **86**, 119–130 (2016).
431. Soysal, P. *et al.* Inflammation and frailty in the elderly: A systematic review and meta-analysis. *Ageing Res. Rev.* **31**, 1–8 (2016).
432. Zelová, H. & Hošek, J. TNF- α signalling and inflammation: interactions between old acquaintances. *Inflamm. Res. Off. J. Eur. Histamine Res. Soc. Al* **62**, 641–651 (2013).
433. Lam, J. *et al.* TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J. Clin. Invest.* **106**, 1481–1488 (2000).
434. Lu, X., Gilbert, L., He, X., Rubin, J. & Nanes, M. S. Transcriptional regulation of the osterix (Osx, Sp7) promoter by tumor necrosis factor identifies disparate effects of mitogen-activated protein kinase and NF kappa B pathways. *J. Biol. Chem.* **281**, 6297–6306 (2006).
435. Osta, B., Benedetti, G. & Miossec, P. Classical and Paradoxical Effects of TNF- α on Bone Homeostasis. *Front. Immunol.* **5**, (2014).
436. Ferrari, R. The role of TNF in cardiovascular disease. *Pharmacol. Res.* **40**, 97–105 (1999).
437. Prediction of coronary disease incidence by biomarkers of inflammation, oxidation, and metabolism | Scientific Reports. <https://www.nature.com/articles/s41598-018-21482-y>.
438. Ahima, R. S. Adipose tissue as an endocrine organ. *Obes. Silver Spring Md* **14 Suppl 5**, 242S–249S (2006).

439. Rosenbaum, M. & Leibel, R. L. Role of leptin in energy homeostasis in humans. *J. Endocrinol.* **223**, T83–T96 (2014).
440. Upadhyay, J., Farr, O. M. & Mantzoros, C. S. The role of leptin in regulating bone metabolism. *Metabolism.* **64**, 105–113 (2015).
441. Cirmanová, V., Bayer, M., Stárka, L. & Zajícková, K. The effect of leptin on bone: an evolving concept of action. *Physiol. Res.* **57 Suppl 1**, S143–151 (2008).
442. Isaia, G. C., D’Amelio, P., Di Bella, S. & Tamone, C. Is leptin the link between fat and bone mass? *J. Endocrinol. Invest.* **28**, 61–65 (2005).
443. Turner, R. T. *et al.* Peripheral Leptin Regulates Bone Formation. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* **28**, 22–34 (2013).
444. Ducy, P. *et al.* Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell* **100**, 197–207 (2000).
445. Koh, K. K., Park, S. M. & Quon, M. J. Leptin and Cardiovascular Disease. *Circulation* **117**, 3238–3249 (2008).
446. Wallace, A. M. *et al.* Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation* **104**, 3052–3056 (2001).
447. Andrade-Oliveira, V., Câmara, N. O. S. & Moraes-Vieira, P. M. Adipokines as Drug Targets in Diabetes and Underlying Disturbances. *J. Diabetes Res.* **2015**, (2015).
448. Shetty, S., Kapoor, N., Bondu, J. D., Thomas, N. & Paul, T. V. Bone turnover markers: Emerging tool in the management of osteoporosis. *Indian J. Endocrinol. Metab.* **20**, 846–852 (2016).
449. Greenblatt, M. B., Tsai, J. N. & Wein, M. N. Bone Turnover Markers in the Diagnosis and Monitoring of Metabolic Bone Disease. *Clin. Chem.* **63**, 464–474 (2017).
450. Muscedere, J. *et al.* Proceedings of the Canadian Frailty Network Workshop: Identifying Biomarkers of Frailty to Support Frailty Risk Assessment, Diagnosis and Prognosis. Toronto, January 15, 2018. *J. Frailty Aging* **8**, 106–116 (2019).

451. Blodgett, J. M. *et al.* Prognostic accuracy of 70 individual frailty biomarkers in predicting mortality in the Canadian Longitudinal Study on Aging. *GeroScience* (2024) doi:10.1007/s11357-023-01055-2.
452. Wołoszyn-Horák, E. *et al.* Frailty syndrome in daily practice of interventional cardiology ward- rationale and design of the FRAPICA trial: A STROBE-compliant prospective observational study. *Medicine (Baltimore)* **99**, e18935 (2020).
453. Korzonek-Szlacheta, I. *et al.* The Association between Circulating Cytokines and Body Composition in Frail Patients with Cardiovascular Disease. *Nutrients* **16**, 1227 (2024).
454. Me, S. *et al.* Frailty and the Interactions between Skeletal Muscle, Bone, and Adipose Tissue- Impact on Cardiovascular Disease and Possible Therapeutic Measures. *Int. J. Mol. Sci.* **24**, (2023).
455. Calvani, R. *et al.* Identification of biomarkers for physical frailty and sarcopenia through a new multi-marker approach: results from the BIOSPHERE study. *GeroScience* (2020) doi:10.1007/s11357-020-00197-x.
456. Pedersen, B. K. & Saltin, B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand. J. Med. Sci. Sports* **25 Suppl 3**, 1–72 (2015).
457. Liu, H. *et al.* Myokines and Biomarkers of Frailty in Older Inpatients with Undernutrition: A Prospective Study. *J. Frailty Aging* **13**, 82–90 (2024).
458. Searle, S. D., Mitnitski, A., Gahbauer, E. A., Gill, T. M. & Rockwood, K. A standard procedure for creating a frailty index. *BMC Geriatr.* **8**, 1–10 (2008).
459. Rose, A. V. *et al.* Protocol for the WARM Hearts study: examining cardiovascular disease risk in middle-aged and older women - a prospective, observational cohort study. *BMJ Open* **11**, e044227 (2021).
460. Heidari, S., Babor, T. F., De Castro, P., Tort, S. & Curno, M. Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use. *Res. Integr. Peer Rev.* **1**, 2 (2016).

461. Heinze-Milne, S. D., Banga, S. & Howlett, S. E. Frailty and cytokines in preclinical models: Comparisons with humans. *Mech. Ageing Dev.* **206**, 111706 (2022).
462. Lombardi, G., Sansoni, V. & Banfi, G. Measuring myokines with cardiovascular functions: pre-analytical variables affecting the analytical output. *Ann. Transl. Med.* **5**, (2017).
463. Janssen, I., Katzmarzyk, P. T. & Ross, R. Waist circumference and not body mass index explains obesity-related health risk. *Am. J. Clin. Nutr.* **79**, 379–384 (2004).
464. Canadian Society of Exercise Physiology. CSEP Certified Personal Trainer® Update to Pre-Participation Screening Procedures CSEP Certified Personal Trainer®. 2–4 (2017).
465. Ling, C. H. Y. *et al.* Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. *Clin. Nutr.* **30**, 610–615 (2011).
466. Sato, S., Demura, S., Kitabayashi, T. & Noguchi, T. Segmental body composition assessment for obese japanese adults by single-frequency bioelectrical impedance analysis with 8-point contact electrodes. *J. Physiol. Anthropol.* **26**, 533–540 (2007).
467. Anderson, T. J. *et al.* 2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult. *Can. J. Cardiol.* **32**, 1263–1282 (2016).
468. Hametner, B. *et al.* Oscillometric estimation of aortic pulse wave velocity: comparison with intra-aortic catheter measurements. *Blood Press. Monit.* **18**, 173–176 (2013).
469. Nerenberg, K. A. *et al.* Hypertension Canada’s 2018 Guidelines for Diagnosis, Risk Assessment, Prevention, and Treatment of Hypertension in Adults and Children. *Can. J. Cardiol.* **34**, 506–525 (2018).
470. Wei, W., Tölle, M., Zidek, W. & van der Giet, M. Validation of the mobil-O-Graph: 24 h-blood pressure measurement device. *Blood Press. Monit.* **15**, 225–228 (2010).

471. Fujiwara, Y. *et al.* Brief screening tool for mild cognitive impairment in older Japanese: Validation of the Japanese version of the Montreal Cognitive Assessment. *Geriatr. Gerontol. Int.* **10**, 225–232 (2010).
472. Nasreddine, Z. S. *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* **53**, 695–699 (2005).
473. Tate, C. C., Ledbetter, J. N. & Youssef, C. P. A two-question method for assessing gender categories in the social and medical sciences. *J. Sex Res.* **50**, 767–776 (2013).
474. Craig, C. L. *et al.* International physical activity questionnaire: 12-Country reliability and validity. *Med. Sci. Sports Exerc.* **35**, 1381–1395 (2003).
475. Herdman, M. *et al.* Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual. Life Res. Int. J. Qual. Life Asp. Treat. Care Rehabil.* **20**, 1727–1736 (2011).
476. Feng, Y., Parkin, D. & Devlin, N. J. Assessing the performance of the EQ-VAS in the NHS PROMs programme. *Qual. Life Res.* **23**, 977–989 (2014).
477. Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R. & Kupfer, D. J. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 1989;28:193–213. (1989).
478. Keller, H. H., Goy, R. & Kane, S. L. Validity and reliability of SCREEN II (Seniors in the Community: Risk evaluation for eating and nutrition, Version II). *Eur. J. Clin. Nutr.* **59**, 1149–1157 (2005).
479. Kroenke, K., Spitzer, R. L. & Williams, J. B. The PHQ-9: validity of a brief depression severity measure. *J. Gen. Intern. Med.* **16**, 606–613 (2001).
480. Song, X., Mitnitski, A. & Rockwood, K. Prevalence and 10-year outcomes of frailty in older adults in relation to deficit accumulation. *J. Am. Geriatr. Soc.* **58**, 681–687 (2010).
481. Radloff, L. S. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Appl. Psychol. Meas.* **1**, 385–401 (1977).

482. Nowak, Z. *et al.* Paffenbarger Physical Activity Questionnaire as an additional tool in clinical assessment of patients. *Kardiol. Pol.* 32–39 (2010).
483. Clegg, A., Young, J., Iliffe, S., Rikkert, M. O. & Rockwood, K. Frailty in elderly people. *The Lancet* **381**, 752–762 (2013).
484. Harrell Jr., F. E., Lee, K. L., Califf, R. M., Pryor, D. B. & Rosati, R. A. Regression modelling strategies for improved prognostic prediction. *Stat. Med.* **3**, 143–152 (1984).
485. Blodgett, J., Theou, O., Kirkland, S., Andreou, P. & Rockwood, K. Frailty in NHANES: Comparing the frailty index and phenotype. *Arch. Gerontol. Geriatr.* **60**, 464–470 (2015).
486. Intlekofer, K. A. *et al.* Exercise and sodium butyrate transform a subthreshold learning event into long-term memory via a brain-derived neurotrophic factor-dependent mechanism. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* **38**, 2027–2034 (2013).
487. Pedard, M., Demougeot, C., Prati, C. & Marie, C. Brain-derived neurotrophic factor in adjuvant-induced arthritis in rats. Relationship with inflammation and endothelial dysfunction. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **82**, 249–254 (2018).
488. Lau, H., Mat Ludin, A. F., Rajab, N. F. & Shahar, S. Identification of Neuroprotective Factors Associated with Successful Ageing and Risk of Cognitive Impairment among Malaysia Older Adults. *Curr. Gerontol. Geriatr. Res.* **2017**, 4218756 (2017).
489. Boyuk, B. *et al.* Relationship between levels of brain-derived neurotrophic factor and metabolic parameters in patients with type 2 diabetes mellitus. *J. Diabetes Res.* **2014**, 978143 (2014).
490. Lee, S.-M. *et al.* FABP3-mediated membrane lipid saturation alters fluidity and induces ER stress in skeletal muscle with aging. *Nat. Commun.* **11**, 5661 (2020).
491. Lee, S.-M. *et al.* FABP3-mediated membrane lipid saturation alters fluidity and induces ER stress in skeletal muscle with aging. *Nat. Commun.* **11**, 5661 (2020).
492. Domaniku, A., Bilgic, S. N. & Kir, S. Muscle wasting: emerging pathways and potential drug targets. *Trends Pharmacol. Sci.* **44**, 705–718 (2023).

493. Szaroszyk, M. *et al.* Skeletal muscle derived Musclin protects the heart during pathological overload. *Nat. Commun.* **13**, 149 (2022).
494. Kattih, B. *et al.* Low Circulating Musclin is Associated With Adverse Prognosis in Patients Undergoing Transcatheter Aortic Valve Implantation at Low-Intermediate Risk. *J. Am. Heart Assoc.* e022792 (2022) doi:10.1161/JAHA.121.022792.
495. Nakamura, K., Nakano, S.-I., Miyoshi, T., Yamanouchi, K. & Nishihara, M. Loss of sparc in mouse skeletal muscle causes myofiber atrophy. *Muscle Nerve* **48**, 791–799 (2013).
496. Nakamura, K., Yamanouchi, K. & Nishihara, M. Secreted protein acidic and rich in cysteine internalization and its age-related alterations in skeletal muscle progenitor cells. *Aging Cell* **13**, 175–184 (2014).
497. Tai, I. T., Dai, M., Owen, D. A. & Chen, L. B. Genome-wide expression analysis of therapy-resistant tumors reveals SPARC as a novel target for cancer therapy. *J. Clin. Invest.* **115**, 1492–1502 (2005).
498. Ghanemi, A., Melouane, A., Yoshioka, M. & St-Amand, J. Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Leads to an Accelerated Ageing Phenotype Which Is Improved by Exercise Whereas SPARC Overexpression Mimics Exercise Effects in Mice. *Metabolites* **12**, 125 (2022).
499. Ghanemi, A., Melouane, A., Yoshioka, M. & St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* **117**, 105627 (2019).
500. Toba, H. & Takai, S. Exploring the roles of SPARC as a proinflammatory factor and its potential as a novel therapeutic target against cardiovascular disease. *Am. J. Physiol.-Heart Circ. Physiol.* **327**, H1174–H1186 (2024).
501. Chen, W., Wang, L., You, W. & Shan, T. Myokines mediate the cross talk between skeletal muscle and other organs. *J. Cell. Physiol.* **236**, 2393–2412 (2021).

502. Sanchis-Gomar, F. *et al.* Neuromuscular Electrical Stimulation: A New Therapeutic Option for Chronic Diseases Based on Contraction-Induced Myokine Secretion. *Front. Physiol.* **10**, (2019).
503. Chu, W. *et al.* Identifying the Biomarker Profile of Pre-Frail and Frail People: A Cross-Sectional Analysis from UK Biobank. *Int. J. Environ. Res. Public. Health* **20**, 2421 (2023).
504. Picca, A., Coelho-Junior, H. J., Calvani, R., Marzetti, E. & Vetrano, D. L. Biomarkers shared by frailty and sarcopenia in older adults: A systematic review and meta-analysis. *Ageing Res. Rev.* **73**, 101530 (2022).
505. Bettariga, F. *et al.* Exercise training mode effects on myokine expression in healthy adults: A systematic review with meta-analysis. *J. Sport Health Sci.* **13**, 764–779 (2024).
506. Farmawati, A. *et al.* Characterization of contraction-induced IL-6 up-regulation using contractile C2C12 myotubes. *Endocr. J.* **60**, 137–147 (2013).
507. Furuichi, Y., Manabe, Y., Takagi, M., Aoki, M. & Fujii, N. L. Evidence for acute contraction-induced myokine secretion by C2C12 myotubes. *PloS One* **13**, e0206146 (2018).
508. Stubbings, G., Farrell, S., Mitnitski, A., Rockwood, K. & Rutenberg, A. The Quantile Frailty Index: A Cutpoint-Free Approach to Biomarker-Based Health Assessment. *Innov. Aging* **4**, 180–180 (2020).
509. Kōks, S. *et al.* Mouse models of ageing and their relevance to disease. *Mech. Ageing Dev.* **160**, 41–53 (2016).
510. Liu, H., Graber, T. G., Ferguson-Stegall, L. & Thompson, L. V. Clinically relevant frailty index for mice. *J. Gerontol. A. Biol. Sci. Med. Sci.* **69**, 1485–1491 (2014).
511. Seldeen, K. L., Pang, M. & Troen, B. R. Mouse Models of Frailty: an Emerging Field. *Curr. Osteoporos. Rep.* **13**, 280–286 (2015).
512. Heart & Stroke 2018 Heart Report. *Ms Understood Women's Hearts Are Victims of a System That Is Ill-Equipped to Diagnose, Treat and Support Them.* (2018).

513. Norris, C. M. *et al.* State of the Science in Women's Cardiovascular Disease: A Canadian Perspective on the Influence of Sex and Gender. *J. Am. Heart Assoc.* **9**, (2020).
514. Rodondi, N. *et al.* Framingham risk score and alternatives for prediction of coronary heart disease in older adults. *PLoS ONE* **7**, (2012).
515. Shlipak, M. G. *et al.* The Incidence of Unrecognized Myocardial Infarction in Women with Coronary Heart Disease. *Ann Intern Med* **134**, 1043–1047 (2001).
516. O'Callaghan, K. M. Solutions for disparities for women with heart disease. *J Cardiovasc. Transl. Res.* **2**, 518–525 (2009).
517. Afilalo, J. *et al.* Frailty Assessment in the Cardiovascular Care of Older Adults. *Jac* **63**, 747–762 (2014).
518. Farooqi, M. A. M., Gerstein, H., Yusuf, S. & Leong, D. P. Accumulation of Deficits as a Key Risk Factor for Cardiovascular Morbidity and Mortality: A Pooled Analysis of 154 000 Individuals. *J. Am. Heart Assoc.* **9**, (2020).
519. Wang, J. *et al.* Novel biomarkers for cardiovascular risk prediction. *J. Geriatr. Cardiol.* **14**, 135–150 (2017).
520. Boreskie, K. F. *et al.* Myokines as biomarkers of frailty and cardiovascular disease risk in females. *Exp. Gerontol.* **133**, 1–7 (2020).
521. Boreskie, K. F. *et al.* BMJ open protocol for the HAPPY Hearts study: Cardiovascular screening for the early detection of future adverse cardiovascular outcomes in middle-aged and older women: A prospective, observational cohort study. *BMJ Open* **7**, 1–9 (2017).
522. Chan, A. W. *et al.* SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* **346**, 1–42 (2013).
523. von Elm, E. *et al.* The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Int. J. Surg.* **12**, 1495–1499 (2014).

524. Heidari, S., Babor, T. F., De Castro, P., Tort, S. & Curno, M. Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use. *Res. Integr. Peer Rev.* **1**, 1–9 (2016).
525. Park, A. Reachable : Data collection methods for sexual orientation and gender identity. *Williams Institue* **2016**, 1–8 (2016).
526. Bauer, G. No Title. *CIHR Inst. Gend. Health-Webinar Ser. Integrating Gend. Sex Health Res.* (2012).
527. Pelletier, R., Ditto, B. & Pilote, L. A composite measure of gender and its association with risk factors in patients with premature acute coronary syndrome. *Psychosom. Med.* **77**, 517–526 (2015).
528. Pelletier, R. *et al.* Sex Versus Gender-Related Characteristics Which Predicts Outcome after Acute Coronary Syndrome in the Young? *J. Am. Coll. Cardiol.* **67**, 127–135 (2016).
529. O’Neil, A., Scovelle, A. J., Milner, A. J. & Kavanagh, A. Gender/sex as a social determinant of cardiovascular risk. *Circulation* **137**, 854–864 (2018).
530. Hernandez-Martinez, A., Martinez-Rosales, E., Alcaraz-Ibañez, M., Soriano-Maldonado, A. & Artero, E. G. Influence of body composition on arterial stiffness in middle-aged adults: Healthy UAL cross-sectional study. *Med. Lith.* **55**, (2019).
531. Ittermann, T. *et al.* Changes in fat mass and fat-free-mass are associated with incident hypertension in four population-based studies from Germany. *Int. J. Cardiol.* **274**, 372–377 (2019).
532. Srikanthan, P., Horwich, T. B. & Tseng, C. H. Relation of Muscle Mass and Fat Mass to Cardiovascular Disease Mortality. *Am. J. Cardiol.* **117**, 1355–1360 (2016).
533. Rothman, M. D., Leo-Summers, L. & Gill, T. M. Prognostic significance of potential frailty criteria. *J. Am. Geriatr. Soc.* **56**, 2211–2216 (2008).
534. Fried, L. P. *et al.* Frailty in Older Adults Evidence for a Phenotype. *J. Gerontol. A. Biol. Sci. Med. Sci.* **56**, M146–M157 (2001).
535. Mitnitski, A. B., Mogilner, A. J. & Rockwood, K. Accumulation of deficits as a proxy measure of aging. *ScientificWorldJournal* **1**, 323–336 (2001).

536. Rikli, R. E. & Jones, C. J. Development and Validation of a Functional Fitness Test for Community-Residing Older Adults. *J. Aging Phys. Act.* **7**, 129–161 (1999).
537. Beatty, A. L., Schiller, N. B. & Whooley, M. A. Six-minute walk test as a prognostic tool in stable coronary heart disease: Data from the heart and soul study. *Arch. Intern. Med.* **172**, 1096–1102 (2012).
538. Balady, G. J. *et al.* AHA Science Advisory. **8721**, 1591–1597 (2000).
539. Redberg, R. F., Whiteman, M. K., Flaws, J. a & Blumenthal, R. S. Cardiovascular and All-Cause Death in Asymptomatic Women. *Methods* **290**, 1600–1607 (2007).
540. Wilson Tang, W. H. & Hazen, S. L. The Gut Microbiome and Its Role in Cardiovascular Diseases. *Circulation* **135**, 1008–1010 (2017).
541. Ahmad, A. F., Dwivedi, G., O’Gara, F., Caparros-Martin, J. & Ward, N. C. The gut microbiome and cardiovascular disease: current knowledge and clinical potential. *Am. J. Physiol. Heart Circ. Physiol.* **317**, H923–H938 (2019).
542. Lau, K. *et al.* Bridging the gap between gut microbial dysbiosis and cardiovascular diseases. *Nutrients* **9**, (2017).
543. Jones, C. R., Taylor, K., Chowienczyk, P., Poston, L. & Shennan, A. H. A validation of the Mobil O Graph (version 12) ambulatory blood pressure monitor. *Blood Press. Monit.* **5**, 233–238 (2000).
544. Fuchs, F. D. & Whelton, P. K. High Blood Pressure and Cardiovascular Disease. *Hypertens. Dallas Tex 1979* **75**, 285–292 (2020).
545. Wu, C. Y. *et al.* High blood pressure and all-cause and cardiovascular disease mortalities in community-dwelling older adults. *Med. U. S.* **94**, e2160 (2015).
546. Agabiti-Rosei, E. *et al.* Central blood pressure measurements and antihypertensive therapy: A consensus document. *Hypertension* **50**, 154–160 (2007).

547. Waddell, T. K., Dart, A. M., Medley, T. L., Cameron, J. D. & Kingwell, B. A. Carotid pressure is a better predictor of coronary artery disease severity than brachial pressure. *Hypertension* **38**, 927–931 (2001).
548. Roman, M. J. *et al.* Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: The strong heart study. *Hypertension* **50**, 197–203 (2007).
549. Hametner, B. *et al.* Oscillometric estimation of aortic pulse wave velocity: Comparison with intra-aortic catheter measurements. *Blood Press. Monit.* **18**, 173–176 (2013).
550. Feistritzer, H. J. *et al.* Comparison of an oscillometric method with cardiac magnetic resonance for the analysis of aortic pulse wave velocity. *PLoS ONE* **10**, 1–12 (2015).
551. Weiss, W. *et al.* Oscillometric estimation of central blood pressure: Validation of the Mobil-O-Graph in comparison with the SphygmoCor device. *Blood Press. Monit.* **17**, 128–131 (2012).
552. Wassertheurer, S. *et al.* A new oscillometric method for pulse wave analysis: Comparison with a common tonometric method. *J. Hum. Hypertens.* **24**, 498–504 (2010).
553. Shoji, T. *et al.* Arterial stiffness predicts cardiovascular death independent of arterial thickness in a cohort of hemodialysis patients. *Atherosclerosis* **210**, 145–149 (2010).
554. Pereira, T., Maldonado, J., Pereira, L. & Conde, J. Aortic stiffness is an independent predictor of stroke in hypertensive patients. *Arq. Bras. Cardiol.* **100**, 437–443 (2013).
555. Choi, C. U. *et al.* Impact of Aortic Stiffness on Cardiovascular Disease in Patients With Chest Pain. Assessment With Direct Intra-Arterial Measurement. *Am. J. Hypertens.* **20**, 1163–1169 (2007).
556. Cruickshank, K. *et al.* Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: An integrated index of vascular function? *Circulation* **106**, 2085–2090 (2002).
557. Mattace-Raso, F. U. S. *et al.* Arterial stiffness and risk of coronary heart disease and stroke: The Rotterdam Study. *Circulation* **113**, 657–663 (2006).
558. Kim, D. & Ha, J.-W. Hypertensive response to exercise: mechanisms and clinical implication. *Clin. Hypertens.* **22**, 16–19 (2016).

559. Schultz, M. G., Hare, J. L., Marwick, T. H., Stowasser, M. & Sharman, J. E. Masked hypertension is ‘unmasked’ by low-intensity exercise blood pressure. *Blood Press.* **20**, 284–289 (2011).
560. Singh, J. P. *et al.* Blood pressure response during treadmill testing as a risk factor for new-onset hypertension. The Framingham heart study. *Circulation* **99**, 1831–1836 (1999).
561. Oguma, Y. & Shinoda-Tagawa, T. Physical activity decreases cardiovascular disease risk in women: Review and meta-analysis. *Am. J. Prev. Med.* **26**, 407–418 (2004).
562. Rodriguez, B. L. *et al.* Physical activity and 23-year incidence of coronary heart disease morbidity and mortality among middle-aged men: The Honolulu Heart Program. *Circulation* **89**, 2540–2544 (1994).
563. Kim, T. H., Jo, M. W., Lee, S. Il, Kim, S. H. & Chung, S. M. Psychometric properties of the EQ-5D-5L in the general population of South Korea. *Qual. Life Res.* **22**, 2245–2253 (2013).
564. Koh, D., Abdullah, A. M. K. Bin, Wang, P., Lin, N. & Luo, N. Validation of Brunei’s Malay EQ-5D questionnaire in patients with type 2 diabetes. *PLoS ONE* **11**, 1–9 (2016).
565. Dyer, M. T. D., Goldsmith, K. A., Sharples, L. S. & Buxton, M. J. A review of health utilities using the EQ-5D in studies of cardiovascular disease. *Health Qual. Life Outcomes* **8**, 1–12 (2010).
566. Nolan, C. M. *et al.* The EQ-5D-5L health status questionnaire in COPD: Validity, responsiveness and minimum important difference. *Thorax* **71**, 493–500 (2016).
567. Kwok, C. S. *et al.* Self-reported sleep duration and quality and cardiovascular disease and mortality: A dose-response meta-analysis. *Journal of the American Heart Association* vol. 7 Preprint at <https://doi.org/10.1161/JAHA.118.008552> (2018).
568. Manuscript, A. of Disease Study 2010. **380**, 2224–2260 (2014).
569. Kroenke, K., Spitzer, R. L. & Williams, J. B. W. The PHQ-9. **46202**, 606–613.
570. Blumenthal, J. A. Depression and coronary heart disease: Association and implications for treatment. *Cleve. Clin. J. Med.* **75**, 48–53 (2008).

571. Fiedorowicz, J. G. Depression and Cardiovascular Disease: An Update on How Course of Illness May Influence Risk. *Curr. Psychiatry Rep.* **16**, (2014).
572. Doherty, A. *et al.* Large scale population assessment of physical activity using wrist worn accelerometers: The UK biobank study. *PLoS ONE* **12**, 1–14 (2017).
573. Full, K. M., Kerr, J. & Grandner, M. A. Validation of a Physical Activity Accelerometer Device Worn on the Hip and Wrist Against Polysomnography. *Sleep Health* **4**, 209–216 (2018).
574. Hajduk, A. M. & Chaudhry, S. I. Sedentary behavior and cardiovascular risk in older adults: A scoping review. *Curr Cardiovasc Risk Rep* **1**, 139–148 (2017).
575. Bertisch, S. M. *et al.* Insomnia with objective short sleep duration and risk of incident cardiovascular disease and all-cause mortality: Sleep Heart Health Study. *Sleep* **41**, 1–9 (2018).
576. Gottlieb, D. J. *et al.* Association of usual sleep duration with hypertension: The Sleep Heart Health Study. *Sleep* **29**, 1009–1014 (2006).
577. Wolk, R., Gami, A. S. & Garcia-touchard, A. Sleep and Cardiovascular Disease. *Curr Probl Cardiol* 625–662 (2005) doi:10.1016/j.cpcardiol.2005.07.002.
578. Rikli, R. E. & Jones, C. J. Development and Validation of a Functional Fitness Test for Community-Residing Older Adults. *J. Aging Phys. Act.* **7**, 129–161 (1999).
579. Kehler, D. S. *et al.* Association Between Cardiac Rehabilitation and Frailty. *Can. J. Cardiol.* **36**, 482–489 (2020).

Appendix A – Fried and Standardized Fried Frailty Phenotype

Total Fried scores range from 0 to 5 depending on the number of variables present. These total scores are then used to stratify individuals into frailty groups (i.e. 0 = robust; 1-2 = pre-frail; ≥ 3 = frail).

	FPC	S-FPC
Weight loss	<i>In the last year, have you lost more than 10 pounds (4.54 kg) unintentionally? If answer is yes, this criterion is positive.</i>	

Exhaustion	<i>Using the CES-D, the following two statements are read asking how often in the last week did you feel this way?</i> - I felt that everything I did was an effort - I 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 <i>Persons answering “2” or “3” to either of these questions are categorized as positive.</i>	
Slowness	<i>Gait speed stratified by height</i> Height ≤ 159cm... ≤ 0.65 m/s Height ≤ 161cm... ≤ 1.10 m/s Height > 159cm... ≤ 0.76 m/s Height > 161cm... ≤ 1.15 m/s <i>If gait speed is lower than these cut-offs, the criterion is positive</i>	
Weakness	<i>Grip strength stratified by gender and BMI quartiles</i> BMI ≤ 23... ≤17 kg BMI ≤ 23.3... ≤21 kg BMI 23.1-26... ≤17.3 kg BMI 23.4-26.1... ≤22 kg BMI 26.1-29... ≤ 18 kg BMI 26.2-29.6... ≤ 22 kg BMI >29... ≤ 21 kg BMI >29.6... ≤ 22 kg <i>If grip strength is lower than these cut-offs, the criterion is positive</i>	
Low activity	<i>Kcal of leisure physical activity stratified by gender:</i> <270 Kcal <640.1 Kcal <i>If Kcal of leisure physical activity are under these cut-offs, the criterion is positive</i>	

Appendix B – WARM Hearts Protocol Manuscript

Protocol for the WARM Hearts study - Examining cardiovascular disease risk in middle aged and older women: A prospective, observational cohort study

Authors: Alexandra V Rose, Kevin F Boreskie, Jacqueline L Hay, Liam A. Thompson, Rakesh C Arora, Todd A Duhamel

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ABSTRACT

Introduction:

Cardiovascular disease (CVD) is a leading cause of death for women. Novel approaches to detect early signs of elevated CVD risk are needed. Although traditional CVD risk assessment approaches are effective, enhancement through the addition of non-invasive procedures to assess physical function or frailty as well as novel biomarkers of cardiovascular, gut and muscle health could improve early identification. The Women's Advanced Risk-assessment in Manitoba (WARM) Hearts prospective, observational cohort will examine a variety of non-invasive procedures and novel biomarker analyses to identify middle-aged and older women who are an elevated risk for adverse cardiovascular events in the 5-year period post-screening.

Methods and analysis:

One thousand women 55 years of age or older will be recruited using a convenience sampling approach and screened by the WARM Hearts protocol. The screening will include an assessment of medical history, body composition, cognition, frailty status through Modified Fried and Frailty Index approaches, functional fitness assessments, a 6-minute walk test, heart rate variability assessment, physical activity, sedentary behaviour and sleep patterns assessed by accelerometry, blood analyses to enable the measurement of lipid and novel biomarker profiles, stool samples to support the characterization of gut microbiota, resting blood pressure (BP), BP response to 3-minutes of moderate intensity exercise, a non-invasive measure of arterial stiffness measured by a Mobil-O-Graph device, physical activity questionnaires (Paffenbarger Physical Activity Scale, and the International Physical Activity Questionnaire), symptoms of depression through the Patient Health Questionnaire-9, quality of life through questionnaires like the EUROQOL Five Dimension Five Level and EuroQol Visual-Analog Scale, self-identified gender identity and gender variables collected through the GENESIS-PRAXY Gender Questionnaire, sleep behaviour assessed through the Pittsburgh Sleep Quality Index, and risk for impaired nutritional states assessed using the Seniors in the Community: Risk Evaluation for Eating and Nutrition Version II (SCREEN II) tool. The Framingham Risk Score will be calculated as the current clinical standard of assessing CVD risk in Canada. A data management plan will be implemented so the electronic data and bio-banked biological samples are accessible to collaborators. The incidence of the adverse cardiovascular outcomes (namely, ischemic heart disease, acute myocardial infarction,

stroke, percutaneous coronary intervention, coronary artery bypass surgery, congestive heart failure, and new cases of hypertension) will be assessed 5-years after screening using health administrative records housed at the Manitoba Centre for Health Policy Population Health Research Data Repository.

Ethics and dissemination

The University of Manitoba Health Research Ethics Board (Version 4.0 dated October 7th, 2019) and the St. Boniface Hospital Research Review Committee (Version 4.0 dated October 7th, 2019) approved the trial (ClinicalTrial.gov identifier NCT03938155). Recruitment for the cohort started October 10, 2020 and will be completed prior to September 30, 2021. Study status will be reported annually. A final study notification will be forwarded at completion of the study or in the event of early termination. The tenets of Good Clinical Practice will be followed. Data gathered from the WARM Hearts study will be published in peer-reviewed journals and presented at national and internal scientific conferences.

STRENGTHS AND LIMITATIONS

1. Strength-The Women's Advanced Risk-assessment in Manitoba (WARM) Hearts study will identify adverse cardiovascular outcomes 5-years after screening using the Personal Health Information Number of each participant and the administrative records housed by the Manitoba Centre for Health Policy Population Health Research Data Repository. This approach is superior to self-reported methods to document adverse CVD outcomes.

2. Strength- The WARM Hearts study will extensively characterize 1000 middle-aged and older women using traditional CVD risk scores, as well as additional non-invasive techniques to assess physical function, frailty status, physical activity, sedentary behaviour and sleep behaviour patterns, BP and arterial stiffness at rest and following moderate intensity exercise, as well as biomarkers of cardiovascular, gut and muscle health.

3. Strength- The WARM Hearts study will be recruiting exclusively women as well as measures of gender roles, relations and identity. This aligns with the recommendations made by the Heart and Stroke Foundation of Canada to improve heart health research by focusing on sex and gender in cardiovascular disease research where women have been underrepresented.

4. Limitation- The WARM Hearts study will use convenience sampling which has the inherent recruitment bias, in that participants who already may be active health consumers are more likely to participate.

Trial registration number: This trial has been registered on ClinicalTrials.gov, a registry and results database of privately and publicly funded clinical studies (NCT03938155).

Keywords: Cardiovascular disease, Risk factors, Gender, Women, Females, Frailty, Biomarkers, Accelerometers, Screening program, Early detection.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death for Canadian women¹³⁷. In the province of Manitoba, women tend to develop heart disease approximately 10-15 years later than males, with a prevalence rate similar between sexes after women turn 55 years of age¹⁴². Even so, 78% of women who report to a hospital with symptoms go undetected for early signs of a myocardial infarction (MI)⁵¹². Additionally, Canadian women, who have previously had an acute MI, were more likely to die within a 1 year period compared to men⁵¹³.

Current efforts in Canada for identifying people with an elevated risk of experiencing a future adverse CVD event (i.e. 10-year estimate of risk for a future heart attack or stroke) focus on traditional risk factors such as age, sex, smoking, cholesterol and blood pressure (BP)⁵¹⁴ using the Framingham Risk Score (FRS) approach⁵¹⁵. However, there is room to improve these methods as the FRS poorly discriminated between those who had onset CVD and those who did not, in an eight year follow up study of older adults^{514,516}. In fact, the absolute risk was markedly underestimated in women (51%) versus men (8%)^{514,516}. There is inadequate evidence and a lack of research that supports sex and gender specific guidelines for the treatment of CVD. This results in women being under-represented, underdiagnosed and under treated in Canadian healthcare system⁵¹³.

Implementing prognostic tools that examine parameters of physical functioning or frailty, assessment of cardiovascular function through pulse wave velocity (PWV) and central blood pressure, as well as novel biomarker analyses of cardiovascular, gut and muscle health may enhance CVD risk profiling. In fact, evidence indicates there is a relationship between frailty and CVD⁵¹⁷. Moreover, a recent pooled analysis in a cohort of 154,000 with a mean age of 71 years identified that frail older adults were at higher risk for CVD morbidity (RR 1.91, 95% CI 1.85-2.08) and CVD mortality (HR 1.91, 95% CI 1.77-2.05) over a median 3.2 year follow-up as compared to robust older adults⁵¹⁸. Additionally, novel blood-based biomarkers of CVD risk may provide additional value to risk prediction by identifying pathophysiological processes earlier⁵¹⁹. For example, our previous work identified myokines that may act as biomarkers of frailty-related CVD progression⁵²⁰. However, evidence is needed to establish that parameters such as these have prognostic value in CVD risk profiling.

The Women's Advanced Risk-assessment in Manitoba (WARM) Hearts study is a CVD screening program adapted from the Assessment of Large and Small Artery Elasticity for the Early Detection of Cardiovascular Disease trial (see ClinicalTrials.gov Identifier: NCT02863211) to include a broader number of non-invasive procedures to assess physical function, frailty status, physical activity, sedentary or sleep behaviour patterns as well as novel blood and stool-based biomarkers⁵²¹. Although the procedures utilized in the WARM Hearts screening protocol were selected based on literature, it is not known if the approaches are feasible and have sufficient predictive value to potentially implement within the Canadian healthcare system. Therefore, the objective of this prospective, observational cohort is to: (1) determine if individual markers of CVD risk collected in the WARM Hearts cardiovascular screening protocol identify women 55 years of age and older who are at an elevated risk for experiencing adverse cardiovascular outcomes within a 5-year period after screening; (2) to create a novel CVD risk score for women incorporating the best markers of CVD risk collected in the WARM Hearts study; (3) compare the predictive ability of the WARM Hearts protocol and the FRS for predicting future adverse cardiovascular outcomes in women. We hypothesize that the WARM Hearts protocol will provide better diagnostic accuracy in identifying middle-aged and older women at an elevated risk for experiencing adverse cardiovascular outcomes in the 5-year period post-screening, as compared with the FRS.

Potential impact of study:

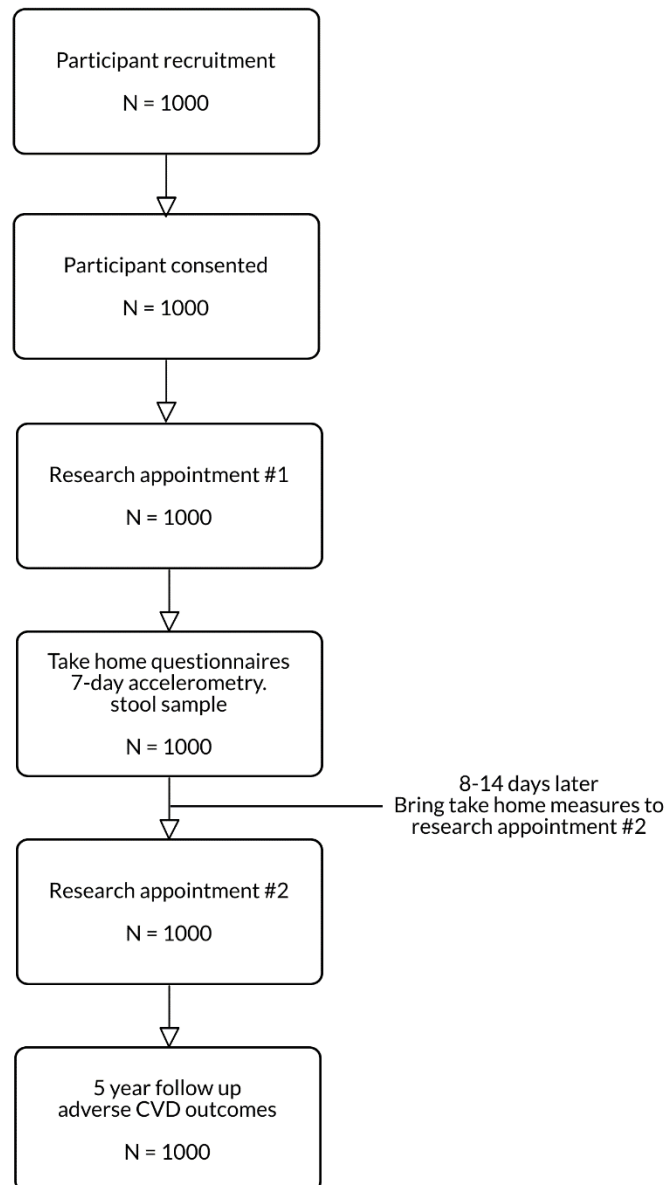
The WARM Hearts study may provide a novel CVD risk prognostic tool specifically for women. This prognostic tool could be considered for implementation in primary care healthcare settings if it was to demonstrate superior ability to screen women at elevated risk for future CVD compared to traditional methods.

METHODS AND ANALYSIS

Study design

The WARM Hearts protocol paper was developed based on the Standard Protocol Items: Recommendations for Intervention Trials (SPIRIT) guidelines⁵²². WARM Hearts publications will follow the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)⁵²³

and the Sex and Gender Equity in Research (SAGER)⁵²⁴ reporting guidelines. This research is using a prospective, observational design to determine if the use of novel CVD prognostic tools identifies women that are at high risk for experiencing an adverse cardiovascular event in the 5-year period after screening (Figure 1). A description of the trial was registered on ClinicalTrials.gov (NCT03938155).



Patient and Public Involvement- Patient engagement

A formal participant engagement process was not utilized in the design of the trial.

Study setting

The WARM Hearts study will be conducted in Winnipeg, Manitoba, Canada. Data collection appointments will be conducted at two sites to support the scheduling of appointments, namely the University of Manitoba Active Living Centre and at the St. Boniface General Hospital Asper Clinical Research Institute.

Recruitment

One-thousand women, aged 55 years or older will be recruited through newspaper articles, an online webpage describing the project, media interviews, poster advertisement or presentations at community events to generate a convenience sample. Women who are interested to learn more about the research will be instructed to contact the WARM Hearts team by email or telephone. The research team will then provide an overview of the study and assess potential participants for eligibility. Table 1 identifies the inclusion and exclusion criteria of the study.

Table 1. Inclusion and exclusion criteria

Inclusion criteria <i>(both need to be fulfilled for eligibility)</i>	Exclusion criteria <i>(any of the following result in ineligibility)</i>
3) Women aged 55 and older 4) Possess a Manitoba Personal Health Information Number	Pre-hospitalisation for: 1) Ischemic heart disease 1) Acute myocardial infarction 2) Stroke 3) Percutaneous coronary intervention 4) Coronary artery bypass surgery 5) Congestive heart failure 6) Peripheral artery disease Additional exclusion: 7) Previous participant in the Assessment of Large and Small Artery Elasticity for the Early Detection of Cardiovascular Disease (NCT02863211)

*Previous hospitalization or diagnosis for the listed conditions

WARM Hearts Protocol

Study Procedures

Eligible participants will be invited to attend two research appointments. The first appointment will be scheduled for 90 minutes at the University of Manitoba and used to secure written informed consent and Day 1 data collection. At the end of this first appointment, each participant will be provided an accelerometer, a stool sample collection kit and a Take-home assessment package. The participants will be asked to complete these protocols on their own schedule at home. A second research appointment will then be scheduled to occur 8-14 days after the first appointment at the St Boniface Hospital Asper Clinical Research Institute. This approach enables the participants to return the accelerometer, stool sample and Take-home assessment to the research team and to complete the Day 2 data collection protocol, which requires approximately 45 minutes to complete. Table 2 summarizes the data collected at each visit and in the Take-home assessment.

Table 2. WARM Hearts data collection design

Day 1 (University of Manitoba Active Living Centre)

Variable	Instrument/questionnaire
Consent process	Protocol and biological sample consent forms
Demographic information	Questionnaire (age, sex, gender)
Medical history	Questionnaire (past medical history, and cardiovascular related testing)
Cognition	Montreal Cognitive Assessment (MoCA) ¹¹
Body composition	Bioelectrical impedance analysis (InBody 270) ⁹ Waist circumference Body mass index
Grip strength	Hand dynamometer
5-meter walk test	Gait speed test
Fried Frailty Phenotype variables	Questions about self-reported exhaustion, unintentional weight loss. Physical activity levels assessed by the Paffenbarger Physical Activity Scale.
Physical function	Senior Fitness Test ¹²
Aerobic fitness	Six-minute walk test ¹⁰ with heart rate variability assessed with heart rate monitor and accelerometer
Personal Health Information Number	Collected via questionnaire

Take-home assessments*

Variable	Instrument/questionnaire
Demographic information	Questionnaire
Cardiovascular risk factors	Questionnaire (smoking status, medications, menopause status, history of cardiovascular disease)
Physical activity characteristics	Accelerometer worn for approximately one week
Physical activity levels	International Physical Activity Questionnaire ¹³
Gut microbiome	Stool collection kit sent home with participants
Quality of life	EuroQol Five Dimension Five Level ¹⁴ & EQ-VAS
Self-esteem	Self-Esteem Scale ²¹
Gender information	GENESIS-PRAXY Gender Questionnaire ¹⁵
Sleep quality	Pittsburgh Sleep Quality Index (PISQI) ¹⁶
Risk for impaired nutritional states	SCREEN II ¹⁷
Self-compassion	Self-compassion Scale ¹⁹
Health anxiety	Health anxiety subscale of the Multidimensional Health Questionnaire ²⁰

**completed on paper or via REDCap online survey*

Day 2 (Asper Clinical Research Institute)

Variable	Instrument/questionnaire
Collect accelerometer and stool samples	
Fasting blood sample	Phlebotomy
Depression symptoms	Patient Health Questionnaire-9 ²²
Arterial stiffness	Mobil-O-Graph® NG ²³
Resting blood pressure	Mobil-O-Graph® NG ²³
Blood pressure response to moderate exercise	Mobil-O-Graph® NG ²³

Demographic, medical history and gender

Demographic and medical history data will be collected via questionnaire. A process recommended by the Williams Institute ⁵²⁵ and supported by the Canadian Institute for Health Research (CIHR) Institute of Gender and Health ⁵²⁶ is used to collect sex and gender identity data. Specifically, a 2 question format similar to Tate et al.⁴⁷³ is used to ask participants: (1) What is your assigned sex at birth?; and (2) What is your gender Identity? [man, woman, transgender, two spirit or prefer to self-identify (open-ended response)]. Participants will also complete the validated gender-related score questionnaire ⁵²⁷ previously used by the GENESIS-PRAXY investigators to examine gender as a continuous variable⁵²⁸. Pelletier et al. have previously used this tool to identify the impact of gender and its association with risk factors in patients with premature acute coronary syndrome^{527,528}. It was found when categorizing according to gender-related scores, characteristics traditionally ascribed to women had a 5% rate of recurrent acute coronary syndrome, compared to 2% in the other two groups (masculine characteristics vs. masculine and feminine characteristics)⁵²⁸. These approaches are also being utilized because gender identity affects disease risk, health outcomes and how individuals seek health care independently of sex⁵²⁴. Gender differences in the CVD risk factor profiles have been historically under examined⁵²⁹.

Anthropometry and body composition

Body composition impacts CVD risk factors^{530,531} and cardiovascular mortality⁵³². Participant height will be measured on a portable Seca stadiometer to the nearest 0.5cm. Waist circumference will be measured in accordance with the Canadian Society for Exercise Physiology (CSEP) guidelines at the level of the superior iliac crest^{463,464}. Body composition, including skeletal muscle

mass and body fat mass, will be assessed through bioelectrical impedance analysis using the InBody 270 body^{465,466}.

Frailty status

Frailty will be assessed using two approaches. The Modified Fried Criteria (MFC)⁵³³ will utilize the traditional measures previously described by Fried et al.⁵³⁴, along with measures of depression and cognitive impairment. Briefly, this phenotypic MFC approach to frailty assessment classifies participants as frail if three or more of the following criteria are met: (1) unintentional weight loss, (2) self-reported exhaustion, (3) weakness, (4) slow walking speed, (5) low physical activity, (6) depression, and (7) cognitive impairment. Exhaustion is assessed by the Center for Epidemiologic Studies – Depression Scale⁴⁸¹. Self-reported physical activity levels are assessed by the Paffenbarger Physical Activity Scale⁴⁸² and unintentional weight loss is measured via a questionnaire. Gait speed is assessed by the 5-meter walk test⁴⁸³. Weakness is assessed by grip strength dynamometer⁴⁸³. Cognitive impairment is assessed using the Montreal Cognitive Assessment (MoCA)⁴⁷¹.

The second assessment of frailty will involve the creation of a frailty index (FI) using variables collected in WARM Hearts study. This assessment methodology is based on frailty being an accumulation of deficits⁵³⁵. The FI will be created according to the general guidelines discussed by Searle et al.⁴⁵⁸ Briefly, these guidelines involve the following criteria for variable selection: (1) deficits must have an association with health status; (2) selected deficit prevalence should increase with chronological age; (3) selected health deficits should not saturate too early; (4) selected deficits should cover a broad range of physiological systems; (5) frailty indices should be kept the same if planned to be used on the same cohort at multiple time points.

Functional Fitness Assessments

The Senior Fitness Test will be used to assess functional ability⁵³⁶. This test measures upper and lower body strength through arm curls and 30 second chair stand respectively. The 8-foot up-and-go measures agility and dynamic balance. Upper body flexibility is measured by the back-scratch test, while lower body flexibility is measured by chair sit and reach. Each test has an associated

risk zone threshold. Participants scoring below this threshold are considered at risk of loss for the associated functional ability.

6-minute walk test (6-MWT)

Participants are instructed to walk as much distance as possible on a 172.5 meter track over a 6-minute period⁵³⁷. The distance covered during the 6-MWT test is recorded. Walk-based testing is an assessment of functional exercise capacity⁵³⁸, which has been shown to influence risk of CV mortality in adult populations⁵³⁹.

Blood Sampling

A blood sample will be collected to examine traditional CVD risk biomarkers for use in the FRS as well as novel biomarkers of cardiovascular and muscle health. Fifteen (15) mL of blood will be drawn by a phlebotomist and separated into 4 tubes: a serum silica tube (1x4mL), a lithium heparin tube (1x4mL), and two EDTA tubes (2x3mL). Two aliquots of whole blood from the serum silica tube will be snap frozen and stored at -80°C. After allowing the whole blood to coagulate and centrifuging at 2000x g for 10 minutes, 2 aliquots of serum will be collected, snap frozen and stored at -80°C. Blood samples in the EDTA and lithium heparin tubes will be centrifuged at 2000 x g for 10 minutes and then the plasma will be snap frozen and 8 aliquots will be stored at -80°C. The planned blood analyses include: (1) total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and fasting blood glucose using one of the lithium heparin plasma samples. The additional blood samples of serum, plasma and whole blood will be stored at -80°C and saved in a biobank for the future analysis of novel biomarkers of CVD risk.

Stool sample collection

Emerging evidence suggests a link between the gut microbiome and the progression of atherosclerosis, hypertension, and heart failure^{540–542}. To characterize the gut microbiome in the cohort, stool will be collected using an EasySampler® stool sample kit (American Laboratory Products Company, NH, USA). Participants will be provided a stool sample collection kit following the completion of the Day 1 research appointment and asked to collect a small sample

in the provided container at some point during the following 8-14 days. Participants will transport their stool sample with an ice pack and then return the stool sample to the research team at the second appointment. The sample container will then be stored at -80°C prior to being aliquotted and processed. Stool sample aliquots will be extracted from the sample container using a disposable biopsy punch (Miltex®, Integra LifeSciences, NJ, USA). One aliquot will be processed and stored in a ZR BashingBead Lysis Tubes (Zymo Research, CA, USA), and sent to the National Microbiology Laboratory (Winnipeg, MB) to characterize gut microbiota via 16S rDNA sequencing. Three additional stool aliquots will be created and stored at -80°C.

Resting Blood Pressure

In accordance with Canadian Cardiovascular Society guidelines⁴⁶⁷, the BP measurement will be made at the left upper arm while the participant is seated using a validated oscillometric device (Mobil-O-Graph)⁴⁶⁹. The Mobil-O-Graph ambulatory BP monitor is validated for the non-invasive assessment of systolic, diastolic, and central BP⁵⁴³. Systolic and diastolic BP is a predictor of CVD risk⁵⁴⁴ and CV mortality⁵⁴⁵. Central BP, an indirectly assessed measure of BP at the ascending aorta, is thought to potentially be a more important measure of BP as it better reflects the pressure exerted on target organs^{137,546–548}.

For adults, a resting BP of 120/80 mmHg or less is considered normal, 120-139/80-89 mmHg is considered pre-hypertensive, and greater than 140/90 mmHg is classified as hypertension⁴⁶⁹. A resting BP of 160 mmHg systolic and 90 mm Hg diastolic and a heart rate of 100 beats per minute is being used as screening cutoffs for proceeding with the data collection appointments as recommended by the CSEP guidelines⁴⁶⁴. Participants whose values exceed these cutoffs are given 5 minutes to rest before the measurement is retaken. Scoring above the cutoff on two consecutive measurements terminates the appointment and participants are then recommended to visit their primary healthcare provider for an assessment.

Non-invasive measure of arterial stiffness

In addition to BP measurements, the Mobil-O-Graph assesses arterial stiffness. Using brachial oscillometry the Mobil-O-Graph calculates aortic pulse wave velocity (PWV) and additional central hemodynamic indices like augmentation index, the augmentation pressure divided by pulse

pressure multiplied by 100. PWV and the measures of central hemodynamics are estimated through analysis of the brachial cuff waveform and have acceptable accuracy as compared with intra-aortic readings⁵⁴⁹ and cardiac magnetic resonance imaging⁵⁵⁰. The Mobil-O-Graph's use has been validated in healthy individuals as well as patients with essential hypertension^{551,552}. Arterial stiffness is associated with CVD risk and is predictive of CVD mortality in a variety of populations^{553–557}.

Blood pressure response to 3-minutes of exercise

An exaggerated BP responses to exercise may be an indicator for new onset hypertension^{558–560}, CVD-related morbidity and mortality⁵⁵⁸. Following the measurement of resting BP as already described, the participant will stand up and complete 3-minutes of moderate intensity exercise (i.e.; a 5 metabolic equivalent (MET) workload) on a treadmill. Immediately following the exercise protocol (within 30 seconds), the participants will move to a seated position and have their BP measured again using the Mobil-O-Graph device. A normal BP response to exercise is defined as a rise in systolic BP of under 30 mmHg and an absolute systolic BP of less than 169 mmHg. A borderline response is defined as a rise of 30-39 mmHg and an absolute systolic BP of 170-179; whereas, an abnormal response is defined as a rise of 40 mmHg or more in systolic BP and an absolute systolic BP of 180 mmHg or higher. All participants with an abnormal BP response to 3-minutes of exercise will be advised to speak with their primary healthcare provider.

Questionnaires

Participants will complete a series of questionnaires:

7. The International Physical Activity Questionnaire (IPAQ) short version⁴⁷⁴ will be used to collect self-reported amount and intensity of physical activity completed in the last 7 days. Low levels of physical activity are linked to elevated CVD risk⁵⁶¹, CVD morbidity, and CVD mortality⁵⁶².
8. The EUROQOL Five Dimension Five Level (EQ-5D-5L) will be used to evaluate the dimensions of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. It is routinely used for this purpose in the general population⁵⁶³ and a variety of disease states^{564–}

- ⁵⁶⁶. The EuroQol Visual-Analog Scale (EQ-VAS) will also be used, as it provides complementary information of the patients' views of their health⁴⁷⁶.
9. Sleep quality and disturbances over a 1-month time interval are assessed using the Pittsburgh Sleep Quality index (PSQI)⁴⁷⁷. Previous research has suggested that abnormal sleep is associated with elevated risk for CVD⁵⁶⁷
 10. The Seniors in the Community: Risk Evaluation for Eating and Nutrition Version II (SCREEN II) will be utilized for the identification of risk for impaired nutritional states in community-living older adults⁴⁷⁸. Diet contributes to hypertension, diabetes, obesity, and CVD ⁵⁶⁸. Healthy dietary patterns influences the prevention of CVD⁵⁶⁸.
 11. The Patient Health Questionnaire-9 (PHQ-9) will be used to screen for depressive symptoms⁵⁶⁹. Depression as an independent risk factor for CVD⁵⁷⁰, and an association between depression and CV mortality⁵⁷¹ and CV morbidity⁵⁷⁰. PHQ-9 scores of 5, 10, 15, and 20 represent mild, moderate, moderately severe and severe depression respectively. Question 9 on the PHQ-9 screens for the presence of and duration of suicidal ideation. Participants with scores on this question in the moderately severe to severe range will receive a recommendation to contact a primary healthcare provider. Participants with scores in the severe depression range or whose response indicate thoughts of self-harm will be immediately directed to a healthcare provider. If the participant does not have access to a healthcare provider, they will be escorted to emergency care at the St. Boniface General Hospital.

Actigraphy

Actigraph wGTx+ accelerometers worn on the non-dominant wrist over a 7-day period will be used to measure physical activity, sedentary and sleep behaviour^{572,573}. Parameters collected by the Actigraph wGTx+ accelerometer will include raw acceleration, activity counts, steps taken, energy expenditures, body position, and sleep/wake measurements. Physical activity ⁵⁶¹, sedentary⁵⁷⁴ and sleep behaviour⁵⁷⁵⁻⁵⁷⁷ are associated with CVD risk or CVD outcomes in adults.

Framingham Risk Score

A multivariate risk factor algorithm described by D'Agostino *et al*¹³¹ is used to calculate the FRS for each participant. This tool is recommended for use in the clinical setting in Canada by the

Canadian Cardiovascular Society⁴⁶⁷. Age, sex, and smoking status are collected by questionnaire; whereas, fasting glucose, HDL, and total cholesterol measured from blood samples. BP is recorded as already described using the Mobil-O-Graph.

Adverse cardiovascular outcomes: 5-year follow-up

Five years after screening, the incidence of adverse cardiovascular outcomes will be assessed in the cohort using Personal Health Information Numbers (PHINs) and the health administrative records housed by the Manitoba Centre for Health Policy Population Health Research Data Repository. De-identified data from administrative records of Manitobans are stored in the repository with a corresponding scrambled identifier which allows the record to be linked in each of the datasets. The specific databases that will be accessed are outlined in Table 3. Resources including hospital separation abstracts, medical claims, and the Drug Program Information Network will also be accessed to provide information on medications usage, hospitalisations, diagnoses, and procedures related to cardiovascular disease. In addition, vital statistics from the repository will provide information on cause of death to allow for mortality rate tracking. We have defined adverse cardiovascular outcomes as hospitalization or death due to ischemic heart disease, acute myocardial infarction, stroke, percutaneous coronary intervention, coronary artery bypass surgery, congestive heart failure, or new cases of hypertension. International Classification of Disease (ICD) Codes will be used to identify the adverse outcomes listed in Table 4 as recommended by the MCHP. The data will then be used to determine if any of the screening approaches are predictive of elevated risk for experiencing an adverse cardiovascular event in the 5-year period after screening.

Table 3. Manitoba Centre for Health Policy data acquisition

Hospital Abstracts	Separation	Hospitalisation for cardiac diseases and cardiac procedures performed	Capture adverse cardiac events to test diagnostic accuracy of WARM Hearts risk score variables versus the Framingham Risk Score
Medical claims (Physician billings)		Diagnosis with cardiac diseases and procedures performed	Track adverse cardiovascular events
Other: vital statistics		Cause of death	Track mortality rates

Drug program information network	Cardiac and other medications used	Track the purchases of cardiovascular medications
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Table 4. Primary outcomes – adverse cardiovascular outcomes

Adverse cardiovascular outcomes	Example ICD codes
Ischaemic heart disease	ICD-9-CM codes 410 or ICD-10-CA codes I20-I22, I24, I25
Acute myocardial infarction	ICD-9-CM code 410 or ICD-10-CA code I21
Stroke	ICD-9-CM codes 431,434,436 or ICD-10-CA codes I61, I63, I64
Coronary artery bypass surgery	ICD-9-CM codes 36,10-36, 14 or CCI code 1.IJ.76
Congestive heart failure	ICD-9-CM codes 428 or ICD-10-CA code I50
Hypertension*	ICD-9-CM codes 401-405 or ICD-10-CA codes I10-I13, I15
Prescription of cardiac and other medications	ATC codes

ICD-9-CM, International Classification of Diseases, 9th revision, clinical modification; ICD-10-CA, International Classification of Diseases, 10th revision, Canada

** this outcome will only be used for those participants with no prior diagnosis of hypertension*

Data management

Initial WARM Hearts study data collection is expected to be complete in late 2021. Deidentified data will be available 6-months after that. Researchers wishing to access data should contact Dr. Todd Duhamel (tduhamel@sbr.ca; orcid.org/0000-0001-8467-4800). A data dictionary will be available upon request. An itemized list of the data being requested along with a brief proposal that outlines the rationale, hypotheses, and proposed analyses will be required. Since biological samples represent a finite resource, the proposed analyses will be considered with regard to ethical approval, feasibility and novelty. A Data Sharing Transfer Agreement or a Material Transfer Agreement must be signed and adhered to by collaborating parties and their respective institutions. Electronic data formats will be made available through the University of Manitoba Dataverse repository, following policies and procedures consistent with Tri-Council requirements.

Statistical analysis

Using estimations of adverse outcomes from published literature used in our previous large cohort CVD screening project, we predict 187-241 adverse health outcomes to occur in the 5-year period after the data collection in the cohort⁵²¹. This estimate is based on rates of ischemic heart disease, acute MI, stroke, congestive heart failure, new prescriptions for cardiac medications (ACE inhibitors and statins), and new diagnoses of hypertension from the Sex Differences in Health Status, Health Care Use, and Quality of Care: A Population-Based Analysis for Manitoba's Regional Health Authorities document⁴⁶⁷ and age stratified data from the Chronic Disease and Injury Indicator Framework⁴⁶⁷. Stepwise regression will be used to identify variables collected from the WARM Hearts study that provide additional statistically significant improvement of the model fit for predicting adverse cardiovascular events in a risk score tool at the end of the follow-up period. Comparative predictability between the WARM Hearts CVD risk tool and the FRS will be assessed using survival analysis techniques, such as Kaplan-Meier methods or a Cox proportional hazards model and calculating c-statistics. Model discrimination will be assessed using techniques like receiver operating characteristic curves and integrated discrimination improvement. Net Reclassification Improvement will be used to assess change in risk category for participants between our developed risk score and the FRS screening protocol. The number of estimated CVD-related outcomes will allow us to conservatively use 10 variables in our model. Distribution and end results may dictate analyses used.

Informed consent

The University of Manitoba Health Research Ethics Board (REB; Version 4.0 dated 7 October 2019) and the St. Boniface Hospital Research Review Committee (Version 4.0 dated 7 October 2019) approved the trial (ClinicalTrial.gov identifier NCT03938155). Minor editorial revisions to the protocol documents were approved by the REB on 22 November 2019, 27 February 2020 and 1 April 2020.

Individuals will be verbally informed of the study procedures by a research assistant and provided a copy of the patient information and consent forms. Each individual will have an adequate amount of time to consider their participation in the WARM Hearts study and will be given the opportunity to ask questions. After this process, if the individual decides to participate in the study, they will be asked to provide written consent upon arrival at their first research appointment. Participants

can decide not to participate in the study or to withdraw from the study at any time. Participants will be asked if they are willing to be contacted about future research studies. If they agree, they will be added to a database for this purpose.

Trial Monitoring and Safety

The research team is responsible for the study design, management, ethical conduct, analysis and dissemination of the WARM Hearts results. If a participant has a serious adverse event during the testing period, the protocol will be stopped. The participant will then be informed of the reasons for stopping the protocol. The appropriate steps will then be made to report the event to the University of Manitoba Health Research Ethics Board and the St. Boniface Hospital Research Review Committee.

End of study

The WARM Hearts recruitment and CVD risk screening data collection phases of the research will end when 1000 participants have been successfully enrolled, which should be accomplished prior to September 30, 2021. Cross-sectional data analysis to characterize the cohort and examine associations between variables will be explored at this time. Adverse cardiovascular event data will be accessed through the MCHP five years after the completion of screening. Analyses of the adverse cardiovascular event data will then occur.

Study status

Recruitment for the WARM Hearts study began in October 10, 2019. Four hundred and eighty (480) individuals were recruited to participate in the WARM Hearts trial as of March 23, 2020. Of these individuals, a total of 240 completed data collection. This date is relevant because it is when the University of Manitoba and St. Boniface Hospital suspended research activities with human participants due to the COVID-19 public health pandemic. Recruitment and data collection will recommence when public health officials in Manitoba and the institutions deem it is appropriate to relaunch the research. The cohort recruitment will be completed prior to September 30, 2021.

Author's Contributions:

TAD, KFB and JLH were involved in conception and design of research. TAD secured funding for the project. KFB, JLH, RCA and TAD obtained ethics approval. AVR, KFB, JLH and LAT drafted the manuscript. All authors edited and revised the manuscript. All authors approved the final version of the manuscript. AVR, LAT and research staff are assisting with data collection. TAD, KFB and JLH are managing the research. AVR and research staff are assisting with administration support.

Competing Interests:

The authors declare no conflicting interests.

Funding and Disclosures:

The study is being financially supported by an operating grant from the St-Boniface Hospital Foundation. KFB is supported by a Frederick Banting and Charles Best Canada Graduate Scholarship – CIHR Doctoral Award. JLH is supported by CIHR Vanier Scholarship. AVR is supported by a University of Manitoba Faculty of Kinesiology and Recreation Management Graduate Scholarship.

Data Sharing Statement:

Electronic data and biological samples can be made available to researchers following the completion of a data sharing agreement. Researchers with an interest to access WARM Hearts data or biological samples should contact Dr. Todd Duhamel.

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Appendix D – WARM Hearts Frailty Index

#	Domain	WARM Hearts assessment tool	Variable	Cut offs	Reference
1	Physical	Hand Dynamometer	Grip Strength (kg)	[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) ¹⁵
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) ⁵⁷⁸
3	Physical	5 M Gait Speed Test	Gait Speed (m/s)	[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) ¹⁵
4	Physical	Paffenbarger PA Questionnaire	Self Report PA (kcal/week)	1= < 270 kcal/week, 0= > 270 kcal/week	Fried (2001) ¹⁵
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) ⁵⁷⁸
6	Physical	Seniors Fitness Test	Back Scratch (cm)	[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) ⁵⁷⁸
7	Physical	Seniors Fitness Test	Sit and Reach (cm)	[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) ⁵⁷⁸
8	Physical	Seniors Fitness Test	8Ft Up and Go (s)	[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) ⁵⁷⁸

9	Physical	Seniors Fitness Test	6 Min Walk Test (m)	[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) ⁵⁷⁸
10	Body composition	Tape Measure	Waist Circumference (cm)	1=>88 cm, 0=< 88 cm	Kehler (2020) ⁵⁷⁹
11	Body composition	InBody	Fat Mass (kg)	1=<18.2 or >36.1, 0=18.2-36.099	Kehler (2020) ⁵⁷⁹
12	Body composition	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) ⁵⁷⁹
13	Psychological	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) ⁴⁷⁹
14	Psychological	Medical History	Clinical Diagnosis of Depression/Anxiety	1=yes, 0=no	
15	Cognition	MoCA	MoCA	1= < 25, 0= > 26	Nasreddine (2006) ⁴⁷²
16	Nutrition	Self Report Questionnaire	Unintentional Weight Loss	1=yes, 0=no	Fried (2001) ¹⁵
17	Nutrition	Self Report Questionnaire	Decline in Food intake	1=yes, 0=no	
18	Nutrition/QOL	SCREEN II	Is biting or chewing food difficult for you?	1=Often or always, .6666=Sometimes, .3333=Rarely, 0=Never	Keller (2005) ⁴⁷⁸
19	Nutrition/QOL	SCREEN II	Do you have any problems getting your groceries?	1=Always, .6666=Often, .3333=Sometimes, 0=Never or Rarely	Keller (2005) ⁴⁷⁸
20	Exhaustion	CES-D	Feel everything was an effort	1=Most (3), .6666=Moderate (2), .3333=Sometimes (1), 0=Rarely (0)	Fried (2001) ¹⁵
21	Exhaustion	CES-D	Could not "get going"	1=Most (3), .6666=Moderate (2), .3333=Sometimes (1), 0=Rarely (0)	Fried (2001) ¹⁵
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,	Janssen (2008)

				0.25=Slight, 0=None	
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) ²⁶
28	Comorbidities	Medical History	Arthritis	1=yes, 0=no	Kehler (2017) ²⁶
29	Comorbidities	Medical History	Sleep Apnea	1=yes, 0=no	Kehler (2017) ²⁶
30	Comorbidities	Medical History	Cancer	1=yes, 0=no	Kehler (2017) ²⁶
31	Comorbidities	Medical History	IBD	1=yes, 0=no	
32	Comorbidities	Medical History	Asthma	1=yes, 0=no	Kehler (2020) ⁵⁷⁹
33	Lab values	Blood Pressure	Resting Systolic BP	1= <90 or >140 mmHg, 0= 90-140 mmHg	Kehler (2020) ⁵⁷⁹
34	Lab values	Blood Pressure	Pulse Pressure	1= <30 or >60 mmHg, 0= 30-60 mmHg	Kehler (2020) ⁵⁷⁹
35	Lab values	Heart Rate Monitor	Resting HR	1= <60 or >99 bpm, 0= 60-99 mmHg	Kehler (2017) ²⁶ Kehler (2020) ⁵⁷⁹
36	Lab values	Blood Draw	Glucose (Fasting)	1=<3.9 or >6.1 mmol/L, 0=3.9-6.1 mmol/L	Kehler (2017) ²⁶ Kehler (2020) ⁵⁷⁹
37	Lab values	Blood Draw	Total Cholesterol	1= > 6.2 mmol/L, 0= < 6.2 mmol/L	Kehler (2020) ⁵⁷⁹
38	Lab values	Blood Draw	Triglycerides	1= > 1.67 mmol/L, 0= <1.67 mmol/L	Kehler (2020) ⁵⁷⁹
39	Lab values	Blood Draw	HDL Cholesterol	1= < 1.03 mmol/L, 0= > 1.03 mmol/L	Kehler (2020) ⁵⁷⁹

40	Lab values	Blood Draw	LDL Cholesterol	1= <0.98 or >3.36 mmol/L, 0= 0.98-3.36 mmol/L	Kehler (2020) ⁵⁷⁹
41	Sleep quality	PSQI	PSQI	1=5-21, 0=0-4	Buysse (1989) ⁴⁷⁷
42	Medications	# of Medications	# of Medications	1=>5, 0=0-4	

BMI, body mass index; **PA**, physical activity; **PHQ-9**, Patient Health Questionnaire-9; **QOL**, quality of life; **MoCA**, Montreal Cognitive Assessment; **SCREEN II**, Seniors in the Community: Risk evaluation for eating and nutrition Version II; **CES-D**, Center for Epidemiologic Studies Depression Scale; **EQ-5D-5L**, EuroQol 5-Dimension 5-Level; **EQ VAS**, EuroQol Visual Analogue Scale; **IBD**, inflammatory bowel disorder; **BP**, blood pressure; **HR**, heart rate; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein; **PSQI**, Pittsburgh Sleep Quality Index.