THE IMPACT OF ACUTE RESISTANCE TRAINING ON IRISIN IN YOUNGER AND OLDER ADULTS LIVING WITH OVERWEIGHT OR OBESITY

by

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Abstract

BACKGROUND: Exercise is a cornerstone for the prevention and management of overweight and/or obesity (OW/OB). Studies suggest that exercise-induced irisin impacts metabolism and health. However, no study has quantified the impact of biological aging on resistance training (RT)-induced increase in irisin.

OBJECTIVES: The purpose of this study was to determine whether irisin concentration would increase during an acute RT bout and to compare irisin release between younger and older adults living with OW/OB.

METHODS: Adults aged between 19-35 (25.9 ± 5.0; n=15) and 60-80 years old (67.7 ± 4.1; n=14) living with OW/OB participated in this study. The primary exposure variable was an acute bout of RT, which consisted of 3 sets of 12-15 repetitions at 65-70% of 1-Repetition Maximum and 3 minutes each of squats and step-box. The primary outcome measure was the concentration of irisin quantified by ELISA before, during, and after the acute bout of RT.

RESULTS: Significant differences were observed between younger and older adults in waist circumference, body fat, fitness levels, and muscle strength (all $p < 0.05$). However, no differences were observed in physical activity levels (young: 46.0 ± 45.5 vs. older adults: 31.2 ± 30.8 min.; $p > 0.05$) nor body mass index (young: 28.6 ± 4.0 vs. older adults: 29.8 ± 4.7 kg/m^2; $p > 0.05$). Repeated measures analyses showed no effect of time on irisin during acute RT, and no interaction effect between age and time ($p > 0.05$).

CONCLUSIONS: The results of the current study suggest that there is no impact of biological aging on the acute release of irisin during RT in individuals living with

OW/OB. Further studies are needed to elucidate the irisin response to acute exercise with different modalities/intensities of exercise.

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List of Abbreviations

ACSM	American College of Sports Medicine
ANOVA	Analysis of variance
AVO_{2diff}	Arterial-venous oxygen content difference
BMI	Body mass index
BP	Blood pressure
CCHS	Canadian Community Health Survey
CHMS	Canadian Health Measures Survey
CSEP	Canadian Society for Exercise Physiology
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FFM	Fat-free mass
FNDC5	Fibronectin type III domain-containing protein 5
FTO Gene	Fat mass and obesity-associated gene
GWAS	Genome-wide association studies
HDL cholesterol	High-density lipoprotein cholesterol
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Heart rate
IGF-1	Insulin-like growth factor 1
Kcal	Kilocalorie
LDL cholesterol	Low-density lipoprotein cholesterol
METS	Metabolic Equivalent of a Task

MVPA	Moderate-to vigorous-intensity physical activity
NAFLD	Non-alcoholic fatty liver disease
NEAT	Non-exercise activity thermogenesis
NHANES	National Health and Nutrition Examination Survey
OW/OB	Overweight or obesity
PPAR- γ	Peroxisome proliferator-activated receptor-gamma (γ)
PGC1-α	Peroxisome proliferator-activated receptor-gamma (γ) co-activator-1α
PGC1-α4	Peroxisome proliferator-activated receptor-gamma (γ) co-activator-1α isoform 4
RMR	Resting metabolic rate
RT	Resistance training
UCP-1	Uncoupling protein 1
VO_{2max}	Maximal oxygen consumption
VO_{2peak}	Peak oxygen consumption
WAT	White adipose tissue
WHO	World Health Organization
1-RM	1-repetition maximum

CHAPTER 1: Introduction

The growing epidemic of obesity has led to the substantial challenge of preventing, managing, and treating this chronic condition. Despite the overwhelming attention overweight and obesity has received in recent decades, the overall prevalence continues to rise worldwide. Today, approximately one in four Canadians are considered to be living with obesity, while 60% are overweight or obese. Unfortunately, these numbers translate to large costs to our health care system. As obesity is an important risk factor for further health complications, and the prevalence of the disease is expected to continue to rise worldwide, novel strategies to slow this rapid increase must be evaluated.

Physical activity and exercise has been recognized as a cornerstone in the management of excess body weight for individuals living with overweight or obesity. However, research shows that Canadian adults do not perform enough physical activity to reach the Canadian Physical Activity Guidelines. This may be related to the fact that the response to exercise interventions is impacted by substantial inter-individual variation. Although exercise generally leads to a number of cardio-metabolic health benefits, some individuals experience greater benefits than others. Furthermore, some individuals, when performing identical exercise regimes to those achieving benefits, will actually see declines in their cardio-metabolic health. As of now, the mechanisms underlying the cardio-metabolic response to exercise have not been fully elucidated. A number of factors, including myokines, contribute to the variation in the response. Myokines are contraction-induced cytokines that provide a mechanistic explanation for the benefits associated with exercise and physical activity in the prevention of metabolic diseases. A recently

discovered myokine, irisin, is regulated by an overexpression of peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC1-α) that is released from the skeletal muscle during exercise into the blood. This release activates thermogenic function in subcutaneous adipose tissue, which therefore increases energy expenditure, reduce body weight, and improve glucose tolerance. As such, it has been recognized as an attractive target in the treatment of obesity, diabetes, and other related metabolic disorders that are improved by exercise.

Since the discovery of this myokine, many studies have emerged that observe irisin and its relationship with exercise. Contradictory results regarding the impact of chronic exercise have led to the notion that irisin may respond to acute exercise rather than chronic. The physiological adaptations to chronic exercise may not be sufficient to keep irisin levels elevated beyond the acute effect of exercise. Furthermore, although it has been established that irisin production is mediated by mitochondrial biogenesis due to an increase in PGC1-α (which is principally observed during aerobic exercise), resistance training also induces this production, but to a smaller degree. As such, this suggests that other physiological pathways must be involved in its release, such as the increase of skeletal muscle mass. Interestingly, irisin has been shown to be associated with a number of key factors which must be accounted for in exercise studies, such as age, BMI, and physical activity level. Previous studies demonstrate that irisin is associated with age; however, current evidence demonstrates that it is inconclusive as to whether irisin increases with age or if it decreases. Similarly, evidence demonstrates that BMI is associated with irisin; but, again, the direction of the relationship is unclear. Furthermore, as physical activity levels are related to irisin release, disregarding this factor could affect

the observed results in previous studies. The current literature involving exercise studies with irisin is limited by the dismissal of many of the above key points. Disregarding these factors may have impacted the observed results in previous studies. As such, to avoid any potential confounding effects, these factors were accounted for in the proposed study. The current study involved both younger and older adults (to compare the effects of age), who were of similar physical activity level and BMI weight class.

The purpose of this study was to determine: 1) whether irisin release increases during an acute session of resistance exercise training in individuals living with overweight or obesity, and 2) whether changes in irisin concentration were different according to age.

CHAPTER 2: Review of the Literature

2.1 Obesity

2.1.1 Definition

In its simplest form, obesity is recognized as an excess proportion of adipose tissue relative to other tissues of the body. However, this newly defined disease [1,2] is, in fact, much more complex, and reflects the extreme difficulties that are being faced in combatting this worldwide epidemic. According to the World Health Organization (WHO), obesity is defined as an excessive accumulation of adipose tissue, which may lead to adverse health effects [3]. Recently, the American Medical Association declared obesity as "a disease state with multiple pathophysiological aspects requiring a range of interventions to advance obesity treatment and prevention" [1]. A number of organizations soon after followed suit by recognizing obesity as a disease, including the Canadian Medical Association [2] and the World Obesity Federation [4].

2.1.2 Body Mass Index

Overweight and obesity are quantified by an equation created by Keys et al. (1972), which was originally known as the Quetelet Index [5]. The term was further coined as Body Mass Index (BMI). BMI is calculated as a ratio of weight to height (kg/m^2), and is a measure that is correlated with an individual's total percentage of body fat content [6]. BMI was developed primarily to identify those who are at an increased risk of morbidity and mortality, and therefore to identify the according interventions required for treatment

[3]. Furthermore, the importance of a high BMI is emphasized by a greater risk of associated comorbidities and conditions. Adults who are classified as overweight (BMI of 25.0-29.9 kg/m^2) have an elevated risk of comorbidities, while adults who are classified as living with obesity (BMI of ≥30 kg/m^2) have a moderate to very severe risk of health complications [3], as demonstrated in Table 1. Obesity is further distinguished by three categories of increasing BMI: classes I-III. As an increased BMI translates to an increased risk of comorbidities and pre-mature mortality, the classification of BMI status is an integral clinical tool for the treatment of this disease. It is important to note that the BMI classification outlined below is specified for adults aged between 18 and 65 [3]. Beyond the age of 65, this classification system still applies; however, should be used with caution, as the 'normal' classification for this population may begin slightly above 18.5 kg/m^2 [7]. To elaborate, the optimal weight for survival increases with age [8], which suggests that an 'obesity paradox' may exist in older adults.

Table 1. BMI classification of adults (aged 18-65)

Classification	**BMI (kg/m^2)**	**Risk of comorbidities**
Underweight	<18.50	Low (but risk of other clinical problems increased)
Normal range	18.50 - 24.99	Average
Overweight:	≥25.00	
Pre-obese	*25.00 - 29.99*	Increased
Obese:	≥30.00	
Obese class I	*30.00 - 34.99*	Moderate
Obese class II	*35.00 - 39.99*	Severe
Obese class III	*≥40.00*	Very severe

WHO, 2000[3]

Although BMI provides a good indication of health on a population level, some limitations exist with respect to this clinical tool for assessing individual health risks. First, BMI provides information about the amount of adipose tissue in the body; however, it does not consider the wide variation in the distribution of adipose tissue [3]. Health risks vary greatly according to the distribution of fat, such that individuals with android obesity (excessive visceral fat), have different risks than those with a dissimilar distribution, such as gynoid obesity (excess fat in the lower extremity or periphery of the body) [3]. Second, BMI does not take into account the relative proportions of skeletal muscle mass compared to adipose tissue [3,9]. Skeletal muscle mass is considered part of an individual's fat-free mass, which also consists of non-skeletal muscle, organs, connective tissue, and bone [10]. Proportions of skeletal muscle mass vary according to the individual, especially within both the athletic and older aged populations [11]. Each of these factors contribute to inter-individual differences in the associated health consequences of obesity [3]. As such, the National Heart, Lung, and Blood Institute recommends that, in addition to BMI, waist circumference measurement should be included during the screening of adults living with obesity [12]. Waist circumference provides a more precise indication of the distribution of detrimental adipose tissue in the body (visceral fat); therefore, a high waist circumference increases an individual's risk of chronic disease [13]. Other methods may also be used for a more comprehensive assessment of health risks, including waist-to-hip ratio and skinfold measures [7,14], and furthermore the measurement of body composition, body fat distribution, energy intake and energy expenditure [3].

The relationship between BMI and all-cause mortality is demonstrated by a U- or J-shaped curve [15,16], demonstrating that not only do individuals living with obesity have an increased risk of mortality than normal weight individuals, but a lower BMI is also

associated with pre-mature mortality [17]. As BMI does not account for racial differences [11], separate BMI cut-points for different populations have been developed to consider the racial/ethnic differences in adipose tissue. Using a single BMI threshold would lead to overestimates or underestimates in the associated risks according to racial or ethnic group [18]. Separate BMI classification cut-points do not alter the individual's actual risks associated with obesity, but demonstrate that the risks are obtained at different cut-points. As such, individuals may experience obesity-related complications at lower/higher thresholds. For instance, the health risks and mortality associated with obesity in Asian populations occur at a lower BMI (≥ 23.0 kg/m^2 [19]), which shows that the relationship between BMI and all-cause mortality resembles a U-shape [16]. In summary, different BMI cut-points according to population should be recognized and the comparison of cross-sectional analyses of BMI should be analyzed with caution [3].

2.1.3 Prevalence

By the year 1983, the London Royal College of Physicians claimed that obesity had become a "substantial public health problem" [20]. Despite the overwhelming attention obesity has received in recent decades since this declaration, the overall prevalence of the disease continues to rise worldwide [21]. Between the years of 1980 and 2014, the world prevalence of obesity increased greater than 2-fold [22]. In the latter year, the WHO reported that approximately 13% of the world's adult population was living with obesity, while 39% (1.9 billion) was overweight [22]. Today, the epidemic of overweight and obesity remains substantial as it is recognized as the third greatest risk factor to health [23].

According to the 2008 Canadian Community Health Survey (CCHS) [24] and the 2007-2009 Canadian Health Measures Survey (CHMS) [25], one in four Canadians are living with obesity (24.1%) [26]. Furthermore, data from the 2012-2013 CHMS revealed that 26% of Canadians were classified as living with obesity [27]. The doubled prevalence in overall obesity observed in Canadians throughout the past 30 years [14,28] accurately reflects the worldwide trends. The prevalence of Canadians living with class III obesity ($\geq$40 kg/m^2), though, has tripled [14,28]. As the risk of comorbidities and pre-mature mortality rises with an increasing BMI [29], this statistic proves to be significant. Canadian obesity trends show that in 2011-2012, not only was the prevalence of obesity in New Brunswick (33.2%) greater than the national average, but this province was among the top three provinces with the highest obesity rates in the country[30]. At this time, New Brunswick had the highest rate of class II (6.1%) *and* class III (2.8%) individuals living with obesity in the country [31].

Among Canadians, variation in obesity status exists with respect to gender, age, and ethnicity. A greater proportion of men living with obesity is reported in comparison to women across all ages, with the exception of those aged 20-39 [32]. This trend is dissimilar to the global prevalence, in which men traditionally present a lower prevalence of obesity than women [22]. It may be argued that this trend occurs because women are evolutionarily predisposed to higher body fat contents to prepare for reproduction and lactation [33]. As for age differences, trends demonstrate that the prevalence of obesity steadily increases as one ages, until the age of 65, thereafter which the prevalence then proceeds to decline [14]. Data pertaining to differences between ethnicity show that obesity is higher among Aboriginal populations compared to non-aboriginal populations [34,35].

Approximately 37.8% [24] of aboriginal adults were considered to be living with obesity, which was higher than the remainder of the population at the time (22.6%) [24,34]. Data from earlier versions, such as the CCHS 2000-2001 reported significant differences between ethnic groups. Tremblay et al. (2005) [35] demonstrated that Aboriginal individuals had increased odds of overweight and obesity compared to white individuals (independent of age, income, education, and physical activity), and had the highest prevalence of all ethnicities.

When comparing the prevalence of obesity among Canadians to citizens of other developed countries, such as the United States, a number of differences exist. Data from the National Health and Nutrition Examination Survey (NHANES) between 2011-2014 demonstrate that approximately 36.5%[36] of Americans are classified as living with obesity compared to 26.0% of Canadians [27]. Similar to the global trends, but dissimilar to Canadian trends, obesity within Americans is seen at a higher rate in women than in men [36]. The most troubling statistic lies within the marked increase in individuals living with class III obesity that has been seen in both Americans (1.7-fold increase between 2000-2010 [37]) and Canadians (3-fold increase between 1979-2004[14,28]). Collectively, both the prevalence and severity of obesity has increased significantly globally [22], while certain sub-populations have experienced with evidence of "exacerbated" growth in certain sub-populations".

2.1.4 Etiology

2.1.4.1 Energetic Balance

Obesity is a complex and still poorly understood disease. The diverse etiology of obesity is clearly demonstrated within the "Obesity System Influence Diagram" [38,39]. This obesity map encompasses components which have a prominent role in influencing the current obesity epidemic. The mechanisms involved are "interactive, homeostatic, and still poorly understood" [6]. Although the causes for obesity are multifactorial, the basic foundation of body weight regulation can be credited to energy imbalance [38-40]. When energy consumption is greater than energy expenditure, the net result is an energy imbalance resulting in weight gain [11]. This imbalance, more specifically, generates a greater energy storage in the form of triacylglycerol in adipocytes (fat cells).

The concept of energetic balance is demonstrated through the first thermodynamic law: energy cannot be created or destroyed, but is conserved, and can only be transformed from one form of energy to another [41]. This applies to weight regulation, in the sense that "body weight cannot change if, over a specified time, energy intake and energy expenditure are equal" [42]. In order to lose body weight to counteract obesity, the energy balance must shift negatively, which requires energy to be metabolized.

2.1.4.2 Food Consumption

A large contributor to the obesity epidemic, diet, has created a shift to a positive energy balance in individuals [43]. Over the last few decades, problematic dietary behaviour has emerged as a result of changes in food consumption, production, and availability [44]. There has been an increase in the consumption of sugar-sweetened beverages, refined

carbohydrates [40], and fats [44]. The choice to consume these foods has been promoted by the increased production of these foods, along with a greater availability of a variety of these unhealthy foods. Further contributing factors include the growth of the fast food industry, the proliferation of social media providing an enhanced vehicle for advertisers, as well as the larger portion sizes and lower cost associated with these food items [44]. The low cost and high convenience of dense, calorie-rich food has contributed to consumption pattern changes thereby leading to unhealthy diets and increased energy intake [43]. Food environments have been proposed as a significant negative contributor to the obesity epidemic, with the expansion of food availability and marketing occurring simultaneously with the global increase in body weight [42].

NHANES [45] data containing information on daily energy intake showed a marked increase between the years of 1971 and 2000 [42]. An average increase of 168 kilocalories (kcal)/day for men and 335 kcal/day for women was observed within this time frame [45]. According to Hill et al. (2012), this alteration could translate to a weight gain of 18 lbs for men and 35 lbs for women each year [42]. Evidently, a change of this magnitude has had a substantial effect on the shift in energetic balance observed throughout this period in time.

An important regulator of food consumption relates to satiety signalling. Satiety - the sensation of fullness - is regulated through the communication of the visceral sensory thalamus with the visceral sensory cortex through projections of the nucleus of the solitary tract [46]. It is theorized that a feedback loop regulates feeding, where the hypothalamus provides long-term regulatory input to the nucleus of the solitary tract (the set-point). Satiety signals, on the other hand, act as direct short-term regulators for feedback input to the nucleus [47]. These factors cause the rate at which one eats to be higher or lower (positive or negative) than the set-point [48]. The set-point satiety center in the brain detects alterations

in energy stores which then cause metabolic responses to maintain energy balance [46]. As demonstrated by Zanutto et al. (2007), long-term regulation with hypothalamic input differs from short-term regulation with satiety signals. Long-term regulation allows the control of body weight; however, as satiety signals vary frequently, they control the patterns of one's meal [47]. Adults tend to have a constant body weight; therefore, in order to make alterations, a large change in this set-point would be required [49]. In conclusion, although this theory has been well documented, some demonstrate that the set-point is not very tightly controlled in humans [49], and advanced theories for weight control regulation have been proposed in its place.

2.1.4.3 Energy Expenditure

Energy expenditure is one of the basic components of energy balance [50]. Individuals expend energy through three main categories: resting metabolic rate (RMR), the thermic effect of food, and physical activity [42]. RMR is the largest contributor to total daily energy expenditure, with a 60-75% influence [51]. Essentially, an individual's RMR is the amount of energy (kcals) required to fuel the body at rest to support basic functions, which is proportional to an individual's body mass [42]. Past the age of 20, RMR decreases two percent in women and three percent in men each decade; however, women physiologically have a 5-10% lower RMR than males of the same weight and height due to their decreased proportion of fat-free mass and corresponding greater proportion of fat mass [51,52]. In an average 70-kg man, ~1,500 kcals would be expended each day due to RMR [53]. This demonstrates the significance of RMR on energy expenditure, as this individual's total energy expenditure (including all components) is ~2900 kcals/day [54].

The thermic effect of food represents the energy expenditure above the RMR in response to food consumption [53] that increases energy metabolism [51,55]. The increased energy expenditure is generated as a result of digestive processes associated with eating, including the energy costs of absorption, metabolism, and the storage of the food within the body [53,55]. This process contributes to about 8-10% of total daily energy expenditure [42,51,56], depending on the macronutrient ingested. Variations in the energy cost of food processing occur due to differing metabolic rates, where the cost is the lowest for fats, and highest for protein and lipogenesis from carbohydrates [53]. The thermic effect of food is categorized as an obligatory metabolic process of overall thermogenesis. However, the overall thermogenic response to food also includes a distinct facultative metabolic process which is controlled by the nervous system that expends energy for thermogenesis in brown adipose tissue: diet-induced thermogenesis [57].

A large body of evidence has explored the controversial relationship between the thermic effect of food and obesity. Many studies have reported that this component of energy expenditure is typically lower in individuals living with obesity compared to lean individuals [58-63]. However, many other studies have shown that the thermic effect of food is not reduced in individuals living with obesity [64-66]. Therefore, no clear consensus exists as to whether the thermic effect of food is reduced in obesity or not [67].

Finally, energy expenditure is affected by the energy cost of physical activity. This component is the most variable part of energy expenditure [51] as it is dependent upon individual activity [42]. It is suggested that the energy cost of physical activity can range from 5-40% [51]. Activity thermogenesis can be separated into two subcategories, which include the energy expended as part of physical activity or of non-exercise activity

thermogenesis (NEAT) [68]. Regardless of activity level, NEAT is the predominant component of this category, and is recognized as the energy expended for all activities other than exercise, eating, and sleeping (e.g.: sitting, standing, occupational or leisure activity, etc.) [69]. NEAT likely contributes to the variation in energy expenditure, which is reflected by the fact that minor differences in activity alters daily energy expenditure by as much as 20% [70]. The variability in the energy expenditure of physical activity also relates to body movement and body size [71]. Individuals living with obesity often have less body movement than lean individuals, as a greater amount of energy from the muscles is required to produce movements in a larger sized body [71]. Overall, energy expenditure has a complex interaction with physical activity, body weight, and body composition [71].

It may be argued that energy expenditure has had an impact on the obesity epidemic. Westerterp and Speakman (2008) reported that physical activity energy expenditure did not decrease during the time that obesity increased, proving an unlikely impact of energy expenditure on the obesity epidemic [72]. However, Church et al. (2011) note that although leisure time physical activity has remained unchanged during that time period, this only represents a very small portion of the hours in a week. They did find that occupation-related physical activity energy expenditure has declined by greater than 100 kcals/day in the U.S. throughout the last 5 decades [73]. This value is substantial, considering the fact that the hours at work constitute the largest segment of waking hours during the week. Based on this observation, they claim that this has greatly impacted the corresponding increase in body weight seen over the same time period [73], and has led to obesity and health-related consequences.

In an effort to increase total energy expenditure and decrease body weight, energy balance must be shifted negatively. Although physical activity is not the largest

component of the energy expenditure balance equation, it is the most variable, and is arguably the most easily modifiable in most individuals [51,73] (with the exception of those who are impacted by exercise intolerance, medications, or chronic conditions that impact physical activity energy expenditure). For instance, an energy output 3500 kcals greater than intake leads to a weight loss of 1 pound [51]. Exercise programs can be designed using this ratio in an attempt to increase energy expenditure, which leads to changes in weight. On the other hand, altering energy expenditure through RMR and the thermic effect of food is an inferior strategy. The thermic effect of food only accounts for a small portion of energy expenditure [53] and increasing energy expenditure through RMR requires a substantial increase in muscle mass, as it is the main contributor of RMR [68,74].

2.1.4.4 Genetics & Heredity

The etiology of obesity can also be, in part, explained by genetics and heredity. Genetics have a substantial contribution in the pathogenesis of the disease, with a ~40-70% influence [75-78]. Initially, obesity was thought to stem from Mendelian inheritance; however, further developments demonstrated that genetics play a far more complex role in the disease [79]. In terms of genetics, obesity is classified according to three categories: monogenic, syndromic, and polygenic (common) obesity [80]. The monogenic form of obesity (of Mendelian inheritance) is extremely rare (affects 5% of the population [81]) and results from a single gene defect [79]. Syndromic obesity, on the other hand, involves genetic defects or chromosomal abnormalities from multiple gene sources [80]. Polygenic obesity, the most common form of obesity, results from defects in several genes/loci, such that there is a "simultaneous presence of DNA variations in multiple genes" [80]. The loci

involved have considerable inter-individual variation, increasing the complexity of the study of common obesity [82,83].

Through the use of genetic epidemiological approaches, such as genome-wide association studies (GWAS), a number of genes associated with obesity have been identified. The fat mass and obesity-associated (FTO) gene was the first locus unequivocally associated with obesity with a GWAS [82,84]. FTO is specifically associated with an increase in BMI. Since this discovery, enhanced GWAS were performed to search for more loci susceptible to obesity. Today, more than 52 genetic loci are associated with at least one obesity-related trait [82,85,86] (BMI, body fat percentage, or abdominal obesity itself [82,87,88]). However, of all loci identified, FTO remains the locus the most susceptible to obesity [82]. Another gene recognized to impact obesity is the *'ob'* gene that codes the hormone leptin (key hormone in weight regulation) at chromosome 7, which suggests obesity is in part regulated by genetics when mutations of this gene occur [89,90]. Although genetics studies have advanced, there is still little known about the *specific* genes involved in causing polygenic forms of obesity, and which mechanism(s) leads to the expression of the disease [82]. Nevertheless, the expression of these genes in humans prove that obesity is, in part, mediated by genetics or epigenetics.

In an attempt to demonstrate the genetic influence of obesity, twin studies have been performed to model the genetic component of specific traits [82]. Feinleb et al. (1977) compared 250 monozygotic twin pairs (who are genetically identical) and 264 dizygotic twin pairs (who share 50% genetic material) and from these results, they provided the first evidence that the "familial aggregation for obesity was due to genetic factors rather than the environment" [91,92]. A similar study with a larger cohort [78] confirmed these results, while adoption studies strengthened these results by showing a strong genetic influence

on body weight between children and their biological parents (compared to their adoptive parents) [92,93]. Finally, Bouchard et al. (1990) observed the effects of over-feeding on weight gain, determining that the correlation of weight gain between monozygotic twins was high (r >70%) [92,94], furthermore demonstrating the strength of genetics and heredity. In each of these studies, although data showed strong evidence for the effect of genetics on obesity, some monozygotic twins responded differently. As their genetic makeup is identical, this proves that other factors influence obesity. Regardless, having a greater understanding of the genetics of polygenic obesity allows for an increased understanding of the pathogenesis of the disease and advances the potential for pharmaceutical treatments to be made [92].

Finally, the impact of genetics on the development of obesity is highlighted by the role of the gastrointestinal (gut) microbiome [95,96]. The gut microbiome, which consists of microorganisms encoded by our genes, performs specific gastrointestinal functions that are unable to be performed by the host (i.e.: human) themselves [95]. A large body of evidence suggests that alterations to the normal composition and function of the gut microbiome predispose their host to the development of obesity [95,97,98]. In a meta-analysis, Okeke et al. (2014) found that alterations to the gut microbiome have been involved in a number of processes which impact obesity and adiposity: the development of chronic inflammation, fat storage, and abnormal glucose response [95]. A number of mechanisms have been proposed to contribute to the development of obesity, including the enhanced absorption of nutrients and reduced activity of fasting-induced adipose factor [99], AMP-activated protein kinase protection in germ-free adult mice [100], as well as inflammation and increased gut permeability [97,101]. Strong evidence demonstrates that the gut microbiome has a significant role in the development of obesity [102]. Intriguingly, Bäckhed

et al. (2004) found that within two weeks of transferring normal cecal microbiota from conventionally raised mice to germ-free adult mice, the recipients experienced a 57% increase in total body fat content and 61% increase in epididymal fat weight, despite reduced food intake [99]. Nevertheless, more research is required to further understand the role of the gut microbiome in obesity.

2.1.4.5 Obesogenic Environment

Major environmental changes that individuals have experienced over the last few decades have been recognized as one of the main determinants of the increase in prevalence of obesity [40]. Obesogenic environments are recognized as physical environments that promote weight gain and obesity through the normalization of increased caloric intake and decreased energy expenditure [103]. These types of environments have resulted from a combination of behavioural and environmental determinants regarding energy imbalance, which, as a result, have negatively shifted the energy balance in individuals and populations across the globe. The negative dietary and physical activity behaviours of those immersed within obesogenic environments have been impacted by both the changing built environment and food environment in today's society [104].

The effects of diet and physical activity on an individual's predisposition to obesity have been confirmed through interesting findings from Ravussin et al. (1994) with the lifestyle of Pima Indians. Individuals of this heritage were compared within extremely contrasting environmental conditions and lifestyles. More specifically, Pima Indians of Arizona living in an "affluent" environment, who are in a high-risk group of obesity [92], were compared to members of Pima ancestry in a remote location in Mexico who live a

"traditional" Pima Indian lifestyle. The group who followed the traditional lifestyle, characterized by a healthy diet (low in animal fat and with more complex carbohydrates) and an increased energy expenditure in positive environmental conditions had a significantly lower BMI and a more favorable metabolic profile compared to their counterparts, regardless of their similar genetic predisposition to obesity [105].

Obesogenic environments have also contributed to an increased prevalence of sedentary behaviours [106] and decreased physical activity, especially through changes within the built environment. Factors contributing to this change include: increasing industrialization and urbanization, a greater reliance of mechanized transportation rather than walking, advancing technology, and the increasing sedentary nature of work forms [22,42].

Other contextual factors that impact the negative health behaviours within an obesogenic environment include: social economic status, geographical location, gender, age, cultural identity, and family compositions [11].

2.1.5 Metabolic Consequences

Obesity in and of itself is associated with a higher risk of mortality; however, it is also related to an increased risk of numerous health risk factors [107]. The development of obesity can be counteracted with some lifestyle modifications. Physical activity, for instance, is used as both a preventative measure, to reduce the chances of developing obesity [108], and as a form of treatment, to reduce body weight [109]. The greatest benefits are seen when combining a healthy diet with lower kcals and performing physical activity to expend more kcals, which create a caloric energy deficit, therefore promoting weight loss.

Maintaining or increasing proper physical activity and diet behaviours are essential, not only to prevent obesity, but also obesity-related comorbidities, including: cardiovascular and endocrine disease, as well as certain cancers [110].

Cardiovascular – Excess weight creates an increased cardiovascular risk due to metabolic changes affecting lipid metabolism and chronic inflammation [110]. Adipocytes exert pro-inflammatory endocrine effects [111], and abnormal lipid metabolism increases the risk for cardiovascular events [110]. Individuals living with obesity typically have increased low-density lipoprotein (LDL) cholesterol particle numbers, and decreased high-density lipoprotein (HDL) cholesterol particle size. The Framingham Heart Study showed that individuals living with overweight or obesity had an increased risk of both cardiovascular disease and cardiovascular risk factors (hypertension, hypercholesterolemia, and Type 2 diabetes (T2D) [112]. Increased blood pressure in overweight individuals increases the risk of hypertension [113], which may lead to cardiac failure [114]. Moreover, Meigs et al. (1997) showed that any increase in weight increases an individual's risk of heart disease, regardless of the initial BMI [115]. Furthermore, in individuals with a BMI ≥ 29 kg/m^2, the risk of coronary artery disease increases by 3.3-fold compared to individuals with a normal body weight [114,116]. Conclusively, this demonstrates that obesity leads to a greater risk of cardiovascular events. However, many studies have found that an 'obesity paradox' may exist with respect to cardiovascular health [117-121], where cardiovascular disease patients living with overweight or obesity have a better prognosis than their leaner counterparts. For example, in a large meta-analysis, Sharma et al. (2015) observed the highest risk of adverse cardiovascular events and hospitalization in heart failure patients with a low BMI (BMI < 20 kg/m^2) while the lowest risk was observed in those who were overweight (BMI

= 25.0-29.9 kg/m2) [122]. Lavie et al. (2009, 2013, 2014) also strongly suggests that heart failure patients living with obesity have a better prognosis than their leaner counterparts [117,120,121].

Endocrine – The relationship between obesity and T2D has been clearly documented [110,114,123]. BMI may be a significant predictor in the development of T2D [110,124]. Furthermore, T2D is strongly associated with overweight across all ethnic groups and sexes [125,126], such that risk increases with increasing BMI. Women who had a BMI ≥ 35 kg/m^2 in the Nurses' Health Study [126] had a 40-fold (4000%) increase in risk of T2D.

The pro-inflammatory endocrine effects of adipokines have the potential to increase the risk of T2D [123]. Insulin resistance in the liver, muscle, and adipose tissue may result from an increase in these pro-inflammatory cytokines [111]. For example, the adipokine interleukin-6 exacerbates insulin resistance [123,127], and tumour necrosis factor-α reduces insulin sensitivity [128]. The levels of both of these adipokines are increased in those with visceral obesity, and therefore might support an increased risk of T2D.

Cancer – The risk of developing certain forms of cancer is augmented in individuals living with obesity [110]. A significant amount of research has analyzed the risk of cancer and excess body weight. A prominent meta-analysis in the *Lancet* performed by Renehan et al. (2008) observed the risk of cancer with an increase in BMI. Each 5 kg/m^2 unit increase in BMI showed strong associations with colon, and renal cancers in men, and endometrial, gallbladder, and renal cancers in women [110,129]. Furthermore, in 2016, the International Agency for Research on Cancer confirmed that the absence of excess body fat decreases an individual's risk of cancer [130]. When compared to the individuals of normal body weight, individuals living with overweight and obesity individuals had a 20

to 50% increased risk of colon, gastric cardia, liver, gallbladder, pancreas, and kidney cancer [129,131-136]. More substantially, those with a BMI ≥ 40 kg/m^2 had an increased risk of oesophageal adenocarcinoma of about 5-fold [137]. Although individuals living with obesity are more likely to be diagnosed with certain types of cancer as compared to normal weight individuals, dissimilar trends have been observed in terms of prognosis and efficacy of cancer treatment. Paradoxically, some studies suggest that high BMI is associated with improved survival in cancer patients [138-140]. However, others reported the opposite [141-143]. As such, the role of BMI and this obesity paradox in the prognosis of cancer remains inconclusive [144].

In conclusion, obesity is a complex and incompletely understood disease. If not treated properly, obesity puts an individual at an increased risk for premature mortality and other chronic diseases [107]. Not only is obesity associated with increased cardiovascular risk, T2D, and certain cancers, but it is also associated with a multitude of other metabolic consequences [114]. For the purposes of this thesis, the remaining diseases and metabolic consequences were not discussed in detail.

2.2 Physical Activity

2.2.1 Definition

Although often used interchangeably, the terms physical activity, exercise, and physical fitness describe different concepts [145]. As such, it is important to understand the distinction of these terms.

Physical activity refers to all body movements produced by skeletal muscles resulting in energy expenditure above the resting state [29,145]. An individual who is walking, swimming, or gardening, is said to be performing physical activity. Physical activity may be performed with the intention of improving their health, fitness, or performance and is done at differing intensities [146]. The amount of energy expended through physical activity is most commonly expressed according to kcals or in metabolic equivalent of a task (METS) [145,147]. In terms of METS, the Compendium of Physical Activities is a resource used to better estimate and classify the energy costs of physical activity. For instance, light-intensity physical activities are those that don't reach sweat production or shortness of breath [148], and are 1.6-2.9 times the intensity of rest in both adults and older adults, which range between 1.6-2.9 METS [147,149]. Moderate-intensity and vigorous-intensity physical activities reach higher METS (moderate = 3.0-5.9 METS; vigorous = ≥6.0 METS) [150] and are the intensities which are recommended in the physical activity guidelines (see section 1.2.2 below).

Exercise is recognized as a sub-category of physical activity [145], and is defined as planned, structured, and repetitive body movements done to improve or maintain one or more components of physical fitness [145,146]. Physical fitness refers to a set of attributes that people have or achieve that are either health- or performance-related [145,146]. The health-related components of physical fitness include improving one's cardiorespiratory endurance, muscular strength and endurance, or flexibility [146]. Physically fit individuals are described as those who have "the ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy leisure-time pursuits and to meet unforeseen emergencies" [151]. Both exercise and physical fitness fall under the category of physical activity for fitness [146]. Most sports and athletic conditioning programs

aim to improve physical fitness; however, when they are planned, purposeful, and repetitive, this is constituted as exercise [145]. Structured physical activities for adults and older adults may include yoga, running/hiking, water aerobics, or fitness classes, etc.[148].

2.2.2 Physical Activity Guidelines

Since the year 1995, the Canadian Society for Exercise Physiology (CSEP) and the Public Health Agency of Canada have collaborated to develop guidelines in an attempt to promote healthy active living among Canadians [152]. The most recent update was released by CSEP in 2011, which included recommendations for children, youth, adults, and older adults, respectively. For the purposes of this thesis, only the latter two will be discussed.

The current physical activity guidelines for adults (18-64 years old) consist of the following: 150 minutes of moderate-to-vigorous-intensity aerobic physical activity per week (in bouts of 10 minutes or more) and two days per week of muscle and bone strengthening activities using major muscle groups. Although these recommendations are in place, they encourage that more physical activity leads to even greater health benefits [153]. These guidelines were established with the goal of reducing the risk of premature mortality, as well as a number of conditions associated with physical inactivity (coronary heart disease, stroke, hypertension, colon cancer, breast cancer, T2D, osteoporosis, and indicators of mental health) [108]. Individuals living with overweight or obesity are recommended to follow these guidelines to enhance overall health outcomes.

Similar to the guidelines for adults, those for older adults (≥ 65 years old) also recommend 150 minutes of moderate-to-vigorous-intensity aerobic physical activity per

week (in bouts of 10 minutes or more) with two days per week of muscle and bone strengthening activities using major muscle groups. However, CSEP suggests that balance-enhancing activities should be incorporated into the guidelines for older adults as well, especially in those with poor mobility in order to prevent falls [154,155]. By following these guidelines, older adults can maintain functional independence and mobility as they age, as well as decrease the risk of chronic diseases and premature mortality. Furthermore, they may improve fitness, body composition, bone health, cognitive function, and indicators of mental health [155]. The addition of balance exercises to these guidelines is imperative, as older adults have the highest risk of fall-related injuries or death, which increase with age [156].

According to data from the 2012-2013 CHMS, only 1 in 5 (20%) of Canadian adults (aged 18-79) achieved the recommended guidelines of aerobic physical activity described above during this time period [157]. Further examinations revealed that 25 minutes were spent each day in moderate-to-vigorous intensity physical activity (MVPA), but with just 12 of those being in a 10-minute bout. Furthermore, this analysis showed that adults aged 18-39 were the most active (34 minutes MVPA), with older adults the least (14 minutes MVPA). In terms of proportions, 32% of adults aged 18-39 reached the Canadian Physical Activity Guidelines, 18% of those aged 40-59, with just 12% of those aged 60-79 [157]. These statistics emphasize the fact that the majority of the Canadian population is physically inactive and live sedentary lifestyles. Interestingly, when comparing these results to the 2007-2009 CHMS, the physical activity of Canadian adults appears to be on the rise. In this short time span, the prevalence of those reaching the Canadian Physical Activity Guidelines increased from 15% to 20% [106].

2.2.3 Aerobic Exercise and Aging

Aerobic exercise improves an individual's cardiorespiratory fitness level by building aerobic capacity through the enhancement of both the cardiovascular and pulmonary systems [158]. Aerobic exercise includes activities that are typically performed for extended periods of time, such as jogging, walking, swimming, cycling, etc. [159] and last a minimum of 2 minutes. On average, individuals reach their peak cardiorespiratory fitness at approximately 25 years of age; cardiorespiratory fitness then declines at a rate of ~1% each year in untrailed adults [160,161]. However, when adults perform regular aerobic exercise, this decline in cardiorespiratory fitness is lowered to a rate of ~0.5% each year [162]. This decline in cardiorespiratory fitness observed with aging appears to be the result of both central and peripheral factors, which include a decrease in cardiac output, muscle oxidative capacity [163] and metabolically active tissue, with a corresponding increase in metabolically inactive tissue (fat mass) [161,162,164,165].

Although cardiorespiratory fitness decreases with aging, data suggests that regular aerobic exercise counteracts this decline and increases cardiorespiratory fitness in older adults [166-168]. Significant improvements in cardiorespiratory fitness of up to 15% have been documented in older adults between 79-91 years of age, when training for 6 months (3 days per week) at a rate of perceived exertion between 13 and 15 [167,169]. Improvements in cardiorespiratory fitness are extremely beneficial to one's health during aging, as improvements have been associated with reduced cardiovascular disease and events [170], blood pressure, and body weight, as well as increased HDL-cholesterol [171]. Interestingly, in the Aerobics Center Longitudinal Study, the most fit men and women had a 43% and 53% lower risk for all-cause mortality, and a 47% and 70% lower risk of cardiovascular disease mortality, as compared to the least fit men and women [172]. Similarly, in a

prospective study of 5.1 years, Blair et al. (1995) found a significant reduction in premature mortality risk in unfit individuals who became fit [173]. These results have been confirmed in all age groups including in older adults [172]. These data are not trivial, as studies have reported that for each increase of 1-METS, the reduction in premature mortality ranges between 10 and 25% (in individuals without cardiovascular disease) [174]. Furthermore, recent evidence revealed that cardiorespiratory fitness notably modifies the association between cardiovascular mortality and traditional risk factors for cardiovascular diseases. Consequently, the *American Heart Association's Statement* promotes the addition of the assessment of cardiorespiratory fitness in clinical settings to improve the management of cardiovascular diseases [175]. However, assessing cardio-respiratory fitness in all patients at risk of cardiovascular diseases may not necessarily be feasible due to the burdens it imposes on time, cost, risk, and resources [176]. As such, formulas to predict cardiorespiratory fitness should be used in the clinical setting as part of health assessments [177].

In summary, aging is associated with a decrease in peak cardiorespiratory fitness. However, regular aerobic exercise improves cardiorespiratory fitness and slows down this age-related decrease. Furthermore, increases in cardiorespiratory fitness by as low as 1-METS are associated with significant reductions in many health risks, and therefore contribute to a greater independence as one ages, and a better quality of life [166].

2.2.4 Resistance Exercise

Resistance exercise training is defined as a "form of physical activity that is designed to improve muscular fitness by exercising a muscle of a muscle group against

external resistance" [178]. Until the year 1990, resistance training was not part of any physical activity recommendations for health. Once the American College of Sports Medicine (ACSM) suggested that resistance training should be included as a significant component of fitness programs for all healthy adults [179], further research emerged regarding the benefits of resistance training on health and disease. Of most significance, resistance training has been proven to favorably impact cardiovascular function, metabolism, and coronary risk factors, in addition to it's well-known effects on muscular strength, mass, and endurance [170]. It also generates a myriad of health benefits for older adults and diseases associated with aging.

Resistance training is useful to improve not only cardiovascular disease, but also cardiovascular risk factors, including: insulin sensitivity, glucose tolerance, blood pressure, and cholesterol levels [180]. Increased skeletal muscle mass has been hypothesized to impact insulin sensitivity because insulin-like effects on glucose uptake occur during isometric contractions and glucose disposal takes place in skeletal muscle [181,182]. Indeed, resistance training has been shown to enhance insulin sensitivity in both younger [183] and older [184] adults in 16-week long interventions [185]. Furthermore, Miller et al. (1984) reported a significant decrease in basal insulin levels which was significantly correlated with fat-free mass [186]. Smutak et al. (1993) showed that resistance training decreased the total area under the curve for both glucose and insulin response [187]. Resistance training also improves insulin sensitivity in individuals with hypertension [188] and with T2D [189]. More recently, Cornelissen et al. (2013) published a meta-analysis including >5000 participants which demonstrated that both dynamic and isometric resistance training (≥ four weeks) significantly reduces both diastolic and systolic blood pressure [190]. It was also found that dynamic resistance training lowers blood pressure 2-3 mmHg, which is similar

to the magnitude obtained with antihypertensive medication [191,192]. Furthermore, just one day a week of exercise can be just as effective, and sometimes more effective, than pharmacotherapy in the reduction of all-cause mortality in hypertensive patients [193]. Finally, Roberts et al. (2013) examined the functional properties of HDL-cholesterol. They found that muscular fitness and training status, independently of body weight, improved HDL-cholesterol function. Also, they observed that chronic resistance training may mediate a decrease in the risk of cardiovascular disease through an increase in HDL-cholesterol [194]. Lira et al. (2010) reported significantly greater increases in HDL-cholesterol during low-intensity acute resistance training [195] compared to higher intensities, while Vatani et al. (2011) reported significant reductions in LDL cholesterol, total cholesterol, and total: HDL-cholesterol ratio in both the moderate- and high-intensity groups during six weeks of resistance training [196]. Additionally, resistance training is also beneficial in the prevention of metabolic syndrome, which is the product of a combination of both cardiovascular and T2D risk factors (abdominal obesity, high triglycerides, low HDL-cholesterol, hypertension and high glucose) [197]. Sénéchal et al. (2014) found that those with low muscle strength had a 2.2-fold increased risk of metabolic syndrome in adults < 50 years of age, with similar trends in those ≥ 50 years of age [198].

Interestingly, resistance training also positively benefits obesity and body composition [170,180]. Although aerobic training burns more calories, resistance training contributes to the total caloric energy expenditure by increasing resting metabolic rate [170]. As resting metabolic rate is mainly determined by fat-free mass [199], an increase in muscle mass causes a greater resting metabolism. Therefore, resistance training is suggested to complement aerobic training programs for weight control [170]. Similarly, although aerobic

training is more likely to increase one's cardiorespiratory fitness, circuit training (resistance training with shorter rest periods than traditional weight lifting) has the potential to elicit a modest effect on cardiorespiratory fitness [180] as well as endurance performance time [200]. Additionally, possessing greater muscle strength favorably impacts cardiovascular fitness and endurance, especially in older adults who have limited muscle capacity to be able to perform aerobic work [159,170]. As such, resistance training for strength is an important contributor to improved cardiovascular health.

2.2.5 Resistance Exercise and Aging

The age-related decline in muscle mass is known as *sarcopenia* and impacts 5-45% of older adults [201,202] depending on the population studied and the definitions used. Sarcopenia is characterized first by a slow phase (10% loss in muscle mass between the ages of 25-50), which is then followed by a rapid phase (40% loss in muscle mass between the ages of 50-80), leading to a total decrease in muscle mass of 50% by the age of 80 [51]. Aging is associated with a loss in both the number and size of type II fibers [51,161,203]. As type II fibers produce more force than type I, this reduction is related to the loss of muscle force production capabilities with aging [161]. Furthermore, the loss of muscle tissue with aging is typically accompanied by an increase in fat tissue, thus altering body composition as well [159].

On the other hand, the age-related decline in muscle strength and power is recognized as *dynapenia* [204], which is more related to the quality of the muscle [203]. Delmonico et al. (2009) reported a 1% decrease per year of thigh muscle area in older men, and a 0.65% decrease per year in older women, over a period of five years (at

baseline age of 70-79) [205]. The decline in the quality of the muscle with muscular atrophy with age is primarily caused by a loss of fibers [203]. However, other physiologic functions contribute to the development of dynapenia with aging. For instance, in terms of muscular physiology, impairment in excitation-contraction coupling processes during muscle contraction may result in the suboptimal activation of muscle and thus a decreased muscle quality. In addition, neurologic factors lead to dynapenia, including decreased motor cortical excitability [206] and cortical plasticity [207], increased activation in areas of the brain which control sensorimotor processing and integration [208], and negative adaptations to motor units [203]. These factors contribute to age-related declines in muscle performance and decreased functional properties of aged skeletal muscle [203]. Similarly to the muscular atrophy observed with age, muscle disuse associated with physical inactivity in older adults also contributes to the atrophy of skeletal muscle [51]. During prolonged periods of muscle disuse, muscle atrophy results from a reduction in muscle protein synthesis, with a subsequent increase in the rate of muscle protein breakdown [51,209]. Consequently, decreased muscle mass and strength in older adults leads to functional impairments [210,211], physical disability [212], and premature mortality [213,214].

Although aging and physical inactivity has negative effects on muscle mass and quality, the skeletal muscle of older individuals is capable of adapting to resistance training programs [51]. Resistance training helps to manage and treat the losses in muscle mass and strength associated with normal aging by increasing muscular fitness [159,179]. The plasticity of skeletal muscle tissue responds to resistance training in a number of ways, which restores the losses in muscle size and function from prolonged muscle disuse. First of all, older adults who engage in resistance training benefit from increases in strength. Lambert et al. (2005) report that the average increase in muscle strength of nine resistance

training studies was 75.9% [161]. These studies ranged between 10-12 weeks long for three days/week at 80% of one-repetition maximum (1-RM). Second, older adults also benefit from increases in muscle size (hypertrophy) with resistance training programs [179], which help to combat the losses associated with sarcopenia. Numerous studies have shown increases in muscle cross-sectional area and fiber area with resistance training [161]. For instance, Fiatarone et al. (1990) published a study in JAMA which showed that only eight weeks of resistance training in a group of older adults increased quadriceps muscle area by 10.9% [215]. However, the older adults included in this study were, on average, 90.2 years of age, which is considered much older in comparison to similar studies of older adults. Finally, within the skeletal muscle, resistance training causes an increased size of both type I and type II muscle fibers in older adults [215,216]. As there is a large reduction in type II fibers with age [51], the increase in type II fibers with resistance training offsets this age-related change.

Studies assessing increases in muscle strength and size have also compared the differences in these factors between younger and older adults. First, in terms of strength gains, neither Hakkinen et al. (2001) nor Joszi et al. (1999) observed a significant difference in the response of muscle strength to resistance training according to age [216,217]. Second, in terms of muscle size gains, Welle et al. (1996) found less hypertrophy in older adults (aged 62-72) compared to younger adults (aged 22-31) during 12 weeks of resistance training [218]. Ivey et al. (2000), on the other hand, and found no difference in the muscle volume response to resistance training between younger and older adults with only nine weeks of resistance training [219]. Finally, resistance training has similar effects on muscle fiber percentages in both younger and older participants [220,221].

Resistance training among older adults also has benefits on physical function [161] and metabolic health. The decrease in strength with age is associated with decreased mobility and functionality, as well as an increased risk of falls [180]. In the U.S., falling is identified as the number one cause of injury/death of injury among older adults [222], while it is the second leading cause of injury deaths worldwide [156]. Nevitt et al. (1991) showed that both upper and lower extremity strength were associated with the risk of falls and injury [223]. The ability of the older adult to protect themselves during a fall also impacted the risk of injury. Evidence has also shown that resistance training leads to improvements in bone health, which can help to prevent fractures and reduce the risk of osteoporosis [159]. Furthermore, increased bone mineral density helps to reduce the severity of falls in older adults. As such, resistance training to improve strength should be performed in those at risk of falls. Additionally, Fiatarone et al. (1994) showed that 10 weeks of resistance training in older adults (mean age = 87.1) increased strength, which translated to improvements in physical function, including gait velocity, stair climbing power, and spontaneous physical activity [224]. Finally, Dionne et al. (2004) demonstrated that metabolic adaptations to resistance training may be altered by age, as younger women (age = 27.8 ± 3.5) had greater changes in body composition, resting energy expenditure, and insulin sensitivity compared to older women (age = 66.6 ± 4.9) in response to 6 months of resistance training. Nevertheless, older women lost fat mass and tended to gain fat-free mass, which had positive impacts on their metabolic health [225].

In conclusion, resistance training provides many health benefits for individuals as they age. The increases in muscle mass and strength combat sarcopenia and dynapenia, while increased strength prevents falls. Furthermore, improvements in bone mineral density decrease the risk of fractures. Resistance training also has favorable impacts on

cardiorespiratory fitness and body composition. Most importantly, performing regular resistance training may enable older individuals to regain their independence while restoring their health [159].

2.2.6 Exercise Intolerance

Although exercise is known to lead to countless health benefits, not all individuals achieve appropriate physical activity levels or intensities to obtain the associated health benefits. In some cases, this limitation is caused by exercise intolerance. Exercise intolerance is defined as "the reduced ability to perform activities involving dynamic movement because of symptoms of dyspnea or fatigue" [226].

Exercise intolerance etiology can be explained by diminished capacity of the cardiovascular system to supply oxygen through a reduced heart function and cardiac output, and the inability of the skeletal muscles to utilize the delivered oxygen, or both [227]. However, McCoy et al. (2017) determined that the pathophysiological mechanisms of exercise intolerance differ significantly in individuals with chronic conditions [228]. For example, individuals with stroke, diabetes, cardiovascular, pulmonary, or neuromuscular disorders may experience a variety of central and/or peripheral factors that limit exercise in these populations [229-233], which suggest different mechanisms exist and inter-individual variability also has an impact. Therefore, data on exercise intolerance has not been equivocal as to what the clear mechanisms are at play. Understanding the factors which lead to exercise intolerance is essential, as it is a severe symptom of many chronic

conditions, and exercise intolerance increases an individual's risk of mortality and disease progression [234-237].

2.2.6.1 Heart Failure

Exercise intolerance has been extensively studied in chronic heart failure [238-243] as it is a primary symptom of the condition [244]. Chronic heart failure is a condition in which impaired cardiac function leads to a mismatch between cardiac output and the metabolic demand [244], which results in inadequate tissue oxygenation [242]. This decreased cardiac function translates to a direct reduction in exercise tolerance in this population [240]. In fact, the primary symptom of both heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF) is severe exercise intolerance [245-249]. Exercise intolerance can be objectively measured as a reduction in peak oxygen consumed during maximal effort exercise, otherwise known as VO_{2peak} [247,249]. VO_{2peak} is defined by the Fick principle, which is the product of cardiac output and the arterial-venous oxygen content difference (AVO_2diff). As such, a decrease in VO_{2peak} may be caused by a decrease in cardiac output, a decrease in the oxygen delivery to exercising muscles, or in impaired oxygen utilization by the exercising skeletal muscles.

The physiological mechanisms regulating exercise intolerance in HFrEF have been extensively studied [243,250], while less is known of the regulating mechanisms in HFpEF. Recent studies demonstrate that multiple cardiovascular [251,252] and peripheral factors [253] impact an individual's tolerance and capacity to exercise. The cardiac factors impacting HFpEF were demonstrated in a number of studies by Borlaug et al. (2006, 2010) and Abudiab et al. (2013). In these studies, the authors compared HFpEF to age-matched healthy controls or comorbidity matched controls without heart failure [251,254,255]. They

observed a decreased VO_{2peak} in individuals living with HFpEF. Decreased VO_{2peak} was found to be associated with a decreased peak cardiac output, mostly attributed to a blunted heart rate response, myocardial contractility, and peripheral vascular vasodilation. Furthermore, Bhella et al. (2011) observed a decrease in VO_{2peak} in individuals with HFpEF compared to healthy control but no significant difference in peak cardiac output between groups [252]. Haykowsky et al. (2011) further added that peripheral factors also play a role in limiting exercise performance in HFpEF [253]. In this study, they found that in addition to cardiac output, the change in AVO_2diff from rest to peak exercise was a strong independent predictor of VO_{2peak} in HFpEF and in healthy controls [253]. Houstis et al. (2018) found that the oxygen pathway step with the largest impact on exercise capacity was skeletal muscle diffusion [256]. The diffusion capacity for oxygen is dependent on muscle capillarity and muscle fiber size [256,257]. Although this study didn't include data that could identify the specific pathways causing the diminished diffusion capacity, others have shown that individuals living with HFpEF have lower capillary to fiber ratios [256,258]. All together, these studies and others [249,259] confirmed that peripheral factors also play a major role in exercise intolerance in HFpEF, including: peripheral circulation, autonomic dysfunction, vascular dysfunction, decreased AVO_2diff, and skeletal muscle abnormalities [239,246,248,253]. In HFpEF, peripheral factors actually limit exercise to a greater extent than central factors, especially compared to HFrEF, which is more centrally driven [246].

2.2.6.2 Skeletal Muscle Abnormalities

Many studies have demonstrated that a significant relationship exists between exercise intolerance and skeletal muscle abnormalities [238,260-262]. Of significant note, nearly all individuals living with HFpEF are older adults, with the majority being females [246,247]. Aging is associated with a decrease in muscle mass [203], which contributes to a person's intolerance to exercise and leads to an increased risk of chronic heart failure [238,261]. The shift in muscle fibers from slow oxidative to fast form in patients with chronic heart failure is associated with exercise intolerance [248]. This shift is also associated with a decrease in mitochondrial volume and density [263] as well as a decrease in aerobic enzymes with a corresponding increase in glycolytic enzymes [264]. These alterations demonstrate the change from aerobic to anaerobic metabolism [248]. Furthermore, along with this shift in muscle fibers, individuals living with chronic heart failure have a different skeletal muscle fibre type composition and capillarization – characterized by a higher percentage of type IIB skeletal muscle fibers, a larger relative type IIB fiber area, and fewer capillaries per fiber – which also contributes to exercise intolerance [265]. Taken together, these changes lead to the early onset of fatigue and contribute to exercise intolerance. Early fatigue is also caused by the decreased blood flow in exercising muscles, creating a greater reliance on anaerobic glycolysis, thereby decreasing VO_{2peak} [249].

2.2.6.3 Adiposity

Increased adiposity is a key contributor to exercise intolerance [266,267]. In fact, obesity is one of the strongest risk factors for the development of HFpEF, with approximately 85% of all HFpEF patients living with overweight or obesity [266]. According

to Kitzman et al. (2016), obesity is part of the pathogenesis of the disease [268]. As previously mentioned, HFpEF is predominantly found in older individuals and aging is characterized by significant changes in body composition, including increased fat mass and decreased muscle mass and strength [269]. Furthermore, aging is associated with an increased percentage of intermuscular adipose tissue [270]. Increased adiposity in skeletal muscle can impair perfusive oxygen delivery and impair mitochondrial function [271,272], negatively impact muscle strength and mobility in older adults [210], and also leads to abnormalities in skeletal muscle composition and function [266], which in turn contributes to exercise intolerance in HFpEF.

Haykowski et al. (2013) analyzed the difference between older HFpEF and healthy age-matched controls with dual-energy x-ray absorptiometry. HFpEF had significantly increased total percent body fat and percent leg fat along with a decreased percent lean body mass and percent lean leg mass [273]. Later, with magnetic resonance imaging, they found greater intermuscular fat in the thigh and intermuscular fat to skeletal muscle ratio in the thigh, which was associated with a decreased VO_{2peak} in HFpEF [272]. Furthermore, it is suggested that in individuals with increased intermuscular fat, blood is diverted to the fat that would normally be delivered to the active muscles during exercise [249]. This observation came about from a study by Heinonen et al. (2012) who observed a seven-fold increase in the blood flow to the adipose tissue adjacent to active muscles during exercise [274]. Moreover, fat infiltration in skeletal muscle is related to a number of factors which decrease VO_{2peak} in older adults with HFpEF, including: decreased muscle strength [272], muscular dysfunction [275], and decreased mitochondrial mass, biogenesis, and oxidative metabolism [271].

Evidence suggests that obesity is a modifiable risk factor for HFpEF, and reduced adiposity is a potential treatment for patients with HFpEF. Other treatments including surgical weight loss [276], caloric restriction [277], and exercise [278], have been proven to be successful in increasing VO_{2peak} in patients with HFpEF, thus improving tolerance to exercise in HFpEF [266].

2.3 Myokines

2.3.1 Discovery of Myokines

Prior to the discovery of myokines, extensive research regarding the biological function of cytokines had become a frontier in medical research [279]. Cytokines represent a collective group of biological substances, among which include adipokines and myokines. Although cytokines represent a diverse group of proteins, they are most accurately characterized as "soluble factors produced by one cell that act on another cell, in order to bring about a change in the function of the target cell" [279]. These changes occur once the cytokine's binding to the cell receptors initiate a cascade of intracellular signalling. Research within this field eventually revealed that cytokines are a part of the endocrine system [279]. This idea revolutionized the way that the hormonal regulation of metabolism in health and disease was viewed [280].

Upon this discovery, further extensive research in hormonal regulation revealed that adipose tissue acts as an endocrine organ, releasing cytokines into the systemic circulation [281-284]. In addition to its main functions of excess energy storage, heat

insulation, and mechanical protection [285], adipose tissue also secretes cytokine bioactive factors from adipocytes which have been coined adipokines [286]. Most adipokines are renowned for their pro-inflammatory responses in adipose tissue, while a small number are known as anti-inflammatory adipokines [287]. Adipokines are involved in the regulation of numerous physiologic functions, including energy metabolism and obesity-induced insulin resistance [284,285].

Ground-breaking research demonstrated that skeletal muscle, like adipose tissue, is an active endocrine organ that secretes muscle-derived bioactive factors named myokines [280,288]. This discovery revealed a mechanistic explanation for the benefits associated with exercise and physical activity in the prevention of metabolic diseases [280,285]. Myokines are defined as a group of "cytokines or other peptides that are produced, expressed, and released by muscle fibres and exert paracrine or endocrine effects" [289]. Pederson et al. (2012) described the interaction between adipokines and myokines as symbolic of a "yin-yang balance", as shown in Figure 1 [280]. Thus, myokines are indeed proteins which act to balance and counteract the negative effects of pro-inflammatory adipokines.

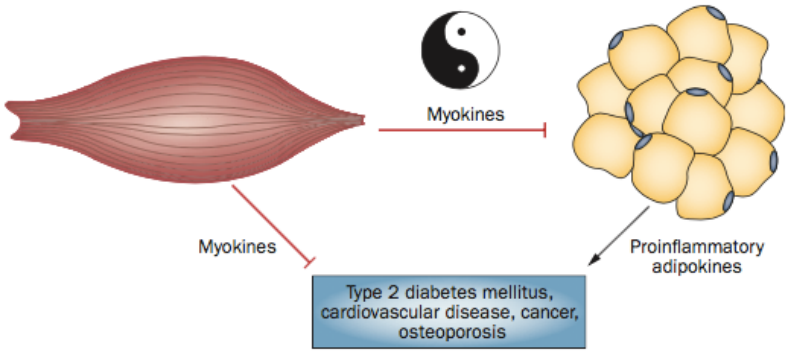

Figure 1. The Role of Pro-inflammatory Adipokines and Myokines on Chronic Diseases. Pederson et al. (2012) [290]

The production and secretion of myokines within skeletal muscle are initiated by contractile activity [291]. During exercise, the skeletal muscle responds with increased levels of myokine secretions [285] due to the accumulation of subsequent muscular contractions which increase the metabolic demand. When released from the muscle cells during contraction, myokines exert endocrine effects on distant organs [280]. This highlights the cross-talk between skeletal muscle and non-muscle tissues [292], or not anatomically linked cells [293], including: adipose tissue, liver, brain, cardiovascular system, intestine, pancreas, and bone, to name a few [290]. Some myokines also exhibit paracrine effects by working within signalling pathways locally, or autocrine effects directly within the skeletal muscle cells itself [280,291,294-298]. Some myokines have also been found to exert both endocrine and paracrine effects within the skeletal muscle and on distant organs. Considering that skeletal muscle is the largest organ in the human body – accounting for 40-50% of the non-obese human's total body mass – it's secretory capacity is significant [280,288]. The secretion of these factors mediate the metabolic changes observed from exercise and the associated training adaptations [299] which establishes the clinical relevance of myokines, especially with regard to chronic disease prevention [280,285].

2.3.2 Key Myokines

The emergence of skeletal muscle as an active endocrine organ was largely due to the identification of myostatin in 1997 [300] and interleukin-6 (IL-6) in 2000 [285,301]. Myostatin, otherwise known as growth differentiation factor 8, was the first secreted muscle factor that suited the criteria for myokines [290]. Myostatin binds to the

transmembrane activin receptor type IIB to inhibit muscle growth. The suppression of this pathway stimulates muscle growth in animal models, where myostatin knockout mice have extensive skeletal muscle hypertrophy compared to wild-type mice [300], as well as in humans [280,302]. Myostatin is also involved in the maintenance of metabolic homeostasis and modulation of adipose tissue function and mass [303-306]; moreover, myostatin may be protective against the development of obesity and diabetes [307]. At first, IL-6 was formerly classified as a cytokine, but was deemed a myokine when Pederson et al. (2008) concluded that IL-6 was also secreted from the skeletal muscle during muscular contraction [297]. In fact, during exercise, IL-6 increases exponentially [290,308], and exerts autocrine, paracrine, and endocrine effects [309,310]. It's main functions within the skeletal muscle consist of glucose uptake and fat oxidation [310], as well as increasing hepatic glucose production during exercise or lipolysis in adipose tissue [311]. Therefore, IL-6 has been recognized as a key myokine involved in the cross-talk between skeletal muscle and adipose tissue.

The discovery of these key myokines led to the identification of a cascade of factors secreted from the skeletal muscle. Several research groups attempted to identify the skeletal muscle cell secretome (all proteins synthesized/processed by the secretory pathway [312]), which revealed several hundred secreted myokines [313-316]. Most recently, Norheim et al. (2011) detected 236 proteins in the secretome [316], and further identified 15 novel contraction regulated myokines [280,288,316]. Although the physiologic and pathologic effects of myokines are not yet completely understood [11], the identification of these factors may potentially describe a molecular link between the function of skeletal muscle and whole body physiology [292].

2.3.3 Irisin

Previous research has identified that the many known beneficial effects of exercise are mediated by the transcriptional co-activator, peroxisome proliferator-activated receptor-gamma (γ) (PPARγ) coactivator-1α (PGC1-α) [317]. As mice with transgenically increased PGC1-α in the muscle are resistant to age-related obesity and diabetes [318], Boström et al. (2012) attempted to isolate which secreted muscle factor(s) mediates an alteration in systemic energy balance [317]. They first identified fibronectin type III domain-containing protein 5 (FNDC5) as a gene target of PGC1-α [317]. In a different experiment, adipocytes that were treated with FNDC5 showed an increase in uncoupling protein 1 (UCP-1) adipocytes and displayed an increased expression of genes involved in thermogenesis [317,319]. This confirms that FNDC5 induces thermogenesis and may impact systemic energy balance through energy expenditure. Furthermore, FNDC5 was determined to be a secreted protein, which is then cleaved and further modified as a distinct hormone [317].

Boström et al.'s (2012) analysis detailed above revealed the discovery of a novel myokine, irisin. Due to its cross-talk between muscle and tissue, it was named irisin after the Greek messenger goddess, Iris [317]. The release of this myokine is regulated by an over-expression of PGC1-α. PGC1-α stimulates the expression of FNDC5 (a transmembrane protein), which became recognized as the precursor of irisin. FNDC5 is synthesized as a type I membrane protein, which is then proteolytically cleaved at amino acid position 30 and 140, at the C-terminus (cytoplasmic-terminus), to release the amino (N)-terminal part of the protein into the extracellular space [151]. The specific protease that cleaves the protein has not yet been identified in the literature [320-322]. Once the signal peptide is removed, this

cleavage and glycosylation allows the release of the 112-amino acid polypeptide irisin into the blood [317]. Figure 2 demonstrates a schematic representation of the FNDC5 protein structure, which is then cleaved and releases irisin. Irisin corresponds to the crystal structure of the FNDC5 extracellular receptor ectodomain, which is the part of the protein that extends beyond the membrane and makes contact with the surface of target cells [323].

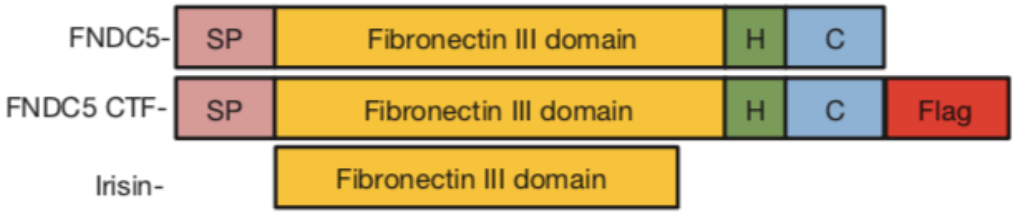

Figure 2. Schematic representation of the FNDC5 protein structure (top), Flag-tagged FNDC5 protein (middle) and irisin (bottom). *C, C-terminal domain; H, hydrophobic domain; SP, signal peptide* [317].

Upon the discovery of irisin, Boström et al. (2012) performed exercise studies in both humans and mice, and demonstrated that irisin was increased in the plasma of both [317]. It was found that mice significantly increased plasma irisin (65%) after three weeks of free wheel training, while humans had a 2-fold increase after 10 weeks of endurance training performed at 65% of their maximal cardiorespiratory fitness. Thus, circulating irisin increases with exercise, and it is this discovery that led to the concept that the expression and release of irisin is principally regulated by continuous muscular contractile activity [292]. Although there are significant differences between mice and humans, the conservation of irisin between the two is identical (100%) [317]. Exercise-induced irisin has been recognized as a method that allows individuals to attain some of the most important

benefits of exercise and muscle activity [317]. As such, increases in circulating irisin have been suggested to have an enormous therapeutic potential. For instance, through the process of browning of white adipose tissue (WAT) and thermogenesis, Boström et al. (2012) suggests that just a moderate increase in this myokine "increases energy expenditure, reduces body weight, and improves diet-induced insulin resistance" [317]. This conclusion came about from the injection of irisin into C57BL/6 mice who are highly prone to diet-induced obesity and diabetes. It was found that moderate increases in circulating irisin stimulated large increases in UCP-1, which led to significant improvements in the glucose tolerance of mice fed a high fat diet compared to control mice [317].

Prior to being released by the skeletal muscle, irisin is synthesized in the following three components of skeletal muscle (in murine models): the perimysium, endomysium, and nucleus [324]. Aydin et al. (2013) also found that high concentrations of irisin were found in the peripheral nerves of the skeletal muscle [324]. These data demonstrate that irisin is likely released by various cell types within the skeletal muscle, and not only by skeletal muscle cells themselves. Structurally, irisin is recognized as a protein dimer [323], which is the "formation of a functional protein complex composed of two subunits" [325]. The FNDIII domain of irisin is a common molecular protein building block [323], where the FN is part of the extracellular matrix that provides connections to cells through receptors and also regulates cell adhesion, migration, and differentiation [326]. However, irisin's structure is the first to form a continuous β-sheet formed between two FNDIII domains to form an extracellular matrix protein [323]. Specifically, using x-ray crystallography, Schumacher et al. (2013) observed that there are 8 irisin subunits in the crystallographic asymmetric unit

that interact to form 4 identical dimers. The two 4-stranded β-sheets (two monomers) combine to create the continuous, anti-parallel 8-stranded β-sheet [323]. This structure impacts the receptor activation and signalling to different targets from the skeletal muscle cells [323]. The intramolecular interactions between the two monomers involved hydrophobic contacts located in the interface of the dimer and the continuous β-sheet interactions contribute 10 backbone hydrogen bonds [323,327]. On the hydrophobic face of the dimer lies the protein N-terminus and flexible loops (residues 55–58 and 106 –108) which may be candidates that allow the interaction of irisin with other proteins and receptors [323]. Specifically, loop/residue 106-108 of irisin corresponds to the RGD loop in FNfnIII10, which is a ligand for many cell surface integrin receptors [328]. Integrins are membrane glycoproteins that facilitate extracellular matrix or cell-cell interactions, like the adhesion between the extracellular matrix and the target cell from the skeletal muscle [329,330].

The skeletal muscle mechanics involving the release of irisin are not clearly indicated in the literature. However, the knowledge surrounding the secretion of cytokines from skeletal muscles may be applied to help understand how irisin is released [331]. It appears as though the mechanisms rely on intracellular signalling factors [332-340] and calcium signalling, similarly to other cytokines [339,341].

2.3.4 Clinical Implications of Irisin

Irisin has become an attractive target in the treatment of obesity, diabetes, and other related metabolic disorders that are improved through exercise [317,342]. BMI has been shown to be associated with basal irisin levels [342-348], with inconclusive evidence as to

whether the relationship is positive or negative. A definitive negative relationship would suggest that basal irisin would be lower with an increasing BMI, which was observed in a number of studies [342,346,347]. This would align with the notion that the weight loss observed with exercise is mediated through the cross-talk between skeletal muscle and adipose tissue with irisin release [285]. However, a number of studies suggest otherwise, that individuals living with obesity tend to have a higher irisin concentration compared to more lean individuals [343,344,348-351]. Not only does irisin target adipose tissue upon release from the skeletal muscle, but adipose tissue also expresses FNDC5 in adipocytes and releases irisin, exerting it's actions within the adipose tissue itself [347,352]. Furthermore, irisin positively regulates FNDC5 in adipose tissue, thereby inhibiting adipogenesis [353]. Therefore, irisin also exerts paracrine/autocrine effects acting as an adipokine, but is secreted in a much lesser extent than from the muscle [285,352]. Other adipokines are also involved in the regulation of irisin, including leptin. Leptin upregulates FNDC5 within the skeletal muscle [285], while it downregulates FNDC5 in the subcutaneous adipose tissue of mice and humans [354], and negatively regulates irisin induced fat browning [354,355]. The inhibitory effect of leptin on irisin in adipose tissue may perhaps explain the decreased serum irisin in individuals living with obesity [285].

In addition to improvements in adiposity, a key factor of irisin release is the resulting improvement in glucose homeostasis [317]. Irisin has been shown to play a role in glucose uptake in the skeletal muscle through calcium- or reactive oxidative species-mediated downstream pathways [356]. GLUT4 is the main glucose transporter protein that mediates the uptake of glucose into skeletal muscle [357]. Intriguingly, the translocation of GLUT4 has intriguingly been found to be stimulated by irisin to a similar degree of insulin [358]. As GLUT 4 plays a large role in glucose homeostasis, irisin has a large therapeutic

potential. Individuals living with T2D have been shown to have significantly lower levels of irisin [343,359], to an extent of 40-50% lower basal irisin than individuals without T2D [360]. These studies "confirmed the potential role of irisin in glucose metabolism regulation and diabetes occurrence" [361]. Moreover, Yan et al. (2014) observed a negative association between irisin and insulin resistance indicators (fasting insulin, Hemoglobin A_{1c}, and waist circumference) [362], demonstrating irisin's key role in glucose metabolism. Furthermore, irisin has been shown to improve the prognosis of T2D [363]. Conversely, a meta-analysis performed by Qiu et al. (2015) observed that irisin was positively associated with insulin resistance. As such, further studies are required to provide a better understanding of the relationship between irisin and glucose metabolism.

Irisin has also been suggested to be linked to other organs that perhaps extends its therapeutic potential. Irisin has been shown to be associated with chronic diseases that are associated with a sedentary lifestyle [292] and that can be improved with exercise, including: chronic kidney disease, non-alcoholic fatty liver disease (NAFLD), cancer, and osteoporosis, etc [361]. First, common risk factors for chronic kidney disease (cardiomyopathies, bone mineral metabolism interference, inflammation, oxidative stress, etc.) are also known to lead to insulin resistance and hyperinsulinemia. As such, it can be expected that since irisin impacts insulin resistance and glucose homeostasis, this myokine may be therapeutic for those with chronic kidney disease [361]. Second, it has been suggested that irisin is related to NAFLD as those with both NAFLD and obesity have significantly lower irisin than lean controls [364]. However, another study reported higher levels of irisin in those with NAFLD compared to controls [365]. This result was inconsistent with previous literature, demonstrating the need for more research. Third, the mechanisms in which irisin improves cancer is unknown, but it is hypothesized that beneficial effects on breast

cancer are created by an anti-inflammatory response, apoptosis, or improved sensitivity of the tumor to common antineoplastic agents [366]. One study found that a one unit increase in irisin concentration (μg/ml) led to a reduction in the probability of invasive ductal breast cancer by nearly 90% [367]. It is not yet determined whether irisin is related to other types of cancer, though. Irisin is also lower in patients with osteoporotic fractures, and a large body of evidence proves its association with bone health [290,368]. Finally, irisin also exerts cross-talk with other organs, such as the brain and heart. Through exercise and irisin release, FNDC5 is expressed and secreted within the hippocampus, which induces brain derived neurotrophic factor expression and neurogenesis [292,369,370]. Further research has also suggested that irisin may be cardio protective due to the browning of WAT [371]. For instance, Kuloglu et al. (2014) found that irisin may be reflective of myocardial infarction as a diagnostic biological marker [372]. Decreased serum irisin has also been found to be associated with the presence and severity of coronary artery disease [373].

Irisin has been shown to promote skeletal muscle growth [374], which is meaningful as it is important to maintain and/or enhance skeletal muscle mass throughout the aging process. In addition, not only Irisin induces skeletal muscle hypertrophy, but it also partially rescues the denervation-induced atrophy of the skeletal muscle [374]. Insulin-like growth factor-1 (IGF-1), whose expression stimulates skeletal muscle hypertrophy, is associated with the expression of PGC1-α and FNDC5 [375]. Furthermore, expression of some PGC1-α isoforms blocks the effects of myostatin (negative muscle growth regulator) [376]. Furthermore, irisin signals through the downstream target IL-6, which is an important regulator of muscular hypertrophy through satellite cell activation [377]. As aging

is associated with a reduction in skeletal muscle mass [202], this feature of irisin release could help to reduce the amount of muscle mass that is lost.

In conclusion, the expression of myokines, like irisin, with exercise help to understand the role of physical activity in health and chronic disease. As discussed, the release of irisin leads to increased energy expenditure, a reduced body weight, and an improvement in insulin resistance [317]. As such, irisin may play a large role in the treatment and prevention of obesity, diabetes, and other chronic diseases related to exercise.

2.3.5 Controversy

A main concern that exists among researchers is the missing explanation as to why irisin is not released in humans in all exercise studies [292]. Boström et al. (2012) indicated that irisin may be affected by the metabolic response to exercise, such that it's beneficial roles may only apply to a select population [317,342]. Many individual factors may impact irisin regulation, including age [378], fitness [379], and perhaps the individual's genetic make-up [342]. This variation has created an incomplete understanding of irisin's effects, which has been subject to controversy since it's discovery.

First, the physiological role of irisin in humans is uncertain. A number of published studies suggest that exercise does not increase irisin concentration in humans [348,380,381], and that the beneficial effect seen in animal models cannot be translated to humans [382] while others demonstrate that circulating irisin levels are certainly upregulated during and after exercise in humans [317,348,350,383-388]. This therefore leaves questions regarding the strength of the data; however, these exercise studies differed in their training protocols (exercise intensity, endurance vs. resistance, acute vs. chronic, or the timing of blood

draws after exercise, etc. [292]), thereby altering the irisin concentrations observed within their samples.

Second, the core idea of the existence of circulating irisin has been debated, as some research groups argue that it does not exist in humans [380,382,389]. This debate arose due to the fact that a functional start codon is absent from the human FNDC5 gene, and is transcribed from an atypical start-codon: ATA (rather than ATG) [382]. This finding could indicate that a mutation may exist that prevents irisin transcription. Yet, 2-4% of eukaryotic genes harbour an atypical start codon as well and are transcribed regardless [292,390,391].

Finally, the measurement and detection method of irisin has been controversial within the literature. The commercial antibodies used in enzyme-linked immunosorbent assays (ELISA) kits have been thought to have prominent cross-reactivity with non-specific proteins [380]. Thus, the role of irisin in humans was called into question due to previous versions of ELISA kits that used commercial antibodies that were invalidated in biological fluids [380]. For these reasons, existing literature that involves previous versions of ELISAs draw discrepancies within results. Nevertheless, updates to current ELISA kits, such as Phoenix Pharmaceutical's (EK-067-29), have recently shown similar accuracy to liquid chromatography mass spectrometry methods, which helped to resolve inconsistencies. Lee et al. (2014) were the first to detect irisin using mass spectrometry [392], which is a highly specific and sensitive gold-standard measurement technique which does not rely on antibodies. The existence of irisin in humans was further validated when Jedrychowski et al. (2015) unequivocally detected and quantified the change in circulating irisin in humans during exercise with liquid chromatography mass spectrometry [388]. For the reasons outlined above, further studies are needed to investigate this controversy to

add to the literature.

2.3.6 Irisin Exercise Studies

2.3.6.1 Chronic Aerobic

Following the discovery of irisin [317], contradictory results emerged regarding the impact of chronic aerobic training on the release of this myokine. Some studies suggest that irisin increases after a chronic aerobic training intervention [317,351,381] , while others report a clear decrease [393-395]. Moreover, the majority of the evidence shows that there were no changes in circulating irisin; therefore, the effect of chronic aerobic training on irisin remains inconclusive. However, it is important to note that in order to draw a unanimous conclusion, trials of similar characteristics must be compared. Qiu et al. (2015) did not account for this factor, and used trials ranging from 8 to 26 weeks with varying intensities and protocols [394], which did not allow for appropriate comparisons. Therefore, trial characteristics make the interpretation of and comparison between studies challenging.

2.3.6.2 Chronic Resistance

It has been established that irisin release is mediated by mitochondrial biogenesis [317] due to an increase in PGC1-α through nitric oxide-dependent skeletal muscle mechanisms [354], which is typically observed with aerobic exercise. However, resistance exercise has been shown to increase irisin concentration [381,383-385,396]. This form of exercise does induce mitochondrial biogenesis, but not to the same degree as with aerobic exercise.

Therefore, this suggests that other pathways are also involved. Skeletal muscle mass is a strong predictor of circulating irisin [387,397]; therefore, resistance training that aims to increase muscle mass and strength might lead to increased circulating irisin [360,383]. Muscle strength has also been shown to be positively associated with irisin concentration when measuring hand grip strength [398] – a proxy measure for overall strength.

The effect of chronic resistance training on irisin release was then explored in a number of studies [342,381,383,394,396,399-403]. Similar to aerobic training, chronic resistance training also yielded inconsistent results in the literature. Increased irisin release has been reported in young (age = 26.4 ± 2.9) [383], middle-aged (age = 48.0 ± 7.0) [381], and older adults (age = 74.5 ± 0.62) [396] after resistance training interventions ranging from 8-26 weeks. However, Tibana et al. (2017) compared the effect of resistance training on irisin in inactive older women (aged >65) stratified by obesity status. A decrease in irisin was seen in normal weight participants after six weeks of resistance training, while no change was seen in their counterparts of individuals living with obesity [342], which may be related to the fact that some studies observed that individuals living with obesity have higher basal irisin [345,348]. A number of other chronic studies have also shown no effect of resistance training on irisin [342,399,400,402,403]. Qiu et al. (2015) reported a decrease in irisin concentration after a resistance training intervention using a meta-analytic approach [394]. In a sub-analysis of randomized controlled trials, they observed a significant decrease in irisin after chronic exercise programs (including aerobic and/or resistance training) (effect size d = -0.46 (95% CI [-0.76, -0.15])), with a specific clear decrease in chronic resistance training. The sub-analysis of non-randomized studies had a trivial decrease in irisin. This meta-analysis is of significance though, as it highlights the importance of study design on the observed results. By performing randomized controlled studies, it increases the

likelihood of observing significant changes on irisin compared to non-randomized trials. Furthermore, it is essential to compare similar exercise protocols, as they studied protocols of varying intensities and program lengths, which did not allow for accurate comparisons. Another flaw in Qiu et al.'s (2015) study, was that they did not account for the time point of irisin assessment post-exercise. The time point of irisin measurement is crucial for accuracy, as irisin is a molecule with a high rate of degradation [379]. Hecksteden et al.'s (2013) results also demonstrate this property of the molecule, as they observed a negative association between irisin and storage duration of frozen samples [381]. Due to these findings, it is suggested that irisin may respond to acute exercise, rather than chronic, as the "physiological adaptations of chronic exercise aren't sufficient to keep irisin levels elevated beyond the acute effect of exercise" [379]. This aligns with the concept that chronic adaptations to exercise may be the result of an accumulation of acute bouts [299]. Furthermore, this idea was validated in a meta-analysis performed by our group, which analyzed the effect of acute exercise with irisin measured immediately post-exercise. A significant increase of 15.0% (95% CI [10.8, 19.3]) was observed in a mixture of acute aerobic and/or resistance exercise sessions, demonstrating the positive impact of acute exercise on irisin [379].

2.3.6.3 Acute Resistance Training

The majority of the literature demonstrates that there is a significant increase in irisin during an acute bout of aerobic training [348-350,384,386,387,394,401,404-410]. It is also suggested that there is a dose-response component to irisin with acute aerobic exercise intensities, where greater exercise intensities result in higher irisin concentrations

[386,387,407]. However, the impact of acute resistance exercise has not yet been extensively explored in the literature. First, Nygaard et al. (2015) examined a small group of young adults (age = 32.0 ± 9.0) with a normal BMI (24.5 ± 2.4 kg/m^2) during a 60-minute resistance training session (3 sets; 10-12 repetitions; 8 exercises). Irisin was not significantly increased after acute exercise ($p > 0.05$), but it was increased 1 hour post-exercise [384]. Second, Tsuchiya et al. (2015) also analyzed irisin in a group of young adults (age = 23.0 ± 1.0) during a 60-minute resistance training session (4 sets; 12 repetitions; 8 exercises; 65% 1-RM). Again, they did not observe significant differences between pre- and post-exercise irisin concentrations ($p > 0.05$) [385]. Although both Nygaard and Tsuchiya's studies were designed as acute resistance training bouts, no acute effect was observed during or immediately post-exercise. It is also important to note that in both of these studies, the population of adults studied were deemed active or moderately physically active. This could have altered their results, as fitness level has been reported to be associated with irisin [379,406]. In our meta-analysis, there was a 2-fold increase in plasma irisin in fit compared to unfit individuals. Therefore, fitness level may have been an important moderator of irisin concentration in these studies. Third, Pekkala et al. (2013) compared the effects of a high intensity acute resistance training session between younger (age = 27.0 ± 3.0; BMI = 23.0 ± 2.0) and older (age = 62.0 ± 5.0; BMI = 25.0 ± 2.0 kg/m^2) adults. There was no change in irisin release during this bout of exercise; however, a contributing factor to this result may have been that the subjects had a normal to slightly overweight BMI [378]. Irisin is typically higher in adults living with obesity compared to more lean individuals [343,344,348-351], which may have predisposed those in this study to a lower irisin level already, thereby potentially explaining why irisin was not significantly

increased. Furthermore, all three of the described acute resistance training studies had a fairly small sample size, ranging from 9 to 21 participants.

2.3.6.4 Aging and Irisin

It has also been suggested that circulating irisin concentration may be affected by the aging process [351,378,379,386,387,410], due to age-related declines in skeletal muscle mass [201,202,411] and strength [204], and increases in adiposity [412]. The degeneration of skeletal muscle mass with age may have a powerful impact on the human exercise response to irisin, as skeletal muscle mass is recognized as a strong predictor of irisin [387,397]. Indeed, previous literature has demonstrated associations between irisin, or it's precursor FNDC5, and age [351,378,379,386,387,410,413]. Huh et al. (2012) studied a group of 117 middle-aged women with BMI ranging from 20.0 to 47.7 kg/m^2 aged 24 to 69 years old and found that age was negatively associated with irisin (r = -0.28, $p < 0.01$) [387]. In 2014, Huh et al. confirmed this result, as this analysis reported that basal irisin was lower in older adults (age = 67.9 ± 5.0) than in younger adults (age = 26.7 ± 4.1) [386].To the contrary, another study found that irisin was significantly increased in older adults (age = 65.0 ± 8.0), but not in younger adults (age = 21.0 ± 1) of similar BMI [351]. Further studies are required to confirm the association between basal irisin concentrations and age.

Aging does not only impact basal irisin concentrations, but it may also impact the acute exercise response to this myokine. Timmons et al. (2012) reported a greater FNDC5 expression after exercise in highly active older adults (1.3-fold increase) compared to sedentary controls, and that FNDC5 gene activation was absent in younger subjects [413]. However, they did not observe an increase in PGC1-α in their cohort, which questions the

result that was observed. Fox et al.'s (2017) meta-analysis showed a negative association between acute change in irisin and age ($p = 0.07$). Using meta-regression analyses, they observed that age, along with BMI and fitness level, explain 23% of the variation in the change in irisin release after acute exercise [379]. Finally, only Pekkala et al. (2013) studied the effect of age on acute resistance training, through the comparison of younger and older adults. Their results show no change in irisin after acute resistance training in either age group. However, this was not surprising as the utilized protocol was very intensive, which consisted of performing 5 sets of 10 repetitions of leg press until failure, with two minutes of recovery time in between sets. Interestingly though, this bout of resistance training caused a significant increase in PGC1-α in older men (2-fold increase) and an even greater increase in younger men (4-fold increase). They also showed that FNDC5 mRNA was increased by 1.4-fold, but only in younger men [378]. Further testing is required with an appropriate load and exercise protocol that would allow participants to gain muscle mass and strength, as to trigger irisin release.

2.4 Gaps in the Literature

The current literature surrounding irisin and exercise presents a number of limitations that will be addressed by the proposed study. First, controversies in the literature appear to result from the utilization of different research questions to investigate irisin during chronic or acute bouts of exercise. Second, previous literature addressing acute bouts of exercise did not quantify the changes of irisin within that acute bout. They

simply analyzed irisin at the beginning and at the end of exercise, rather than using multiple time point measurements during the acute bout. The current study will analyze an acute bout of resistance exercise with multiple time point measurements of irisin within the bout, in order to offer a more comprehensive understanding of the changes of irisin during exercise. Third, the comparison of between different exercise protocols has also led to controversial results regarding the role of irisin. Different exercise parameters, such as exercise type (chronic vs. acute/resistance vs. aerobic), intensity (light/moderate/vigorous), duration (length of exercise), or volume (length of exercise program), may have a large impact on irisin concentrations. Hence, in order to provide accurate comparisons between studies, similar exercise protocols must be used. Fourth, the literature to date regarding the acute impact of resistance exercise training is limited by: 1) underpowered studies, and 2) not accounting for BMI and physical activity level. In fact, most of the studies involved a sample of moderately physically activity to active individuals. To the best of our knowledge, only one study has accounted for the effect of aging on an acute bout of resistance exercise training. However, this study included participants who varied within a wide range of BMI (which is known to impact irisin[343,344,348-351]), and involved a more intensive exercise protocol compared to what was performed by participants in previous studies. Consequently, based on the gaps in the literature, this study involved an acute bout of resistance exercise training in older adults living with overweight or obesity that was compared to a group of younger adults living with overweight/obesity matched for physical activity and BMI.

2.5 Study Objectives and Hypothesis

Based on the gaps in the literature, the purpose of this study was two-fold: 1) to determine whether irisin release increases during an acute bout of resistance exercise training in individuals living with overweight or obesity; 2) to determine whether changes in irisin concentration during an acute bout of resistance exercise training were observed in different age groups of adults living with overweight or obesity. It was hypothesized that: 1) irisin concentration would increase during an acute bout of resistance exercise training; 2) a significant difference in response of irisin release to resistance exercise training would be observed between the two age groups.

CHAPTER 3: Article

Title: The Impact of Acute Resistance Training on Irisin in Younger and Older Adults Living with Overweight or Obesity

Authors: Rioux BV, Brunt KR, Bouchard DR, Fox J, Sénéchal M

3.1 Abstract

BACKGROUND: Exercise is a cornerstone for the prevention and management of overweight and/or obesity (OW/OB). Studies suggest that exercise-induced irisin impacts metabolism and health. However, no study has quantified the impact of biological aging on resistance training (RT)-induced increase in irisin.

OBJECTIVES: The purpose of this study was to determine whether irisin concentration would increase during an acute RT bout and to compare irisin release between younger and older adults living with OW/OB.

METHODS: Adults aged between 19-35 (25.9 ± 5.0; n=15) and 60-80 years old (67.7 ± 4.1; n=14) living with OW/OB participated in this study. The primary exposure variable was an acute bout of RT, which consisted of 3 sets of 12-15 repetitions at 65-70% of 1-Repetition Maximum and 3 minutes each of squats and step-box. The primary outcome measure was the concentration of irisin quantified by ELISA before, during, and after the acute bout of RT.

RESULTS: Significant differences were observed between younger and older adults in waist circumference, body fat, fitness levels, and muscle strength (all $p < 0.05$). However, no differences were observed in physical activity levels (young: 46.0 ± 45.5 vs. older adults: 31.2 ± 30.8 min.; $p > 0.05$) nor body mass index (young: 28.6 ± 4.0 vs. older adults: 29.8 ± 4.7 kg/m^2; $p > 0.05$). Repeated measures analyses showed no effect of time on irisin during acute RT, and no interaction effect between age and time ($p > 0.05$).

CONCLUSIONS: The results of the current study suggest that there is no impact of biological aging on the acute release of irisin during RT in individuals living with OW/OB. Further studies are needed to elucidate the irisin response to acute exercise with different modalities/intensities of exercise.

3.2 Introduction

The increased prevalence of obesity observed over the last few decades has led to a global epidemic [414]. One in four Canadian adults are classified as living with obesity (26.0%) [27], while recent data demonstrates that 36.5% of adults in the United States are living with obesity [36]. This is not trivial, as overweight and obesity are associated with an increased risk of cardio-metabolic risk factors and chronic conditions, such as diabetes, hypertension, and high cholesterol [107]. Furthermore, aging is also associated with increased cardio-metabolic risk factors [415]; therefore, the concomitant effect of overweight/obesity and aging increases the likelihood of experiencing a worse cardio-metabolic profile.

Recently, it has been demonstrated that skeletal muscle is an active endocrine organ that secretes muscle-derived bioactive factors named myokines [288,310]. Myokines have been described as a potential mechanistic explanation for the health benefits associated with exercise, including weight loss and metabolic disease prevention [285,290]. Recently, Boström et al. (2012) discovered a myokine named irisin. Irisin release is regulated by an over-expression of the transcriptional co-activator: peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC1-α) [317]. PGC1-α stimulates the expression of a transmembrane receptor, fibronectin type III domain-containing protein 5 (FNDC5), which is then proteolytically cleaved to allow the release of irisin into the bloodstream [317]. Their analysis demonstrated that the release and expression of irisin is primarily regulated by continuous muscle contractile activity and, in addition, increased exercise-induced irisin concentrations lead to increased energy expenditure, decreased

body weight, and improvements in glucose tolerance [317]. Consequently, this myokine has positive implications for individuals living with overweight/obesity or diabetes.

A meta-analysis from our group suggested an increase of irisin of 15.0% (95% CI [10.8, 19.3]) following acute exercise, which was independent of exercise type [379]. Skeletal muscle mass and muscle strength are strong predictors of circulating irisin levels [387,398], which suggests that irisin might be impacted by resistance training (RT). However, data on the impact of chronic RT on irisin levels are inconclusive [342,383,396,400]. To the best of our knowledge, only a few studies have explored the impact of acute RT on irisin levels [350,378,384,385], but these studies failed to account for a number of key factors. First, they involved a sample of varying age and BMI. Second, they utilized a wide range of exercise protocols of differing intensities, sets, and repetitions. Finally, they did not analyze the acute response of irisin during the acute RT bout, and the blood draws were obtained at dissimilar time points that limit comparison between studies. Therefore, the purpose of this study was to determine whether 1) irisin concentration increases during an acute bout of RT in individuals living with overweight or obesity; and 2) changes in irisin concentration during an acute bout of RT are observed between younger and older adults living with overweight or obesity.

3.3 Data and Methods

3.3.1 Participants

A total of 26 participants including younger (n = 13) and older (n = 13) adults took part in this acute exercise study (The REACTION Study). Figure 3 represents the recruitment process and exclusion criteria which led to the final study sample size. Initially, 137 participants expressed interest in the study. After applying the exclusion criteria, 38 participants remained eligible and completed the first visit. After this visit, four individuals were considered to be "dropouts", as they wished to terminate their completion in the study due to discomfort with intravenous procedures or were deemed not fit to participate in the study by a medical professional. Four more participants were excluded due to high physical activity levels. A total of 30 participants completed the three visits of the study. However, two participants were excluded due to missing irisin values and two others were excluded due to missing accelerometer data (did not wear the accelerometer for four valid days even after multiple attempts of wearing the device). Finally, a total of 26 participants were included who provided information for the primary outcome and exposure variables in addition to the potential confounders.

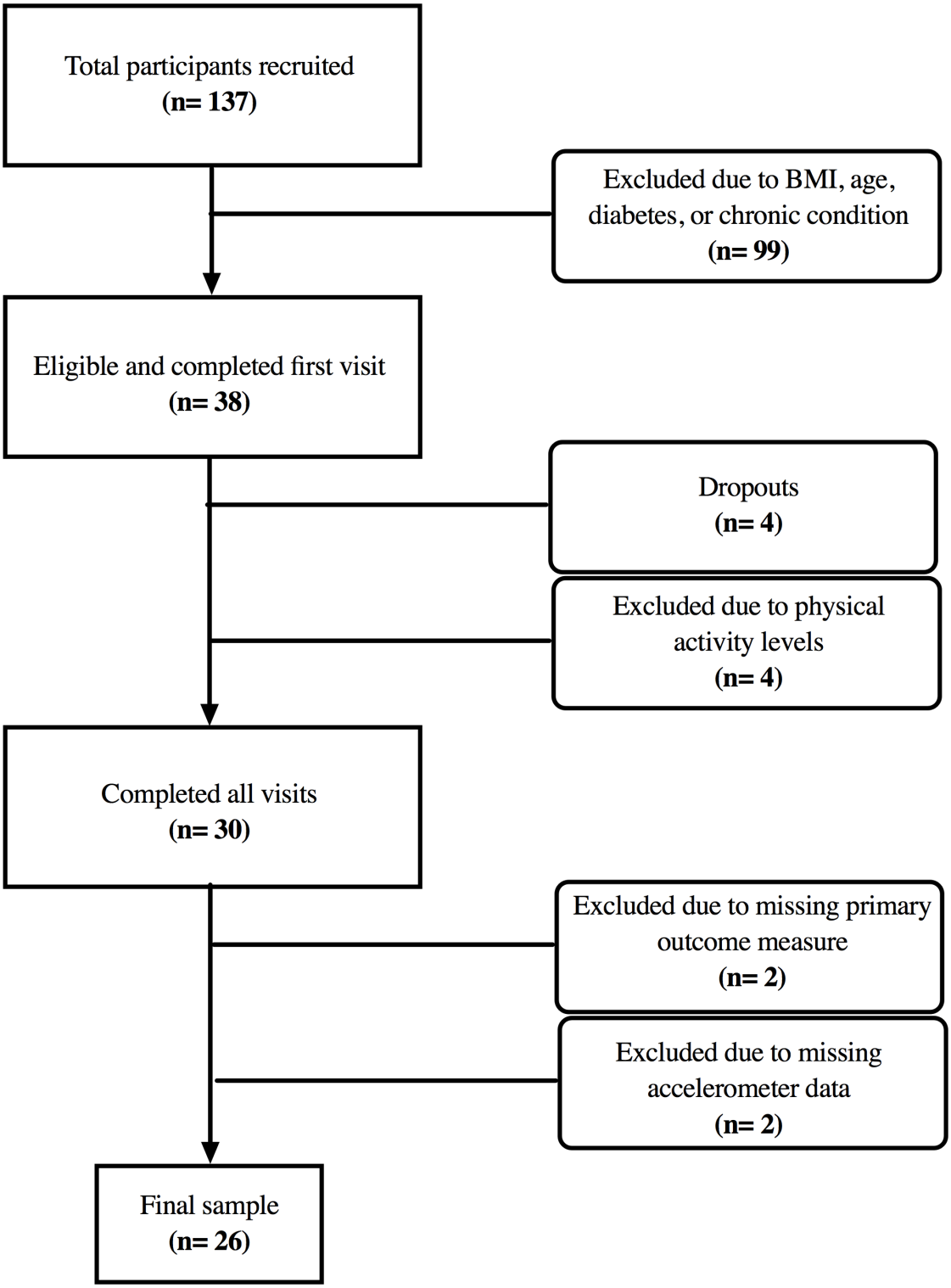

Figure 3. Flowchart describing the recruitment process and exclusion characteristics of the sample in the randomized controlled trial

Inclusion criteria for the participants were: aged between 19-35 years old (younger adult) or 60-80 years old (older adult); classified as overweight or obese (BMI ≥25 kg/m^2); and considered inactive (must not reach the Canadian Physical Activity Guidelines of engaging in less than 150 minutes of moderate-to-vigorous physical activity (MVPA) per week in 10-minute bouts as measured by accelerometers). The age ranges were selected to analyze two extremes of age that exist on the wide continuum of aging – from younger to older adults, so as to avoid any overlapping effect of middle-age on the primary outcome measure. Medical history was obtained along with a list of medications consumed. A CSEP Physical Activity Readiness Questionnaire was obtained to determine whether participants had a chronic condition that would impact the maximal oxygen consumption (VO_{2max}) test or prevent them from performing the acute exercise session.

Participants were excluded if they were: engaging in a regular exercise training program; had a previous diagnosis of Type 2 diabetes; had any injuries or conditions that would prevent them from performing a maximal oxygen consumption (VO_{2max}) test or the acute resistance exercise training session. Participants with non-controlled chronic conditions (e.g. hypertension) were cleared by a physician or were otherwise excluded. The study was approved by the University of New Brunswick Research Ethics Board (UNB 2015-115).

3.3.2 Overview of Protocol

Participants underwent a screening and baseline assessment of body composition, fitness, and medical history during the first visit. One week later, the second visit involved physical activity level and muscle strength assessments. After completing the screening visits, participants performed an acute bout of resistance training during the third visit.

Changes in irisin were quantified and compared to the baseline concentrations of irisin in younger and older adults.

3.3.3 Primary Outcome Measure

3.3.3.1 Change in Plasma Irisin Concentration

Blood samples were obtained by standard intravenous punctures in the forearm by a registered nurse in accordance with the following timeline: before the exercise session (pre) = at 0 minutes (T0); during the exercise session (after each set) = after 15 minutes (T15), 30 minutes (T30), and 45 minutes (T45) of exercise; and after the exercise session (post) = after 45 minutes of rest (T90). Blood was drawn into 3 mL tubes coated with an anticoagulant, ethylenediaminetetraacetic acid (EDTA), and was then pipetted (~1.5 mL) into 1.5 mL microcentrifuge tubes in duplicate in a Biosafety Cabinet (Thermo Fisher Scientific, 1300 Series A2, MA, USA). Each microcentrifuge tube was centrifuged at 1600g at 4 degrees Celsius for 15 minutes to separate the plasma, which was collected and stored at -80°C until analysis.

Irisin concentration was analyzed using enzyme-linked immunosorbent assay (ELISA) kits (EK-067-29) according to the manufacturer's protocol (Phoenix Pharmaceuticals, Inc., CA, USA). These kits have been validated against both liquid chromatography mass spectrometry [388] and western blotting [416]. The manufacturer reported a coefficient of variation of <10% (intra-assay) and <15% (inter-assay) with this ELISA kit. The optical density for each ELISA plate was determined by a microplate reader set at 450 nm using the Gens5 software, which was then converted into irisin concentration using Prism version 7 (GraphPad Software, CA, USA).

3.3.4 Primary Exposure Variable

3.3.4.1 Acute Resistance Training Session

As suggested by previous studies, plasma irisin may be altered by food and exercise; therefore, participants were asked to refrain from exercise 24 hours prior to the exercise session and from eating food or drinking caffeine two hours prior [417]. The resistance exercise session involved the use of free weights, resistance exercise machines, and the participant's own body weight for a total of six exercises. Each participant performed 3 sets of 12-15 repetitions of the exercises with weight at 65-70% of their 1-repetition maximum (1-RM). Sub-maximal 1-RM was calculated for the following three exercises with weight: 1) incline dumbbell bench press, 2) latissimus pull down, and 3) bicep curl using a standing pulley machine; the three exercises without weight included: 4) 3 minutes of step-box (consecutive step-up and down on a York Barbell – Plyo/Step-Up Box at a height of 30 cm for women, and 38 cm for men), 5) 3 minutes of squats (to a near seated position over a bench), and 6) 1 minute of the plank. There was 55 seconds of rest between each exercise. A total of three sets of all six exercises were performed with 3 minutes of rest in between to obtain blood draws.

3.3.4.2 One-Repetition Maximum

To prepare a proper individualized protocol for each participant for the resistance exercise session during the third visit, each participant's 1-RM was calculated using the American College of Sports Medicine's predictive equation: 1-RM = weight lifted/[1.0278-(repetitions*0.0278)] [418]. The following resistance exercises were completed during the second visit: incline dumbbell bench press, latissimus pull down, and bicep curl. Participants were instructed to perform 10 repetitions with an increase in

weight at each set for a maximum of 3 sets. Once the participants could properly complete 6-10 maximum repetitions, the value of repetitions from the last set was used in the equation to estimate their 1-RM. Thereafter, each participant's 65-70% 1-RM weight was calculated and practiced so that they could effectively perform the acute resistance training session.

3.3.5 Potential Confounders

3.3.5.1 Anthropometric Measures

BMI was calculated using the following formula: weight (kg)/ height (m^2). Body weight was measured to the nearest 0.1 pound on a calibrated balance (SECA707, Hambourg, Germany) which was converted to kilograms and height was obtained to the nearest 0.5 cm with a standardized stadiometer. Waist circumference was measured to the nearest 0.5 cm twice on the right side of the body with a measuring tape according to the Canadian Society of Exercise Physiology protocol [419]. If measurements were more than 1 cm apart, a third measurement was taken. Briefly, a measuring tape was placed at the top of the iliac crest where the assessor placed landmarks, and the cross-handed technique was used to ensure enough pressure on the measuring tape.

3.3.5.2 Body Composition

Body composition was assessed by using the BodPod version 1.69 (COSMED, California, USA). The BodPod uses air displacement plethysmography to calculate body density by measuring the volume of air being displaced in the chamber. Subsequently, both fat-free mass and fat mass are calculated with the Siri equation [420]. The BodPod involves highly reliable measurements with minimal error, ranging from ± 1 to 2.7%

[417,421]. Participants were instructed to refrain from eating and exercising 3-4 hours prior to testing [420] and adhered to the recommended attire for the standard protocol. Based on fat-free mass calculated with the BodPod, muscle quality was quantified as muscle strength divided by total fat-free mass: 1-RM (kg)/fat-free mass (kg). Relative fat-free mass was calculated as a function of fat-free mass relative to height: fat-free mass (kg)/height^2 (m). A composite score of strength relative to body weight was calculated by adding each of the 1-RM measurements in kilograms (dumbbell press, latissimus pull-down, bicep curl) divided by body weight in kilograms: total 1-RM (kg)/body weight (kg). Similarly, strength relative to fat-free mass was calculated as: total 1-RM (kg)/fat-free mass (kg).

3.3.5.3 Cardiorespiratory Fitness

The TrueMax 2400 Metabolic Measurement Cart (ParvoMedics, Utah, USA) was used to evaluate cardiorespiratory fitness by a graded exercise test. The protocol for older adults was designed as follows: three minutes of walking (3.0 mph; 2.0% slope), with an increase in slope of 2.0-3.0% and speed of 0.5-1.0 miles per hour every two minutes until exhaustion. Younger adults followed a similar protocol, with the exception of the three-minute walking stage at 3.5 miles per hour rather than 3.0. The volume of oxygen was scaled to kilograms of body weight and fat free mass as some studies have suggested that scaling to fat free mass was the best scaling factor in individuals living with overweight and obesity [422].

3.3.5.4 Physical Activity Level

Participants wore an ActiGraph GT3X accelerometer (ActiGraph, Florida, USA) on their left hip for seven consecutive days during waking hours. A minimum of four valid

days (minimum wear time of 10 hours) was required to be included in the analyses. The ActiLife Software Version 5 (ActiGraph, Florida, USA) was used to determine the following variables: total number of 10-minute bouts in MVPA, and minutes spent in bouts of MVPA per week; total time and percentage of time spent in sedentary, light, moderate and vigorous activity; total time of physical activity (light + moderate + vigorous) per week; and the sum/average of steps taken. Physical activity intensity was determined by activity counts, which used validated age and sex-specific cut-points to quantify the intensities [423].

3.3.6 Statistical Analysis

Descriptive statistics were performed to present baseline characteristics stratified per age group (young vs. old). Continuous and categorical variables were presented as mean ± SD or median (25th and 75th percentiles), and n (%). Mann-Whitney U tests were performed to compare the means of the general and physical activity/fitness characteristics of the sample according to age group. Spearman correlations were performed to determine the association between irisin and body composition, physical activity, and strength measures. To determine whether changes in irisin concentration increased during an acute bout of resistance training, and whether differences were observed according to the different age groups of adults living with overweight or obesity, a two-way analysis of variance (ANOVA) using repeated measures was used unadjusted and adjusted for the main potential confounders (waist circumference, percent body fat, cardiorespiratory fitness with relative VO_{2peak}, and strength with 1-RM), with Bonferroni post-hoc tests if necessary. A power analysis calculation was performed to determine our sample size. For a two-way ANOVA with repeated measures, assuming a power of 0.80 and an alpha level

of 0.05, a total sample size of 20 participants (younger adults: n=10; older adults: n=10) was required in order to observe a significant difference between groups. Exercise interventions typically observe a 30% dropout rate. Although this is not an exercise intervention, we accounted for this proportion of dropouts in our calculation by recruiting a total of 5 more participants in each group. The significance level was accepted at $p < 0.05$, and analyses were performed using IBM SPSS statistics version 22.0.

3.4 Results

3.4.1 General Characteristics

Table 2 describes the baseline characteristics of the sample. There were no sex nor ethnicity differences between age groups ($p > 0.05$). Although BMI was not significantly different between groups (younger: 28.7 ± 3.8 kg/m^2 vs. older: 30.2 ± 4.7 kg/m^2; $p > 0.05$), waist circumference (younger: 97.9 ± 7.4 cm vs. older: 107.8 ± 11.4 cm; $p < 0.01$) and total fat mass were significantly different between age groups (younger: 27.9 ± 10.1 kg vs. older: 34.5 ± 11.0 kg; $p < 0.05$). Total fat free mass was significantly different between age groups (young: 59.8 ± 11.7 kg vs. older: 48.3 ± 11.4 kg; $p < 0.05$). Significant differences were observed between younger and older adults living with overweight or obesity in all other body composition measures ($p < 0.05$).

Table 2. Baseline General Characteristics of the Sample

Variables	Younger Adults (n=13)	Older Adults (n=13)	*p*-value
Age (years)	25.54 ± 4.79 24.00 (22.00-30.50)	67.92 ± 4.17 68.00 (64.50-69.50)	**0.000**
Female n (%)	5 (38.46)	8 (61.54)	0.249
Caucasian n (%)	12 (92.31)	12 (92.31)	0.956
Resting HR (beats/min)	71.96 ± 11.01 71.50 (68.25-76.00)	68.02 ± 12.75 67.00 (57.75-74.50)	0.293
Systolic BP (mmHg)	124.85 ± 10.79 124.50 (119.50-133.50)	127.50 ± 12.43 126.50 (114.50-138.25)	0.798
Diastolic BP (mmHg)	74.63 ± 9.94 76.50 (64.50-83.75)	82.02 ± 6.58 81.50 (76.50-88.75)	0.081
Medications			
NSAIDS n (%)	0 (0.0)	2 (15.4)	0.149
BP Medication n (%)	0 (0.0)	5 (38.5)	**0.015**
Anthropometrics/Body Composition			
Weight (kg)	88.58 ± 12.28 85.62 (80.06-98.43)	82.82 ± 14.00 78.58 (75.86-86.98)	0.158
Waist Circumference (cm)	97.99 ± 7.83 97.00 (91.71-104.75)	107.63 ± 11.88 103.50 (99.25-110.63)	**0.026**
Body Mass Index (kg/m^2)	28.67 ± 4.06 27.16 (25.77-30.95)	29.84 ± 4.70 28.42 (27.15-31.00)	0.343
Fat Mass (%)	30.87 ± 10.11 27.80 (23.15-38.30)	41.01 ± 10.49 45.50 (29.10-50.00)	**0.020**
Fat Mass (kg)	27.34 ± 10.70 27.79 (19.35-30.79)	33.72 ± 10.98 33.98 (25.05-38.40)	**0.048**
FFM (kg)	61.04 ± 12.14 61.49 (51.02-70.53)	48.68 ± 11.80 46.58 (37.72-57.46)	**0.022**
Relative FFM (kg/m^2)	19.54 ± 2.36 19.35 (17.91-21.13)	17.33 ± 2.94 17.48 (14.72-19.12)	**0.033**
Muscle Quality (kg/kg)	1.12 ± 0.11 1.09 (1.03-1.18)	0.98 ± 0.18 0.98 (0.83-1.12)	0.054

Continuous data are presented as means ± SD and median (25th and 75th), while categorical variables are presented as n (%); HR = Heart Rate; BP = Blood Pressure; FFM = Fat Free Mass; NSAIDs = non-steroidal anti-inflammatory drugs.

3.4.2 Cardiorespiratory Fitness and Training Characteristics

Table 3 describes cardiorespiratory fitness and training variables. Younger adults displayed greater absolute, relative to body weight, and relative to fat-free mass VO_{2peak} compared to older adults (p <0.05). MVPA performed in 10-minute bouts was not significantly different between age groups (young: 54.6 ± 54.1 minutes vs. older: 31.3 ± 30.9 minutes; p >0.05). Similarly, both groups displayed similar amounts of time spent sedentary (young: 614.2 ± 172.9 minutes vs. older: 622.0 ± 93.6 minutes; p >0.05), and in the number of steps taken each day (young: 6264.1 ± 1576.7 minutes vs. older: 5854.0 ± 2538.6 minutes; p >0.05). However, significant strength differences were observed within the sample. Older adults had significantly lower muscle strength measured by 1-RM in each of the exercises in addition to a weaker relative strength to body weight and fat free mass (all p <0.05).

Table 3. Baseline Physical Activity and Fitness Characteristics of the Sample

Variables	Younger Adults (n=13)	Older Adults (n=13)	*p*-value
Absolute VO_{2peak} (L/min)	3.82 ± 0.95 3.83 (2.98-4.71)	2.00 ± 0.54 1.91 (1.51-2.50)	**0.000**
Relative VO_{2peak} ($mL \cdot kg^{-1} min^{-1}$)	43.01 ± 8.13 47.10 (36.51-49.92)	24.17 ± 5.69 24.23 (19.54-28.62)	**0.000**
Relative VO_{2peak} FFM ($mL \cdot kg^{-1} min^{-1}$)	62.13 ± 5.25 62.27 (58.94-66.58)	41.45 ± 7.88 40.49 (37.79-46.82)	**0.000**
MVPA in 10-minute bouts (mins/week)	46.05 ± 45.50 46.43 (0.00-76.06)	31.26 ± 30.87 19.83 (0.00-61.42)	0.482
Steps (# per day)	6205.12 ± 1624.90 6644.33 (4496.48-7715.76)	5854.02 ± 2538.56 5365.86 (4656.17-5729.64)	0.343
Sedentary Time (mins/day)	617.51 ± 179.52 589.36 (492.24-674.42)	621.99 ± 93.55 641.11 (591.51-682.44)	0.369
1-RM Dumbbell Press (kg)	24.61 ± 12.42 22.68 (12.76-36.97)	10.04 ± 5.01 9.07 (5.82-13.61)	**0.002**
1-RM Latissimus Pull Down (kg)	68.81 ± 18.46 70.31 (52.39-75.90)	47.58 ± 13.93 45.96 (38.59-57.72)	**0.004**
1-RM Bicep Curl (kg)	45.95 ± 15.87 48.84 (28.35-54.20)	29.85 ± 12.61 29.48 (19.20-37.42)	**0.024**
Strength Relative To Body Weight (kg/kg)	1.57 ± 0.43 1.58 (1.18-1.98)	1.06 ± 0.36 0.99 (0.78-1.29)	**0.007**
Strength Relative to Fat Free Mass (kg/kg)	2.24 ± 0.33 2.20 (1.95-2.52)	1.78 ± 0.40 1.80 (1.55-2.02)	**0.007**

Continuous data are presented as means ± SD and median (25th and 75th), while categorical variables are presented as n (%); VO_{2peak} = Peak Oxygen Consumption; MVPA = Moderate-to-Vigorous Physical Activity; 1-RM = 1-Repetition Maximum.

3.4.3 Association Between Percent Change in Irisin and Body Composition, Physical Activity, and Strength Measures

No association was observed between the percent change in irisin during exercise and BMI (r = -0.10, p = 0.73), fat mass (r = 0.05, p = 0.82), nor fat-free mass (r = -0.16, p = 0.44) (Table 4). As for physical activity and fitness variables, the percent change in irisin was not significantly associated with MVPA performed in 10-minute bouts (r = 0.02, p = 0.91), nor with sedentary time (r = 0.15, p = 0.48), nor with relative VO_{2peak} (r = -0.16, p = 0.43) (Table 5). The composite score of muscle strength relative to body weight was not associated with the percent change in irisin (r = -0.13, p = 0.51) (Table 6).

Table 4. Association Between the Percentage Change in Irisin and Body Composition Measures

	Younger Adults (n=13)	**Older Adults (n=13)**	**Whole sample (n=26)**
Body Mass Index (kg/m^2)	-0.27 (0.374)	0.10 (0.748)	-0.10 (0.734)
Fat Mass (%)	-0.18 (0.565)	0.11 (0.721)	0.06 (0.758)
Fat Mass (kg)	-0.20 (0.517)	0.12 (0.707)	0.05 (0.820)
Fat Free Mass (kg)	-0.01 (0.972)	-0.30 (0.316)	-0.16 (0.438)
Relative Fat Free Mass (kg/m^2)	0.00 (1.000)	-0.35 (0.247)	-0.19 (0.351)
Muscle Quality (kg/kg)	-0.10 (0.748)	-0.01 (0.972)	-0.22 (0.282)

Data are presented as r (p-value).

Table 5. Association Between the Percentage Change in Irisin and Physical Activity Measures

	Younger Adults (n=13)	Older Adults (n=13)	Whole sample (n=26)
Absolute VO_{2peak} (L/min)	0.10 (0.748)	-0.42 (0.156)	-0.20 (0.321)
Relative VO_{2peak} ($mL \cdot kg^{-1} min^{-1}$)	0.34 (0.255)	-0.39 (0.188)	-0.16 (0.434)
Relative VO_{2peak} FFM ($mL \cdot kg^{-1} min^{-1}$)	0.31 (0.306)	-0.42 (0.150)	-0.13 (0.515)
MVPA in 10-minute bouts (mins/week)	0.32 (0.290)	-0.12 (0.690)	0.02 (0.914)
Steps (# per day)	0.42 (0.156)	-0.20 (0.517)	0.22 (0.273)
Sedentary Time (minutes/day)	0.14 (0.655)	0.37 (0.209)	0.15 (0.479)

Data are presented as r (*p*-value); VO_{2peak} = Peak Oxygen Consumption; MVPA = Moderate-to-Vigorous Physical Activity.

Table 6. Association Between the Percentage Change in Irisin and Strength Measures

	Younger Adults (n=13)	Older Adults (n=13)	Whole sample (n=26)
1-RM Dumbbell Press (kg)	0.07 (0.830)	-0.17 (0.589)	-0.16 (0.439)
1-RM Latissimus Pull Down (kg)	-0.15 (0.621)	-0.34 (0.252)	-0.27 (0.175)
1-RM Bicep Curl (kg)	-0.01 (0.964)	-0.29 (0.344)	-0.22 0.272
Strength Relative to Body Weight (kg/kg)	0.12 (0.694)	-0.15 (0.629)	-0.13 (0.513)
Strength Relative to Fat Free Mass (kg/kg)	0.12 (0.707)	-0.03 (0.929)	-0.12 (0.557)

Data are presented as r (*p*-value); 1-RM = 1-Repetition Maximum.

3.4.4 Impact of Time and Age Group on Irisin

Unadjusted repeated measures analyses demonstrated that there were no time effect on irisin concentration during the acute bout of RT between T0 and T90 ($p = 0.702$). In addition, no group effect was found ($p = 0.851$) nor was there a time vs. group interaction ($p = 0.493$; Figure 2) (Figure 4). Figure 5 shows adjusted analyses for percent body fat, waist circumference, relative VO_{2peak}, and muscle strength. In this analysis, similar results were observed without an effect of time between T0 and T90 ($p = 0.453$), nor an effect of age group ($p = 0.418$), nor an interaction effect ($p = 0.582$).

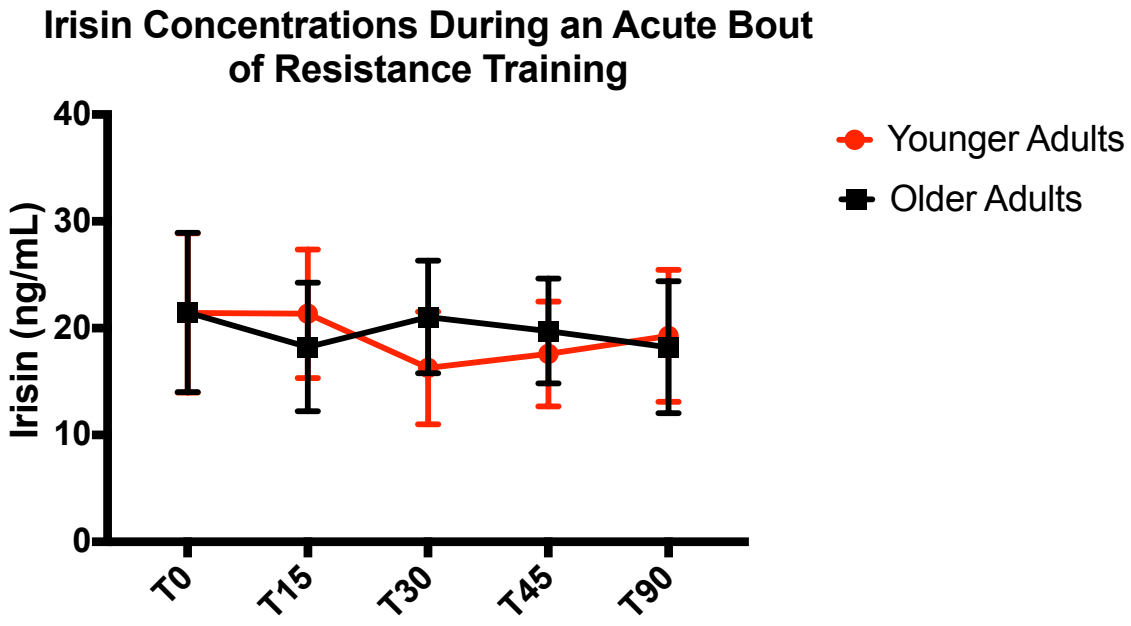

Figure 4. Irisin concentrations during an acute bout of resistance training in younger and older adults living with overweight or obesity.

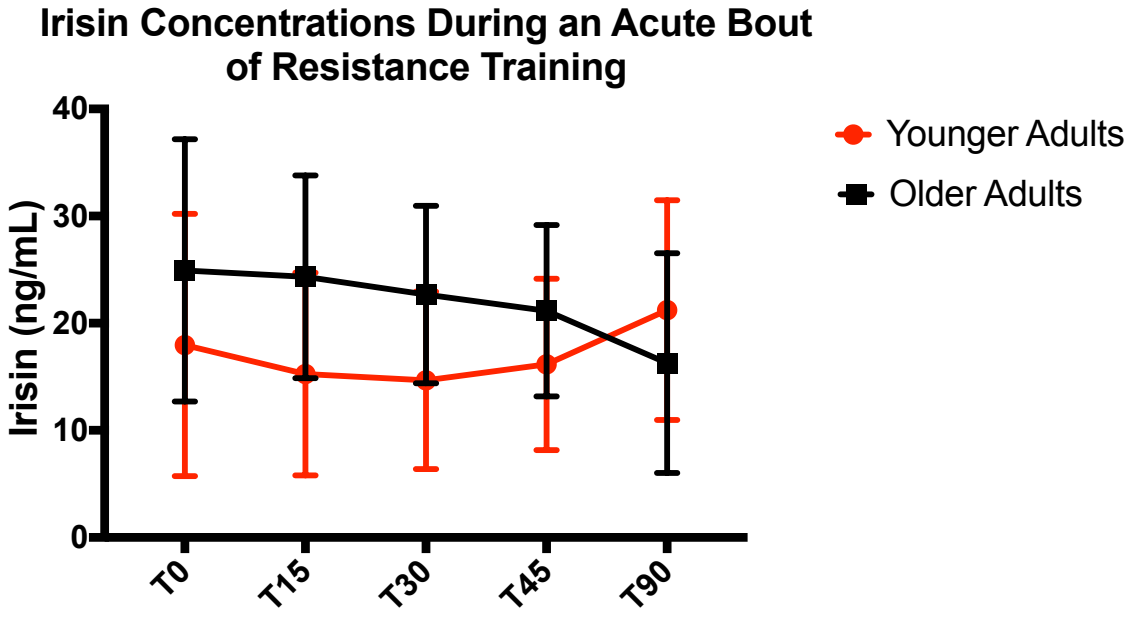

Figure 5. Irisin concentrations during an acute bout of resistance training in younger and older adults living with overweight or obesity, adjusted for % body fat, waist circumference, relative VO2peak, and 1-repetition maximum.

CHAPTER 4: Discussion

4.1. Does Irisin Increase During an Acute Bout of Resistance Training in Younger and Older Adults Living with Overweight or Obesity?

The first objective of this study was to identify whether irisin concentration increases during an acute bout of resistance training in individuals living with overweight or obesity. The second objective was to investigate whether differences in irisin were observed between younger and older adults living with overweight or obesity. Unexpectedly, our results showed that an acute bout of resistance training did not raise circulating irisin concentrations in individuals living with overweight or obesity. Furthermore, in individuals of similar BMI and physical activity levels, no differences were observed in the acute release of irisin after resistance training between younger adults living with overweight or obesity compared to older adults living with overweight or obesity. These findings are relevant from both a clinical and an exercise standpoint as they provide new insight into the role of exercise and myokine release in individuals living with overweight or obesity. As irisin has been proposed to play a key role in the treatment of obesity and diabetes, and exercise is the first line of treatment for individuals living with overweight, obesity, and diabetes, these findings are meaningful from a treatment and management perspective.

Although irisin has been shown to transiently increase during both aerobic and resistance exercise, irisin is mainly regulated by an overexpression of PGC1-α [317]. Typically, PGC1-α is known to be induced by aerobic exercise [424,425]; however, recently, it has been shown that resistance exercise might stimulate PGC1-α as well [426-428]. However, the intensity of exercise also seems to impact irisin. It may be hypothesized that

the intensity of resistance exercise may play a role in the expression of this protein. According to Burd et al. (2012), exercising at a low intensity causes a significant induction in PGC1-α. They observed a 3-fold increase in PGC1-α mRNA expression 6 hours post-exercise after an acute bout of resistance training (3 sets of leg extension to failure at 30% 1-RM) [426]. Based on these data, it is believed that the program that was utilized in the current study was performed at a load that was too high (65-70% 1RM), such that it may have inhibited the PGC1-α pathways with resistance exercise in this sample.

While limited research exists surrounding the impact of acute resistance training on irisin release, the results of the available studies emphasize that irisin concentration increases following the completion of the acute resistance training session [350,378,384,385]. However, in these studies, blood samples were taken after the exercise sessions while no blood draws were taken during the exercise session. Therefore, our results add to the body of evidence on acute resistance training by documenting the transient changes in irisin during resistance training in two distinct age groups. The current study aimed to provide a more comprehensive understanding of the changes of irisin during exercise; therefore, multiple measurement time points of irisin were taken during the acute bout (15, 30, and 45 minutes) in addition to pre- and post-exercise (after 45 minutes of rest). Differences between our results and those observed may have been driven by a delayed effect of PGC1-α expression following exercise. In fact, some data suggest that PGC1-α peaks 3 hours post-exercise [299,348,429] and, therefore, it is possible that the immediate post-exercise blood samples were taken too early to detect any changes in irisin. In a different population, both Nygaard et al. (2015) and Tsuchiya et al. (2015) observed a significant increase in irisin one hour following an acute bout of resistance training [384,385], which

supports a potential delayed effect of PGC1-α rather than an acute increase immediately post-exercise. In the current study, irisin concentration began to increase linearly in younger adults living with overweight or obesity leading up to one-hour post-exercise, although non-significantly. The final time point was collected 45 minutes post-exercise, which may not have been long enough of a duration to observe changes in irisin according to data showing that PGC1-α is upregulated maximally 2-3 hours post-exercise [299,348,429]. However, some studies suggest that changes in PGC1-α are not consistently accompanied by changes in FNDC5, the precursor of irisin [378,413]. Pekkala et al. (2013) observed a 4-fold increase in PGC1-α and only a 1.5-fold increase in FNDC5 one hour following an acute bout of resistance training [378]. As the authors observed an unmatched increase in PGC1-α and FNDC5 expression, they suggested that another pathway may be involved in the release of irisin [378]. Furthermore, Raschke et al. (2013) prompted that a "profound induction of PGC1-α may be required to activate the downstream target of FNDC5" [382], which therefore may be needed in order to cause a meaningful alteration in irisin concentration. In resistance training, significant increases in PGC1-α expression have been reported [426,427]; therefore, aerobic training is not the only form of exercise to induce the expression of this transcriptional co-activator and could potentially lead to irisin release.

Interestingly, Ruas et al. (2012) identified a different isoform of PGC1-α, termed PGC1-α4, which is expressed abundantly in skeletal muscle during resistance training. These authors showed that this isoform regulates different genes than PGC1-α itself, and instead, PGC1-α4 increases insulin-like growth factor 1 (IGF-1) and reduces myostatin, leading to increased muscle mass and strength [427]. In another study using primary cultured

human myocytes, cells treated with irisin upregulated PGC1-α4, and increased IGF-1 and decreased myostatin gene expression [430]. All together, these results suggest that PGC1-α4 expression is related to muscle growth and is an important regulator during resistance training. Considering that it has been established that aerobic exercise performed simultaneously with resistance exercise counteracts each other [431], it is possible that the step-box and squat exercises performed over a period of three minutes each in the current study may have negatively impacted the release of PGC1-α4. These resistance exercises involve a strong component of aerobic exercise, and skeletal muscle adaptations are specific to the mode performed [432,433]. As such, a simultaneous stimulation of both aerobic and resistance exercise pathways might have led to a sub-optimal activation of each, reducing the capability of skeletal muscle to express PGC1-α4 and FNDC5. This hypothesis cannot be ruled out since we did not measure PGC1-α4.

A number of factors relating to type, intensity, and duration may have influenced the observed results. According to the findings of the current study, the notion that a single acute bout of exercise may not be enough to stimulate the signaling pathways during exercise is supported. In addition, the intensity might have been too high to meaningfully induce irisin, as the high load may have inhibited the pathways that regulate irisin release. In aerobic exercise studies, a dose-response exists in which greater exercise intensities result in greater irisin concentrations [386,387,407]. For instance, Daskalopoulou et al. (2014) compared the effects of aerobic intensity exercises on irisin in three separate conditions: maximal workload (VO_{2max}), relative workload (70% VO_{2max}), and an absolute workload (10-minute cycling bout at 75W). They reported a dose-response increase in irisin concentrations ($p = 0.001$), with the greatest increase occurring after the maximal exercise

condition [407]. However, greater metabolic stress to skeletal muscle with higher intensity resistance exercises might actually hinder irisin release, as high loads of resistance exercise may inhibit the PGC1-α4 pathways involved in irisin release. Lastly, as an accumulation of subsequent muscle contractions increases the metabolic demand, a longer duration of resistance training may be required – which might allow enhanced muscular endurance.

Although our hypothesis was not confirmed, this may have been partially due to the fact that the sample involved in the study was considered to be very inactive. The participants were far from reaching the Canadian Physical Activity Guidelines, with the younger adults performing only 46.05 ± 45.50 minutes per week and older adults just 31.26 ± 30.87 minutes per week. Interestingly, there were no differences ($p > 0.05$) in physical activity levels between younger and older adults, nor in the amount of steps taken per day, nor in sedentary time. Results from a meta-analysis performed by our group indicate that fitness level was the best predictor of irisin, such that fit individuals had a 2-fold greater irisin concentration compared to their unfit counterparts following an acute bout of exercise [379]. However, there was no relationship between fitness and the percent change in irisin in our sample ($r = -0.16$, $p > 0.05$). In Nygaard et al. (2015) and Tsuchiya et al. (2015)'s studies, the participants involved were moderately physically active to active [384,385]. As higher fitness levels have been associated with higher irisin concentration [379], participant differences might explain discrepancies observed between studies.

4.2 Are Differences in Irisin Observed Between Younger and Older Adults Living with Overweight or Obesity During an Acute Bout of Resistance Training?

We hypothesized that there would be a significant difference in the irisin response to acute resistance training between the age groups in individuals living with overweight or obesity. The data from the current study do not support our hypothesis, as no differences were observed between the age groups of younger and older adults. As such, biological aging does not appear to alter the acute response to irisin with resistance training. This observation might have been impacted by age-related alterations in skeletal muscle and adiposity.

In our study, although irisin did not change throughout the acute bout of resistance training, we observed a trend toward a decrease in irisin in older adults. This finding may have been impacted by the age-related loss in skeletal muscle mass known as *sarcopenia* [434]. Individuals living with sarcopenia have been shown to have lower basal irisin concentrations compared to those without sarcopenia [398]. Interestingly, in a cross-sectional analysis, circulating irisin concentrations were associated with a 80.0% reduced risk of sarcopenia when adjusted for age, sex, BMI, and waist-to-height ratio [398]. Irisin induces the release of an adipo-myokine, interleukin 6 (IL-6), which is an essential regulator of skeletal muscle hypertrophy [374]. Irisin has been shown to promote myogenesis through IL-6 signaling [374]. It may be hypothesized that the lack of irisin release during RT with aging leads to a reduced IL-6 release, thereby blunting increases in muscle mass, which might lead to sarcopenia [398]. By the age of 80 years old, about 50% of original skeletal muscle mass is lost [51]. As lower muscle mass is associated with lower irisin [387], it is logical to believe that losses of this magnitude may largely reduce irisin concentrations in older adults. However, according to our findings, fat-free mass was not associated with the

percent change in irisin (r = -0.16, p = 0.44). This was surprising, as there was a significant difference in baseline fat-free mass between age groups (p = 0.022) but no difference in baseline irisin concentrations (p >0.05). This finding provides new data to suggest that fat-free mass may not be an important regulator of irisin release. Our data adds to this literature by providing insight into the acute irisin response of older adults with low muscle mass.

Reductions in skeletal muscle mass might contribute to the age-related losses of skeletal muscle strength [435,436]. In fact, older adults in our study displayed a reduced fat-free mass as well as a reduced muscle strength compared to younger adults. Fat-free mass was correlated with muscle strength in each of the 1RM strength tests in older adults (bicep curl: r = 0.82, p = 0.001; lateral pull down: r = 0.77, p = 0.002; dumbbell press: r = 0.59, p < 0.05), suggesting that reduced muscle mass contributed to lower muscle strength in older adults. Although this was observed, no relationship was observed between muscle strength and irisin (r = -0.27.; p >0.05). These results are surprising, as many studies observed a positive relationship between muscle strength and irisin [374,398]. For instance, Chang et al. (2017) observed a positive relationship between irisin and muscle strength measured by handgrip strength using a dynamometer (r = 0.22 (men); r = 0.312 (women), all p < 0.01) [398]. Based on these observations, it was logical to hypothesize that there would be a significant difference in the irisin response between individuals of different ages during the acute bout of resistance training.

Finally, the muscle quality of participants in the current sample might, in part, explain why acute resistance training did not increase irisin. Muscle quality is defined by muscle strength per volume of fat-free mass [437] and was calculated as: 1-RM (kg)/fat-free mass (kg). Some data suggest that muscle quality is a potentially better indicator of muscle

function than muscle strength alone [438,439]. Proper muscle function may have been impaired in our participants living with overweight or obesity, which could weaken the skeletal muscle's ability to secrete irisin. Both aging and obesity are associated with an increased amount of skeletal muscle fat infiltration [440-443]. Increased fat infiltration in the skeletal muscles impairs force production [270,444], decreases strength and mobility in older adults [445,446], and is associated with poor muscle quality [447]. Older adults had a lower fat-free mass and muscle strength, demonstrating that younger adults were stronger than their counterparts. Although younger adults have a better muscle quality, higher fat-free mass and muscle strength, they also likely had high amounts of fat infiltration due to their weight status [440]. Possessing an equal amount of skeletal muscle fat infiltration might have impacted the contractility of the skeletal muscles in both groups, which may have reduced their capability to exercise at the proper intensity to stimulate irisin release beyond resting concentrations. Although it has been reported in the literature that skeletal muscle fat infiltration is involved in contraction impairment, our study did not assess this variable.

During aging, there is also an increase in adiposity [269]. Although a similar BMI was observed between the two groups in this study ($p > 0.05$), older adults had a significantly higher amount of fat mass (33.7 ± 10.9 kg) compared to their younger (27.3 ± 10.7 kg) counterparts ($p < 0.05$). The lack of a relationship between irisin and BMI might be partially explained by different amounts of adipose tissue. In mice and humans, leptin has been shown to downregulate FNDC5 in the adipose tissue [354], which negatively regulates irisin-induced fat browning [354,355]. Older adults who possess greater amounts of adipose tissue may express more leptin [448], and therefore less FNDC5 in the adipose tissue. However, leptin upregulates FNDC5 in skeletal muscle [285]. As such, this upregulation in

skeletal muscle by leptin may have compensated for the potentially lower FNDC5 due to lower fat-free mass in older adults. This may have led to similar amounts of FNDC5 being expressed between younger and older adults, and therefore potentially similar irisin. Furthermore, irisin also acts as an adipokine, and exerts paracrine and autocrine effects from the adipose tissue [285]. Due to leptin's downregulating action on FNDC5 in adipocytes, it is possible that irisin released from the adipose tissue acts locally and is not cleaved and released in the blood. Accordingly, no association was observed between irisin and adiposity in the current study, nor was there an association between irisin and BMI. Although the relationship between BMI and irisin is unclear [342,449,450], many have reported a negative association [342,346,347]. However, a study found that skeletal muscle FNDC5 is higher (non-significantly) in those living with obesity [360]. The discrepancy between FNDC5 and irisin according to BMI suggests that an event may occur between the cleavage of FNDC5 and the release of irisin that alters the concentrations. Differing adiposity levels might have also led to a discrepancy between FNDC5 cleavage and irisin release in our study.

Thus far, the relationship between biological aging and irisin has not been extensively explored in a meaningful way. In a study by Miyamoto-Mikami et al. (2015), they compared the difference in irisin release between younger and older adults before and after 8 weeks of chronic aerobic training [351]. They observed a significant increase in irisin in older adults, but no changes were seen in younger adults. This trend was dissimilar to what was seen in our study, as older adults tended to have a non-significant decrease in irisin over time and younger adults non-significantly increased irisin over time. Nevertheless, fundamental differences existed in the design of their study compared to ours that could help explaining the difference in results. First, the study focused on aerobic

training, which would induce mitochondrial biogenesis to a greater extent than resistance training, thereby upregulating PGC1-α and FNDC5. Second, their protocol involved chronic training, and adaptations to chronic exercise are different than the adaptations seen with acute exercise. Finally, both groups were healthy and of normal BMI, indicating that basal irisin concentrations were more than likely different than the sample in our study. In fact, basal irisin was considerably higher in the study by Miyamoto-Mikami et al. (2015) (younger adults = 155.3 ± 17.3 ng/mL; middle-aged/older adults = 140.6 ± 26.7 ng/mL) compared to ours (younger adults = 21.4 ± 14.4 ng/mL; older adults = 21.5 ± 11.5 ng/mL). In addition, it should be noted that the results observed by Miyamoto-Mikami et al. (2015) should be interpreted with caution, as the baseline values obtained were substantially higher than ours, and the version of the ELISA that was used in their study (EK-067-16) may have been part of the ELISA kits that were utilized prior to the validation of these assays [380]. Our study involved an ELISA kit that was validated against liquid chromatography mass spectrometry and western blotting (EK-067-29) [388,416]. Thus, these factors might have also explained the different response to irisin that they observed during exercise compared to our study. Another study, by Timmons et al. (2012), reported a greater FNDC5 expression after exercise in highly active older adults (1.3-fold increase) compared to sedentary controls [413]. These results would suggest that irisin and FNDC5 increase with aging, which was not observed in our data. A number of studies have demonstrated the negative relationship between aging and irisin, though [386,387]. To the best of our knowledge, Pekkala et al. (2013) is the only study that investigated the impact of aging on acute resistance training [378]. No changes in irisin were observed in their sample; however, it should be noted that this cohort involved those with a normal to slightly

overweight BMI. As these participants were leaner than those of our study, they may have already had a higher irisin concentration, potentially explaining the lack of increase in irisin observed in their study. In addition, although no changes in irisin were seen in either group, they reported a lower increase in PGC1-α in older adults (2-fold) compared to younger (4-fold) adults, and a significant increase in FNDC5 only in the younger adults. This aligns with the suggestion that in order to activate FNDC5 downstream and observe a significant alteration in irisin in older adults, a strong overexpression of PGC1-α4 would be required [382]. No data was obtained to measure PGC1-α4 and FNDC5 in our study, thus comparisons may not be made in this regard; however, neither our study or that of Pekkala et al. (2013) observed a significant increase in irisin following an acute bout of resistance training in younger or older adults.

4.3 Strengths and Limitations

The current study has a few limitations that must be highlighted and taken into consideration for the interpretation of the data. First, diabetes occurrence within the sample was self-reported. A direct measure would have allowed us to control for those who had high levels of glucose (living with pre-diabetes) or those who may be un-diagnosed. This is important, as individuals living with diabetes have lower basal irisin [360] and those with pre-diabetes have higher concentrations [451]. Second, although a measure of irisin was taken 45 minutes post-exercise, based on more recent data it is possible that irisin might have increased after this measurement and we missed the kinetic peak for our population. As a number of studies observed increased irisin a few hours after the completion of an acute resistance training bout, it may have been important to include

timepoints similar to these. Third, irisin was measured by ELISA, which is not the gold standard for this measurement. Although the ELISA utilized was validated with the gold standard, a number of flaws remain with ELISA analysis. Although a number of limitations exist, this study is strengthened by the use of strong measurement tools. Body composition was assessed with the gold standard measurement of the BodPod system, which determines precise measures with minimal error. Cardiorespiratory fitness was assessed with the use of a gold standard VO_{2max} test to make associations with irisin and aging. Physical activity levels and fitness were directly measured using accelerometers, rather than self-reported measures. Furthermore, the ANOVA statistical analysis was adjusted for the main potential confounders, including adiposity, waist circumference, cardiorespiratory fitness, and strength. Finally, the participants included in our study included two distinct age groups who were of similar physical activity levels and BMI.

4.4 Conclusion

In conclusion, an acute bout of resistance training did not increase plasma irisin concentrations in individuals living with overweight or obesity. Furthermore, biological age differences were not associated with a different irisin response to an acute bout of resistance training in younger nor older adults of similar fitness and BMI levels. However, the findings of the current study help to elucidate the effect of acute resistance training on irisin release and provide relevant insight into the biological impact of aging on acute exercise and myokine response. Additional studies are needed to further understand the mechanisms of the irisin response to acute exercise using different modalities/intensities

of exercise, and to directly measure PGC1-α4 and FNDC5 expression in the skeletal muscle to confirm the pathways involved in the release of irisin.

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Appendix A

Enzyme-linked Immunosorbent Assay Procedure

(Phoenix Pharmaceuticals, Inc. EK-067-29)

General Design:

The plate is pre-coated with a secondary antibody, which finds to the primary antibody. The primary antibody is bound by the biotinylated peptide and the targeted peptide in either the peptide solution or the unknown sample through a competitive process. The biotinylated peptide interacts with streptavidin-horseradish peroxidase (SA-HRP). Then, the substrate solution is catalyzed by the SA-HRP. The intensity of the yellow color from this reaction is directly proportional to the amount of biotinylated peptide-SA-HRP complex, and is inversely proportional to the amount of targeted peptide. Unknown peptide concentration from the samples is extrapolated by a standard curve based on O.D. absorbance and known standard peptide concentrations.

Summary of Protocol:

1. Add 50 μL/well of standard, sample, or positive control, along with 25 μL/well of primary antibody and biotinylated peptide to each well
2. Incubate at room temperature (20-23°C) for 2 hours
3. Wash immunoplate 4 times with 350 μL/well of 1x assay buffer
4. Add 100 μL/well of SA-HRP solution
5. Incubate at room temperature (20-23°C) for 1 hour
6. Wash immunoplate 4 times with 350 μL/well of 1x assay buffer
7. Add 100 μL/well of Tetramethylbenzidine substrate solution
8. Incubate at room temperature (20-23°C) for 1 hour

9. Terminate reaction with 100 μL/well of 2N Hydrochloric Acid

10. Read absorbance O.D. at 450nm and calculate result

Curriculum Vitae

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Publications:

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50:396-397. (American College of Sports Medicine: 65th Annual Meeting, 9th World Congress on Exercise is Medicine, World Congress on the Basic Science of Muscle Hypertrophy, Minneapolis, MN, United States).

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