Exercise Response: Is Irisin a Novel Factor That Explains the Metabolic Adaptation?

by

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A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of

Doctor of Philosophy

in the Graduate Academic Unit of Interdisciplinary Studies

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This dissertation is accepted by the
Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

December, 2025

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ABSTRACT

Exercise is a cornerstone in the management of metabolic conditions like obesity and type 2 diabetes (T2D), yet substantial variation exists in individual responses. Irisin, an exercise-induced myokine involved in regulating adipose tissue and glucose metabolism, may contribute to this heterogeneity. However, its precise quantification and exercise-induced responses remain unclear due to analytical discrepancies and inconsistent findings across study designs and populations. To address these gaps, this dissertation comprehensively examined exercise-induced irisin responses across metabolic health statuses and exercise intensities. The objectives were to: evaluate the analytical performance of irisin ELISA kits; assess acute irisin responses across different exercise intensities in individuals of varying obesity and insulin sensitivity status; and investigate the impact of increasing intensity on chronic exercise adaptations, along with irisin's predictive role in glycemic improvements. This dissertation consisted of three projects. First, the agreement, between-day reliability, and within-subject precision of two commercially available irisin ELISA kits was evaluated. Second, acute irisin responses were assessed in a randomized controlled crossover trial, where adults with overweight/obesity (OW/OB) or a healthy weight completed acute bouts of moderate-intensity continuous training (MICT), high-intensity interval training (HIIT), and rest. Third, a secondary analysis of the INTENSITY trial examined the impact of increasing exercise intensity on irisin and its association with individual glycemic responses in individuals with prediabetes/T2D who completed a 28-week aerobic training (AT) intervention. Significant bias was identified between ELISA kits, with R&D Systems demonstrating superior reliability and precision compared to Phoenix Pharmaceuticals. OW/OB exhibited lower circulating irisin that only increased

after acute HIIT but not MICT. Irisin was positively correlated with insulin sensitivity in healthy weight and not OW/OB. Circulating irisin remained unchanged across the AT intervention at the group-level; minor, individual increases in irisin predicted glycemic improvements. This dissertation offers novel insights into irisin's role in exercise metabolism, revealing distinct acute and chronic adaptations with robust quantification. Higher exercise intensities may be necessary for acute irisin responses in OW/OB. In individuals with prediabetes/T2D, no chronic adaptations were observed regardless of intensity; yet, minor individual increases predicted glycemic improvements. These findings support the development of targeted strategies for personalized exercise prescription.

DEDICATION

For my mother, Michelle, who instilled in me a love for learning from my earliest days. Your guidance, tireless devotion, and profound influence ignited a sense of curiosity and perseverance that made this dissertation possible. You are, and always will be, my biggest role model.

For my brother, Zachary, whose brilliance never ceases to inspire me, and for my father, Yvano, whose uplifting spirit motivates me to be resilient and determined.

To my husband, Colin, for always being a calm in the storm. Your steadfast presence provides an essential balance, bringing laughter and lightness in even the most arduous of times, and offers the vital reminder not to sweat the small stuff. Thank you for your unwavering belief in my capabilities and for inspiring me throughout this journey.

To the many incredible friends I've made at the Cardiometabolic Exercise and Lifestyle Laboratory (CELLAB) since its establishment, who have truly felt like family.

And for Martin, whose mentorship has profoundly shaped my development both as an academic and as a person over the past decade. Thank you for your invaluable guidance, for every teaching moment shared and opportunity provided, and for your endless supply of dad jokes. The culmination of this work stands as a testament to your extraordinary commitment to fostering student success, a legacy I am honoured to contribute to.

Lastly, to my loving family, encouraging friends, and all those whose support helped me navigate the ups and downs throughout this journey. My gratitude is eternal.

"The best view comes after the hardest climb."

ACKNOWLEDGEMENTS

I'd like to extend my sincere appreciation to my supervisor, Dr. Martin Sénéchal, for his exceptional guidance, trusted insight, and invaluable expertise that were instrumental throughout every stage of this dissertation. I am also indebted to my supervisory committee members, Dr. Neil Johannsen and Dr. Stephen Cornish, for their input and thought-provoking discussions throughout this process. I am grateful to each of them, and to Dr. Maryam Kebbe and Dr. Normand Boulé, for their generous contributions of time and critical evaluations of my dissertation.

This research was made possible through financial support from the Canadian Institutes of Health Research (CIHR) (Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award) and the University of New Brunswick (Dr. William S. Lewis Doctoral Fellowship, Canadian Federation of University Women Scholarships, and a New Brunswick Innovation Foundation CIHR Top-Up Award). Project-specific grant funding was generously provided by Research New Brunswick, Diabetes Action Canada, the Canada Foundation for Innovation, and the Heart and Stroke Foundation of New Brunswick. Furthermore, my gratitude goes to the dedicated students and staff at the CELLAB for their assistance with data collection and analysis of the projects involved in this dissertation (particularly Travis J. Hrubeniuk, Benjamin H. Colpitts, Martine Girard and Spencer Smith), and to the many volunteers for their essential participation in the iMET and INTENSITY studies.

My deep appreciation also extends to the UNB Faculty of Kinesiology, for their exceptional facilities, supportive staff, and dedicated faculty. Their collective efforts were instrumental in the successful completion of this research.

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LIST OF ABBREVIATIONS

1RM	One-repetition maximum
ACSM	American College of Sports Medicine
ADA	American Diabetes Association
ADAM	A disintegrin and metalloproteinase
AMPK	AMP-activated protein kinase
AT	Aerobic training
ATP	Adenosine triphosphate
AUC	Area Under the Curve
BMI	Body mass index
CCHS	Canadian Community Health Survey
CELLAB	Cardiometabolic Exercise and Lifestyle Laboratory
CHMS	Canadian Health Measures Survey
CI	Confidence interval
CSEP	Canadian Society for Exercise Physiology
CV	Coefficient of Variation
DARE Trial	Diabetes Aerobic and Resistance Exercise
DPP-IV	Dipeptidyl peptidase-4
EDTA	Ethylenediaminetetraacetic acid
E-MECHANIC	Examination of Mechanisms of Exercise-Induced Weight Compensation
ELISA	Enzyme-linked immunosorbent assay

ERK	Extracellular signal-regulated kinase
EXIT	EXercise and Irisin sTudy
FFM	Fat-free mass
FNDC5	Fibronectin type III domain-containing protein 5
FTO	Fat mass and obesity-associated
GBD	Global Burden of Disease
GDP	Gross domestic product
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLUT	Glucose transporter
GWAS	Genome-Wide Association Study
HART-D	Health Benefits of Aerobic and Resistance Training in Individuals with Type 2 Diabetes
HbA_{1c}	Glycated hemoglobin
HDL	High-density lipoprotein
HERITAGE	HEalth, RIsk factors, exercise Training And Genetics Family Study
HIIT	High-intensity interval training
HOMA-IR	Homeostatic model assessment of insulin resistance
HR	Hazard ratio
HRR	Heart rate reserve
ICC	Intraclass Correlation Coefficient
IDES	Italian Diabetes and Exercise Study

IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL	Interleukin
iMET	Irisin and Metabolic Exercise Training Study
INTENSITY	Improving Individual Glycemic Response with Exercise Intensity
LDL	Low-density lipoprotein
LMM	Linear mixed modeling
Look-AHEAD	Action for Health in Diabetes
MCID	Minimal clinically important difference
METs	Metabolic equivalents of a task
MHO	Metabolically healthy obese
MICT	Moderate-intensity continuous training
mRNA	Micro ribonucleic acid
MPH	Miles per hour
MU	Metabolically unhealthy
MUO	Metabolically unhealthy obese
MVPA	Moderate-to-vigorous intensity physical activity
NAFLD	Non-alcoholic fatty liver disease
NCD-RisC	NCD Risk Factor Collaboration
NCEP:ATPIII	National Cholesterol Education Program: Adult Treatment Panel III

NEAT	Non-exercise activity thermogenesis
OGTT	Oral glucose tolerance test
OR	Odds ratio
OW/OB	Overweight/obesity
p38 MAPK	p38 mitogen-activated protein kinase
PI3K/Akt/mTOR	Phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin
PGC-1α	Peroxisome proliferator-activated receptor-γ coactivator 1α
PPARγ	Peroxisome proliferator-activated receptor-γ
QUICKI	Quantitative insulin sensitivity check index
RCT	Randomized controlled trial
RMR	Resting metabolic rate
RNS	Reactive nitrative species
ROS	Reactive oxidative species
RR	Relative risk
RT	Resistance training
SGLT2	Sodium-Glucose Transport Protein 2
Si	Insulin sensitivity
SNP	Single nucleotide polymorphism
STRRIDE-AT/RT	Studies of a Targeted Risk Reduction Intervention through Defined Exercise-Aerobic and Resistance Training

T2D	Type 2 diabetes
TNF-α	Tumour necrosis factor-α
UCP1	Primarily uncoupling protein 1
VO_{2max}	Maximal oxygen consumption
VO_{2peak}	Peak oxygen consumption
WAT	White adipose tissue
WBISI	Whole-body insulin sensitivity index
WHO	World Health Organization

1.0 INTRODUCTION

Despite the unequivocal evidence of the health benefits of physical activity and exercise, global physical inactivity rates remain a critical public health challenge.[1] The consequences of physical inactivity are substantial, leading to a higher incidence of chronic diseases[2,3] and a marked reduction in lifespan.[4] Despite the alarming levels of physical inactivity worldwide, it is estimated that current rates of physical activity avert approximately 3.9 million premature deaths annually.[5] This emphasizes the critical need for evidence-based physical activity guidelines and effective public health messaging to translate scientific recommendations into clear, actionable steps that individuals can take to improve their health.[6] While guidelines established by global[7] and federal agencies[8,9] specify recommended amounts and types of exercise to maximize health benefits, it has become increasingly apparent that not all individuals experience these gains to the same extent.[10-12]

Data from the seminal HEalth, RIsk factors, exercise Training And GEnetics (HERITAGE) Family Study initially brought attention to the substantial interindividual heterogeneity that exists in response to exercise training.[13] This finding triggered a subsequent proliferation of research investigating the variation in individual responses to exercise, revealing notable interindividual heterogeneity across a broad range of health outcomes.[11,12,14,15] The observed differences in exercise responses may arise from a combination of intrinsic and extrinsic factors, including age, sex, genetics, sleep patterns, and health status, etc.[16] Additionally, the specific parameters used in exercise interventions can also significantly impact these individual responses.[17] Crucially, as only 20-30% of the variation in the cardiometabolic response to exercise training has been elucidated, even

when accounting for genetic factors,[18] which suggests that other factors may play important roles in influencing exercise response heterogeneity, including irisin.

Irisin, an exercise-induced myokine, has attracted considerable attention for its potential role in mediating some of the beneficial effects of exercise and physical activity on cardiometabolic health. Its association with adipose tissue browning and contributions to increased energy expenditure, weight loss, and improved glycemia suggest its involvement in the pathophysiology and treatment of metabolic conditions, such as obesity and type 2 diabetes (T2D).[19] However, inconsistent findings in irisin quantification due to methodological discrepancies have compromised the precise characterization of its role in exercise research. Acute bouts of exercise have been shown to transiently increase circulating irisin concentrations and protein expression,[20-24] although its effects may vary in clinical populations.[25,26] Additionally, conflicting and often inconsistent evidence exists regarding irisin adaptations in response to chronic exercise interventions,[20-24] particularly concerning the influence of differing exercise parameters.[23,27,28]

Interestingly, studies across various health outcomes, including cardiorespiratory fitness, suggest that modifying exercise stimuli (e.g. intensity, duration, frequency, or mode) may reduce the variability in individual responses and increase the likelihood of experiencing beneficial physiological adaptations.[12,29,30] This raises the possibility that irisin may serve as a pivotal factor in explaining individual variability in cardiometabolic exercise responses, thereby providing further understanding of the mechanisms involved in these adaptations.

Given the substantial interindividual variability in exercise responses and irisin's potential role in influencing these effects, this dissertation aims to address these gaps in the

literature by investigating the exercise-induced irisin responses across diverse metabolic health statuses and exercise intensities. Specifically, the objectives of this research are to: 1) comprehensively evaluate the analytical performance of commercially available irisin ELISA kits to ensure reliable and precise quantification of circulating irisin in human plasma; 2) examine the impact of exercise intensity on acute irisin responses in individuals with and without overweight/obesity, while exploring its association with insulin sensitivity; and 3) investigate the impact of increasing exercise intensity on chronic circulating irisin adaptations in individuals with prediabetes or T2D and assess irisin's predictive role in achieving individual and clinically meaningful glycemic improvements. The insights gained from these works will contribute to a more nuanced understanding of irisin's complex role in exercise responses and provide a foundation for the development of personalized exercise strategies that tailor interventions to individual needs.[31,32] Such advancements could have significant implications for the prevention and management of chronic conditions like obesity and T2D.

1.1 Physical Activity

1.1.1 Physical Activity Guidelines

The *Canadian Physical Activity Guidelines*[33] recently transitioned to the *24-Hour Movement Guidelines* in 2020.[8] Experts provided updated recommendations to maximize the benefits of physical activity alongside other health-related movement patterns. The culmination of evidence led to the recommendation that adults aged 18-64 should accumulate at least 150 minutes per week of moderate-to-vigorous intensity physical activity (MVPA) and a minimum of two days per week of muscle-strengthening activities using major muscle groups. Notably, the previous requirement for MVPA to be

accumulated in bouts of 10 minutes or more has been eliminated due to a lack of supporting empirical evidence.[34] Identical recommendations apply for adults aged 65 and over, but with an added focus on physical activities that challenge balance.[8] New to these guidelines is the incorporation of light physical activity, including standing, to further induce health benefits,[8] as several hours per day can improve glucose and insulin levels,[35] lipid profiles, body composition, blood pressure,[36] and can even reduce the risk of premature all-cause mortality.[37] Altogether, these findings reinforce the messaging in the updated guidelines that adults should be active in any way they can throughout the day, and that some is better than none.

The *Canadian 24-Hour Movement Guidelines* were among the first in the world to adapt their physical activity recommendations to incorporate a range of movement-related behaviours that contribute to a healthier 24-hour day.[38,39] In this context, physical activity is seen as one component of the integrated system of health-related movement patterns, which also includes sedentary behaviour and sleep.[40-42] In this all-encompassing view of health, Ross et al. (2020) suggest that "replacing sedentary behaviour with additional physical activity, and trading light physical activity for more MVPA, while preserving sufficient sleep, can provide greater health benefits".[8] Although 24-Hour Movement Guidelines are not yet implemented universally, many countries have developed similar frameworks following Canada's lead, highlighting the beginning of a new era in public health interventions and physical activity research. While the physical activity components of the guidelines from global[7] and federal agencies[8,9] may differ slightly, each relies on the central principle of reaching a minimum of 150 minutes per week of MVPA, along with

muscle- and bone-strengthening activities, and limiting time spent engaging in sedentary behaviours.

1.1.2 Impact of Physical Activity on Health

1.1.2.1 Impact of Aerobic Training on General Health

Of central importance, a strong inverse dose-response relationship exists between the amount of MVPA performed in a week and all-cause mortality, which is consistent across age, sex, race, and weight status.[8] In a large pooled analysis, including data from six population-based prospective cohort studies (n = 661,137), Arem et al. (2015) found that performing even less than the recommended MVPA guidelines still led to a decreased risk of premature all-cause mortality, with additional benefits observed at higher levels.[43] The greatest risk reduction was reported at three to five times the recommended MVPA levels (hazard ratio (HR) 0.69, 95% confidence interval (CI) [0.67, 0.70]); however, the risk reduction was not substantially greater than meeting the minimum guidelines (HR 0.61, 95% CI [0.59, 0.62]), suggesting that simply meeting the guidelines may provide the same benefits on mortality as the highest levels of MVPA. They also observed a similar dose-response relationship for MVPA and mortality due to cardiovascular disease, as well as cancer.[43] Their results reflect the inverse dose-response relationship between MVPA and mortality risk initially identified by Paffenbarger et al. (1986) and corroborated by subsequent studies over the years.[43,44]

Previous guidelines emphasized the need to accumulate MVPA in bouts of 10 minutes or more to obtain health benefits.[33] This was refuted by several cross-sectional and prospective studies indicating that MVPA, regardless of how it is accumulated, contributes to health benefits.[34] Research comparing bouts of <10 minutes to bouts ≥10 minutes found

no significant differences in the association between MVPA and improved health outcomes, including fasting glucose,[45] lipid profiles,[45,46] blood pressure,[45,47,48] frailty index,[49] and even metabolic syndrome risk.[50] Some studies even found that shorter bouts (<10 minutes) were more effective than longer bouts (≥10 minutes) for improving markers such as glycated hemoglobin A_{1c} (HbA_{1c}), C-reactive protein, and triglycerides,[47,51] but the effectiveness of MVPA in shorter bouts remains conflicting in terms of body mass index (BMI) and body fat.[46,50,52] Remarkably, some data suggest that even bouts as short as 1 minute or less of vigorous exercise, performed multiple times throughout the day, can benefit cardiorespiratory fitness and cardiometabolic health outcomes.[53] For instance, a randomized controlled trial (RCT) in young, inactive adults revealed that intermittent bouts of three 20-second maximal cycling sprints (separated by one to four hours of rest (sprint snacks)) elicited similar increases in peak oxygen consumption (VO_{2peak}) compared to sprint intervals completed within a single 10-minute session (separated by 3-minute rest intervals (traditional sprint interval training)).[54] Together, these data demonstrate that total MVPA plays a key role in overall health benefits, regardless of how it is accumulated.

1.1.2.2 Impact of Resistance Training on General Health

A minimum of two days per week of muscle-strengthening exercise is also recommended for all adults.[7-9] Resistance training (RT) is well-known for increasing skeletal muscle mass, strength, and endurance,[55] favourably enhancing musculoskeletal health. In the Studies of a Targeted Risk Reduction Intervention through Defined Exercise-Aerobic and Resistance Training (STRRIDE-AT/RT) study, Willis et al. (2012) found that improvements in lean body mass were only observed when RT was part of the intervention.[56] Significant gains in lean body mass were observed in the RT alone (Δ+1.09

kg (1.54), $p < 0.0001$) and combined training (Δ+0.81 kg (1.38), $p = 0.001$) groups, but aerobic training (AT) alone showed no changes (Δ-0.10 kg (1.22), $p = 0.613$). In the *Canadian 24-Hour Movement Guidelines*, Ross et al. (2020) acknowledged that two days per week of RT is a sufficient training frequency, as performing more than two days per week does not appear to lead to significantly greater enhancements in health.[8] This recommendation is supported in an RCT of adults randomized to high frequency (6 times/week) versus low frequency (3 times/week) RT, with equal volume and intensity, that observed similar improvements in strength.[57] Moreover, in a meta-analysis of 370,256 healthy adults, Saeidifard et al. (2019) reported that one to two days per week of RT was associated with 21% lower all-cause mortality (95% CI [0.69, 0.91]), but more than two days per week did not provide significant additional benefits compared to one to two days per week.[58]

RT also exerts favourable impacts on cardiovascular function and metabolism, by regulating various cardiometabolic risk factors.[59,60] Several studies demonstrate that RT is a powerful reducer of blood pressure. In fact, just a single acute bout of RT can reduce blood pressure for up to 24 hours after exercise.[61] In a meta-analysis of 64 RCTs, MacDonald et al. (2016) analyzed the antihypertensive effects of dynamic RT on overweight middle-aged adults living with hypertension.[60] Dynamic RT, performed two to three days per week for 14 weeks at a moderate intensity (65-70% one-repetition maximum (1RM)), reduced systolic and diastolic blood pressure by 3.0 mmHg and 2.1 mmHg, respectively.[60] This was corroborated by a previous meta-analysis that showed that both dynamic and isometric RT lasting ≥ 4 weeks lowers blood pressure by 2-3 mmHg in healthy adults without hypertension.[62] Although reductions of this magnitude appear

modest, they are not trivial, as they are comparable to the effects obtained with antihypertensive medication.[63,64] In a meta-analysis of adults with and without cardiovascular disease, each 5-mmHg reduction in blood pressure reduced the likelihood of cardiovascular events by 10% (95% CI [0.88, 0.92]).[65] Furthermore, RT has been shown to be involved in preventing the progression of metabolic syndrome – a cluster of cardiometabolic risk factors, including abdominal obesity, hypertension, elevated triglycerides and glucose, and reduced high-density lipoprotein (HDL)-cholesterol.[66] In an RCT, 35 postmenopausal women with metabolic syndrome displayed improved cardiometabolic profiles following 12 weeks of resistance band training three days per week.[67] The women significantly reduced glucose, insulin resistance, triglycerides, low-density lipoprotein (LDL)-cholesterol, and blood pressure and increased HDL-cholesterol. A cross-sectional analysis of 5,685 men revealed that those with low muscle strength had a 2.2-fold increased risk of metabolic syndrome in those under 50 years old.[68] Additionally, others have shown that muscle strength may independently contribute to the pathogenesis of metabolic syndrome[69,70] – which subsequently increases the risk of further conditions, including cardiovascular disease and T2D.[71] Together, these studies demonstrate that RT may effectively reduce the risk of these chronic conditions and risk factors. In fact, in a sub-analysis of a large epidemiological meta-analysis, Aune et al. (2015) reported an inverse association between RT and the risk of T2D. Evidence pointed to a non-linear curve with greater risk at low levels of activity.[72]

1.2 Glucose Metabolism

1.2.1 Normal Glucose Physiology

Glucose, a simple carbohydrate required for adenosine triphosphate (ATP) production, is of central importance physiologically as the primary source of energy during physical activity.[73] A homeostatic state of glucose control is maintained primarily by the opposing and balanced actions of insulin and glucagon released from the pancreas, as depicted in Figure 1.[74] Insulin controls glucose physiology through the simultaneous regulation of hepatic glucose production and the rates of its removal from the circulation.[73] Elevated blood glucose concentrations predominantly result from a malfunction in the feedback loops between insulin action and insulin secretion.[75,76] A complex network of hormones and neuropeptides are also involved in the tight regulation of glycemia.[74,77] As disturbances in this system may lead to metabolic disorders, including T2D, a mechanistic understanding of the physiological pathways involved is imperative.

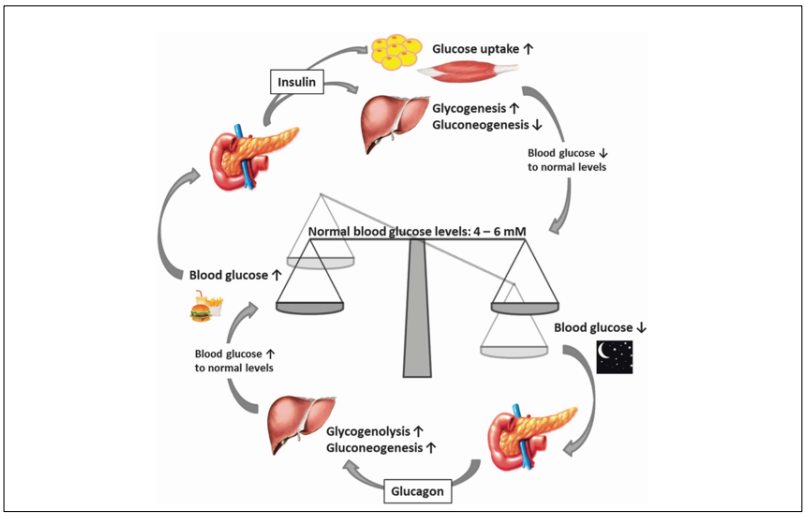


Figure 1. Maintenance of blood glucose levels by glucagon and insulin.[74]

1.2.1.1 Healthy Postabsorptive and Postprandial Glucose Response

Glucoregulatory hormones maintain circulating glucose concentrations within a narrow range to sustain homeostasis.[77] In the *postabsorptive (fasted) state*, after food has

been digested, absorbed, and stored for later use, glucose is then cleared from the circulation at a constant rate as it gets taken up by cells for either energy utilization or storage. As glucose decreases in the blood, circulating insulin concentrations also lower, thereby slowing down the storage of glucose in the form of glycogen (within the liver and muscles) and triglycerides (within the adipose tissue),[78] Without endogenous glucose production, blood glucose concentrations would fall to dangerously low levels (ie: hypoglycemia). Endogenous glucose is produced mainly from the liver through two key mechanisms: glycogenolysis and gluconeogenesis.[79] Glycogenolysis, the breakdown of stored glycogen into glucose to release into the circulation, is the primary source of glucose availability during sleep or in between meals.[77] For longer periods of fasting, the body relies on hepatic (and, to a lesser extent, renal) gluconeogenesis to produce glucose from non-glucose sources,[80,81] including lactate, pyruvate, glycerol, and amino acids.[77,82] Hepatic glucose production by glycogenolysis and gluconeogenesis is partially under the control of glucagon, a hormone released from the α-cells of the pancreas that opposes the action of insulin.[77] The inhibition of glucagon secretion contributes to the suppression of hepatic glucose production and reduction in circulating glucose,[83] thus returning plasma glucose to the normal range.[84,85]

Under steady-state conditions in the *postabsorptive state*, insulin secretion is kept at a baseline rate, with endogenous glucose production matching glucose removal by the tissues.[86,87] Glucose disposal occurs primarily in insulin-independent tissues during fasting – specifically the brain and red blood cells, which rely on extracellular glucose concentrations for survival[88,89] (where glucose transport is facilitated by glucose transporter (GLUT) 1 [73,90]) and to a lesser extent, in insulin-dependent tissues, like skeletal

muscle and adipose tissue.[88] Adipose tissue is vital for maintaining glucose homeostasis in this state, as it can provide free fatty acids as an alternative fuel for both skeletal muscle and liver.[73] These free fatty acids reduce glucose uptake in skeletal muscle and stimulate gluconeogenesis.[91,92]

In response to exogenous glucose appearance, such as after a meal, blood glucose rises, and rapid uptake occurs in the pancreatic β-cells via GLUT2.[74] In this *postprandial state*, this elevation in glucose triggers glycolysis, generating ATP and initiating the release of the anabolic hormone, insulin, whose primary action is to stimulate glucose clearance.[76,77,93] The simultaneous rise in glucose and insulin concentrations activates glucose uptake and suppresses endogenous glucose production by inhibiting glucagon secretion.[94-99] Insulin secretion promotes glucose transport and uptake into insulin-sensitive peripheral tissues (skeletal muscle and adipose tissue) by increasing the translocation of GLUT4 from the intracellular pool to the cell membrane.[90,99,100] Immediately postprandially, glucose is oxidized to replace fatty acid oxidation used for energy during the previous fast; once this pathway is saturated, excess glucose from the circulation is stored in the liver and skeletal muscles as glycogen through glycogenesis.[101,102] When glycogen stores are full, skeletal muscle can no longer release free glucose back into the circulation. Instead, the remaining glucose is converted to gluconeogenic precursors – lactate, alanine, and glutamine – which are directed to the liver to synthesize glycogen through this indirect pathway.[102-104] Any remaining glucose in skeletal muscle can also be disposed of by converting it into fatty acids, which are then stored as triglycerides in adipocytes.[79,105,106] This de novo lipogenesis generates lipids, thereby increasing fat reserves in the liver, adipose tissue[107] and skeletal muscle.[108] Under

normoglycemic conditions, lipolysis is suppressed in response to elevated insulin concentrations following postprandial hyperglycemia. This decreases free fatty acid concentrations[73] and, therefore, promotes glucose uptake by peripheral tissues and inhibits hepatic glucose production.[92] It is important to note that this suppression is attenuated in the presence of impaired glycemia.[92] While skeletal muscle is the main site of glucose uptake by peripheral tissues postprandially (~80-85% total uptake)[88], adipocytes are essential for the maintenance of glucose homeostasis. Not only does adipose tissue regulate the release of free fatty acids from stored triglycerides, but it also produces adipocytokines that influence muscular and hepatic insulin sensitivity.[92,109,110]

Postprandial blood glucose levels, and consequently the degree of hyperglycemia, are further influenced by additional organs and physiological processes beyond insulin and glucose regulation. For instance, the kidneys account for about 10% of total glucose disposal following a typical 75-gram oral glucose load.[104] This process is driven by high glucose and insulin concentrations, which promote glucose uptake in the kidneys, where glucose is anaerobically metabolized into lactate and released into the circulation.[111] The rate of gastric emptying and glucose absorption in the gastrointestinal tract also plays a vital role in mediating the feedback relationship between glucose concentrations and insulin secretion. The gastrointestinal tract absorbs glucose and stimulates insulin release through mechanisms independent of fluctuating glucose concentrations,[73] as evidenced by early studies demonstrating differing insulin secretory responses between oral glucose ingestion and intravenous glucose administration.[112,113] Gastrointestinal hormones, including ghrelin, leptin, and incretin are also integral to the regulation of insulin secretion and the maintenance of glucose homeostasis.[73] The two predominant incretin peptides,

glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), act on pancreatic β-cells and α-cells via specific receptors to stimulate glucose uptake.[73,114,115] Furthermore, over 50% of ingested glucose is initially taken up by the liver, which reduces the amount of glucose entering the peripheral circulation prior to skeletal muscle glucose uptake.[101,116] This hepatic glucose uptake provides marked protection against postprandial hyperglycemia and hyperinsulinemia.[73] Taken together, these interconnected mechanisms operate concurrently, with glucose homeostasis being highly coordinated by the central nervous system.[73]

It should be noted that the amount of insulin released from pancreatic β-cells depends on the metabolic requirements of insulin-sensitive tissues.[117] These β-cells and insulin-sensitive tissues work together in a negative feedback system, which adjusts insulin sensitivity and secretion to maintain glucose homeostasis.[118] Healthy pancreatic cells respond properly to changes in insulin action, such that a decrease in insulin action upregulates its secretion.[76] However, when the feedback loops between insulin action and secretion malfunction, hyperglycemia arises.[76] Defects in any of these mechanisms can lead to metabolic imbalances and further impairments in glucose regulation.

1.2.2 Impaired Glucose Physiology

1.2.2.1 Pathophysiology of Impaired Glycemia

When the feedback loops regulating glucose metabolism are disrupted, a chronic state of hyperglycemia results from hyperinsulinemia, insulin resistance, and pancreatic β-cell dysfunction.[119] This hyperglycemia progresses along a continuum, with various stages characterized by diverse pathophysiology.[120-122] Prediabetes, an intermediate metabolic state between healthy glucose regulation and diabetes, is characterized by impaired fasting

glucose (IFG), impaired glucose tolerance (IGT),[123] or mildly elevated glycemia.[124,125] IFG results from hepatic insulin resistance (characterized by an impaired suppression of hepatic glucose production) with normal muscle insulin sensitivity, while IGT presents with normal or slightly impaired hepatic insulin sensitivity and skeletal muscle insulin resistance.[126] Both IFG and IGT are associated with a decline in β-cell function,[127] which further compromises the body's ability to effectively regulate glucose. Each metabolic state on its own increases the risk of developing T2D, but the risk is most pronounced in combined IFG+IGT (RR: 12.13, 95% CI [4.27, 20.00]).[128] It's estimated that 70% of all individuals with prediabetes will transition to overt T2D throughout their lifetime.[129]

The impairment in insulin secretion from pancreatic β-cells represents a hallmark of the progression from prediabetes to T2D.[130,131] In order for insulin resistance to be associated with T2D, concomitant β-cell dysfunction must be present.[132] When β-cells compensate for insulin resistance, hyperinsulinemia ensues, further deteriorating insulin sensitivity and increasing the workload of β-cells.[133] Eventually, the β-cells will lose their ability to compensate for insulin resistance and, once the β-cells become less functional, β-cell mass declines, thereby paving the way for the progression to T2D.[133] Eventually, β-cells become exhausted towards failure, causing a reduced synthesis of insulin and, therefore, insufficient secretion of insulin (hypoinsulinemia).[134]. At this point, exogenous insulin administration becomes required for the maintenance of glucose homeostasis.[134]

The critical interplay between insulin resistance and β-cell dysfunction is well-documented in the progression from prediabetes to T2D. The early and significant impact is demonstrated in cross-sectional and longitudinal studies in the Pima Indian people of Arizona,[135,136] along with data from the UK Prospective Diabetes Study that suggest that

the onset of β-cell loss may begin as early as 10-12 years prior to the diagnosis of T2D, with reports of 50% β-cell loss at diagnosis.[137,138] Likewise, retinopathy data (a downstream complication of hyperglycemia)[139] indicates that the actual onset of T2D may precede its diagnosis by four to seven years.[140,141] β-cell function continues to deteriorate throughout the progression of the disease, even when patients are treated for hyperglycemia.[142] Age appears to be a factor related to β-cell function, as youth aged 10-17 experience β-cell loss more rapidly than adults,[143] which suggests that youth may experience a more aggressive form of T2D.

1.2.2.2 Measurement of Impaired Glycemia

Prediabetes is defined by established criteria that aim to identify individuals at an increased risk of developing T2D and its associated micro- and macrovascular complications, compared to those at no or only "normal" risk.[123,144,145] The defining features of prediabetes – IFG and IGT[123] – are identified by distinct testing procedures. IFG is typically determined by an overnight fasting plasma glucose test,[146] with Diabetes Canada categorizing IFG between 6.1 to 6.9 mmol/L.[123] While there is no universal definition of IFG,[125,147] this range reflects an increased risk of developing T2D compared to the threshold set by the American Diabetes Association (ADA) (5.6-6.9 mmol/L).[148,149] In contrast, IGT is diagnosed using an oral glucose tolerance test (OGTT), which reflects the body's response to glucose postprandially.[146] If plasma glucose is between 7.8 and 11.0 mmol/L two hours after ingesting a glucose beverage, glucose uptake is not occurring rapidly enough in peripheral tissue to return to baseline levels, resulting in prediabetes. Although IFG and IGT remain the recommended testing procedures for diagnosing prediabetes, mildly elevated HbA_{1c} can also serve as a diagnostic marker for the transition

to T2D.[124,125] According to Diabetes Canada, an HbA_{1c} range of 6.0% to 6.4% is classified as prediabetes,[123] which reflects a higher risk for T2D than the diagnostic range between 5.7% to 6.4% from the ADA.[150] However, an HbA_{1c} <6.0% may still be associated with an increased risk of T2D.[151] Therefore, the commonly accepted lower threshold for classifying prediabetes is 5.7%. Since HbA_{1c} may be a less sensitive test for prediabetes diagnosis (given the decreased specificity to detect lower levels of hyperglycemia as compared to T2D)[150], pairing an HbA_{1c} test with a fasting plasma glucose test or an OGTT may provide more accurate risk estimations.[125] For example, individuals with prediabetes who have a fasting plasma glucose between 6.1 to 6.9 mmol/L, along with an HbA_{1c} of 6.0% to 6.4%, have a 100% cumulative risk of progressing to T2D within five years.[151]

Diabetes Canada defines T2D using the following criteria: 1) fasting plasma glucose ≥7.0 mmol/L, 2) HbA_{1c} ≥6.5%, 3) 2-hour plasma glucose value of ≥11.1 mmol/L following an OGTT, or 4) elevated random plasma glucose of ≥11.1 mmol/L.[123,125] HbA_{1c}, the primary method for T2D management, is generally targeted at ≤7.0% given its association with reduced complications in new T2D cases.[152] HbA_{1c} is most advantageous for management purposes as it reflects the average plasma glucose over the previous three to four months,[123] as glucose concentration in the blood is directly proportional to the rate of HbA_{1c} glycation. Glycation refers to the irreversible attachment of glucose to hemoglobin molecules within red blood cells, a process that lasts for the ~120 day life span of the red blood cells.[153] This is the foundation of HbA_{1c} measurement, as it reflects long-term glucose exposure, and thus accounts for day-to-day variation fluctuations in blood glucose concentrations.[125] In contrast, fasting glucose provides only a snapshot of blood glucose without the process of glycation. As such, HbA_{1c} is recognized as the best method

to establish the risk of T2D-related complications[153,154] and is beneficial for diagnosis, as it is more convenient than OGTT or fasting plasma glucose, and it is used more frequently.[155]

Insulin resistance reflects impairments in insulin action resulting in reduced peripheral glucose uptake, increased hepatic glucose production, increased adipose tissue lipolysis, and reduced responsiveness/sensitivity to insulin-mediated glucose disposal.[156] Insulin sensitivity represents insulin's metabolic actions to promote glucose disposal,[157] which is evaluated by measuring peripheral glucose uptake and glucose production in either fasting or insulin-stimulated conditions.[156] Due to the barriers associated with the gold standard measurement of whole-body insulin resistance – the hyperinsulinemic-euglycemic clamp[158] – simpler methods are often used in clinical and research settings, including indices derived from glucose and insulin levels, either at fasting or in response to an OGTT.[159] One of the most popular methods of insulin resistance assessment is the homeostatic model assessment (HOMA-IR),[160] which relies on single fasting blood samples, similar to the quantitative insulin sensitivity check index (QUICKI),[161] and thus is reflective of hepatic insulin resistance. Other measures incorporate postabsorptive insulin and glucose levels in addition to fasting measures to provide a representation of whole-body insulin sensitivity,[159,162] including the insulin sensitivity index (ISI)[163] and the composite whole-body insulin sensitivity index (WBISI), otherwise known as the Matsuda index.[164]

1.2.3 Glucose Physiology in Response to Exercise

Beyond the immediate initiation of exercise where free ATP is used as a source of energy, muscular contraction relies on the oxidation of carbohydrate and fat to sustain

energy needs for exercise lasting for several minutes to hours.[165] Longer durations of exercise rely primarily on carbohydrates derived from intramuscular and extramuscular stores of glycogen, glucose, and fatty acids[165,166] – especially at higher intensities.[167] The oxidation of muscle glycogen and fatty acids declines during prolonged exercise durations, while there is a simultaneous progressive increase in skeletal muscle glucose and fatty acid uptake.[165,168,169] Skeletal muscle glucose uptake can increase by up to 50-fold during intense endurance exercise,[170] and occurs primarily as a consequence of the increased translocation of GLUT4 from exercise-sensitive intracellular storage sites to the sarcolemma (plasma membrane) and t-tubules.[171] Once glucose is transported across the sarcolemma, it is phosphorylated to glucose-6-phosphate to initiate the glycolytic and oxidative pathways to regenerate energy during exercise or convert to glycogen for storage.[171]

During exercise, skeletal muscle undergoes a rapid insulin-independent increase in skeletal muscle glucose uptake.[172-174] Although GLUT4 translocation is considered insulin-dependent, skeletal muscle biopsies from animal models demonstrate that muscular contractions can recruit GLUT4 to the sarcolemma independently of insulin.[175-178] Insulin is also a potent stimulator of glucose transport in skeletal muscle, but insulin and exercise stimulate GLUT4 translocation via distinct mechanisms.[179] Exercise activates glucose transport mechanisms that can "bypass defects in insulin signalling in skeletal muscle".[179] This is exemplified by data that shows that insulin-stimulated glucose transport is impaired in T2D, but exercise-stimulated glucose uptake is normal.[180] GLUT4 expression was significantly increased in human skeletal muscle following a hyperinsulinemic-euglycemic clamp,[181] but the increase was lower in the skeletal muscle of individuals with T2D.[182,183]

As such, insulin-stimulated GLUT4 translocation is a key step in glucose uptake that is diminished in T2D.[172]

A critical role of exercise in glucose control is its ability to sensitize skeletal muscle to subsequent insulin stimulation post-exercise.[184] A single acute bout of exercise can transiently increase insulin sensitivity for up to 120 hours in healthy adults,[185] and even up to 72 hours in individuals with T2D,[186] likely due to the increased translocation of GLUT4 to the sarcolemma.[187] Repeated bouts of exercise can have a lasting impact on insulin sensitivity[188,189] through the enhancement of skeletal muscle glucose uptake and glycogen storage.[172] These insulin-sensitizing effects contribute to the mechanistic understanding of how exercise improves glycemic regulation and insulin action, and reinforce the importance of regular exercise training for individuals with insulin resistance and/or T2D.[190]

1.2.4 Type 2 Diabetes Mellitus

1.2.4.1 Prevalence of Impaired Glycemia

According to recent global estimates, 529 million people were living with diabetes in 2021,[191] a rise of more than 5-fold since 1980.[192] While an increase of this magnitude is difficult to grasp, experts project that this prevalence will double again by 2050.[191] The rise in diabetes is driven mainly by the continuous increase in the incidence of T2D, which can account for up to 96% of all diabetes cases.[191,193] In the coming decades, nearly 1 in 10 people are expected to live with T2D.[191] This is not trivial, given that diabetes is considered the eighth leading cause of death and disability in the world,[194] which undoubtedly causes immense strain on the healthcare system and global health expenditures (US $966 billion in 2021).[195,196]

Notably, the actual onset of T2D may occur years before diagnosis,[193] a finding consistent with glucose physiology, including that β-cell dysfunction begins as early as 12 years before clinical diagnosis.[197] In fact, half of all cases of diabetes mellitus worldwide are estimated to be undiagnosed.[198] This is problematic given that those who are unaware of their condition and hence are untreated are at significantly greater risk of complications than those who receive treatment.[199] Global estimates suggest that 9.1% of the population was living with IGT and 5.8% with IFG in 2021, reflecting the prevalence of prediabetes.[198]

Diabetes is considered one of the most significant health issues facing Canadians today.[200] As of 2022, nearly 10% of the population was living with the disease (>3.5 million Canadians),[201] and this prevalence is expected to continue to rise. According to data from the most recent Canadian Health Measures Survey (CHMS), just 1% of younger adults (aged 20 to 39) were diagnosed with diabetes compared to nearly 20% of older adults (aged 60 to 79).[202] Together, nearly 30% of the total population may be living with prediabetes or T2D (including undiagnosed cases), highlighting that almost one in three Canadians has impaired glycemia.[203] These estimates and predictions together underscore the severity of the T2D epidemic. This data is immensely consequential, considering the additional health complications associated with chronic hyperglycemia.

1.2.4.2 Etiology

T2D is a complex heterogenous disorder that results from a combination of individual, social, genetic and environmental factors that interact with one another and lead to a greater predisposition for the disease.[204] Some of these factors are modifiable with healthy behaviour changes, while others are predisposed due to biological and

environmental factors. Together, these elements each affect multiple molecular pathways and tissues that contribute to T2D.[205,206]

1.2.4.2.1 Modifiable Risk Factors

Unhealthy lifestyles significantly raise the likelihood of T2D. As these behaviours can often be changed, individuals can modify their lifestyles to reduce the chance of both prediabetes and T2D. Some of the modifiable factors heavily influencing T2D development include obesity, poor diets, and high levels of sedentary behaviours accompanied by physical inactivity and smoking.[207,208]

Obesity, characterized by increased adiposity, is recognized as the strongest risk factor for developing T2D.[191,209] Recently, the term 'diabesity' has gained prominence owing to the parallel rise in T2D and obesity, highlighting the concern due to their combined detrimental effects on health.[209] Excessive adiposity, depending on the location, has the capacity to drastically alter cardiometabolic profiles, and it is believed that these resulting abnormalities are what drive the risk for T2D in individuals with obesity.[210,211] Indeed, individuals with obesity who have excess abdominal or visceral adiposity, including those with metabolically unhealthy obesity (MUO) are more likely to have cardiometabolic complications compared to those considered metabolically healthy obese (MHO).[212] In fact, abdominal/visceral obesity (captured via waist circumference measurement) may be better at predicting the risk of T2D than classical obesity (via BMI), perhaps due to its association with harmful ectopic fat.[213,214] Compared to adults with a normal BMI, individuals classified as obese have a 7- to 12-fold increased risk of developing T2D depending on BMI category (30 kg/m^2 vs 40 kg/m^2),[215,216] however, these data were not adjusted for adiposity distribution. Nevertheless, high BMI remains

positively associated with morbidity and mortality, independent of age, sex, and race/ethnicity, as is increased waist circumference.[217,218]

Sedentary lifestyles and energy-dense diets are also substantial drivers of the T2D epidemic. In Canada, half of all adults are considered physically inactive, with only 49.2% (95% CI [41.6, 56.7]) of those 18 years and older meeting the recommended aerobic portion of the physical activity guidelines [219]. Higher physical activity levels are associated with a reduction in disease risk, as demonstrated by Wahid et al. (2016) who, in an epidemiological meta-analysis (n = 261,618), showed that reaching 150 minutes per week of just moderate physical activity reduced the risk of T2D by 26%.[220] In addition to a lack of physical activity, the average Canadian adult spends 9-10 hours per day engaging in sedentary behaviour,[221] higher than the recommended maximum 8 hours per day.[8] It has been reported that more than 8 hours per day of sedentary time was associated with a 30% increased risk of T2D.[222] Furthermore, most Canadian adults do not have a healthy diet.[223] Data indicates that low quality dietary fats and excessive carbohydrate intake are associated with the incidence of T2D.[224,225] 'Obesogenic' environments, characterized by changes in the food systems, including increased availability and affordability of energy-dense, nutrient-poor foods, as well as reduced physical activity due to changes in the built environment and, therefore, modernization of lifestyles, are recognized as drivers of the parallel T2D and obesity epidemics.[226,227]

Other individual-level modifiable risk factors exist for T2D development. For instance, smokers are significantly more prone to T2D, even those with a high-level exposure of second-hand smoke.[228,229] The association between smoking and T2D may be related to the increased abdominal/visceral fat accumulation in smokers or the

hyperinsulinemia and insulin resistance caused by smoking.[230] The metabolic health risks associated with smoking is troublesome, considering nearly 1 in 5 Canadian adults are current tobacco smokers.[223] Some common medications are also associated with T2D risk, including cholesterol-lowering statins and blood pressure medications like thiazides and beta-blockers.[231] Interestingly, several biomarkers – in addition to glycemic factors – are associated with the risk of T2D. Adipose-derived factors like adiponectin and leptin, hepatic-derived factors including insulin-like growth factor 1, and several inflammatory markers like those in the interleukin family and C-reactive protein, have all been shown to be associated with T2D risk.[232,233]

Increasing evidence demonstrates that social determinants of health can influence individual-level modifiable risk factors.[204] The main determinants of health that play an important role in the risk of T2D and its complications include, but are not limited to: income, employment, education, childhood experiences, physical environments, social supports, food security, access to health services, and racism.[234,235] For instance, in an analysis of the Canadian Community Health Survey (CCHS), individuals in lowest income groups are 4.14 times higher than those in the highest income group.[236] Similar observations are found in those who have not completed high school compared to those with a university education.[237] In a study of diabetes treatment coverage in low-middle income countries, fewer than one in ten people with T2D receive adequate treatment coverage.[196] Socioeconomic factors are incredibly impactful on T2D prevalence, given that individuals with T2D can pay up to 16% of their gross annual income on diabetes medications and devices.[238] As further described in later sections, difficulties in combating the T2D epidemic lie in the fact individual or group efforts alone are not enough to change

many of the risk factors.[239] There is an urgent need for improvement in policies and programs needed to enhance health system infrastructures[240,241] that will impact the ability of individuals and the population to adopt healthy behaviours.

1.2.4.2.2 Non-modifiable Risk Factors

Strong evidence exists for the adoption of a healthy lifestyle to lower the risk of T2D, as detailed in the above sections. However, there is substantial heterogeneity in an individual's predisposition to T2D due to non-modifiable risk factors.

Certain biological factors increase the risk of developing T2D, including age, sex, and race/ethnicity.[208] T2D risk increases with age,[208] with HbA_{1c} values rising by up to 0.1% per decade of life.[242,243] Moreover, adult men in Canada have a greater risk of T2D compared to women[208] due to the fact that males may be more insulin resistant than females and they may develop T2D at lower BMI thresholds.[191,244] Certain ethnicities, like those of African American, Indigenous, Hispanic and Asian descent, have a greater risk of T2D due to HbA_{1c} values that are up to 0.4% higher than non-Hispanic white individuals.[245-248] This is especially concerning given that the population of these ethnicities together represent nearly 30% of the Canadian population.[249] In Canada, the prevalence of T2D is 3 to 5 times higher for Indigenous people,[250] who face more T2D-associated complications than non-Indigenous populations as well as diagnosis at an earlier age.[251,252] Still, further biological risk factors are involved in the development of T2D, including previous gestational diabetes mellitus, the gut microbiome, immune dysregulation and inflammation. Compared to those with a normoglycemic pregnancy, women with gestational diabetes mellitus had a seven-fold increased risk of T2D[253] and make their offspring prone to T2D in later life due

to exposure to intrauterine hyperglycemia.[254] Finally, certain variations in gut microbiome diversity and composition are also associated with T2D.[255]

Exposure to certain environmental factors play a major role in the development of T2D due to the impactful interaction between genes and the environment. Yet, even with identical environmental exposures, some individuals are more susceptible to T2D than others, which highlights the genetically inherited nature of the disease.[256] Data from various population, family, and twin-based studies suggest that variation in DNA sequence contributes to T2D risk.[257] Family history of T2D predisposes individuals to the development of the disease,[258] such that the risk of developing T2D from an affected first-degree relative is 3- to 6-fold higher than those without familial T2D.[259] Furthermore, twin studies indicate that the probability that a pair of twins will both develop T2D is far greater for monozygotic twins, who share 100% of their DNA, compared to dizygotic twins.[260] Estimates of T2D heritability range from 25-72%,[259-262] implying a large genetic component, and causing a search for genetic predictors of T2D.

Large-scale genomic studies have revealed both known and novel molecular pathways associated with T2D,[263] allowing an enhanced knowledge of its pathophysiology. Genome-wide association studies have identified hundreds of loci linked to T2D, which may help explain the heredity of the disease.[264-266] Notably, 90% of these genomic variants are found in regions that influence the function of β-cells and, reinforcing that β-cell dysfunction is a key determinant of the development of T2D.[267-269] Some studies posit that genetic factors underlying T2D are more closely related to impairments in insulin secretion from the β-cells than to insulin sensitivity.[270-272] Specifically, genetic risk scores are most commonly associated with the prediction of β-cell dysfunction, while circulating

metabolites (lipids, amino acids, etc.) are strongly associated with insulin resistance.[273,274] Together, metabolites and genetic information improve the accuracy of T2D risk predictions beyond what is determined by clinical traits alone.[274] However, rare variants and lifestyle factors may have a greater impact on insulin sensitivity.[256]

As a mostly polygenic disease, T2D is determined by the interaction of thousands of gene variants scattered across the genome. Polygenic risk scores are generated from genome-wide association studies to determine the "combined influence of many variants with small effects",[122] thus improving the prediction of disease risk and, in turn, the likelihood of preventing T2D.[275] For instance, individuals in the upper vs lower 2.5% of the polygenic risk score distribution have a 9.4-fold increased T2D risk.[264] Yet, some argue that the genetic variants identified in these studies do not improve T2D risk prediction beyond those of risk scores based on traditional risk factors, including age, sex, dyslipidemia, fasting glucose, BMI, etc.,[276,277] highlighting the uncertainty in the usefulness of genetic markers in disease prediction thus far.[257] In any case, the identified genetic variants associated with T2D account for only a small portion (~20%) of T2D heritability,[278] suggesting that other factors contribute to T2D risk. Various theories exist for the unexplained heritability of T2D, among which include the complex interaction between genetics and the environment.[193,279] Not only can genetic variants directly impact disease pathogenesis, but they can also alter how one interacts with the environment.[280] Genetic variants can affect metabolic processes that increase an individual's susceptibility to adopting an unhealthy lifestyle by influencing diet, physical activity, and sedentary behaviour patterns,[281,282] which have dire consequences in obesogenic environments. Similarly, exposure to obesogenic environments can trigger underlying genetic factors to

exert their effects.[119] Given that increased adiposity is one of the main risk factors for T2D,[283] and that obesity is one of the main drivers of T2D in every population,[256] it is not a surprise that the heritability of T2D significantly reflects the heritability of obesity.[256]

Altogether, the etiology of T2D is multifaceted, with factors that lead to substantial variability in patient phenotypes, including impacting how an individual responds to treatment.[120] While utilizing genomic studies on their own to identify T2D risk is limited, using a whole systems physiological approach by combining polygenic risk scores with metabolomics, transcriptomics, proteomics, etc. may improve the understanding of T2D pathophysiology.[284,285] Such knowledge is imperative in order to pinpoint pathogenic mechanisms that may be targeted for enhanced prevention and treatment strategies that account for individual variability. Furthermore, given the strong link with environmental factors, lifestyle implementation strategies are needed to combat the current obesogenic environmental landscape.

1.2.4.3 Associated Cardiometabolic Consequences of Impaired Glycemia

The upsurge in T2D prevalence and incidence over the last four decades has had severe consequences for adults living with the condition. It is estimated that diabetes is the eighth leading cause of death and disability combined worldwide.[191] T2D is widely known for its detrimental effects on both micro- and macrovascular complications, which are considered the leading causes of morbidity and mortality in individuals with T2D.[286]

In 2021, the International Diabetes Federation (IDF) estimated that each year, 6.7 million adults lost their lives to diabetes and its complications.[287] T2D is associated with a 15% increased risk of all-cause mortality in adults, which is twice as high as those without T2D.[288] In a meta-analysis of ~700,000 adults, diabetes accounted for 10-12% of all

vascular-related deaths.[289] Age appears to impact the magnitude of mortality risk in those with T2D compared to healthy adults, with the likelihood of both all-cause and cardiovascular mortality being two times higher in younger adults (<55 years old) but a slight reduction in the likelihood of mortality in older adults (>75 years old).[288] In a large-scale observational analysis of individual participant data in adults with T2D in high-income countries, each decade of earlier diagnosis was associated with 3-4 years of reduced life expectancy.[290] The higher rate of premature mortality experienced in those with young-onset T2D (<45 years old) may be a consequence of a more aggressive phenotype in this population, one that is characterized by more severe metabolic complications.[291-293]

Approximately 50% of all patients with T2D will present with microvascular complications, including retinopathy, neuropathy, and nephropathy in their lifetime.[139,294] While macrovascular complications are slightly less common (27%),[294] their consequences are more deleterious in terms of their contribution to T2D-related mortality.[193] Macrovascular complications of T2D include cardiovascular, cerebrovascular, and peripheral vascular disease.[139] Cardiovascular disease is the leading cause of both morbidity and mortality in those with T2D,[119,193] and occurs two to four times more often than in those without T2D.[295] According to data from the Emerging Risk Factors Collaboration, T2D is associated with a two-fold risk of coronary heart disease (HR: 2.00, 95% CI [1.83, 2.19]) and ischaemic stroke (HR: 2.27, 95% CI [1.95, 2.65]).[289] Furthermore, T2D is the leading cause of: end-stage renal disease (those with T2D are 12 times more likely to be hospitalized due to this complication); new cases of blindness due to retinopathy; and non-traumatic limb amputation caused by foot ulceration.[296,297]

Although not all individuals with prediabetes will develop T2D, many will progress along the continuum of chronic hyperglycemia. Compared to adults with normoglycemia, those with either impaired glucose tolerance or impaired fasting glucose had a 5.8-fold increase in the risk of developing T2D; however, when they co-occurred, the relative risk rose to 12-fold.[128] As prediabetes is associated with the simultaneous presence of insulin resistance and β-cell dysfunction,[298] the risk of macrovascular complications can be just as detrimental as those with T2D.[299] According to a meta-analysis of prospective cohort studies with 1.6 million adults, prediabetes significantly elevated the risk of cardiovascular disease, coronary heart disease, stroke, and all-cause mortality.[300] Those with prediabetes still can experience microvascular complications, but not to the same degree as seen in T2D.[125,299]

The metabolic syndrome – a complex condition characterized by a cluster of interconnected abnormalities – represents risk factors for the development of both T2D and cardiovascular disease.[298,301,302] Although the explicit criteria for the metabolic syndrome has been debated over the years, the most widely used definitions are those of the IDF[301] and the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (NCEP:ATPIII)).[303] The NCEP:ATPIII criteria define the metabolic syndrome as a manifestation of any ≥3 of the 5 following components: abdominal obesity measured by ethnicity-specific waist circumference (F: ≥88 cm, M: ≥102 cm), high blood pressure (systolic: ≥130 mmHg or diastolic: ≥85 mmHg, elevated triglycerides (≥1.7 mmol/L), reduced HDL-cholesterol (F: <1.3 mmol/L, M: <1.03 mmol/L), and raised fasting glucose (≥5.6 mmol/L).[301,304] The IDF, however, requires abdominal obesity as part of the diagnosis (in addition to meeting any ≥2 of the additional criteria), highlighting its role in

metabolic dysfunction and its relationship with T2D. Under the IDF definition, this cluster of risk factors increases the risk of T2D by 5-fold compared to those without the metabolic syndrome.[301] Furthermore, Church et al. (2009) demonstrate that individuals with T2D who also have metabolic syndrome do not have a higher risk of cardiovascular mortality compared to those with T2D alone. This finding suggests that while metabolic syndrome is associated with cardiovascular mortality, it does not add any additional risk once an individual has developed T2D.[305]

The culmination of these metabolic complications has contributed tremendously to the global burden of T2D in terms of disability, premature mortality, and associated health costs.[193] T2D and its complications accounted for 11.5% of the total global health expenditure in 2021,[287] which is expected to have risen along with the increased prevalence of the disease. Together, the data presented above emphasize the importance of interventions that aim to prevent T2D, delay disease onset, and manage the condition, especially in those diagnosed earlier in life.

1.2.4.4 Prevention of Impaired Glycemia

The rise in the prevalence and incidence of T2D calls for an urgent need for intervention. Accumulating evidence of RCTs and prospective observational studies have demonstrated that T2D can be prevented through a variety of lifestyle modifications. Different levels of treatment may be necessary depending on the individual risk of T2D, with more aggressive approaches undertaken for those at highest risk.[155]

1.2.4.4.1 Lifestyle Interventions

1.2.4.4.1.1 Physical Activity

Several key RCTs have demonstrated the efficacy of lifestyle interventions for T2D prevention centered around engaging in regular physical activity and maintaining a healthy diet.[306-308] The Diabetes Prevention Program was a formative trial in determining how to slow the progression of the increasingly prevalent chronic condition.[306] In this large, multi-center RCT of 3,234 adults (>25 years) of various ethnicities at risk for T2D, participants were randomized into one of three conditions for the 24-week intervention, including: 1) 150 minutes per week of MVPA; 2) metformin medication; 3) placebo. Each group received lifestyle advice for diet, weight loss, and physical activity. At follow-up, the incidence of T2D was significantly reduced in the exercise group, such that it was 58% lower (95% CI [48, 66]) than the placebo group, and was even 39% lower (95% CI [24, 51]) than the metformin group. The results of this trial suggested that exercise could be superior to medication in reducing the risk of T2D, and that exercise is a very effective means of preventing T2D. Follow-up studies of the Diabetes Prevention Program reported that 2-year weight loss was the strongest predictor of T2D incidence[309] and that benefits were sustained for up to 10 years.[310] After 15 years of follow-up, diabetes incidence reduced in those who received lifestyle interventions and those in the metformin treatment by 27% and 18%, respectively.[311] Another landmark trial, the Finnish Diabetes Prevention Program,[308] also observed a 58% reduction in T2D risk in overweight adults with prediabetes after 4 years of a structured program of at least 150 minutes of MVPA along with a low-calorie, low-fat diet that aimed for weight loss of approximately 5%. Similarly, the Da Qing IGT and Diabetes Study in China, 110,660 adults were subjected to one of three treatment arms of physical activity, diet, or both. A portion of this group (577 individuals) were prediabetic according to IGT. After 6 years, the physical activity, diet,

and physical activity + diet groups all had a reduction in T2D risk compared to control, with a 46%, 31%, and 42% (all $p < 0.05$), even when adjusted for baseline BMI and fasting glucose.[307]. The benefit of these studies was long lasting, as evidenced by the reduced decreased incidence of T2D in the intervention groups after one to two decades of follow-up.[312-314] Resistance training also has the capacity to prevent T2D, as shown in a prospective cohort study of 32,000 at-risk men.[315] A dose-response relationship was observed between more time spent engaging in resistance training and a lower risk of T2D ($p < 0.001$). Combined training, however, with both aerobic and resistance training had the greatest T2D risk reduction (59%, 95% CI [39, 73]).

Physical activity interventions have also demonstrated the ability to reduce the risk of T2D-related cardiometabolic complications. Hypertension, an independent risk factor for T2D,[316] can be managed by aerobic training, even when performed under 150 minutes per week. In a recent RCT, Lopes et al. (2021) demonstrated that 120 minutes per week of AT for 12 weeks significantly reduced systolic blood pressure by 10 mmHg (95% CI [-17.6, 2.5).[317] This is not trivial, as those with hypertension have a nearly 50% increased risk of developing T2D (HR 1.51, 95% CI [1.29, 1.76]) as compared to normotensive adults ($p < 0.001$).[316]

1.2.4.4.1.2 Dietary Interventions

Diet is an essential component of managing glycemia and weight loss, which are integral to lifestyle interventions for T2D prevention.[306-308] While dietary recommendations are typically paired with physical activity, data from the Da Qing IGT and Diabetes Study demonstrate that eating a healthy balanced diet sufficient in all macronutrients can reduce the risk of T2D by 31%, independent of physical activity.[307]

Individuals at risk of T2D are encouraged to consume a diet rich in high-quality fats (polyunsaturated fatty acids) and low in carbohydrates (low glycemic index and load).[318] Additionally, whole grains, dairy products, fruits, vegetables, nuts, and legumes are promoted for T2D prevention,[319] as well as limited red or processed meat, saturated fat, refined grains, and sugar-sweetened beverages.[318] Individuals with prediabetes may benefit from nutrition therapy and counselling to prevent the progression of hyperglycemia to T2D. After 12 weeks of treatment from a registered dietitian, focusing on self-management, weight loss, and dietary instructions, participants significantly decreased HbA_{1c} -0.19 ± 0.41%.[320]

1.2.4.4.1.3 Other Interventions

In cases of severe obesity, bariatric surgery has been used as a means of weight loss management in T2D.[321] While lifestyle interventions are the preferred T2D prevention strategy, they are not feasible in all cases. For these individuals, clinicians may decide that pharmacological approaches may be the best prevention strategy. Several clinical trials have demonstrated the efficacy of metformin in reducing the risk of T2D.[306,322] Other medications, including thiazolidinediones,[323], have also been proven to reduce the risk of T2D. In Canada, metformin is recommended by Diabetes Canada for those with IGT and for those with IFG or a HbA_{1c} between 6.0% and 6.4%.[155] According to data from the Diabetes Prevention Study, metformin treatment reduced the progression from prediabetes to overt T2D by 31% compared to placebo.[306]

Conclusions

Prevention of T2D is important at the individual and population level. If primary prevention would prove to be effective, patients would avoid potentially decades of

pharmacotherapy and T2D-related complications. At the population level, T2D prevention would halt downstream complications and result in fewer cases of cardiovascular disease, end-stage renal disease, blindness and non-traumatic limb amputation, all of which place a significant burden on healthcare systems.[204] Moreover, according to an epidemiological analysis in Caucasian males in the US, all-cause mortality could be reduced by up to 6.2% if all T2D cases were to be avoided.[324] Yet, implementing impactful population level preventative approaches remains a challenge for healthcare systems, which has led to a predominant culture of reactive treatment rather than proactive. However, early implementation of T2D management strategies increase the chances of reducing the burden of the disease. This is essential, considering that increases in disability due to T2D have outpaced T2D-related mortality.[325,326]

1.2.4.5 Managing Type 2 Diabetes Mellitus

Initially, T2D management involves intensive lifestyle modifications as a first-line strategy, including weight loss, by increasing physical activity and eating a healthy diet.[193] Over time, however, medication may be unavoidable and often escalates to treatment with exogenous insulin[327] as secretory capacity progressively declines with time.[328] In order to improve glycemia and prevent complications caused by chronic hyperglycemia, management is focused on meeting individualized optimal blood glucose targets.[329] Alarmingly, only half of all Canadians reach their target range of glycemia.[202]

1.2.4.5.1 Lifestyle Interventions

1.2.4.5.1.1 Physical Activity

Physical activity is an essential component of T2D management due to its *ability* to manage the disease and its related cardiometabolic consequences. Regular physical

activity enhances β-cell function and insulin sensitivity,[330,331] effectively improves glycemic control,[332] and is associated with a decreased risk of both cardiovascular and all-cause mortality in those with T2D.[333]

Given the increasing prevalence of T2D and the urgent need for strategies to reduce the burden of this disease,[334,335] experts put together a series of physical activity guidelines to maximize the benefits that may be obtained for this population. Individuals with T2D and healthcare professionals alike can look to recommendations from *Diabetes Canada*, the *ADA*, and the *American College of Sports Medicine (ACSM)*[336-338] to gain valuable insight on how best to manage their chronic condition. These guidelines focus on the same components as those of the traditional physical activity guidelines for all healthy adults (AT, RT, functional fitness, and flexibility training),[7-9,55] but with additional guidance tailored to the needs of this population.

1.2.4.5.1.1.1 Aerobic Training

In line with the guidance of physical activity for all healthy adults, the physical activity guidelines for T2D also recommend a minimum of 150 minutes per week of MVPA.[336-338] However, individuals with T2D can still gain beneficial results in glycemic control when exercising at lower volumes, albeit to a reduced magnitude[332,339] – highlighting that some is better than none. This prompted the experts composing the physical activity guidelines for T2D to acknowledge that exercising at a lower volume should still be performed (i.e. 90-140 minutes/week).[336]

AT benefits individuals with T2D through enhancements in cardiorespiratory fitness, glycemic control and overall reductions in cardiometabolic risk.[332,340] Several influential RCTs in individuals with T2D with multiple treatment arms have shown that

AT (alone or in combination with RT) improves HbA_{1c}.[341-343] A meta-analysis of 47 RCTs and a total of 8,538 adults with T2D by Umpierre et al. (2011) reported that ≥12 weeks of AT with volumes corresponding to the physical activity guidelines decreased HbA_{1c} by 0.73% (95% CI [-1.06, -0.40]) compared to controls.[332] Furthermore, meta-analytic data has demonstrated the ability of AT to effectively reduce blood pressure and improve lipid profiles in this population.[340] Improvements in cardiorespiratory fitness result from AT, even in individuals with T2D,[340,342,344,345] however, the improvements are inferior to healthy individuals. This is of particular relevance for reducing the risk of cardiovascular disease and all-cause mortality, as individuals with T2D who have low cardiorespiratory fitness have a substantially greater likelihood of cardiovascular mortality in those with normal weight (HR 4.2, 95% CI [2.0, 8.6]), overweight (HR 4.3, 95% CI [2.3, 7.9]) and obesity (HR 4.5, 95% CI [2.3, 8.7]), as compared to those with normal weight and high cardiorespiratory fitness.[346] These data are especially important considering that cardiovascular disease is the major cause of morbidity and mortality in this population.[347,348] Moreover, in a retrospective analysis of the Look-AHEAD (Action for Health in Diabetes) trial, greater cardiorespiratory fitness was associated with a 30% reduction in all-cause mortality, even when controlling for age, sex, race/ethnicity, BMI, and medications.[349]

The intensity at which the AT is performed can have significant implications for the health outcomes of interest for individuals with T2D. In the physical activity guidelines for T2D, they suggest that high-intensity interval training (HIIT) can be performed in the place of continuous moderate-intensity exercise to increase gains in cardiorespiratory fitness.[336] For instance, Winding et al. (2018) assessed the impact of 11 weeks of AT on

cardiorespiratory fitness in individuals with T2D randomized to HIIT, moderate-intensity continuous training (MICT), or control groups.[350] HIIT involved 20 minutes of cycling where individuals performed 10 one-minute intervals (95% maximum workload) interspersed with one-minute of active recovery (20% maximum workload). The continuous moderate group cycled for 40 minutes at 50% maximum workload. Although HIIT involved half the total weekly exercise volume and a significantly lower overall energy expenditure, it improved VO_{2peak} to a greater extent (20 ± 20%) than continuous moderate-intensity exercise (8 ± 9%) ($p < 0.05$).[350] In another study, unsupervised HIIT walking exercise in individuals with T2D elicited a significant increase in maximal oxygen consumption ($VO_{2max)}$ (16.1 ± 3.7%) as compared to controls, while continuous moderate-intensity walking did not.[351] Both groups performed the same volume of exercise (300 minutes per week – well above the recommendations for MVPA), supporting that exercise intensity is a vital factor to consider when deciding on what type of exercise to perform to increase cardiorespiratory fitness. As only the HIIT group improved glycemic control, this suggests that intensity may be involved in glycemic control as well. Several groups have demonstrated that HIIT produces greater changes in glycemic control than continuous moderate training. In the same trial by Winding et al. (2018), they observed similar or greater improvements in glycemic control in the HIIT group than the MICT group, as measured by reductions in HbA_{1c}, insulin resistance, and both fasting and postprandial glucose.[350] In a crossover RCT of treadmill walking in 15 individuals with T2D, Mendes et al. (2019) randomized each participant to perform three separate conditions: 1) HIIT walking (five intervals: three minutes at 70% heart rate reserve (HRR) followed by three minutes at 30% HRR); 2) continuous moderate-intensity walking (30 minutes at 50%

HRR); 3) control session of 30 minutes of rest. They measured glucose at various time points during the acute sessions, where they observed a greater overall effect of HIIT on immediate acute glycemic control through time-point blood glucose measurements than with continuous moderate-intensity walking ($p < 0.05$).[352] Moreover, a meta-analysis of 13 RCTs in individuals with T2D by Liu et al. (2019) demonstrated that HIIT elicited significantly greater reductions in HbA_{1c} of 0.37% compared to moderate continuous intensity exercise (95% CI [-0.55, -0.19], $p < 0.0001$).[353] In this analysis, they also reported a greater effect of HIIT with respect to cardiorespiratory fitness (3.37 ml/kg/min, 95% CI [1.88, 4.87]) as well as reductions in BMI (-0.40 kg/m^2, 95% CI [-0.78, -0.02]) (all $p <$ 0.05) compared to MICT. Altogether, these data support the importance of higher intensity AT for T2D exercise management.

An additional recommendation for individuals with T2D is that no more than two days should elapse without AT.[336-338] The basis of this recommendation is due, in part, to the time course of insulin action following exercise.[354,355] Depending on the intensity and duration of the exercise undertaken, insulin-dependent mechanisms stimulate glucose uptake for 24 to 48 hours following exercise. Exercise of a shorter duration (~20 minutes) can enhance insulin action if the activity is particularly intense[356] and low-intensity AT in long enough durations (≥ 60 minutes) elevates glucose uptake for 24 hours.[357] Therefore, prolonged exercise is preferred to achieve the benefits for a longer duration[358] and the effects are optimized if they are at moderate or high intensities.[359] In order to maintain the benefits obtained from enhanced insulin action, AT should be performed regularly.[354] Over time, chronic AT leads to increased skeletal muscle mitochondria, as well as greater

glucose uptake via increased expression and translocation of GLUT4, which are associated with improved insulin sensitivity.[179]

1.2.4.5.1.1.2 Resistance Training

The physical activity guidelines for T2D also call for a minimum of twice-weekly RT on non-consecutive days – ideally reaching three times, however, for maximum benefit.[336-338] RT elicits changes in skeletal muscle physiology and induces signalling pathways that reduce the complications associated with T2D. For instance, RT has the capacity to enhance insulin sensitivity, through the increase of skeletal muscle mass and the concomitant increase in glucose storage and uptake, as well as via improvements in muscle oxidative capacity.[360-362] Furthermore, RT improves glycemic control by increasing muscle mass and strength, decreasing fat mass, and reducing inflammation.[363-367] As such, exercise that increases skeletal muscle mass and strength are of great importance for those with T2D.

Several studies have been conducted on individuals with T2D that demonstrate the benefits of RT in this population. Most importantly, RT has been proven to have a glucose lowering effect.[368] In a group of older adults with T2D, a RCT of six months of RT progressing from moderate- to high-intensity led to a 3-fold reduction in HbA_{1c} of 1.2%.[369] This underscores the power of RT as a means to treat T2D, as reductions of HbA_{1c} of 1% are associated with a 14% reduction in the risk of cardiovascular events and a 21% reduction in mortality in T2D.[370]. Others have shown that moderate-intensity RT can also reduce HbA_{1c} after just three months ($0.54 \pm 0.4\%$).[371] In a RCT of adults with T2D randomized to four months of AT vs RT, the RT group improved glycemic control to a similar extent as those in the AT group (-0.40% vs -0.35%, $p > 0.05$).[372] These data, along

with other meta-analyses, confirm that RT, undertaken in any intensity, favourably reduces HbA_{1c} in adults with T2D.[339,361] However, more recently, a meta-analysis by Liu et al. (2019) challenged this notion when comparing two intensities of RT in 962 adults with T2D.[373] They demonstrated that high-intensity RT was significantly better than low- to moderate-intensity RT at reducing HbA_{1c}, (-0.61, 95% CI [-0.90, -0.33] vs. -0.23, 95% CI [-0.41, -0.05]). Furthermore, it should be noted that in their analysis, only RT trials with higher intensities were sufficient to significantly reduce insulin levels. These findings suggest that higher intensity exercise in general may be needed to elicit changes in insulin, as these findings are also reflected in AT, as higher intensity AT produces optimal benefits on insulin action.[356]

While higher intensities of RT may be best to improve glycemic control, all intensities of RT efficiently benefit muscular strength, balance, and functional fitness.[338] RT interventions ranging from 8 to 26 weeks involving various intensities improved muscle strength in adults with T2D by greater than 50%.[368] In a retrospective analysis of the Diabetes Aerobic and Resistance Exercise (DARE) trial, a large RCT in middle-aged adults with T2D (aged 39-70; n = 251), the observed increases in skeletal muscle strength were associated with a concomitant improvement in glycemic control.[374] Furthermore, as one ages, muscle mass and strength decrease,[375] which have significant implications on health as well as functional fitness and mortality in older adults.[376,377] As T2D is recognized as an independent predictor of low muscle strength,[378] RT should be performed in those with T2D to reduce the complications associated with T2D and low muscle strength. Additionally, RT should be performed as it is an important means of improving functional fitness and balance.[379-381]

1.2.4.5.1.1.3 Combined Aerobic and Resistance Training

Although myriad benefits arise from AT and RT on their own, the benefits for individuals with T2D are maximized when performing both together. Several large-scale RCTs have explored the effects of exercise when combining AT and RT together, with the DARE, Health Benefits of Aerobic and Resistance Training in individuals with T2D (HART-D), and the Italian Diabetes and Exercise Study (IDES) trials being the largest of those involving individuals with T2D.[341-343] In the DARE trial (n = 251), the middle-aged adults with T2D were randomized to a combined training group, AT and RT only groups, and a control group.[341] For nearly six months (three times per week), AT progressed to 135 minutes per week (60-75% maximum HR), RT progressed to three sets of seven exercises at the maximum weight that could be lifted 7-9 times, and the combined training group performed twice the volume, as they underwent both of the full interventions of AT alone and RT alone, together. On their own, both AT and RT led to significant improvements in glycemic control, with AT reducing HbA_{1c} by 0.51% more than controls and RT by 0.38% (both $p < 0.05$). The combined training group, however, experienced additional gains over either mode on their own. Combined training conferred a 0.46% greater reduction over the AT group, and 0.59% over the RT group (both $p < 0.05$). Given the sheer difference in training volumes, the difference in HbA_{1c} may have been attributed to the substantially greater amount of physical activity performed each week. The HART-D trial accounted for this limitation by standardizing exercise volume across the AT and combined training groups according to body weight to equate to 150 minutes per week of moderate-intensity physical activity. In HART-D, 261 participants were randomized to the same four conditions as the DARE trial for a total of nine months.[342] However, a broader age range was captured, including both younger and older adults with T2D (ages 30-75 years old).

RT involved 10-12 repetitions of exercises targeting all major muscle groups for one to two sets, according to whether they were in the combined training group or RT alone group or combined training. Interestingly, neither the AT nor the RT groups on their own improved glycemic control significantly compared to controls, but a significant reduction in HbA_{1c} was observed in the combined training group (-0.34%, 95% CI [-0.64, -0.03]). In the IDES trial (n = 606), authors examined the effect of combined training as compared to usual care in middle-aged and older adults with both T2D and metabolic syndrome, but without intervention arms of AT or RT alone.[343] Exercise training involved two supervised sessions per week of mixed AT and RT to a total of 150 minutes per week. The results of this trial also produced a significant decrease in HbA_{1c} compared to the control group (-0.30%, 95% CI [-0.49, -0.10], $p < 0.001$). Two recent meta-analyses have corroborated these findings by confirming that combined training is the best mode of exercise for glycemic control.[382,383] In addition, Zhao et al. (2021) reported an enhanced effect of combined training for reducing insulin resistance, blood pressure, and BMI,[383] which are all essential components of the management of T2D. Together, these data, along with others,[361,384] led experts to recommend that RT be performed in addition to AT in those with T2D to gain further health benefits.[336-338]

1.2.4.5.1.2 Dietary Interventions

Several dietary recommendations for those with T2D have been put forth by Diabetes Canada,[385] including restricting carbohydrates, cholesterol and fat. Low glycemic index carbohydrates and dietary fiber are emphasized for improved glycemia[386,387] and cholesterol[388] and to reduce the risk of cardiovascular complications.[389,390] Saturated fatty acids should be replaced with polyunsaturated fats (including omega-3 sources),

monounsaturated fats from plant sources, and whole grains.[391] Additionally, those with T2D should maintain regular timing and spacing of meals for better glycemia.[392] Diabetes Canada also recommends that individuals with T2D follow dietary recommendations from registered dietitians to not only treat T2D and its complications, but also to improve quality of life and overall health.[385] Nutrition therapy has been shown to reduce HbA_{1c} up to 2.0% in just 3 months, demonstrating its overall effectiveness.[393]

As an estimated 80-90% of adults with T2D are living with overweight or obesity,[394,395] this emphasizes the importance of weight loss for the management of T2D. For these individuals, dietary recommendations place special focus on caloric restriction to achieve a healthy weight to improve glycemia and reduce cardiovascular risk.[385] Dietary interventions for T2D management that implement low-calorie diets report rapid weight loss[396] and a reduction in HbA_{1c} to levels resembling prediabetes or normoglycemia. For instance, in a RCT of individuals with T2D with an average BMI of 35 kg/m^2, a very low-calorie diet of 825-853 kilocalories (kcal)/day for three to five months and 15 kg of weight loss resulted in 46% of the participants maintaining an HbA_{1c} <6.5% at 12 months (even after being off of antidiabetic drugs for a minimum of 2 months).[397] These findings, along with other trials[398,399] demonstrated that T2D remission is possible with aggressive control of caloric intake.

Some of the most effective interventions for weight loss, however, are those that incorporate caloric restriction with physical activity or, sometimes, bariatric surgery.[400,401] The importance of pairing dietary interventions with physical activity is emphasized in the most recently updated PA guidelines for T2D from ACSM that recommend a minimum weight reduction of 5% in order to reduce HbA_{1c}, lipids, and blood pressure,[337] due to

findings from meta-analyses of weight loss interventions in this population.[402,403] The Look-AHEAD trial was the largest RCT (n = 5,145) in older adults with T2D with excess body weight that assessed a physical activity and diet intervention.[404,405] In this study, they targeted a weight loss of greater than 7% through exercise with a minimum of 175 minutes per week of MVPA (unsupervised) and caloric restriction. Following the completion of the trial, those in the intervention group lost 8.6 ± 6.9% of their baseline weight, while the control group (diabetes support and education) only lost 0.7%. As for glycemic control, the intervention group had a substantially greater improvement in HbA_{1c} compared to the control group (mean difference of -0.5%, $p < 0.001$), as well as in other essential risk factors like blood pressure, lipids, and cardiorespiratory fitness.[344,406] The improvements in weight and HbA_{1c} were sustained for several years relative to the control group, as demonstrated at four years of follow-up.[404] Interestingly, in a secondary analysis of the HART-D trial, they showed that if participants significantly reduced body fat mass, they were 3.5-fold more likely to significantly reduce HbA_{1c}.[367] In addition to these studies demonstrating the importance of weight loss in overt T2D, increased adiposity is also recognized as the strongest predictor for the development of T2D.[209] Those with a BMI ≥30 kg/m^2 have a 7.2-fold higher risk of developing T2D than normal weight adults.[216] As such, weight loss in lifestyle interventions are important in both the prevention and management of T2D.

1.2.4.5.2 Pharmacotherapy

When lifestyle interventions are not enough to curb the progression of T2D, clinicians then implement antihyperglycemic pharmacological treatment to prevent symptoms of hyperglycemia and reduce the risk of micro- and macrovascular complications.[407] According to Diabetes Canada recommendations, if after 3 months of

lifestyle intervention, medication should be initiated; however, when patients have a HbA_{1c} ≥1.5% above the individualized target of generally 6.5-7.0%,[329] antihyperglycemic medication is prescribed immediately in combination with diet and physical activity behaviour changes.[407] Immediate insulin therapy is advised in those who have severe hyperglycemia, ketosis, or unintentional weight loss and/or symptomatic hyperglycemia, regardless of HbA_{1c} for a short-term to rapidly stabilize glucose.[408] This early introduction of insulin may preserve β-cell function and reduce glucotoxicity.[409] According to data from Statistics Canada between 2016-2019, nearly three in four adults with diabetes used pharmacotherapy as part of their management plan, with the proportion of medication users rising with age.[410]

The first-line antihyperglycemic medication of choice for T2D is metformin, primarily due to its safety, low cost, and cardiometabolic benefits.[407]. The physiological mechanism by which metformin improves glycemia is primarily through a reduction in hepatic glucose output, along with improved peripheral tissue insulin sensitivity, and enhanced GLP-1 secretion,[411] which can reduce HbA_{1c} by approximately 1.5%.[412] Metformin is preferred to other pharmaceuticals due to the lack of drug-induced weight gain, positive effects on blood pressure and lipids, and because of the low risk of associated hypoglycemia.[413] If metformin and lifestyle changes do not sufficiently control glycemia within optimal blood glucose targets, second-line pharmaceutical agents may be added[407,414] – in addition to metformin – for a 40% greater chance of achieving an HbA_{1c} below 7.0% after 6 months.[408] Several antihyperglycemic agents are now approved for glycemia management, which allows for a more personalized treatment depending on the patients' individual needs.

Each antihyperglycemic medication has associated risks and benefits, which must be carefully weighed by the healthcare provider. Popular second-line treatments paired with metformin include incretin therapies, including GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-IV) inhibitors. GLP-1 receptor agonists (e.g. semaglutide, liraglutide) stimulate GLP-1 effects that trigger insulin secretion and inhibit glucagon secretion, reduce hepatic glucose production, delay gastric emptying and increase satiety.[415] DPP-IV inhibitors enhance the effects of physiological GLP-1.[415] For those with obesity, GLP-1 receptor agonists may be preferable, as they induce up to 3 kg of weight loss compared to DPP-IV inhibitors that are weight neutral.[407] Sodium-Glucose Transport Protein 2 (SGLT2) Inhibitors may also be recommended to those with obesity due to the substantial weight loss associated with the medication.[416] SGLT2 inhibitors increase urinary glucose excretion which reduce body weight by glycosuria and urinary calorie loss.[417] Insulin secretagogues (e.g. sulfonylureas and meglitinides) act on β-cells to stimulate insulin secretion.[418] Sulfonylureas cause weight gain and are associated with cardiovascular risks, while meglitinides have lower hypoglycemic risk than sulfonylureas, but are less effective for glycemic management.[419,420] Thiazolidinediones are peroxisome proliferator-activated receptor-γ (PPARγ) agonists that improve peripheral improve tissue sensitivity to insulin,[407] which has mechanistic similarities to metformin, but they are associated with weight gain, bone fractures, and certain cancers.[421] Finally, although insulin therapy is also effective at managing glycemia, it increases the risk of hypoglycemia and can cause weight gain of up to 4 kg.[422] Altogether, the data presented above demonstrate that various factors must be considered before making an informed choice that requires a knowledge of the specific features of each pharmaceutical.[407] However,

importantly, healthcare providers must also balance the broader social needs of the patients, including affordability and patient preferences, given the disparities in medication coverage.[241]

1.2.4.5.3 Other Interventions

1.2.4.5.3.1 Diabetes Education

Diabetes education is recommended by experts, as they allow individuals with T2D to be informed about their disease so that they can engage in self-management practices and behaviours.[423] Diabetes education is associated with improved glycemia, reduced cardiovascular risk, and fewer limb amputations,[424] as well as improved quality of life, sustained weight loss and improvements in fitness.[405,425] Evidence also points to a potential impact of diabetes education at the population level, as a retrospective study of 28,790 individuals with T2D had significantly lower T2D-related healthcare expenditures if they had at least one diabetes education session.[426]

As T2D is a multifaceted chronic disease, diabetes education has several components. Topics range from making healthy choices like following a proper dietary regimen performing regular physical activity, to self-monitoring of blood glucose, and the importance of medication adherence and how to prevent/manage both hypoglycemia and hyperglycemia.[423] In the diabetes education treatment arm in the above-mentioned Look AHEAD trial, overweight or obese participants with T2D experienced improvements in their fitness (2.0%), weight (-0.9%), and in their cardiometabolic profile, with a significantly greater reduction in LDL-cholesterol than the lifestyle intervention group (-12.84 vs. -11.27 mg/dl, $p = 0.009$).[405] Although the magnitude of the result was lesser than in the lifestyle intervention group, these findings demonstrate the relative importance of

incorporating diabetes education into overall disease management. When paired with lifestyle intervention, diabetes education may lead to meaningful changes in glycemia. For instance, data from our group indicate that T2D education and physical activity utilizing elastic bands for resistance training (The Band-Frail Study) lowered HbA_{1c} by ≥0.3% in 48.6% of participants who have had T2D for more than 10 years.[427]

1.2.4.5.3.2 Bariatric Surgery

For those with T2D and severe obesity, bariatric surgery may be an appropriate option to manage excess weight and adiposity.[321] A follow-up of a pooled analysis of RCTs of intensive lifestyle interventions vs. bariatric surgery by Courcoulas et al. (2024) demonstrated that bariatric surgery was more effective for long-term glycemic control than lifestyle interventions after 12 years of initial randomization.[428] Those who underwent bariatric surgery also had higher rates of diabetes remission and used less T2D medication. Similar reductions in HbA_{1c} were observed in those with T2D with a BMI between 25.0-35.0 kg/m^2 compared to those with a BMI ≥35.0 0 kg/m^2, suggesting that bariatric surgery may be an effective treatment option for T2D in those who would not traditionally be eligible for bariatric surgery.[429] In fact, newer bariatric surgery standards support the use of bariatric surgery for those with T2D with a lower BMI.[430] A retrospective population-based matched cohort study analyzed 3,455 participants with T2D and a BMI ≥ 35.0 kg/m^2 who underwent bariatric surgery. After 4.6 years of follow-up, bariatric surgery was associated with a 47% decrease in all-cause mortality (HR: 0.32, 95% CI [0.15, 0.66]), 68% lower cardiovascular mortality (HR: 0.32, 95% CI [0.15-0.66]) and a 42% decrease in renal complications (HR: 0.58, 95% CI [0.35-0.95]).[431] In this analysis, those who had the greatest absolute benefit, demonstrated by absolute risk reduction, were men, adults

>55 years old, and those with a diabetes duration of more than 15 years. Together, these data support bariatric surgery as a means of managing T2D and reducing its associated complications.

1.3 Obesity

Obesity, an excessive accumulation of adipose tissue that can impair health,[432] is a complex multifactorial disease.[433] Classically, obesity is defined by body mass index (BMI), a ratio of body mass relative to height (BMI = kg/m^2). This indicator of body fat [434] (derived from the Quetelet Index)[435,436] provides a standard reference to classify overall health risk and mortality.[437]

Compared with normal weight adults (BMI = 18.5-24.9 kg/m^2), individuals living with overweight (BMI = 25.0-29.9 kg/m^2) or obesity (BMI ≥ 30.0 kg/m^2) are at greater risk of developing comorbidities, as seen in Table 1.[437] In fact, a BMI ≥30 kg/m^2 is associated with a 5- to 15-fold increased risk of cardiovascular disease and T2D.[438] Most critically, however, is the substantially elevated risk of all-cause mortality observed with increasing BMI, as shown in various large-scale prospective studies and pooled analyses.[439-442] This is highlighted by the finding that, for each 5 unit increase in BMI above 25 kg/m^2, the risk of all-cause mortality increases by approximately 30%.[443]

Table 1. BMI classification of adults (aged 18-65).[437]

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

The dose-response relationship between BMI and mortality illustrates either a J- or U-shaped association, depending on the population analyzed.[439] This indicates that, while the excess adiposity in overweight and obesity increases the risk of mortality, underweight adults (BMI <18.5 kg/m^2) are also at greater risk of premature mortality compared to those in the normal range. Most data has reported a U-shaped association,[440,443] signifying a progressive increase in mortality with lower BMI; however, others have demonstrated that restricting the analysis to healthy participants who never smoked flattens the curve to a J-shape.[199,439,441] Smoking is often associated with lower weight status[444] and various specific causes of mortality,[445] which should be accounted for to reduce the confounding effect on the relationship between BMI and all-cause mortality. Moreover, age and sex have been shown to impact the BMI-mortality association. In a population-based cohort study of 3.6 million adults, Bhaskaran et al. (2018) reported a weaker association between BMI and mortality in older adults as compared to younger adults. They highlighted that the BMI associated with the lowest risk of all-cause mortality (ie: optimal BMI) may rise with age, as the nadir of the curve increases from 23 kg/m^2 in those <70 years old to 25 kg/m^2 in those ≥70 years old.[199] Similar conclusions were drawn from the results of previous studies.[446-448] This may be explained by the reduced effectiveness of using BMI to measure

adiposity later in life, and its reduced ability to account for the pre-existing diseases that become more prevalent with increasing age.[447] Although the optimal BMI may increase with age regardless of sex, the optimal BMI has been shown to be lower in women compared to men.[446] Similarly, higher BMI may be more strongly associated with both all-cause mortality and cardiovascular mortality in men compared to women.[199] On the other hand, in a meta-analysis of older adults (≥65 years old), men and women had similar mortality risks according to BMI,[449] further highlighting the varying effect of age on the BMI-mortality relationship. With respect to race/ethnicity, in a large prospective analysis of the Multiethnic Cohort Study, Park et al. (2012) observed that the association between BMI and all-cause mortality was consistent across ethnic groups studied.[442]

1.3.1 Definition and Prevalence

1.3.1.1 Body Mass Index

Over the past 50 years, the prevalence of overweight and obesity has risen drastically to reach epidemic proportions.[227,450-452] Based on the latest data from the NCD Risk Factor Collaboration (NCD-RisC), each of the 200 countries included in their pooled analysis of 2,416 population-based studies (over 112 million people) had an increased prevalence of obesity between 1975 and 2016.[450] In the latter year, 39% of the world's adult population was estimated to be overweight and 12% was obese – representing a tripling of the prevalence in 40 years.[450] Children and adolescents experienced a steep increase in obesity prevalence, from less than 1% in 1975 to greater than 7% in 2016.[450] This is particularly concerning, as BMI prevalence in children offers an estimation of the effect of obesity in the future.[453] If current trends continue, the world could see more than 50% of its population being overweight or obese by the year 2035 (>4 billion). In 2020,

nearly one billion adults, children, and adolescents were considered obese (14%); by 2035, approximately one in four people could be living with obesity globally (24%).[454]

According to the most recent data from the 2021 CCHS, 35.5% of adults were classified as overweight with an additional 29.2% as obese,[455,456] for a total of 64.7% of Canadians that have increased health risks due to elevated BMI. These statistics reveal a 4.8-fold increase in prevalence since 1985, when just 6.1% of adults were considered obese according to BMI. Even since 2005, the prevalence of obesity has nearly doubled (29.2% vs 15.8%).[457] In Canada, there has been a disproportionate increase in the prevalence of adults with severe obesity (classes II and III), who saw a 455% increase between 1985 and 2016.[458] Alarmingly, projections from the World Health Organization (WHO) and NCD-RisC estimate that in Canada, obesity rates alone may continue to rise to as much as 50% of the population by 2035.[454] Alternatively, some data suggest that the prevalence of obesity has remained relatively stable between 2007 and 2017, which may mitigate these projections.[458]

Although there has been a global increase in overweight and obesity across all age, sex, geographic location, race/ethnicity, and socioeconomic groups,[459,460] wide variance exists in the prevalence and overall burden of these groups. According to data between 1980 and 2015 from the Global Burden of Disease (GBD) Study,[460] Chooi et al. (2019) showed that the prevalence of overweight and obesity was greater overall in women than in men.[459] From another GBD Study, Afshin et al. (2017) observed – in 67.8 million people from 195 countries – that obesity prevalence peaked at age 60-64 for women and at age 50-54 for men,[227] and trended downwards thereafter into late adulthood. Obesity rates also vary geographically, with the greatest prevalence observed in North America, South

America, and Europe.[459] Historically, the United States has been known to have the highest obesity prevalence[461]; however, recent international obesity rates indicate that many countries now meet or exceed those of the United States.[450] In 2015, the United States had the highest level of childhood and adolescent obesity (12%), but Egypt had the highest in adults (34.9%).[227] BMI prevalence varies within a country itself, according to the local environment in which one resides. A recent study from the NCD-RisC (2019) reported that BMI is rising at a faster rate in rural areas in compared to urban cities – especially in women,[462] which contradicts some claims of urbanization as the driver of the obesity epidemic. Furthermore, in low-income countries, middle-aged adults from urban environments with high socioeconomic status have higher obesity rates; but in high-income countries, the greatest obesity prevalence shifts to those from low socioeconomic status in rural areas.[226,463,464] Finally, obesity disproportionately affects those from minority populations. In the United States, the highest prevalence of obesity by race/ethnicity was reported in Black, Hispanic, and Native American men and women.[465] In Canada, some on-reserve indigenous communities report that one in two Indigenous adults are living with obesity (51%).[466] The critically elevated rates of overweight and obesity presented here, across all groups, are a great cause for concern given the grave impact of obesity and its related comorbidities.

The deleterious effect of the increased prevalence of overweight and obesity extends beyond an individual's personal health, by having a substantial impact on the economy. Although the prevention of obesity requires financial investment, the consequences of failing to prevent and treat obesity will be significantly more detrimental economically, as a result of the inflated direct and indirect healthcare costs of treating

obesity as well as its influence on economic productivity.[467] According to Krueger et al. (2015), the annual economic burden attributed to excess adiposity in Canada is more substantial than that of smoking; overweight and obesity has a 25% greater cost to the economy.[468] On a global scale, current trends indicate that the cost of overweight and obesity will more than double by 2035: up to US$4.32 trillion as compared to US$1.96 trillion in 2020. This will translate to nearly 3% of global gross domestic product (GDP) annually.[467,469] To put this into perspective, this is comparable to the estimated impact of COVID-19 during the calamitous first year of the pandemic, which caused the global economy to shrink by 3% of GDP.[467,470] Altogether, these data highlight the global health priority of overweight and obesity and the urgent need to attenuate current trends.

1.3.1.2 Adiposity

Although obesity in adults is defined using BMI, it has been criticized for its use at the individual level due to its inability to differentiate between the weight of fat mass and that of lean mass,[434] which varies significantly according to age, sex, and race/ethnicity.[434] Despite the strong correlation between BMI and body fat percentage (r = 0.72-0.86),[471,472] the distribution of fat is not captured by BMI, which makes it an imperfect measure of adiposity.[473] The common belief is that this distinction becomes important when assessing the cardiometabolic health risks associated with excess body weight; however, several lines of evidence suggest that having a high BMI – irrespective of body fat distribution – still places individuals at a greater risk of adverse cardiometabolic outcomes, metabolic syndrome, and T2D[474] and cardiovascular disease mortality[475] in adults. Some, however, suggest that greater proportions of visceral fat with central obesity (fat accumulated around and between the organs in the abdomen)[476] are associated with worse cardiometabolic

health outcomes than traditional obesity characterized by subcutaneous fat.[477] Waist circumference and waist-to-hip ratio more accurately depict visceral fat accumulations[478] that are positively associated with the incidence of both all-cause and cardiovascular mortality, even when accounting for BMI.[479,480] These indicators are also relevant for those with a low BMI, as increased waist circumference increases the risk of morbidity and mortality even in underweight or normal weight BMI categories.[481,482] Data from the CHMS in 2007-2009 suggest that 2.6% of normal weight adults, 35.3% of overweight adults, and 93.0% of obese adults had a waist circumference indicative of excessive visceral fat and, consequently, abdominal obesity.[483] As such, pairing waist circumference with BMI is advised when assessing obesity for better disease management.[213]

1.3.2 Etiology

1.3.2.1 Energetic Balance

Although its etiology is complex, obesity primarily results from a chronic imbalance of energy owing to a greater consumption than expenditure of energy.[484] When the energy gained from consuming calories matches the energy lost due to expenditure, energy homeostasis is maintained, and body mass remains stable.[485] Changes in body mass, whether positive or negative, only occur following a prolonged disruption in energy balance. Each of the physiological, social, and environmental determinants of obesity is fundamentally mediated through this alteration in energy balance,[485] which is primarily affected by individual eating behaviours and physical activity patterns. The enormous complexity of the systems causing obesity and their interactions are depicted in the Obesity System Map.[486]

1.3.2.1.1 Energy Consumption

An overconsumption of energy for a prolonged period of time causes a positive energy balance and, thus, increased body weight.[485] Even small changes in energy homeostasis, as little as 1-2% of daily energy intake, can significantly impact weight gain.[487] Energy in humans is derived from the consumption and subsequent metabolization of carbohydrates, protein, and fat.[488] The excess energy resulting from the overconsumption of these macronutrients is mobilized and converted mostly to triglycerides, which are stored in adipose tissue deposits.[489] This disproportionate storage of energy leads to adipose tissue growth through adipocyte hypertrophy and hyperplasia.[490] This excess adiposity is distributed throughout subcutaneous and visceral adipose tissue depots in the form of white adipose tissue (WAT),[491] which – in addition to its primary function of energy storage – is an active endocrine organ.[492] In fact, the chronic, low-grade inflammation seen in excess adiposity is associated with the infiltration of macrophages into adipose tissue that secrete pro-inflammatory adipokines, including tumour necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8, leptin, and adiponectin, to name a few.[493,494]

According to the energy balance model, the brain is the primary centre for the regulation of energy balance.[495] Several brain regions modulate food intake unconsciously via short-term signals that control meal patterns (e.g. ghrelin, GLP-1, and peptide YY) and long-term signals (e.g. leptin) that mediate short-term system activity,[496] with the hypothalamus playing a critical role.[497] Appetite and satiety regulation takes place in the hypothalamus through the inhibition or excitation of the arcuate nucleus by circulating neuropeptides, including leptin, insulin, glucose and free fatty acids.[495] The importance of the role of the hypothalamus in energy balance was demonstrated from early studies that

showed that hypothalamic brain lesions and tumours in animal and human models develop obesity.[498,499] Furthermore, the observation that mutations in the *ob* gene encoding leptin cause severe obesity in *ob/ob* mice[500,501] highlighted the critical role of leptin in regulating appetite and energy balance. These findings demonstrate the importance of the central mechanisms of energy regulation, where the brain integrates peripheral signals arising from various sources, including the microbiome and cells within the adipose tissue, stomach, pancreas, etc.[502,503] Although day-to-day caloric intake and energy expenditure varies, alterations in the neural regulation of energy balance occur over a prolonged period of time, reflecting the interaction of these signals.[496]

1.3.2.1.2 Energy Expenditure

Reductions in body weight can be achieved by tipping the balance to expend more energy than what is consumed. Energy expenditure is categorized by several components, including resting metabolic rate (RMR), activity-induced thermogenesis, and diet-induced thermogenesis.[495] RMR represents the minimal energy expenditure for living cells and tissues working at rest and is composed of standard metabolic rate (amount of energy at rest in a thermoneutral environment) and the thermic effect of food (heat generated during digestion, absorption, and processing of food).[495] RMR is the largest contributor to energy expenditure, accounting for approximately 60-70% of total daily expenditure,[495,504] and is largely influenced by body composition and hormonal factors.[505] Fat-free mass (FFM), the major predictor of RMR,[505] reduces with age[506] and thus contributes to reductions in energy expenditure at different stages of life [507]. RMR decreases as one ages, but males may begin to experience significant reductions a decade earlier than females (40 vs. 50 years of age), owing to a greater proportion of FFM and less fat mass.[508] These changes translate to a 5-

10% lower RMR in females than males of the same BMI.[508] However, a recent large, diverse cross-sectional analysis (n = 6,421) suggested that RMR is comparable in adulthood across the ages of 20-60, with no differences according to sex.[507]

The energy expenditure resulting from the thermic effect of food, an obligatory metabolic component of RMR,[495] depends on the macronutrient ingested. The cost of food processing is lowest for fat and carbohydrates and is highest for protein,[509] demonstrating the variation in potential expenditure depending on dietary patterns. Overall, the thermic effect of food comprises ~10% of daily energy expenditure which accounts for the energy cost of absorbing and transporting nutrients as well as synthesizing carbohydrate, protein, and fat in tissues.[509] However, diet-induced thermogenesis is a facultative process beyond the thermic effect of food that represents the heat generated in response to excess caloric intake.[509] In addition to the variation in energy expenditure caused by differing macronutrients, the thermic effect of food may also be impacted by age and physical activity levels.[510] Some suggest that insulin sensitivity and obesity may be the principal drivers of the thermic effect of food.[510,511] The thermic effect of food may be reduced in individuals with obesity[510,512] but more research is needed to clarify the role of obesity in energy expenditure from the thermic effect of food.[510,513]

A key portion of total energy expenditure is the energy cost of physical activity, which includes both physical activity-induced thermogenesis and non-exercise activity thermogenesis (NEAT).[495] While resting metabolic rate and diet-induced thermogenesis are relatively static, physical activity and NEAT are the most variable portion of energy expenditure,[485] which accounts for ~15-50% of total daily energy expenditure.[514-516] NEAT refers to the energy expenditure that encompasses the energy expended from occupational

and leisure physical activity, basic activities of daily living, and unconscious or spontaneous activity like fidgeting or maintaining posture.[516,517] These low-intensity physical activities can have a remarkable effect on total energy expenditure. In fact, apart from some vigorous forms of exercise, NEAT is the predominant component of activity-induced energy expenditure.[518] In sedentary individuals, NEAT accounts for only ~15% of total daily energy expenditure, but can contribute ~50% in highly active individuals.[518] These differences translate to a variation of ~2000 kcals per week between individuals of similar size due to NEAT.[519] Several studies have shown that obesity is associated with lower levels of NEAT than their non-obese counterparts, perhaps owing to the additional two hours per day engaging in sedentary behaviours.[520-522] If individuals with obesity were simply to increase NEAT expenditure to that of their counterparts, an additional 350 kcals/day could be expended, which translates to approximately 18 kilograms in one year.[517] Together, these data demonstrate the potential of NEAT as a weight loss strategy for obesity management.

Several cross-sectional and longitudinal studies have analyzed the trends of both physical activity and certain aspects of NEAT throughout the past several decades that may contribute to the parallel rise of the obesity epidemic. For instance, Church et al. (2011) demonstrated that occupational physical activity energy expenditure decreased by more than 100 kcals/day in the US between the 1960s and early 2000s. They posit that this decline has had a large impact on the corresponding increase in body weight over the same time period.[523] Furthermore, Du et al. (2019) showed that activity trends in US adults between 2007 and 2016 did not significantly improve, even when combining leisure-time, work-related, and transportation-related activity.[524] However, as demonstrated by

Canizares et al. (2018), generational differences in activity patterns are emerging in Canada, with younger cohorts more likely to report greater participation in leisure-time and transportation-related activity.[525] While this data is promising, significantly greater levels of activity are required to offset the estimations of future obesity trends.

1.3.2.2 Genetics

Genetics and heritability are among the underlying physiological mechanisms influencing energy homeostasis and thus the maintenance of body weight.[526] The risk of developing obesity may largely depend on individual genetic predisposition, but the contribution of heritability is still being elucidated. Twin, family, and adoption studies estimate that the heritability of obesity ranges from 40 to 70%.[527,528] However, more recent genomic studies suggest that genetic variants may explain less of the heritability. A large-scale Genome-Wide Association Study (GWAS) and meta-analysis of >300,000 adults determined that common single nucleotide polymorphisms (SNPs) only explained 20% of the variation in BMI (when accounting for pathways of insulin action and secretion, energy metabolism, lipid biology, and central nervous system mechanisms)[529] and whole genome sequencing reported 30% heritability.[530]

The genetic basis of obesity is classically considered to be either polygenic or monogenic.[526] Monogenic forms of obesity that stem from mutations in single genes are inherited in a Mendelian pattern and are extremely rare, early-onset, and severe.[526,531] Several candidate genes for monogenic obesity have been identified that play essential roles in appetite control, food intake, and energy homeostasis,[491] with the most common being mutations in leptin and melanocortin 4 receptor genes.[503,532] Polygenic obesity (common obesity) results from hundreds of polymorphisms that each exert small effects.[526]

As such, GWAS strategies became widely used to identify the polygenic mechanisms that confer the susceptibility to polygenic obesity. Over 1,100 loci have been discovered that are associated with BMI and obesity traits (including: body fat percentage, fat-free mass, circulating leptin, etc.),[533-537] with the most prominent signal being from fat mass and obesity-associated fat mass and obesity-associated (*FTO)* gene variants.[491,538,539] Individuals who carry a mutation in the *FTO* gene have a 20-30% increased risk of obesity,[538,540] and are predisposed to T2D through increases in BMI.[538] As discussed in Loos & Yeo (2022), recent findings posit that the line that distinguishes polygenic from monogenic obesity is being blurred due to similar underlying biology. Evidence demonstrates that neuronal pathways controlling hedonic aspects of food intake may be the major drivers of excess body weight for all obesity, irrespective of developmental origins,[526] and that the expression of mutations causing monogenic obesity may be influenced by an individual's polygenic susceptibility to obesity.[541]

Although progress has been made in the identification of genetic variants causing obesity, the search remains for the missing heritability. Evidence points to a large influence of obesogenic environmental changes in recent decades that interact with genes to cause obesity.[526] While there is evidently a significant genetic component to obesity, there is large interindividual variation in how people respond to the environment and whether these genes are activated or not.[542] For instance, epigenetic analyses of gene x environment interactions show that having a healthy diet or being more physically active reduced the effect of the *FTO* locus on the risk of obesity by 30-40%.[543,544] Even differences in how an individual responds to food marketing campaigns may impact an individual's genotype, as those who possess an *FTO* mutation are more susceptible to increasing food intake in

response to exposure to food advertisements than wild-type carriers.[545] Although the role of epigenetics on obesity is an emerging area of research, certain mechanisms have been pinpointed that may influence obesity development, including physical activity and dietary patterns, gut microbiota, and exposure to other environmental factors during fetal and early postnatal development.[546]

1.3.2.3 Obesogenic Environments

The increased prevalence of obesity over the last five decades has coincided with the rise of major environmental changes that make it easier for people to overeat and harder for them to obtain sufficient physical activity levels. It is widely agreed upon that these obesogenic environments are largely to blame for the global surge of overweight and obesity.[453,467,547] Long-term positive energy balance has become possible through the globalization of food systems that have altered the availability and composition of food products. Adverse changes include the promotion of overconsumption[548] and increased availability of inexpensive, energy-dense, nutrient-poor foods and beverages.[226,549,550] When considering activity levels, recent decades have seen trends of reduced occupational physical activity and a substitution of leisure time physical activity for sedentary behaviours.[523] As a reflection of these factors, some point to the urbanization of local environments for the rise in obesity, including increasingly modern built environments, reduced walkability of neighbourhoods, a greater reliance on transportation, greater density of fast-food chains, etc.[226] The suspected impact of urbanization of cities on the obesity epidemic has been challenged by data that demonstrate that BMI is rising at the same rate, or faster, in rural areas – especially in low- and middle-income regions.[462] In fact, this data

from NCD-RisC (2019) demonstrates that rising rural BMI may actually be the largest contributor to the rise in BMI globally.[462]

The effects of obesogenic environments are exemplified by further data investigating the lifestyles of Pima Indians residing in contrasting environmental conditions. The prevalence of obesity (as well as T2D) is significantly lower in Pima Indians of Mexico who live the traditional lifestyles of their ancestry (characterized by a healthy diet low in fat and more complex carbohydrates with increased energy expenditure) compared to Pima Indians of Arizona living in the United States who have the same genetic background.[551-553] As another example, Nigerian men and women living in the United States have a 20-25% higher BMI than people from Nigeria living in Nigeria.[554] Together, these data demonstrate that in genetically related populations who are prone to metabolic conditions, the development of obesity and T2D may primarily be a consequence of environmental circumstances.

Over the years, humans have evolved to store and retain energy as fat in adipocytes, which has conflicted with current physically inactive lifestyles and easy access to abundant nutrient-poor food.[555] As a result, great difficulty has come about in the attempts to curb the obesity epidemic at the population level. As the trends of obesity and T2D largely parallel one another, it is no surprise that many of the crucial risk factors impacting these chronic conditions cannot be changed only by individual or group efforts.[239] Transformations brought about by governments and organizations to shape environments and communities in a way to make individual choices of healthy food and regular physical activity the most accessible, affordable, and easiest ones are necessary to see sustainable population level improvements in obesity.[207,556]

1.3.3 Cardiometabolic Consequences

Overweight and obesity is associated with a cluster of risk factors that are associated with multiple cardiometabolic comorbidities and mortality. The increased risk of all-cause mortality due to obesity has been consistently reported in large-scale prospective studies and pooled analyses.[439-442] Obesity is specifically linked to increased cardiovascular disease mortality as well as cancer mortality.[557] Cardiovascular disease was the leading cause of death related to high BMI followed by T2D, accounting for 41% and 14% of all obesity-related deaths in 2015, respectively.[227] Counterintuitively, data suggests that in patients with established cardiovascular disease, obesity may be associated with improved survival.[558] Despite the adverse effects of obesity on cardiovascular health, individuals with obesity seem to have a better prognosis than their lean counterparts with the same cardiovascular disease.[559] Together, these data showcase the so-called 'obesity paradox'.

Obesity is linked with various cardiovascular risk factors that increase the likelihood of various types of cardiovascular disease, including coronary heart disease and stroke.[560-562] For those with class II and III obesity, the risk of cardiovascular disease is nearly 15-fold (odds ratio (OR): 14.5, 95% CI [10.1, 21.0] $p < 0.0001$).[438] However, it is uncertain whether this association is merely a consequence of the frequent co-existence between obesity and hypertension, hypercholesteremia, and high glucose levels (major risk factors for cardiovascular disease).[563,564] In fact, in a pooled analysis of 1.8 million participants, the presence of the combination of these three risk factors mediated 69% of the association between obesity and cardiovascular disease in both men and women.[563] Additional support for the notion that obesity may not directly cause cardiovascular

disorders is the proportion of individuals considered metabolically healthy obese (MHO) (a subset of individuals free of concomitant cardiometabolic risk despite having an elevated BMI).[565] Nevertheless, even though MHO reduces the risk of cardiovascular disease compared to metabolically unhealthy obesity (MUO), MHO individuals are still at 45% greater risk of cardiovascular disease compared to normal weight individuals.[212] This aligns with the concept that regardless of obesity status, weight gain increases the risk of cardiovascular disease. Moreover, the risk of weight gain may be driven by adiposity, especially when considering individuals with excess fat mass but without BMI-determined obesity: 'normal weight obesity'.[566] In women, normal weight obesity – which is positively associated with visceral adipose tissue – is independently associated with the risk of cardiovascular-related death.[567] According to data from the Framingham Heart Study, direct measures of visceral adipose tissue conferred a more precise estimation of the risk of cardiovascular disease compared to BMI, especially in women.[568] This accumulation of visceral adipose tissue and lipid accumulation may lead to cardiovascular disease through pro-inflammatory macrophage infiltration.[569,570]

Metabolically, obesity is also associated with an increased risk of non-alcoholic fatty liver disease (NAFLD)[571] and even some types of cancer. The prevalence of NAFLD increases with increasing BMI,[572] and this spectrum of liver abnormalities is associated with further cardiometabolic risk factors and chronic conditions such as T2D, metabolic syndrome, and cardiovascular disease.[573,574] Growing evidence demonstrates the heightened risk of certain types of cancer with excess adiposity,[575,576] with the strongest risk reported for pancreatic, esophageal, gallbladder, breast, and colorectal cancer.[576] An umbrella review of 204 systematic reviews and meta-analyses determined that for each 5

kg/m^2 increase in BMI, the risk of cancer ranged between 9 and 56%.[577] For each 0.1 increase in waist-to-hip ratio, the risk of endometrial cancer was raised by 21% (relative risk (RR): 1.21, 95% CI [1.13, 1.29]) and each 5 kg of weight gained in adulthood increased the risk of breast cancer by 11% (RR: 1.11, 95% CI [1.09, 1.13]).[577] Interestingly, the obesity paradox may also apply to the association between obesity and cancer, with some data suggesting a protective effect on treatment and survival outcomes.[578,579] However, not all data support this hypothesis.[439,580]

Given the complexity of obesity and the detrimental effects of excess adiposity systemically, it is not surprising that individuals with obesity are affected by several other comorbidities. Further consequences of obesity include non-overlapping diseases, including digestive, respiratory, neurological, musculoskeletal, and infectious disorders.[581] These components are beyond the scope of this dissertation, but the reader is directed to reviews on these topics for more in-depth information.[581-585]

1.3.4 Management of Obesity

Obesity management primarily involves lifestyle interventions, including both nutrition and physical activity, as well as pharmacotherapy and bariatric surgery.[430,586-588] Furthermore, recent observations highlight that obesity management should focus on patient-centred health outcomes for overall health and well-being.[589,590]

1.3.4.1 Lifestyle Interventions

Clinical practice guidelines in both Canada and the US recommend that all individuals with obesity be referred to intensive, multicomponent behavioural/lifestyle interventions.[586-588] Lifestyle interventions for obesity management center around tipping the scales to a chronic negative energy balance through a greater energy expenditure than

intake. Combined physical activity and dietary interventions often produce 5 to 10% weight loss (mean -2.39 kg, 95% CI [-2.86, -1.93]),[591] with maximal weight loss typically seen between 6 and 12 months.[306,591,592] This is not trivial, as reductions of this magnitude translate to meaningful improvements in cardiometabolic risk.[592]

The Look-AHEAD trial demonstrated that lifestyle interventions can reduce body weight irrespective of obesity classification (class I, II, or III).[593] Through 12 months of caloric intake between 1,200-1,800 kcal/day and a progressive increase of physical activity to 175 mins/week of MVPA, participants achieved 8.6 ± 6.9% weight loss.[406] Further data from this trial showed similar weight loss and cardiometabolic benefits across obesity classification, even in those with a BMI ≥40 kg/m^2.[593] In this cohort, 5 to 10% weight loss was associated with greater odds of achieving a 0.5% reduction in HbA_{1c} (OR: 3.52, 95% CI [2.81,4.40]), a 5-mmHg decrease in systolic blood pressure (1.56 95% CI [1.27,1.91]), a 5 mg/dL increase in HDL-cholesterol (1.69, 95% CI [1.37,2.07]), and a 40 mg/dL decrease in triglycerides (2.20, 95% CI [1.71,2.83]).[594] The Finnish Diabetes Prevention Program was another large RCT that successfully reduced weight through physical activity and dietary interventions.[308] Although the active intervention period only lasted for an average of 4 years, greater weight loss than the control group was sustained after 9 years of follow-up (p = 0.021), as was the incidence of T2D (p < 0.001).[314] Weight loss interventions consistently show their long-term protection against T2D, with the Finnish Diabetes Prevention Program reducing the risk of T2D by 38% after 9 years,[314] the US Diabetes Prevention Program by 34% after 10 years[310] and the China Da Qing Diabetes Prevention Study by 43% after 20 years.[595] The effects of weight loss on T2D outcomes can be explained by the improvement in pancreatic β-cell function and peripheral insulin

sensitivity in addition to fewer adipose-tissue disturbances (including reduced adipose tissue mass, intrahepatic triglyceride content, etc.).[596]

Although lifestyle interventions may provide long-term protection against chronic disease, weight regain is a common issue upon intervention cessation.[597,598] Continued physical activity is imperative to ward off weight regain and improve metabolic health.[599] Some suggest that performing more than the general recommended physical activity guidelines for health may be required for long-term weight loss.[600,601] For example, Jakicic et al. (2008) found that those who performed ≥275 mins/week of MVPA (in addition to caloric restriction) were the ones who were able to maintain a weight loss of ≥ 10% body weight after 24-months in a group of overweight/obese women (n=201).[602] However, data from the Examination of Mechanisms of Exercise-Induced Weight Compensation (E-MECHANIC) study demonstrate that expected weight loss may still not be achieved, even in those prescribed exercise at a dose recommended for weight loss/maintenance.[603] The authors concluded that this may be a consequence of increased energy intake and appetite that occur with exercise training, thereby stressing the importance of using physical activity as a treatment in combination with dietary and/or pharmacotherapy interventions.

The mode of exercise performed may determine the success of weight loss interventions. For instance, in the STRRIDE-AT/RT RCT, investigators sought to determine the effects of different modes of exercise in a study of 119 sedentary, overweight or obese adults (BMI 25-35 kg/m^2).[56] Compared to combined training (AT and RT together) and RT alone, they observed that AT alone was the most effective at reducing body weight (AT = Δ-1.76 kg (3.00), $p = 0.001$; RT = Δ+0.83 kg (2.32), $p < 0.05$; AT/RT = Δ-1.63 kg (3.17), $p < 0.01$) following eight months of exercise.[56] Performing MVPA not

only reduces the risk of weight gain,[604-606] but it also enhances longevity. In a large cohort of adults of various ages and ethnicities, Moore et al. (2012) found that adults who both do not reach the guidelines and are living with severe obesity (BMI ≥35 kg/m^2) have a reduced lifespan of 7.2 years (95% CI [6.5, 7.9]) in comparison to their active, normal weight counterparts.[607]

Obesity management guidelines recommend that individuals with obesity reduce caloric intake by at least 500 to 750 kcals/day to achieve weight loss goals.[592] Additionally, very low-calorie dietary interventions (<800 kcals/day) may be very impactful for rapid weight loss and T2D-related outcomes, as reported in section 2.2.3.5.1.2 above. Although caloric restriction is a foundational component of weight loss, it should not be used in isolation.[587] Without physical activity, compensatory neurological mechanisms may prevent maintained weight loss by encouraging the expression of peripheral modulators of appetite.[608] As such, dietary interventions are recommended in those with obesity in combination with other interventions (psychological, pharmacological, and bariatric surgery).[587]

1.3.4.2 Pharmacologic Interventions and Bariatric Surgery

Achieving weight loss goals in individuals with obesity may be extremely challenging, especially when attempting to reach an ideal BMI. Should further weight loss be required to meet individualized goals beyond what is attainable with lifestyle interventions, more intensive pharmacological and surgical options may be considered.[587]

1.3.4.2.1 Pharmacological Treatment

The advent of a new generation of anti-obesity medications established by the US Food & Drug Administration's approval of semaglutide in 2021 has the potential to

revolutionize the management of obesity.[609] Since then, numerous nutrient stimulated hormone-based therapies have become available and have shown an average weight loss of 15% or greater,[609-611] with a variety of new drug targets being explored.[611] Semaglutide is a GLP-1 receptor agonist that mimics the gut hormone GLP by stimulating insulin secretion and inhibiting glucagon release. Semaglutide reduces energy intake by slowing gastric emptying and enhancing satiety signaling [612]; it has been shown to reduce hunger and food cravings, improve eating control, and reduce the preference for energy-dense foods [613]. Tirzepatide, which combines both GLP-1 and GIP agonism, was also recently approved for the treatment of overweight/obesity.[610] While its mechanisms of action on weight loss are incompletely understood,[614] tirzepatide reduces energy intake to a similar extent of semaglutide and improves appetite regulation.[615] The SURMOUNT trials assess the effects of tirzepatide in adults with obesity; the original trial (SURMOUNT-1) observed that adults with overweight/obesity (but not T2D) lost 20.9% of their baseline weight after tirzepatide treatment for 72 weeks.[616] According to Wadden et al. (2023), they acknowledge that the reduction of hunger, increase of satiety, and lowered hedonic eating associated with the new generation of anti-obesity medication may tackle patients' response to the toxic food environment we currently experience.[617] Moreover, these drugs may have major benefits beyond weight loss itself. Recently, semaglutide has been approved as the first treatment to reduce cardiovascular risk in adults with overweight or obesity[618] after reports showed semaglutide reduced the risk of major cardiovascular events including cardiovascular death, myocardial infarction, or stroke by 20% (HR: 0.80, 95% CI [0.72,0.90]).[619] Furthermore, semaglutide reduces the risk of T2D by 73% (HR: 0.27,

95% CI [0.24,0.31]),[619] which isn't surprising considering that these drugs were developed initially for T2D, yet the results are remarkable.

1.3.4.2.2 Bariatric Surgery

The eligibility criteria for bariatric surgery were recently lowered to include individuals with a BMI of ≥35 kg/m^2 or 30-34.9 kg/m^2 with concurrent metabolic disease given the presence of high-quality data on the safety, efficacy, and cost-effectiveness of surgery.[430] Weight loss at 12-months following laparoscopic sleeve gastrectomy roux-en-Y gastric bypass surgery ranges from 25-30%.[620,621] proving to be an effective obesity management strategy when appropriate. In addition to weight loss, bariatric surgery improves obesity-related comorbidities, increases physical function, enhances quality of life, and reduces the risk of mortality.[622-624] Lifestyle interventions must precede the progression to bariatric surgery for those with a BMI below, but not above 35 kg/m^2.[430] However, post-operative exercise training may be beneficial on many fronts, as meta-analytic data showed additional weight and fat loss, increased cardiorespiratory fitness, and improved muscle strength in those who exercised compared to those who underwent surgery without exercise training.[625]

1.4 Myokines

1.4.1 Skeletal Muscle Physiology

1.4.1.1 Adaptations to Exercise and Molecular Mechanisms Involved

Skeletal muscle contains various cells and fibers that produce bodily force and movement.[626] Among other processes, it functions to produce energy, regulate blood flow, and produce supplies of oxygen and various substrates[627,628] In response to exercise, skeletal muscle undergoes a series of molecular, structural and functional changes, which

lead to many well-known adaptations, including: changes in contractile protein function, mitochondrial function, metabolic regulation, intracellular signalling, and transcriptional responses, etc.[627,629-634] As such, during exercise, adaptations result in enhanced carbohydrate and/or lipid oxidation and transport, mitochondrial metabolism and oxidative phosphorylation, and in the transcriptional regulators of gene expression and mitochondrial biogenesis.[627,634-637]

The molecular adaptations to exercise occur via acute and chronic exercise: acute responses represent the acute molecular changes during and for a short period of time post-exercise, while chronic responses represent the long-term metabolic adaptations. Overall, these changes are the result of exercise-induced muscle contractions that challenge metabolic homeostasis. With exercise, there is a shift in fuel utilization in the muscle that depends on the type of exercise performed.[638] Furthermore, muscle activation differs vastly between aerobic training and resistance training, as the mode of exercise influences distinct intramuscular and hormonal signals from the muscle.[628] As depicted by Egan and Zierath (2013), short bouts of exercise will acutely impact micro ribonucleic acid (mRNA) expression, protein content, and enzyme activity. While these acute molecular responses do not immediately impact performance, these repeated bouts of exercise will lead to chronic adaptations in mRNA activity, protein synthesis, and increased enzyme function. Ultimately, these training-induced adaptations will improve whole muscle metabolism and will contribute to enhanced long-term exercise performance (Figure 2).[627] The precise cellular and molecular mechanisms linking exercise to its health benefits remain poorly understood.[639,640] However, our understanding of the skeletal muscle gene expression response to exercise and their respective signalling pathways has expanded greatly in the

last decade.[641] Myokines constitute a regulatory component that may underlie some of the beneficial effects of exercise.[626,642]

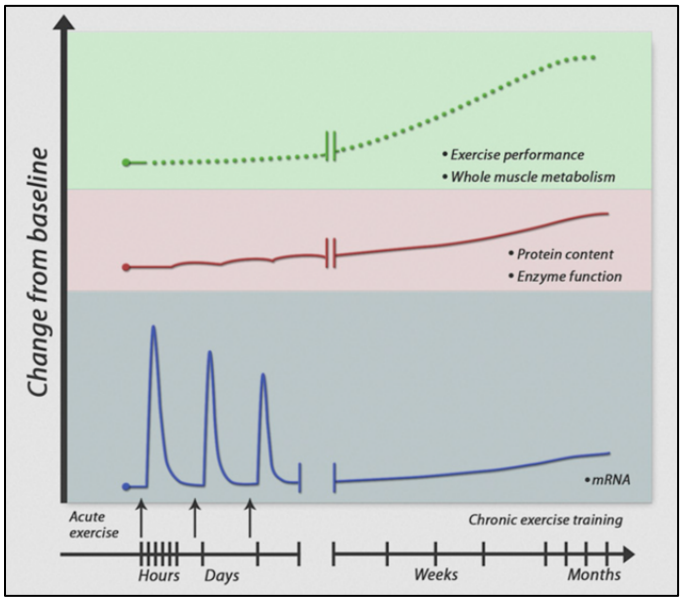


Figure 2. The molecular basis of adaptation to exercise.[627]

1.4.2 Discovery of Myokines

The Pedersen group coined the term 'myokine' to represent cytokines (or other peptides) that are produced, expressed, and released by muscle fibers and exert autocrine, paracrine and endocrine effects.[643,644] These exercise-induced factors have been proposed as mediators of the metabolic benefits of exercise through the regulation of energy homeostasis.[640] Autocrine signalling pathways allow myokines to exert their effects on skeletal muscle function itself through myocyte proliferation, differentiation, and growth to maintain skeletal muscle mass, strength, and function.[626] Myokines can also act locally in a paracrine manner on nearby cells[645] and can communicate with remote organs via endocrine signalling.[646] This crosstalk between skeletal muscle and diverse organs – such as adipose tissue, liver, pancreas, bone and brain – provides a conceptual basis to understanding how exercise lowers the risk of several chronic diseases.[642] This inter-organ

communication gives a metabolic link between skeletal muscle function and whole-body physiology as a result of exercise training.[647]

IL-6 was the first myokine that, in response to muscular contraction, was secreted into the bloodstream.[645] The acute release of IL-6 from skeletal muscle led to a 100-fold post-exercise increase.[648] Various myokines have since been identified that are secreted throughout the body and confer effects on a range of distant tissues.[640,649,650] Recent estimates point to a possibility of over 3000 proteins existing with a known secretory signal peptide,[650,651] which demonstrates the challenge of characterizing the metabolic proteome. In fact, a recent review by Severinsen et al. (2020) highlighted that more than 650 myokines exist,[642] which is likely to have grown in number, since new myokines are being discovered continuously.[652] Yet, in proportion to the amount that exist, only few of the identified myokines have been sufficiently characterised and meet the criteria put forth by Pedersen and Febbraio nearly 15 years ago.[645]

The primary exercise adaptations influenced by myokines include the regulation of skeletal muscle growth and substrate metabolism, which in turn regulate whole-body glucose and lipid metabolism.[653] Myokines are also integral to the processes of adipose tissue browning, hepatic gluconeogenesis, and lipogenesis, which highlight their potential importance in the pathophysiology and treatment of obesity and T2D.[642,654] Furthermore, myokines are also involved in the browning of WAT, a process that catabolizes stored energy in adipocytes to generate heat production through increases in mitochondria, which thereby elevates energy expenditure and leads to a negative energy balance.[655] As a result of the metabolic stress imposed by exercise, adipose tissue browning converts WAT to beige/'brite' (brown in white) adipose tissue through the upregulation of browning genes

and transcription factors.[656] The mechanisms of browning are incompletely understood; however, recent work points to the possible involvement of myokines in the process, including irisin.

1.4.3 Irisin

Skeletal muscle PPARγ coactivator 1α (PGC-1α) is a transcriptional co-activator that plays a crucial role in energy metabolism with exercise.[657] In fact, it is widely regarded as the 'master' regulator of skeletal muscle mitochondrial biogenesis.[658] Its activation depends on signalling from various kinases (AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK)) and the tumour suppressor p53,[659] which together contribute primarily to skeletal muscle adaptations to AT.[647] Intriguingly, transgenic mice overexpressing PGC-1α are resistant to age-related obesity and T2D.[660] As the precise effects of skeletal muscle PGC-1α on systemic metabolism are not yet fully elucidated, Boström et al. (2012) undertook a search for the specific muscle-secreted factor regulated by PGC-1α that induces metabolic effects.[19] Their investigation revealed a novel myokine, irisin, that is mediated by PGC-1α muscle activation and induces the browning of WAT with exercise. Due to its crosstalk between muscle and external tissues, it was named irisin after the ancient Greek messenger goddess, Iris.[19]

Bostrom et al.'s (2012) initial discovery highlighted that irisin has powerful effects on adipose tissue browning both *in vitro* and *in vivo*. Cell culture of primary subcutaneous white adipocytes and irisin administration in C57BL/6 mice (mice highly prone to diet-induced obesity and diabetes) induced thermogenesis through the upregulation of browning-associated genes (primarily uncoupling protein 1 (UCP1)),[19] which is possibly mediated by p38 MAPK and extracellular signal-regulated kinase (ERK) signaling

pathways.[661] Even with moderately elevated circulating irisin (~3-fold), Boström et al. (2012) showed that increased thermogenesis led to increased energy expenditure, reduced body weight, and improved insulin resistance.[19] Through exercise, they demonstrated that both mice and humans raise irisin to a similar extent as seen in their *in vivo* and *in vitro* experiments. After three weeks of free wheel training in mice and 10 weeks of aerobic training in humans (20-35 mins of cycling 4-5X/week, ~65% VO_{2max}), circulating plasma irisin was increased by 65% and 200%, respectively. Together, these data suggest that irisin may be responsible for some of the beneficial effects of exercise on WAT browning and increased energy expenditure.

1.4.3.1 Irisin Synthesis and Release

The synthesis and release of irisin from skeletal muscle is regulated by an overexpression of PGC-1α, which stimulates the precursor of irisin: fibronectin type III domain-containing protein 5 (FNDC5). FNDC5 is a 209 amino acid residue type-I transmembrane protein in mice (212 in humans) consisting of a signal peptide N-terminal, a fibronectin III domain, a linker domain, a hydrophobic transmembrane domain, and a C-terminus cytoplasmic domain,[19,662] as seen in Figure 3. After removal of the N-terminal signal peptide, FNDC5 undergoes glycosylation and is proteolytically cleaved to release irisin into the circulation.[662,663] The exact protease that hydrolyzes FNDC5 to release irisin is currently unknown.[662] Yu et al. (2019) propose that the protease is potentially a member of a disintegrin and metalloproteinase (ADAM) family, such as ADAM-10, and Nie et al. (2020) suggest that there may be various cleavage sites.[662,664]

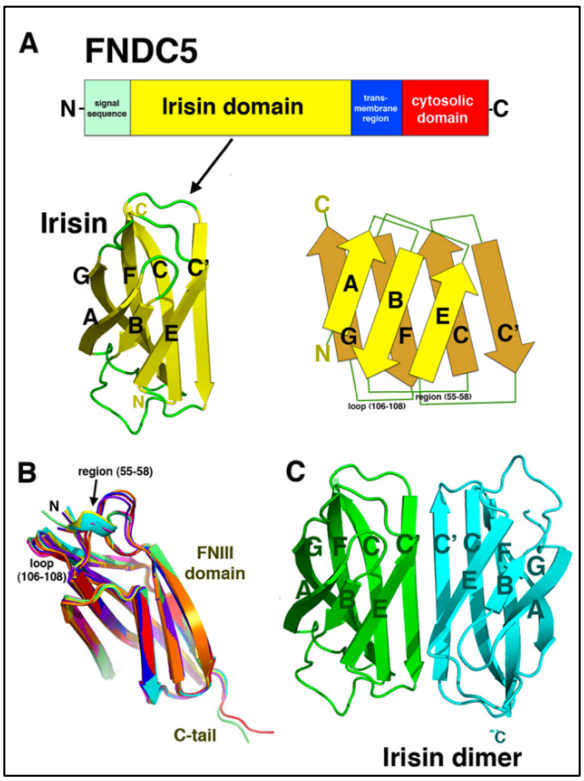


Figure 3. Crystal structure of irisin.[663]

Although the 112 amino acid protein sequence of irisin is identical between humans and rodents (100% conserved),[19] the human *FNDC5* gene has a mutant start codon: ATA rather than the canonical ATG.[665] As constructs with an ATA codon express FNDC5 protein levels that are only 1% of those with ATG codon, some suggest that human *FNDC5* mRNA may have substantially lost the ability to be translated into full-length FNDC5 protein.[665] Nevertheless, several groups have confirmed the existence of circulating irisin in humans by mass spectrometry,[666-668] because full-length FNDC5 could only be detected by a transcript with an ATA start codon being translated into circulation.[669] Jedrychowski et al. (2015) posit that the non-canonical ATA codon may be translated at a higher level *in vivo* in their native environment compared to the *in vitro* conditions reported by Raschke et al. (2013), but this theory is not supported by all.[665,666,669] The reasons why irisin can be detected by mass spectrometry in its native state are not yet fully understood.[669]

Debate continues regarding the expected molecular weight of irisin in biological samples. The originally reported 22 kDa molecular weight of irisin[19] was detected with an antibody raised against the C-terminus of the FNDC5 protein, which is not part of the cleaved irisin peptide.[670] Further studies detected bands from western blots in the range of 20-26 kDa using other antibodies, but they were still generated against this incorrect portion of the peptide.[671-674] Some argue that these bands likely did not detect irisin but probably non-specific cross-reactive proteins.[675] Nevertheless, using antibodies raised against the proper excised irisin, several studies detected bands at the theoretical molecular weight of non-glycosylated irisin (~12 kDa)[666,675,676] and glycosylated irisin (20-25 kDa)[672,675] in human and animal models.

The crystal structure of irisin, as reported by Schumacher et al. (2013) and shown above in Figure 3, consists of the typical structure of an FNIII domain that includes a β-sandwich with 3 β-strands on one side and 4 β-strands on the other.[663] However, in contrast to other FNIII domains, they showed that a tight dimer is formed through the connection of the 4 β-strands of two subunits. Together, these two monomers combine to create the continuous, antiparallel 8-stranded β-sheet across the two domains. This novel inter-subunit β-sheet dimer structure may impact the receptor activation and signalling to different targets from the skeletal muscle cells. Two regions of irisin (residues 55-58 and 106-108) have considerable conformational fluctuations, which suggest that these areas may allow irisin to interact with other proteins.[663] It was not until 2018 that researchers determined that irisin exerts its biological functions via integrin receptors.[677] Integrin receptors are widely distributed throughout the body and, therefore, allow irisin to act on various tissues. Kim et al. (2018) showed that irisin binds to the αV class of integrins, and

further biophysical studies revealed a high binding affinity of the αVβ5 integrin for irisin.[677]

Although irisin is mainly secreted by muscle (both skeletal and cardiac),[678] it belongs to the class of adipomyokines, as it is expressed in both adipose and muscle tissue.[672] Immunohistochemical studies in rats demonstrate that irisin is synthesized in the perimysium, endomysium, and nucleus portions of the skeletal muscle and produced abundantly in the myocardium and connective tissue of cardiac muscle.[678] As an adipokine, irisin is highly synthesized by subcutaneous adipose tissue in rats and, in lower amounts, by visceral adipose tissue.[672] In WAT, FNDC5 expression is 100-200 times lower than in human skeletal muscle, suggesting that WAT contributes minimally to circulating irisin.[679,680] In rats, approximately 70% of irisin is synthesized by muscles[19] with the remaining 30% from adipose tissue.[672] Small amounts of irisin have also been detected in salivary, sebaceous, and sweat glands[681], as well as in the liver, kidney and brain[667,678] and in lesser amounts elsewhere.[680]

1.4.3.2 Clinical Implications of Irisin

Given the biological impact of irisin on various tissues, it is logical to assume that irisin has immense therapeutic potential in treating metabolic disorders like obesity and T2D. Irisin induces mitochondrial biogenesis, regulates oxidative metabolism, and attenuates inflammation in numerous cell types via autocrine, paracrine, and endocrine pathways.[19] It directly acts on adipose tissue through the upregulation of mitochondrial UCP1, which prompts energy-storing WAT to behave more like energy-burning brown adipose tissue.[661] Consequently, increased thermogenesis leads to increased oxygen consumption in adipocytes and reduced body weight.[19] In addition to WAT browning,

irisin also reduces the volume, size, and mass of adipocytes and activates lipolysis.[682-685] Irisin also promotes glucose disposal in adipocytes by inducing GLUT4 expression and stimulating glycolysis.[682]

Along with adipose tissue, skeletal muscle is one of irisin's main glucose regulation targets.[686,687] Several animal and human experiments have shown that irisin-treated skeletal myocytes increase glucose uptake in an autocrine manner by upregulating GLUT4 expression and translocation, likely through the phosphorylation of AMPK and subsequent activation of p38 MAPK,[688-690] even to a similar extent of insulin.[691] This is not trivial, as GLUT4 is the main glucose transporter mediating the uptake of glucose into skeletal muscle.[692] In fact, Huh et al. (2014) showed that irisin led to the uptake of glucose and fatty acids at a similar rate to insulin exposure in primary human skeletal muscle cells.[688] Yano et al. (2020) demonstrated that irisin treatment in murine C2C12 myoblast cell lines preserved insulin activity under palmitic acid-induced insulin resistant conditions.[693] Lee et al. (2015) reported increased glucose uptake following recombinant irisin administration in L6 rat muscle cells after AMPK activation.[691] Further, in human myocytes of individuals with obesity, irisin treatment upregulated glycolysis-related enzymes and downregulated enzymes associated with glycogenolysis and gluconeogenesis, thereby improving glucose homeostasis.[688] These changes may be triggered by declines in intracellular and intra-mitochondrial ATP, which lead to increased AMPK, MAPK, ERK 1/2 and p38 signaling.[693]

Irisin targets hepatic glucose and lipid metabolism by enhancing pancreatic β-cell function, demonstrating irisin's actions as an insulin-sensitizing adipo-myokine.[694] Hepatic-released irisin can also function as a paracrine/autocrine factor, as irisin treatment

in insulin resistant hepatocytes of mice with T2D decreased gluconeogenesis and increased glycogenesis.[689,695] In obese mice fed a high fat diet, irisin reduced plasma and hepatic cholesterol[696] and in a mouse model of non-alcoholic fatty liver disease (NAFLD), irisin inhibited inflammatory pathways in the progression of the disease.[697] Others made similar positive observations in lipid metabolism via exercise-induced irisin *in vitro*.[698] Furthermore, irisin may have a potential effect on the crosstalk between skeletal muscle and pancreas.[699] Natalicchio et al. (2017) demonstrated that irisin increased β-cell proliferation, thereby increasing insulin secretion in both human and animal models.[700] Using cell cultures, they also observed that irisin reduced β-cell apoptosis under lipotoxic conditions.

The anti-inflammatory properties of irisin also help to elucidate its therapeutic potential in metabolic diseases, as obesity and T2D have been associated with an increased expression of genes involved in inflammatory pathways.[701] For instance, the presence of TNF-α in obese adipose tissue disrupts insulin signaling and glucose homeostasis,[702] and reactive oxidative/nitrative species (ROS/RNS) activate further pro-inflammatory pathways that lead to microvascular dysfunction in T2D.[703] Irisin treatment in human adipose tissue and cell lines suppressed the expression of pro-inflammatory cytokines in a concentration-dependent manner, including TNF-α and IL-6, and enhanced the synthesis of anti-inflammatory adiponectin and IL-10.[704,705] Irisin can also stimulate the phenotypic switching of macrophage infiltration in WAT from a pro- to an anti-inflammatory state[706] and can reduce the production of ROS/RNS and increase antioxidant enzymes.[707,708] Additionally, the cell apoptosis and reduced β-cell function that results from prolonged

elevated glucose exposure is attenuated by irisin treatment in pancreatic β-cells and vascular endothelial cells.[694,709]

Together, the data presented above give insight into the role that irisin plays in many of the metabolic pathways affecting obesity and T2D. As such, a large focus of the literature has been on determining how irisin concentrations are altered in these chronic conditions. Circulating irisin concentrations are associated with several obesity indicators; however, the direction of the association is inconclusive. Although a meta-analysis concluded that individuals with obesity have higher circulating irisin, subgroup analyses revealed that irisin was higher only in those of African descent, but not from European, Asian, or American backgrounds,[710] therefore misrepresenting a large portion of the population. Others have also reported positive associations between irisin and measures of adiposity, including BMI.[680,711,712] It has been theorized that elevated irisin concentrations in obesity may correspond to an adaptive compensatory response to obesity-induced glucose metabolism dysfunction that serves to maximize its anti-hyperglycemic effects.[680,699,713,714] This 'irisin resistance' may function similarly to the well-documented leptin resistance in obesity and the metabolic syndrome.[715] On the contrary, data in both human and animal models report that obesity is linked to reduced irisin concentrations. Both Makiel et al. (2023) and Ebert et al. (2016) observed negative associations between circulating irisin concentrations and body fat in humans,[716,717] and Khalafi et al. (2020) showed that plasma irisin concentrations reduce with increasing BMI in rats.[718] Moreover, Yosaee et al. (2020) showed that individuals with MUO had lower irisin than MHO and non-obese healthy participants.[719] Irisin concentrations are also negatively correlated with

human visceral adipose tissue,[720,721] and higher levels of irisin may reduce the risk of having a higher BMI (OR = 0.281, 95% CI [0.09, 0.85], $p = 0.024$).[722]

Zhang et al. (2020) postulate that as individuals with obesity progress to T2D, irisin secretion may begin to decline.[713] Indeed, the majority of findings report that irisin concentrations are reduced in T2D, regardless of the time of diagnosis, whether they are taking anti-diabetic medication, age, sex, or obesity status.[713,723-728] Greater irisin concentrations may be protective against the development of T2D[728,729]; conversely, elevated irisin may predict T2D onset.[730] Whether irisin is positively or negatively associated with markers of impaired glucose homeostasis remains under debate. Studies of varying populations show that irisin is positively associated with markers of insulin resistance and impaired glucose[713,731]; however, when studying T2D participants on their own (who have low baseline irisin), irisin tends to be negatively associated with fasting glucose, fasting insulin, HbA_{1c} and HOMA-IR.[732,733] There is also uncertainty in whether irisin is higher[734-737] or lower[738-740] in those with prediabetes. Irisin appears to play a role in ameliorating several of the cardiometabolic consequences of T2D. In animal studies, irisin lowers blood pressure and lipids, and reduces atherosclerosis – the main pathological mechanism of macrovascular disease in T2D.[741-744] Furthermore, in individuals with T2D, irisin concentrations appear to be lower in those with coronary artery disease or atherosclerosis than those without these complications[727,745] and is lower in those with nephropathy compared to those with normal kidney function.[746] Moreover, a meta-analysis by Hou et al. (2023) demonstrated that individuals with T2D and at least one T2D-related chronic complication had significantly lower circulating irisin concentrations compared not only to the healthy control group (SMD: -2.40, 95% CI [-3.02, -1.77]), but also to the

individuals with T2D without these conditions (SMD: -1.43, 95% CI [-1.76, -1.09]) (both $p < 0.00001$).[26] As such, circulating irisin could potentially be used as a diagnostic marker of macrovascular and microvascular disease progression in those with T2D.

Although the endocrine nature of irisin has largely dominated the enthusiasm for this adipomyokine, irisin's autocrine signaling pathways further convey its therapeutic relevance. Studies suggest that irisin may affect muscle hypertrophy, as it has been shown to upregulate IGF-1 and IL-6 and downregulate myostatin.[682,747] In a mouse model, Reza et al. (2017) reported that irisin injection promoted muscle regeneration and hypertrophy in response to muscle injury, which may occur through the activation of satellite cells and enhanced protein synthesis.[747] They also showed that treatment of C2C12 myotubes with irisin activated the phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/Akt/mTOR) pathway. Huh et al. (2014) demonstrated that irisin treatment in human skeletal muscle cells induced PGC-1α4 (PGC-1α isoform induced by RT that regulates skeletal muscle hypertrophy) via the ERK pathway.[682] The activation of these pathways of muscle hypertrophy implies that irisin may have beneficial roles in sarcopenia and other conditions affected by muscle atrophy.[748]

Finally, irisin is linked to other organs and tissues that may extend its therapeutic potential. Several lines of evidence point to the therapeutic potential of irisin in cancer, such that irisin treatment may inhibit cancer cell proliferation, reduce metastasis, and suppress tumor growth.[749,750] In accordance with this data, lower irisin concentrations are generally observed in cancer patients.[751] Irisin may also be beneficial for those with osteoporosis and other bone diseases, as it is positively associated with bone mineral density[752] and irisin administration enhances bone formation[753] and prevents osteocyte

apoptosis.[677] Finally, the role of irisin in brain function may be substantial, as irisin treatment has been shown to rescue the cognitive decline in mouse models of Alzheimer's disease.[754] Others have also demonstrated irisin's neuroprotective potential both *in vitro* and *in vivo*.[755,756]

Taken together, irisin expression may help elucidate the complex link between skeletal muscle physiology and health in chronic diseases. The data presented above provide evidence of irisin's involvement in the pathogenesis of various metabolic diseases, especially in obesity and T2D. Although much of these data are cross-sectional and more quality studies are required in the form of RCTs and longitudinal designs, these findings suggest that irisin might have tremendous therapeutic potential.

1.4.3.3 Irisin Exercise Studies

Following the initial discovery of irisin and the recognition of its potential therapeutic effect, scientists began exploring its relationship with various forms of exercise. The enthusiasm surrounding this adipo-myokine momentarily waned due to concerns regarding its detectability.[665,675] However, claims of its existence in humans were substantiated with direct evidence for irisin in the circulation[666,671] and in human cerebrospinal fluid[667,757] using tandem mass spectrometry, making it increasingly difficult to dispute. Nevertheless, the literature continues to reveal inconsistent findings on the effects of exercise on irisin release. These discrepancies may be attributed to the limited use of mass spectrometry in the existing literature on irisin, owing to the fact that it requires specialized technical expertise and substantial resources, thereby complicating the accurate detection and quantification of irisin (see section 2.4 above), may reflect the wide range of exercise parameters utilized in irisin exercise studies to date (including intensity, duration,

frequency, and mode), and may also arise from variability in participant demographics, including metabolic health status, which may influence irisin responses to exercise. This underscores the need for a deeper understanding of how these factors may influence the acute and chronic irisin response to exercise.

1.4.3.3.1 Acute Exercise Studies

It is suggested that the cumulative effect of the repeated transient increases in gene expression in skeletal muscle after each successive acute bout of exercise is what leads to the molecular adaptations over time that produce some of the positive effects of exercise.[627,628] While the acute bouts may drive the adaptations to exercise (as seen in Figure 2 above), training-induced changes in protein content generally result from the additive effect of repeated exercise bouts due to repeated, intermittent increases in mRNA expression.[627] Consistent with this physiological perspective, repeated bouts of acute exercise are expected to trigger recurring transient elevations in FNDC5 mRNA expression, leading to training-induced changes in irisin protein content. Acute bouts of exercise may trigger changes in protein levels, provided that the stimulus is sufficiently strong, whether due to high exercise intensities or prolonged durations, etc., as seen with other skeletal muscle adaptations.[628,758] Indeed, it is generally agreed that individual acute bouts of exercise lead to increases in circulating irisin levels. Several meta-analyses have shown this effect across a variety of aerobic- and resistance-based acute exercise bouts performed at moderate-to-high intensities, in both healthy adults[20-24] and clinical populations.[24] Our group showed in a meta-analysis that acute exercise, together with AT, RT, and combined AT+RT, results in an acute increase of circulating irisin of 15.0% (95% CI [10.8,19.3]).[24] However, a moderate to high degree of heterogeneity exists in these

analyses, which likely results from the substantial variation in the protocols used and populations studied.

Extensive research has been conducted to investigate the acute effect of exercise on circulating irisin in humans as well as in animal models. Together, with the pooled data described above,[20-24] the evidence supports a significant rise in irisin concentrations in response to acute aerobic, anaerobic, and resistance exercises of varying intensities, bout durations, and types/modes (including continuous and interval-based cycling, walking/running, and swimming exercise). A predominant trend in the literature suggests that exercise intensity acts as a key factor influencing acute irisin responses.[25,27,28,759,760] In animal models, acute increases in irisin are generally only observed with higher-intensity exercises[676,761,762] and not with moderate intensities.[763] Considering compelling evidence that indicates higher-intensity exercise can generate a greater acute physiological response in general than moderate-intensity exercise within a shorter time frame and reduced exercise volume,[627] acute HIIT may be more effective than MICT in increasing irisin concentrations. Due to the increase in exercise intensity, HIIT may amplify the observed metabolic changes in substrate utilization and mobilization, mitochondrial biogenesis, and insulin sensitivity compared to MICT[764,765] – all of which contribute to the physiology of irisin. Additionally, while PGC-1α (upstream regulator of irisin) may be expressed to a similar extent in acute HIIT as in acute MICT,[766-768] exercise intensity is the main determinant of PGC-1α activation in both human and animal skeletal muscle,[768,769] implying that HIIT could have a stronger influence on irisin release.

Several lines of evidence suggest that exercise intensity may indeed play a pivotal role in regulating irisin release. In fact, some data point to a potential dose-response effect

of irisin concentrations with increasing exercise intensities.[759,760] Randomized crossover studies of healthy adults[770,771] and youth,[772] as well as non-randomized trials of adults[688,773] and youth,[774] demonstrate acute changes in circulating irisin in response to higher intensities of aerobic exercise, with no significant changes induced by moderate intensities. However, some have also observed either no change[775] or a decrease in irisin[776,777] after acute HIIT in healthy adults. Interestingly, in a randomized crossover trial comparing AT vs RT in overweight-obese individuals with and without metabolic syndrome by Huh et al. (2015) (including: 1) an acute bout of HIIT, 2) continuous high-intensity AT, and 3) moderate-to-high intensity RT), the largest increase in irisin was actually produced by RT, highlighting irisin's ability to be induced not just by AT but also by RT.[778] Nygaard et al. (2015) demonstrated that increases in irisin were comparable between the aerobic HIIT and high-intensity RT groups, suggesting that both exercise modes elicit similar irisin responses.[779] Several trials of varying exercise intensities also show a decrease or no effect of RT (either traditional or circuit training) on acute irisin responses.[780-782] However, exercise intensity appears to especially influence acute changes in circulating irisin in AT. Furthermore, the AT mode – whether interval-based as HIIT or continuous high-intensity AT – may further impact the observed acute response. In a meta-analysis of 16 studies in healthy adults (n = 412), Cosio et al. (2023) demonstrated that HIIT did not influence acute irisin responses (d = 0.16, 95% CI [0.12, 0.44], p = 0.20), whereas continuous AT (including mostly high-intensity trials) showed a moderate significant effect of increasing irisin (d = 0.33, 95% CI [0.20, 0.46], $p < 0.001$). In this meta-analysis, they also showed that cycling exercise led to more substantial changes when compared to running exercise.[23]

Several mechanisms could be responsible for this response, including a greater depletion of glycogen due to the localized muscle activation observed with cycling.[688]

Understanding the acute effects of irisin is essential to elucidate its molecular mechanisms and clarify its role in the regulation of metabolic responses and its impacts on energy metabolism. This involves the examination of the kinetic changes of irisin during an acute bout of exercise as well as post-exercise changes during the recovery period. Surprisingly, very little information exists on the dynamic shifts in irisin concentrations during an acute bout of exercise. To the best of our knowledge, our group[772,783,784] is among the few to investigate the kinetic changes of irisin from this perspective.[785,786] However, there has been considerable interest in determining the timing of peak irisin release after an acute exercise bout. While a meta-analysis has shown that circulating irisin rises immediately post-exercise (within 0 to 10 minutes of exercise completion),[23] another meta-analysis points to a delayed effect of irisin, with the timing of peak circulating irisin occurring in the post-exercise recovery period 1-hour post-acute exercise.[22] Additional evidence supporting a potential delay in peak irisin circulation comes from data on upstream regulators of irisin, indicating that both PGC-1α[766] and FNDC5[787] skeletal muscle mRNA expression are not significantly elevated until after 3 hours of rest following an acute bout of HIIT. However, the dynamic changes of PGC-1α/FNDC5 are unknown after the immediate post-exercise period in these studies up until 3 hours post, highlighting that the peak may have been during this period. The delayed effect of peak irisin has led some to hypothesize that irisin release may involve molecular pathways that extend beyond the FNDC5-PGC-1α pathway.[23,788] Bao et al. (2022) hypothesize that FNDC5 expression is not directly proportional to irisin, such that the upregulation of skeletal muscle FNDC5

mRNA expression most likely occurs during the recovery period, while circulating irisin increases during exercise but returns to baseline levels post-exercise,[736,786,788] as can be seen in Figure 4 below. Evidently, the timing of irisin release in response to acute exercise is complex, and further research should include both measures of circulating irisin and mRNA tissue expression (muscle, adipose tissue, liver, etc.) to fully comprehend the mechanisms and regulatory pathways of irisin release both during and post-exercise.

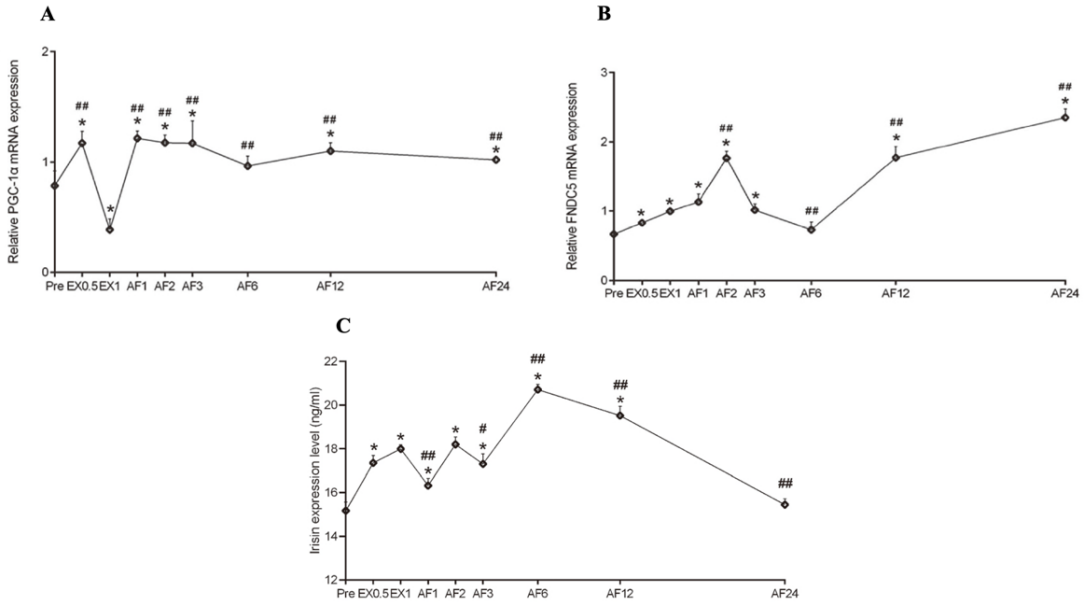


Figure 4. Acute changes in PGC-1a , FNDC5 and irisin in response to moderate-intensity aerobic exercise.

Changes in skeletal muscle PGC-1α (A) and FNDC5 (B) mRNA expression as well as circulating irisin (C) before (pre), during exercise for 30 min (EX0.5) and 1 h (EX1), and post (1, 2, 3, 6, 12 and 24 hours after) an acute bout of moderate-intensity continuous aerobic exercise in healthy, young, male mice.[786]

It is imperative to note that the above-mentioned studies have several limitations that must be accounted for when discussing acute changes in circulating irisin. First, the studies varied in their populations studied in terms of age, sex, cardiorespiratory fitness,

and metabolic status, all of which may have an influence on irisin concentrations and its exercise-induced acute response. While inconsistent findings may simply result from differences in exercise parameters, including intensity and duration, or may be attributed to the varying baseline characteristics, the substantial impact that individual variation has on the results must not be overlooked.[777,782,789-795] Furthermore, attention must also be given to the differences between irisin quantification methods, given the substantial inter- and intra-assay variation in enzyme-linked immunosorbent assay (ELISA) kits used to measure irisin.[668] Nevertheless, while individual studies demonstrate variable results, the meta-analyses conducted thus far highlight the strong potential of acute exercise – especially at higher intensities – to stimulate irisin.[20-24] Moreover, the ability of irisin's upstream regulators to increase following acute exercise is clearly established – especially when considering a meta-analysis of a large transcriptomic profiling analysis (n > 1,100) of skeletal muscle exercise adaptations. Pillon et al. (2020) determined that PGC-1α is increased by 2.3- and 1.8-fold after acute aerobic and resistance exercise, respectively. However, discrepancies remain regarding the direct influence of PGC-1α on irisin, raising further questions on the acute effect of exercise on irisin responses.

1.4.3.3.2 Chronic Exercise Studies

The effects of chronic exercise on irisin responses are less well-established compared to the increases observed following acute exercise bouts.[20-24] While differences in exercise parameters likely contribute to this variability, an alternative hypothesis suggests that differences in response may stem from variations in metabolic profiles. Some data indicate that individuals who are metabolically unhealthy (MU) may respond differently to exercise compared to healthy individuals,[25,26] potentially due to the presence

of altered baseline circulating irisin levels in these clinical populations.[710,713,716,724] Although numerous meta-analyses have demonstrated that irisin increases following chronic exercise interventions across diverse populations and exercise protocols,[21,25,28,796,797] further investigations of the studies reporting non-significant changes involved participants that could be considered MU. Notably, the chronic exercise interventions that failed to increase irisin concentrations in these populations involved exercise parameters that appeared to be of a sufficient stimulus to induce changes in irisin.[759] The vast majority of studies reporting non-significant chronic changes in irisin following high-intensity AT and/or RT were conducted in individuals with obesity[721,790,792,798-802] and/or T2D,[803-806] with a similar pattern observed in moderate-intensity exercise interventions in individuals with obesity[712,736,807-810] and/or T2D.[811-813] Findings from animal models further support these findings, as obese mice and rats subjected to chronic moderate- or high-intensity AT exhibited no significant changes in irisin levels.[814-816] Importantly, the chronic interventions that did report increased irisin levels in MU individuals often required greater exercise intensities to observe this response. Conversely, in studies where irisin concentrations decreased following chronic exercise interventions, the majority of participants were considered healthy.[780,817,818] Altogether, these data suggest that irisin responses to chronic exercise are influenced by a complex interplay between varying exercise parameters and baseline metabolic health.

1.4.3.3.3 Impact of Exercise Intensity

As outlined in previous sections, repeated bouts of acute exercise stimulate gene expression and lead to a gradual accumulation of proteins, culminating in chronic adaptations that affect whole-muscle metabolism.[627] Exercise intensity has been shown to

play a large role in driving these metabolic responses. For example, a recent large meta-analysis (N = ~6,000) by Mølmen et al. (2024) showed that high-intensity exercise elicited a two- to four-fold greater increase in mitochondrial biogenesis and cardiorespiratory fitness compared to low-intensity exercise.[819] Exercise intensity has also been identified as a primary determinant of PGC-1α expression in both human and animal skeletal muscle.[768,769] Given that PGC-1α is the master regulator of mitochondrial biogenesis,[820] its upregulation in response to higher-intensity exercise may, in turn, drive the observed increases in irisin levels in such interventions.

These findings further strengthen the connection between exercise intensity and changes in irisin concentrations from both acute and chronic perspectives. Various meta-analyses support this link, indicating that higher-intensity exercise leads to greater circulating concentrations of irisin in chronic interventions involving AT and/or RT.[28,821] For instance, Torabi et al. (2024) demonstrated that HIIT induced more pronounced changes in irisin compared to high-intensity continuous AT, RT, or combined AT+RT in individuals with overweight or obesity.[28] Similarly, Cosio et al. (2021) highlighted in subgroup analyses of chronic RT interventions that irisin levels were elevated in higher-intensity trials.[821]

Elevated exercise intensities may be particularly beneficial for MU individuals. Studies comparing different exercise intensities in animal models and humans with obesity and/or T2D indicate that high-intensity exercise elicits chronic changes in circulating irisin, while MICT does not result in such adaptations.[772,807,811,816,822] Moreover, data in both humans[27] and animal models[823] of T2D have shown that while chronic MICT can elevate irisin concentrations, chronic HIIT may produce superior responses. Other studies, such as

that of Dünnwald et al. (2019), have shown that chronic MICT does not induce significant changes. In their study of adults with T2D and overweight or obesity, they found that four weeks of HIIT provided a sufficient stimulus to significantly increase circulating irisin levels, whereas four weeks of MICT did not.[822] These findings support the notion that chronic HIIT may lead to the most substantial effects on glycemia, BMI, and cardiorespiratory fitness in individuals with T2D.[350,353]

1.4.3.3.4 Impact of Exercise Duration

The duration of exercise interventions is a critical factor in understanding the discrepancies in the literature regarding the changes in irisin following chronic exercise interventions. Studies that differ in intervention length may contribute to variations in irisin responses in both healthy[824,825] and MU individuals.[826,827] A meta-analysis by Cosio et al. (2021) reported that, in healthy adults, RT interventions shorter than 12 weeks increased irisin concentrations, whereas those lasting longer than 12 weeks led to a decrease. They further identified that higher-intensity RT stimulated greater changes in irisin concentrations.[821] Conversely, a meta-analysis by Motahari-Rad et al. (2021) reported that RT interventions lasting less than 8 weeks had no significant effect on circulating irisin across individuals with varying BMIs, but those lasting 8 weeks or longer resulted in significant increases in irisin.[825]

When considering the influence of metabolic health, it is possible that longer exercise interventions may be necessary to induce significant increases in circulating irisin in MU individuals. For instance, 12 weeks of combined moderate-intensity AT+RT increased circulating irisin in females with metabolic syndrome, but just 8 weeks failed to produce a significant effect.[826,827] Similarly, 12 weeks of MICT increased irisin in males

with metabolic syndrome, while there was no effect observed when combining MICT with moderate-intensity RT.[716] In the majority of the chronic exercise trials that failed to demonstrate increased irisin levels in individuals with T2D, the interventions primarily consisted of moderate-intensity AT and/or RT exercise lasting between 8 and 16 weeks.[806,811-813] Therefore, the absence of chronic increases in irisin levels may be attributed to insufficient exercise durations, intensities, or a combination of both.

A comprehensive understanding of both the acute and chronic effects of exercise, as well as the influence of varying exercise parameters on irisin levels, is vital for developing personalized exercise prescriptions that optimize irisin responses. This is particularly important for enhancing the therapeutic potential of irisin in MU individuals, especially those with obesity and/or T2D.

1.5 Exercise Response Heterogeneity

Overwhelming evidence underscores the extensive health benefits of standardized exercise training.[7,828] Scientific and regulatory bodies worldwide emphasize exercise as a critical component of overall health and provide guidance at the population level.[7,8,829] However, it has become increasingly evident that not everyone benefits from exercise to the same extent as others, even with identical exercise training programs.[11,12] This recognition has contributed to the growing movement toward personalized medicine [32] in an effort to optimize outcomes for each individual.[31]

Following the seminal work of the renowned HEalth, RIsk factors, exercise Training And GEnetics (HERITAGE) Family Study,[13] a large body of research emerged investigating the individual response to exercise. This research initially focused on responses related to cardiorespiratory fitness before expanding to include cardiometabolic

outcomes.[14,830] Building on these findings, researchers began categorizing individuals as 'responders', 'non-responders' or 'adverse responders', based on the magnitude and direction of changes observed in the outcomes of interest after exercise.[831 832]

1.5.1 Factors Influencing Interindividual Response Variation

The mechanisms responsible for the interindividual response to exercise are not fully elucidated. However, the observed heterogeneity may result from various factors that impact an individual's response to exercise, including intrinsic (age, sex, genetics, race/ethnicity, hormonal status, etc.) and extrinsic factors (circadian rhythm, sleep, diet, health status/history, medication use, etc.), that contribute either independently or cumulatively.[16] With respect to cardiorespiratory fitness, genetics appear to contribute the most to variation, explaining ~50% to the interindividual response.[13] Bouchard et al. (1999) observed that age, sex, and baseline fitness together account for less than 10% of the variation. Additionally, the parameters of the exercise program will greatly affect the interindividual variability observed in outcomes of interest, including the intensity, duration, volume, and frequency of exercise, as well as the compliance and adherence to the trial.[16] Together, these findings depict the intricate nature of interindividual variation in response to exercise and the complexity of developing personalized exercise prescriptions.

Emerging research highlights the substantial interindividual heterogeneity in exercise responses to cardiometabolic outcomes. Data from our group demonstrates wide variation in insulin sensitivity responses following a 6-week RT intervention in youth living with obesity, with decreases as low as -19.8% to increases of 85.7%.[784] Stephens et al. (2018) showed that 10 weeks of aerobic training failed to improve insulin sensitivity

and even worsened HbA_{1c} in 35% of participants, despite significant improvements in cardiorespiratory fitness.[833] Moreover, in an analysis of >1,600 adults, 7% of participants experienced adverse responses to exercise in ≥2 of the analyzed risk factors (insulin, triglycerides, HDL-cholesterol, blood pressure).[831] In a sub-group analysis of the HART-D trial in adults living with T2D, it was reported that ~65% of the variation of the change in HbA_{1c} was explained by various biological factors, including a shorter duration of T2D, reduced free-fatty acid concentrations, being male and non-Caucasian, along with increased skeletal muscle PGC-1α protein expression.[834] Other groups have also demonstrated substantial variation in markers of skeletal muscle metabolism.[835-837] For instance, a study assessing muscle strength showed that after 12 weeks of RT, the group mean improvement in one-repetition maximum was 54%, but individual changes varied widely, ranging from 0 to 250%.[838] This variability further emphasizes the concept of response heterogeneity in exercise training, which is present in individuals across all age groups in terms of both muscle strength and size.[839]

1.5.2 Predictors of the Response

Predictors of the exercise response are factors that contribute to interindividual heterogeneity[840] by acting as moderators of the treatment effect.[841] Solomon et al. (2018) postulated that the variability in response in blood glucose control following exercise might be explained by several factors, including exercise parameters, the timing of exercise according to meals, interactions between exercise and certain medications, or genetics.[17] In a crossover RCT,[15] a group analyzed the glucose response to an acute bout of moderate-intensity exercise in healthy adults who either received a glucose infusion or did not. Results indicated that glucose levels were significantly higher in the adults who received a

glucose infusion compared to those who did not. This potentially suggests that: 1) the glucose infusion conditions may have blunted the glucoregulatory benefits of acute exercise; or that 2) exposure to hyperglycemia immediately prior to exercise can contribute to exercise response heterogeneity.[15]

Metformin is the most widely prescribed drug to treat hyperglycemia in T2D and is often co-prescribed with exercise,[842] but without a clear understanding of its interaction with exercise.[843] In a review of the literature performed in 2020, it was concluded that metformin likely blunts the insulin-sensitizing effects of exercise in skeletal muscle. Therefore, it is suggested that metformin could also contribute to heterogeneity in glycemic responses to exercise.

A number of exercise trials have also determined predictors of the response based on participant responder status. For instance, He et al. (2022) identified predictors of the response following two years of exercise in a RCT of 113 older adults with prediabetes.[844] Individuals were classified as responders (those who reversed to normoglycemia or remained in prediabetes) and non-responders (those who progressed to T2D) to the exercise treatment. Reductions in HbA_{1c} (-0.29 ± 0.46%) and BMI (-0.56 ± 2.17 kg/m^2), as well as improvements in β-cell function (0.58 ± 1.23), were predictors of being classified as a responder. In a sub-analysis of the HART-D trial, Stephens et al. (2015) found that exercise non-responders exhibited a unique baseline expression of specific genes involved in muscle metabolism.[845] They postulated that this distinct basal muscle transcriptional profile may predict the lack of exercise response in various metabolic outcomes. Similarly, Liu et al. (2020) demonstrated that twelve weeks of combined high-intensity AT+RT improved glucose homeostasis and insulin sensitivity, with these adaptations being associated with

the gut microbiome profile of the responders.[846] Furthermore, an 8-week RT intervention in post-menopausal women postulated that exercise training frequency and insulin resistance may be determinants of the change in response.[847] In this study, Orsatti et al. (2022) found that lower resistance training frequency and higher HOMA-IR were associated with higher odds of being a non-responder. Investigators have also suggested that responders to exercise may have greater ribosome biogenesis and satellite cell proliferation and also may possess an altered mRNA profile, which enhances the expression of insulin-like growth factor-1.[835-837] Ultimately, identifying factors that are determinants of interindividual response variability is invaluable, as it can aid in determining novel approaches to eliminate non-response in future trials and treat these individuals in clinical practice.

Our team has investigated the individual response to exercise in a variety of analyses based on the EXercise and Irisin sTudy (EXIT) trial. The EXIT trial investigated the effects of exercise training on sedentary youth living with obesity at risk of T2D (age: 15.7 ± 0.5 yrs, BMI ≥95th percentile, n = 11).[784] In this trial, the youth underwent two acute bouts of exercise (one each of AT and RT) followed by six weeks of RT. Participants in the top 50th percentile of change in insulin sensitivity were classified as responders, while those in the lower 50th percentile were considered non-responders. We found that responders exhibited an approximate 90% increase in irisin concentration during an acute bout of AT, compared to only about 20% in non-responders; and a correlation between the change in irisin and the change in insulin sensitivity in responders (r = 0.68; p <0.05).[784] Furthermore, Pierdoná et al. (2022) investigated the potential influence of extracellular vesicles as determinants of individual responses. In this same group of youth, those

classified as responders exhibited a much larger size of extracellular vesicles released during acute exercise.[848]

1.5.3 Effect of Altering Exercise Parameters on Interindividual Variation

Although very few studies have investigated the impact of modifying exercise parameters for non-responders, some suggest that doing so may shift their response categorization to responders. Marsh et al. (2020) demonstrated that previously identified non-responders could become responders upon converting exercise types (modes), such as switching from AT to RT, or vice versa.[30] The concept of 'rescuing' non-responders was demonstrated earlier by Montero & Lundby (2017), who showed that non-responders to a 6-week AT program could become responders if they underwent an additional 6 weeks of training with two extra sessions per week, based on improvements in cardiorespiratory fitness.[29] Furthermore, a 6-month AT trial found that increasing the intensity of exercise (at a fixed amount) completely eliminated the rate of non-response to cardiorespiratory fitness, while increasing the volume of exercise (at a fixed intensity) reduced it by 50%.[12] In the Improving Individual Glycemic Response with Exercise Intensity (INTENSITY) trial conducted by our group, we investigated whether the response categorization to HbA_{1c} could be modified when participants with prediabetes or T2D randomized to either a maintained or increased intensity group completed an additional 12 weeks of AT following an initial 16 weeks of AT. Using a conservative method of response categorization, increasing the intensity or duration of AT improved the response categorization for some participants, but not all. Critically, however, increasing exercise intensity was effective in preventing the regression to adverse responder status in certain participants. These findings

potentially suggest that changing the exercise intensity for pre-identified non-responders could improve their response to exercise.

1.5.4 Potential Role of Myokines in Mediating the Exercise Response

Given their role in cardiometabolic health, myokines have emerged as potential mediators of the interindividual variability observed in exercise responses.[16] These molecules may, therefore, contribute to the unexplained variation in individual responses to exercise. For example, in an acute randomized crossover trial of young, healthy males, He et al. (2018) investigated the response of a range of myokines and found that less than 25% of participants were classified as irisin responders following acute HIIT and RT – which was significantly lower than the other myokines studied.[789] Furthermore, evidence suggests that irisin responses differ according to the parameters of the exercise intervention.[23,27,28] This inherent variability in irisin's response, coupled with its established link to metabolic health, highlights the need for further research to investigate irisin's potential to predict individual metabolic adaptations in response to exercise. A more comprehensive understanding of irisin's exercise-induced response from an individual perspective, and its relationship with cardiometabolic health outcomes, could provide valuable insights into optimizing exercise prescription for individuals living with obesity and/or T2D, who could derive considerable benefit from irisin's positive effects on metabolic health.

1.6 Application of Interdisciplinary Studies

Interdisciplinarity has gained momentum in academia in recent years as a valuable tool for addressing complex issues. This trend has enhanced the generation of knowledge, and both funding agencies and policymakers have placed particular emphasis on research

grants that adopt an interdisciplinary approach.[849,850] Addressing complex issues through a single disciplinary lens limits the breadth, comprehensiveness and realism of the solutions that are possible.[851] Thus, interdisciplinary studies and integrative research projects emerged to advance knowledge beyond the borders of traditional disciplines by incorporating the ideas, theories, and methods/methodologies of two or more disciplines.[852] The innovative value of interdisciplinary studies is clear in that it can generate novel solutions and advance our comprehensive understanding of a topic.[853]

My doctoral research expands beyond the primary focus of exercise physiology to integrate disciplines such as molecular biology, biochemistry, nursing, and biostatistics to develop evidence-based solutions by using an approach that does not sacrifice the depth of knowledge of these individual disciplines. Successful implementation of the exercise interventions within this dissertation was ensured by working closely with experts in conducting large clinical trials and with nursing professionals to conduct bloodwork and allow the measurement of the metabolic variables central to my dissertation. Their expertise proved to be invaluable in designing interventions that align with principles of patient-oriented care, by ensuring that the individual needs and experiences of individuals living with obesity and/or T2D were considered. Furthermore, the quality of findings was considerably enhanced by leveraging external connections to gain access to facilities and experts in molecular biology and biochemistry to support the advanced research methodologies necessary for the projects. Collaborating with exercise physiology specialists was vital to gaining a refined understanding of physiological mechanisms and additional insights and perspectives that enhance the primary focus of my research. Finally, biostatisticians played a pivotal role in ensuring robust data analysis and the appropriate

interpretation of advanced statistical models. Integrating the methodologies and expertise of these diverse disciplines improved the depth and quality of the research conducted, ultimately strengthening the overall scientific rigour of this dissertation. A key distinction of the approach taken for this research involved the integration of the disciplinary knowledge and practical applications of each individual discipline, rather than the simple parallel application, as seen in multidisciplinary or transdisciplinary research.[854] This integrative approach seeks to develop innovative solutions that would not be possible to achieve through a single-disciplinary perspective. Moreover, upon the completion of these projects, insight will be drawn from knowledge translation practices to share the research beyond the scientific community in order to affect meaningful change that positively impacts the individuals for whom this research is ultimately conducted.[855]

Prioritizing an interdisciplinary approach for this doctoral work provides new insights into the metabolic challenges faced by individuals living with obesity and/or T2D and thus may generate "rich, innovative, and relevant data for public health intervention".[856] Given the complex, multidimensional nature of these chronic conditions, as evidenced by the extensive literature review presented above, this integrative approach is crucial to addressing the accelerating prevalence and burden of obesity and T2D. This need for interdisciplinary solutions is highlighted by several public health initiatives. The principles and recommendations for public health interventions outlined in *The Lancet* Commission report by Swinburn et al. (2019) are based on a comprehensive body of evidence spanning nearly 30 individual disciplines – including biological, social, environmental sciences, and more.[857] This extensive undertaking depicts the intricacies of obesity as a chronic disease and reinforces the notion that the obesity epidemic cannot be tackled through a single-

disciplinary lens. Similarly, the multifaceted etiology of T2D – encompassing biological, genetic, environmental, and societal influences – exemplifies the need for interdisciplinary approaches to public health solutions to affect meaningful change for both the individual and for society. At the individual level, involving interdisciplinary care teams is crucial to help individuals with T2D reach their glycemic targets,[858,859] as reflected in the most recent clinical guidelines from Diabetes Canada[208] and the American Diabetes Association.[860] From a public health perspective, the Public Health Agency of Canada's (2022) *Framework for Diabetes in Canada* emphasizes the importance of interdisciplinary collaboration between researchers, practitioners, industry, policy-makers and patient partners to advance diabetes research.[861] Altogether, the complexity of these chronic conditions necessitates a truly interdisciplinary, collaborative approach to deliver innovative, evidence-based solutions that benefit both society and the individual health of those living with obesity and/or T2D.

1.7 Gaps in the Literature and Objectives

Despite considerable evidence supporting exercise-induced irisin responses and its vital role in cardiometabolic health, significant gaps remain in our understanding of the intricate nature of its responses across metabolic health statuses and varying exercise parameters. While acute exercise intensity is frequently suggested as a key determinant of irisin responses, the consistency and magnitude of this response are yet to be fully elucidated. Furthermore, although emerging evidence indicates that increasing exercise intensities can elicit greater adaptations in circulating irisin, these reports are predominantly from studies with limited sample sizes,[822] single-sex cohorts,[811,862] or animal models.[823] Additionally, ambiguities persist regarding irisin's precise role in overall

cardiometabolic adaptations with exercise training. Specifically, irisin's potential as a predictor of individual glycemic improvements in response to exercise is yet to be fully characterized. Considering the substantial interindividual variability observed in exercise-induced metabolic adaptations, a more thorough understanding of irisin's contributions to this heterogeneity is essential. Addressing these unresolved questions will help elucidate irisin's role in the metabolic health benefits of exercise, and ultimately, contribute to the development of more effective, personalized exercise prescriptions for individuals with obesity and T2D.

In order to address these identified gaps, this dissertation comprehensively examines the effects of exercise intensity from both acute and chronic perspectives across various metabolic health statuses through a series of investigations. Specifically, the objectives of this research are to: 1) comprehensively evaluate the analytical performance of commercially available irisin ELISA kits to ensure reliable and precise quantification of circulating irisin in human plasma; 2) assess acute irisin responses across different exercise intensities in individuals with and without overweight/obesity, while exploring its association with insulin sensitivity; and 3) investigate the impact of increasing exercise intensity on chronic circulating irisin adaptations in individuals with prediabetes or T2D and assess irisin's predictive role in achieving individual and clinically meaningful glycemic improvements.

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2.0 PROJECT I

Quantitative Performance of Two Commercial Irisin ELISA Kits: An Assessment of Agreement, Reliability, and Precision

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2.1 Abstract

BACKGROUND: Irisin, a key metabolic myokine, is recognized for its potential role as a molecular link between exercise and its associated health benefits. However, variations in the analytical performance of commercial enzyme-linked immunosorbent assays (ELISAs) highlight the need to comprehensively assess the performance of contemporary ELISA kits.

PURPOSE: This study aimed to rigorously evaluate the agreement, between-day reliability, and within-subject precision of irisin concentrations derived from two commercial ELISAs: Phoenix Pharmaceuticals (EK-067-29) and R&D Systems (DY9420-05).

METHODS: Baseline irisin concentrations were assessed in a sub-sample of 43 participants (34.3 ± 9.0 years old; 28.6 ± 7.9 kg/m^2) from the Irisin and Metabolic Exercise Training Study on two separate days, at least 24 hours apart. Statistical agreement was determined using Bland-Altman analyses, paired-samples t-tests, and Pearson correlations. Between-day reliability was assessed via intraclass correlation coefficients (ICC). Within-subject precision was quantified with coefficients of variation (CV%).

RESULTS: Systematic and proportional bias was identified between kits. Phoenix consistently produced significantly higher irisin mean irisin concentrations than R&D across all measurement days (average: 17.1 ng/mL vs. 5.4 ng/mL, $p < 0.001$), with wide limits of agreement (average: 95%: 5.0-18.4 ng/mL) and increasing variability at higher irisin concentrations, indicating proportional bias. Additionally, the persistent lack of a significant linear relationship (r values ranging from -0.20 to -0.04; $p > 0.05$) exacerbated the inter-kit measurement disagreement. The between-day reliability of average irisin concentrations was considered good with R&D (ICC = 0.81, 95% CI [0.65, 0.90], $p <$

0.001) and moderate with Phoenix (ICC = 0.68, 95% CI: [0.40, 0.83], $p < 0.001$). Both kits demonstrated acceptable within-subject precision (median CV%: R&D = 5.7%; Phoenix = 10.1%).

CONCLUSION: Substantial analytical discrepancies and inconsistent performance observed between two commercial irisin ELISA kits suggest that results between kits may not be interchangeable. R&D demonstrated superior analytical performance compared to Phoenix, with greater between-day reliability and precision. These findings highlight the critical need for reliable and comparable quantification methods to enable accurate cross-study comparisons.

Keywords: Irisin; Myokines; Reliability; Agreement

2.2 Introduction

Irisin, an exercise-induced myokine, has drawn considerable attention since its initial discovery by Bostrom et al. (2012)[1] as a potential molecular link between exercise and its associated health benefits. Its ability to regulate energy expenditure, coupled with its roles in glucose metabolism and insulin sensitivity, has positioned irisin as a promising candidate for the treatment and management of obesity and diabetes.[1] Additionally, its influence on cancer prevention,[2,3] bone metabolism,[4,5] cognitive function,[6] and cardiovascular health[7] has further emphasized its extensive therapeutic potential.

As irisin's diverse physiological roles became clear, analytical challenges concurrently arose, prompting concerns regarding its accurate quantification and detectability in humans.[8,9] Claims of its existence in humans were supported through the use of tandem mass spectrometry,[10-13] a technique that overcomes limitations related to the cross-reactivity and non-specificity of antibody-based measurements, such as enzyme-linked immunosorbent assay (ELISA).[14] Despite these advantages, the high cost and limited accessibility of mass spectrometry have impeded its widespread adoption, leading to the predominant use of ELISA for quantifying irisin in most exercise studies,[10,15] largely due to its affordability and high throughput capabilities. Consequently, persistent concerns regarding its precise quantification remain, as large inconsistencies in irisin concentrations continue to be reported under both basal and exercise conditions.[8,16,17] While largely influenced by diverse exercise protocols[17-19] and differences in study populations (e.g. biological variation due to age, sex, health status, etc.),[13,20,21] a considerable portion of the variability can also be attributed to the measurement error introduced by ELISA.

Inconsistencies in irisin concentrations quantified by ELISA are frequently noted in the literature.[16,22] Direct comparisons reveal significant variability in results between

widely used irisin ELISA kits from different manufacturers,[23-27] different versions from the same manufacturer,[27-29] and even different lots from the same version.[30] For instance, although the Phoenix Pharmaceuticals irisin ELISA (version EK-067-29) is among the most frequently used kits,[15] it has been found to yield 10-25 times higher concentrations than in previous versions from the same manufacturer.[28,29] Furthermore, Winn et al. (2017)[25] reported that the Adipogen irisin ELISA (AG-45A-0046EK-k101) exhibited 230-fold higher irisin concentrations than the Phoenix kit (EK-067-29) in the same group of participants. The substantially elevated irisin concentrations reported in these studies are in significant contrast with the lower physiological levels quantified by mass spectrometry (3-5 ng/mL). While these striking findings continue to illustrate the poor analytical validity of many irisin ELISAs, these comparisons provide limited insight into the actual statistical agreement or degree of measurement error between different kits currently in use, which few groups have adequately quantified.[27,30-32]

To the best of our knowledge, no study has directly compared the agreement between ELISA kits from different manufacturers, nor the between-day reliability of these specific Phoenix and R&D irisin kits. Moreover, many of the previous comparisons utilized older, less specific, or even discontinued kit versions,[16] thereby limiting their relevance to current research and highlighting a critical gap in the literature. As such, this accentuates the need for a rigorous evaluation of the performance of irisin ELISA kits, particularly with more contemporary commercially available versions, to enable appropriate comparisons and meaningful interpretations across studies. The purpose of this analysis is to evaluate the agreement, reliability, and precision between the Phoenix Pharmaceuticals (EK-067-29) and R&D Systems (DY9420-05) immunoassay kits for measuring irisin concentrations

in human plasma. It was hypothesized that these kits would exhibit poor agreement with suboptimal overall performance.

2.3 Materials and Methods

Study Design and Participants

The plasma irisin samples analyzed in this study were obtained from participants who were part of a larger randomized crossover trial (Irisin and Metabolic Exercise Training Study (iMET) Study; NCT03514238). A comprehensive description of the iMET Study trial design has been previously reported.[33] Briefly, participants were aged 19-50 years and had an average body mass index (BMI) of 28.6 ± 7.9 kg/m^2. Exclusion criteria for the main trial included impaired glucose metabolism, injuries or chronic conditions preventing exercise, the use of diabetes-related or heart rate-altering medications, or a significant weight change in the preceding six months. For this secondary analysis, a subset of 43 participants was included based on having at least two baseline irisin concentration measurements that fell within the manufacturer's validated lot-specific linear range for each ELISA kit analysis across the three acute experimental conditions.

The study was approved by the University of New Brunswick Research Ethics Board (UNB-2017-105), and all protocols adhered to the general principles of the Declaration of Helsinki. Each participant provided written informed consent for their participation. Participant demographic, anthropometric, and metabolic profile characteristics collected as part of the original iMET Study are summarized for this sub-group and are summarized in Table 2.

Plasma Sample Collection and Preparation

Blood samples were collected intravenously from the antecubital vein by a registered nurse at baseline immediately prior to each of the three acute experimental conditions on three separate days at least 24 hours apart. Blood was drawn into 3 mL Vacutainer collection tubes coated with an anticoagulant, ethylenediaminetetraacetic acid (EDTA). Samples were then pipetted into 1.5 mL microcentrifuge tubes and centrifuged at 1600g for 15 minutes at 4°C. The plasma was subsequently collected, aliquoted, and stored at -80°C until further analysis.

Plasma irisin concentrations were measured by ELISA using two separate commercially available kits: Phoenix Pharmaceuticals (Cat# EK-067-29, Phoenix Inc., Burlingame, CA, USA) and R&D Systems (Cat# DY9420-05; ancillary reagent kit Cat# DY008B, R&D Systems, Minneapolis, MN, USA); assays were performed according to manufacturer protocols. Optical density was determined using an EPOCH 2 Microplate Spectrophotometer with Gen5 software (BioTek, Winooski, VT, USA), with readings at 540 nm subtracted from those at 450 nm to correct for optical imperfections. Standard curves were generated using a four-parameter logistic curve-fitting method (MyAssays, MyAssays Ltd., Brighton, UK) from which irisin concentrations were extrapolated from the optical density measurements. For each kit, the first assay was performed in duplicate (with a coefficient of variation below 2.0%), while subsequent assays were performed in singlet, as previously established in our laboratory.[33-35] To minimize technical bias introduced by measurement error, a single technician used the same calibrated pipette for all assays.

For this analysis, only samples that fell within the manufacturer's established validated linear range were selected. Specifically, the acceptable ranges for Phoenix were

1.6-24.4 ng/mL (lot #610220) and 1.6-26.9 ng/mL (lot #610250), as clarified by the manufacturer based on a ± 2 standard deviation allowance from the kit's specified linear range. For R&D Systems, the validated linear range was 2.5-8.0 ng/mL. A minimum of two days of in-range concentrations were required for each participant, which were then standardized for the reliability analysis. If a participant had in-range baseline irisin concentrations from all three acute experimental conditions, two were randomly selected and assigned to Day 1 and Day 2. However, if only two of the three irisin concentrations were in-range, these values were directly assigned as Day 1 and Day 2.

Statistical Analysis

Data normality was visually inspected and assessed using Shapiro-Wilk tests. Continuous variables are presented as mean ± standard deviation, median (interquartile range) and categorical variables as n (%). Paired samples t-tests were used to assess the systematic bias between the R&D and Phoenix ELISA kits, conducted for each of the separate days (Day 1, Day 2), as well as a two-day average. Additionally, Pearson correlations were used to assess the linear association between irisin concentrations obtained from each manufacturer's kits. To visualize the systemic bias and limits of agreement between kits, Bland-Altman plots were generated for Day 1, Day 2, and the Two-Day Average irisin concentrations. The between-subject coefficient of variation (CV%) was calculated for irisin concentrations at baseline across all days, reflecting inter-individual biological variability ((average SD/average mean) × 100). To determine the between-day reliability of irisin measurements for each kit across the two measurement days, intraclass correlation coefficients (ICCs) were quantified using a two-way random effects model with absolute agreement, with the two daily baseline concentrations as

repeated measures. Finally, within-subject precision was quantified as intra-individual CV% ((individual SD/individual mean) × 100)), reflecting the consistency of repeated measures for each kit on the same individual and was compared with a Wilcoxon Signed-Rank Test, given the non-normal distribution of these values. All statistical analyses were conducted using SPSS version 22.0 (IBM Corporation, Armonk, NY, USA) with graphs generated with GraphPad Prism version 10.1 (GraphPad Software, LLC., San Diego, CA, USA). The significance level for all analyses was accepted at $p \leq 0.05$.

2.4 Results

Baseline Characteristics

This analysis included 43 participants (55.8% female; 90.7% Caucasian) who were 34.3 ± 9.0 years old. Participants had an average body mass index (BMI) of 28.6 ± 7.9 kg/m^2 with a body fat percentage of 31.5 ± 14.1%. Glucose (5.0 ± 1.3 mmol/L) and insulin concentrations (24.9 ± 31.2 pmol/L) were obtained in a fasted state prior to a standard 75-g oral glucose tolerance test, from which insulin sensitivity was derived (Matsuda index: 31.7 ± 27.9). Participants exhibited a mean relative VO_{2max} of 31.0 ± 9.5 ml/kg/min. Further detailed baseline characteristics are presented in Table 2.

Table 3 depicts the irisin concentrations quantified at baseline on two separate measurement days by the Phoenix and R&D kits. Overall descriptive statistics for the baseline irisin concentrations are presented in Table 4. The between-subject CV% was calculated to reflect the total observed variability within the study population on each day (Day 1: Phoenix = 22.5%, R&D = 24.5%; Day 2: Phoenix = 18.8%, R&D = 18.9%). When considering the Two-Day Average measurement, the between-subject CV% was 18.0% with Phoenix and 20.0% with R&D.

Analysis of the Agreement Between Phoenix Pharmaceuticals and R&D Systems ELISAs

Analysis of agreement between the kits revealed a substantial systematic bias, with Phoenix consistently producing significantly higher mean irisin concentrations than R&D on both Day 1 (17.1 ± 3.9 vs. 5.4 ± 1.3 ng/mL, $p < 0.001$) and Day 2 (17.2 ± 3.2 vs. 5.5 ± 1.0 ng/mL, $p < 0.001$). Large mean differences remained significant when analyzing the two-day averaged concentrations (11.7 ng/mL, 95% CI: [10.4, 13.1], $p < .001$). All mean differences were accompanied by very large effect sizes (Cohen's d ranging from 2.7 to 3.4). Furthermore, correlations between kits were weak and non-significant across all measurement days (Day 1: $r = -0.20$; Day 2: $r = -0.04$; Two-Day Average: $r = -0.17$; all $p > 0.05$).

These findings were supported in Bland-Altman plots, which consistently demonstrated a significant systemic positive bias between the kits for Day 1, Day 2, and the two-day average concentrations (Figure 5). These plots visually confirmed the higher irisin concentrations produced by Phoenix and revealed wide 95% limits of agreement (LoA). On Day 1, the LoA ranged from 3.3 to 20.2 ng/mL; on Day 2, the LoA were 4.9 to 18.4 ng/mL; and the Two-Day Average were from 5.0 to 18.4 ng/mL.

Between-Day Reliability of ELISAs and Kit Precision

The between-day reliability of the Phoenix and R&D kits was assessed using ICC, based on a two-way random effects model with absolute agreement (Table 4). R&D demonstrated good between-day reliability for average measures (ICC = 0.81, 95% CI [0.65, 0.90], $p < 0.001$) and moderate reliability for single measures (ICC = 0.68, 95% CI [0.49, 0.82], $p < 0.001$). Phoenix revealed moderate between-day reliability for both

average (ICC = 0.68; 95% CI [0.40, 0.83], p < 0.001) and single measures (ICC = 0.51, 95% CI [0.25, 0.70], $p < 0.001$). Overall, R&D exhibited greater between-day reliability and absolute consistency across measurement days.

Furthermore, the within-individual precision of each kit was quantified using the intra-individual CV%, reflecting the day-to-day consistency of measurements for each participant. Both kits achieved appropriate reliability, with better precision reflected in R&D (5.7% (4.1-9.0%)) compared to Phoenix (10.1% (4.3-15.1%), indicated by a trend towards a statistically significant difference between kits ($p = 0.07$).

2.5 Discussion

This study investigated the statistical agreement, between-day reliability, and within-subject precision of commercially available irisin ELISA kits from Phoenix Pharmaceuticals and R&D Systems. Our findings reveal substantial analytical discrepancies and a lack of agreement between the two kits, suggesting their results are not interchangeable. Phoenix consistently produced significantly higher irisin concentrations than R&D, whose values are in greater alignment with mass spectrometry-derived human circulating concentrations. Furthermore, R&D demonstrated superior between-day reliability and within-subject precision compared to Phoenix. Collectively, these findings provide insight into how analytical discrepancies and inconsistent kit performance may compromise the direct comparability of irisin concentrations across studies.

Analytical Discrepancies and Lack of Agreement Between Irisin ELISA Kits

Substantial analytical discrepancies observed between Phoenix and R&D irisin ELISA kits in our analysis uncovered a critical lack of agreement and called into question inter-kit comparisons across studies. Not only was a significant systemic bias present, with

Phoenix consistently producing markedly higher irisin concentrations compared to R&D kits across all measurement days, but Bland-Altman plots revealed a clear proportional bias, with the magnitude of disagreement worsening as irisin concentrations increased. Exacerbating this lack of agreement is the absence of a linear relationship between the kits' measurements, implying a non-parallel quantification of irisin concentrations, a finding others have observed.[8] This overall lack of agreement aligns with previous investigations that similarly reported large variations in mean values when comparing ELISA kits. For instance, Hew-Butler et al. (2015) reported mean biases between different kit versions ranging from -89 to 90 ng/mL,[27] while Montes-Nieto et al. (2016) observed a mean bias of 183%.[30] However, it is essential to note that these studies used discontinued kit versions in their comparisons (e.g. the first generation of EK-067-29), limiting their generalizability to novel irisin studies using updated kits. Nevertheless, our findings and the existing literature[8,24-26,28,29] support that irisin concentrations may not be directly interchangeable between different ELISA kits, necessitating caution when comparing results across studies.[8,16,22,36]

The large differences in absolute irisin concentrations produced by the Phoenix and R&D kits provide further evidence of poor agreement, with concentrations diverging from expected human circulating concentrations. Across all measurement days, R&D consistently detected irisin concentrations (~5.4 ng/mL) approximately 11.7 ng/mL lower than those from Phoenix, which are more in line with the lower range typically found in human circulation (3-5 ng/mL), as established by the gold-standard for irisin measurements, mass spectrometry.[10] Despite some studies reporting concentrations closer to the expected range with this Phoenix kit (~8-10 ng/mL),[20,37-39] other results highlight

that even the updated version of the EK-067-29 kit continues to yield inconsistent concentrations.[28,30,40] Inter-kit inconsistencies are further exemplified by results of an Adipogen ELISA kit (AG-45A-0046EK-KI01) that yielded irisin concentrations astonishingly 230-fold higher than Phoenix's (~9-10 ng/mL at baseline).[25] While studies utilizing R&D show some variability in irisin concentrations (as low as 0.2-3.8 ng/mL[41-43] to greater than 10 ng/mL[44]), their range is notably narrower compared to other kits. Nevertheless, the wide ranges reported regardless of manufacturer reiterate the ongoing need for rigorous validation of irisin ELISA kits.[40]

Varying Reliability and Precision of Commercial Irisin ELISA Kits

Our analysis identified varying degrees of between-day reliability and within-subject precision for the Phoenix and R&D irisin ELISA kits. Although both manufacturers' kits displayed appropriate within-subject precision,[45] and reasonable between-day reliability, R&D's intra-individual CV% were lower when compared to Phoenix (median: 5.7% vs. 10.1%), indicating superior precision that enhances its overall reliability. Indeed, our comprehensive reliability assessment using ICCs further supported this. Specifically, R&D demonstrated good between-day reliability with average measures (ICC: 0.81) and moderate reliability with single measures (ICC: 0.68).[46] In contrast, not only did Phoenix remain moderately reliable (single ICC: 0.51; average ICC: 0.68), but its wider 95% confidence intervals imply greater uncertainty in its true reliability. These differences in kit reliability are not trivial, as the accurate interpretation of irisin changes in intervention studies relies on the assumption that baseline concentrations remain relatively stable. Therefore, a reliable ELISA kit is required to disentangle true physiological effects from measurement error.

Adding a critical layer of complexity to irisin quantification is the observation that while individual kits may demonstrate consistent measurements between days, this provides little insight into their validity or accuracy. For instance, Newman et al. (2025) reported excellent between-day reliability (ICC = 0.96) for irisin over a 24-hour period using a MILLIPLEX immunoassay; however, the irisin concentrations were very low (median (IQR): 0.92 (0.96)). Similarly, Panagiotou et al. (2014) noted consistent measurements over a 6-month period (ICC = 0.84) with an Aviscera Biosciences kit (now Phoenix Pharmaceuticals EK-067-52),[36] but exceptionally high mean baseline irisin concentrations (546 ng/mL),[31] suggesting overestimated values characteristic of early irisin kits and concerns regarding their validity. Altogether, the disparate irisin concentrations reported with varying degrees of reliability across manufacturers' kits reflect challenges in inter-kit comparisons between studies.

Strengths and Limitations

Certain limitations of the current analysis should be considered. Primarily, the absence of a direct comparison between ELISA-derived concentrations with mass spectrometry limits critical insight into kit accuracy, particularly given ongoing irisin quantification controversies.[8,16,22] Nevertheless, while mass spectrometry is considered the gold-standard for irisin measurement,[47] its high cost and limited accessibility, along with potential variability introduced from sample preparation that still impacts accuracy.[8] This has led to the predominant use of ELISA for irisin quantification,[15] especially in exercise trials. Additionally, the cohort studied in our analysis, consisting primarily of middle-aged Caucasian adults, limits generalizability to other populations, as irisin concentrations may vary according to individual factors (e.g. age, sex, body composition, health status,

etc.).[13,20,21] Moreover, the inherent risk of bias from non-blinded kit analysis and potential differences in sample degradation must be acknowledged, due to slight variations in storage duration and an additional freeze-thaw cycle for the R&D kit. Despite this disadvantage, the R&D kit demonstrated superior performance than the Phoenix kit. Finally, a lack of detailed nutritional information could influence variability in baseline irisin concentrations;[48] however, participants in the main iMET trial refrained from food and caffeine intake prior to baseline irisin collection. Nevertheless, this study is strengthened by its direct and comprehensive comparison of two contemporary commercially available ELISA kits using a stringent statistical approach for agreement and reliability. The inclusion of a between-day reliability analysis with matched plasma samples across timepoints allowed for the control of inherent individual biological variability, while the use of two different Phoenix kit lots accounts for potential inter-lot variability, a known concern for this kit.[30]

Conclusions

This study provides valuable insights into the performance of Phoenix and R&D irisin ELISA kits, revealing substantial discrepancies. Our comprehensive analysis demonstrated that the concentrations obtained from the kits may not be interchangeable, primarily due to substantial systemic and proportional bias, with Phoenix consistently producing significantly higher irisin concentrations than R&D. While both kits showed acceptable between-day reliability and within-subject precision, R&D exhibited consistently superior analytical performance, providing more precise and reliable measurements that better align with mass spectrometry data and effectively capture between-subject biological variability. Despite the Phoenix's irisin kit's widespread use in

irisin research, its performance was not equivalent to the under-utilized R&D kit, which appeared to offer superior analytical performance. The analytical discrepancies, varying reliability, and precision across commercially available irisin kits reflect a major ongoing challenge in irisin research, and reinforce the need for more robust, standardized, and consistently accurate methods for quantifying irisin. Such limitations impede the accurate interpretation of irisin's physiological role and therapeutic potential. As such, the availability of valid and interchangeable assays is of paramount importance to advance our understanding of this critical metabolic myokine.

2.6 Tables and Figures

Table 2. Baseline characteristics of participants.

Demographics	**Mean ± SD**	**Median (IQR)**
Age (years)	34.3 ± 9.0	35.0 (26.0-41.0)
Sex (Female (%))	24 (55.8)	---
Anthropometrics		
Weight (kg)	86.5 ± 23.1	82.0 (69.0-100.2)
Body Mass Index (kg/m^2)	28.6 ± 7.9	28.4 (22.3-32.3)
Waist Circumference (cm)	98.6 ± 20.0	95.8 (82.3-110.8)
Body Composition		
Fat Mass (kg)	29.3 ± 19.7	26.4 (13.5-38.6)
Fat-Free Mass (kg)	56.9 ± 11.2	56.4 (47.9-66.1)
Fitness		
Relative VO_{2max} (ml/kg/min)	31.0 ± 9.5	29.2 (24.5-37.5)
Metabolic Profile		
Fasting Glucose (mmol/L)	5.0 ± 1.3	5.0 (4.4-5.8)
Fasting Insulin (pmol/L)	24.9 ± 31.2	15.3 (5.3-28.1)
Insulin Sensitivity (Matsuda Index)	31.7 ± 27.9	19.9 (11.2-42.2)
Triglycerides (mmol/L)	1.1 ± 0.6	1.0 (0.7-1.3)
Total Cholesterol (mmol/L)	4.7 ± 0.9	4.9 (4.1-5.4)
HDL Cholesterol (mmol/L)	1.4 ± 0.4	1.4 (1.1-1.7)
LDL Cholesterol (mmol/L)	2.9 ± 0.7	2.8 (2.4-3.4)
LDL/HDL (mmol/L)	2.3 ± 1.1	2.1 (1.6-2.7)

Continuous data are presented as mean ± standard deviation and median (IQR), while categorical variables are presented as n (%). VO_{2max}: maximal oxygen consumption; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 3. Irisin concentrations determined by two irisin ELISA kits across measurement day.

	Irisin Concentrations (ng/mL) (n=43)		
	Day 1	**Day 2**	**Two-Day Average**
R&D Systems	5.4 ± 1.3	5.5 ± 1.0	5.4 ± 1.1
	5.3 (4.6-6.5)	5.5 (4.8-6.2)	5.6 (4.6-6.4)
Phoenix Pharmaceuticals	17.1 ± 3.8	17.1 ± 3.2	17.1 ± 3.1
	17.6 (15.1-20.6)**	17.1 (15.5-19.1)**	17.2 (15.3-19.2)**

Presented as mean ± standard deviation and median (IQR), CV: coefficient of variation. ELISA kits: R&D Systems (Cat# DY9420-05), Phoenix Pharmaceuticals (Cat# EK-067-29). ** Represents significant differences in mean concentrations between kits ($p < 0.001$).

Table 4. Between-day reliability and within-individual precision of irisin concentrations from Phoenix Pharmaceuticals and R&D Systems ELISA Kits.

	ICC	95% CI	*p* value	CV%
R&D Systems				5.7 (4.1-9.0)
Single Measures	0.68	0.49-0.82	<0.001	
Average Measures	0.81	0.65-0.90	<0.001	
Phoenix Pharmaceuticals				10.1 (4.3-15.1)[†]
Single Measures	0.51	0.25-0.70	<0.001	
Average Measures	0.68	0.40-0.83	<0.001	

ELISA kits: R&D Systems (Cat# DY9420-05), Phoenix Pharmaceuticals (Cat# EK-067-29). ICC: intraclass correlation; CI: confidence interval; CV: coefficient of variation. Within-individual CV% are presented as median (IQR).

[†] Difference in within-individual precision between R&D Systems and Phoenix Pharmaceuticals assessed by Wilcoxon Signed-Rank Test, $p = 0.07$

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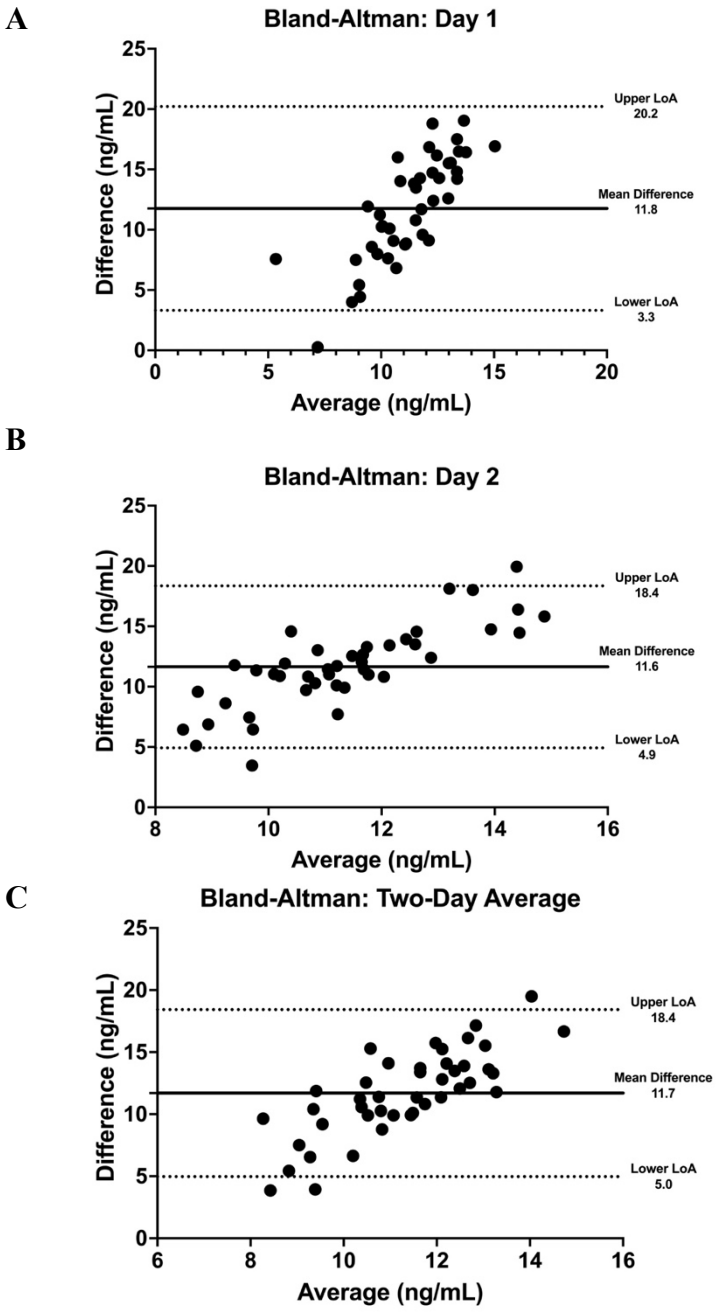


Figure 5. Bland-Altman plots for the agreement between Phoenix Pharmaceuticals and R&D Systems irisin ELISA kits.

Agreement illustrated between irisin concentrations measured by Phoenix Pharmaceuticals (Cat# EK-067-29) and R&D Systems (Cat# DY9420-05) ELISA kits, with the difference (y-axis) and average (x-axis) demonstrated for Day 1 (A), Day 2 (B), and the Two-Day Average (C), (x-axis). Solid horizontal line indicates the mean difference (systematic bias); dashed horizontal lines represent the upper and lower 95% limits of agreement (LoA).

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Acknowledgements: The authors are grateful to the volunteers for their participation in the iMET Study and to the CELLAB students and staff who were involved in data collection and analysis.

B.V.R. was supported by a Canadian Institutes of Health Research Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award and a University of New Brunswick Dr. William S. Lewis Doctoral Fellowship.

Competing interests: The authors declare there are no competing interests.

Author contributions: B.V.R. and M.S. conceived and designed research; B.V.R. performed experiments; B.V.R. and Y.P. analyzed data; B.V.R., Y.P., N.M.J., S.M.C. and M.S. interpreted results of experiments; B.V.R. prepared figures; B.V.R. and M.S. drafted the manuscript; B.V.R., Y.P., N.M.J., S.M.C. and M.S. edited, revised, and approved the final version of the manuscript.

Funding: This research was supported by an Establishment Grant from the New Brunswick Health Research Foundation (Research New Brunswick), an Operating Grant from Diabetes Action Canada, and an Infrastructure Grant from the Canada Foundation for Innovation.

Data availability: All relevant data are reported within the manuscript.

Supplementary material: N/A.

3.0 PROJECT II

An Examination of Exercise Intensity and its Impact on the Acute Release of Irisin Across Obesity Status: A Randomized Controlled Crossover Trial[1]

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[1]The contents of this chapter are published in Applied Physiology, Nutrition, and Metabolism. Rioux BV, Paudel Y, Thomson AM, Peskett LE, Sénéchal M. An examination of exercise intensity and its impact on the acute release of irisin across obesity status: a randomized controlled crossover trial. Appl Physiol Nutr Metab. 2024;49(12):1712-1728. doi:10.1139/apnm-2024-0091

3.1 Abstract

Limited data exist regarding the impact of exercise intensity on irisin release and its association with insulin sensitivity in individuals of differing obesity status. The objective of this study was to investigate the impact of exercise intensity on the acute release of irisin in healthy weight individuals and individuals with obesity, and whether irisin release during acute exercise was associated with greater insulin sensitivity across obesity status. A randomized controlled crossover study was conducted on 26 non-overweight/obese (non-OW/OB) (BMI: 22.2±1.5 kg/m2) and 26 overweight/obese (OW/OB) (BMI: 33.9±6.5 kg/m2) adults who performed an acute bout of moderate-intensity continuous training (MICT), high-intensity interval training (HIIT), and rest. Irisin was quantified via ELISA and western blotting, and insulin sensitivity (Si) was estimated using the Matsuda index. OW/OB displayed a significantly lower level of circulating irisin and protein expression compared to non-OW/OB (p<0.01). Insulin sensitivity was positively correlated with irisin release during MICT and HIIT in non-OW/OB (all p<0.05), but not in OW/OB. Regarding irisin expression, non-OW/OB with high-Si had a 2.03-fold (p<0.05) increase during HIIT, while OW/OB with high-Si had only a 1.54-fold increase (p<0.05). These results suggest that irisin is released differently according to obesity status and varying exercise intensities. OW/OB individuals have a blunted irisin response to acute exercise and lower baseline irisin concentrations compared to non-OW/OB individuals. Although exercise stimulates irisin release in non-OW/OB individuals, only a greater exercise intensity stimulates irisin release in OW/OB individuals. These findings are clinically relevant, as irisin is associated with greater insulin sensitivity. This trial was registered at clinicaltrials.gov (NCT03514238).

Keywords: Obesity; Irisin; Myokines; Insulin Sensitivity; High-Intensity Interval Training; Exercise.

Take-home message:

- Obesity appears to blunt the irisin response to acute exercise, as only a greater exercise intensity stimulates irisin release in individuals with obesity.
- Irisin is associated with greater insulin sensitivity, supporting its role in glucose metabolism.

3.2 Introduction

Exercise is an essential tool used to prevent and manage several cardiometabolic diseases, including type 2 diabetes mellitus (T2DM), obesity, and cardiovascular disease [1], and has favourable impacts on many of their associated cardiometabolic complications [2]. As a result of continuous skeletal muscle contractions, muscular tissue undergoes a series of well-known adaptations [3] through an increase in metabolic demand and changes in molecular signalling. Although more progress has been made in the understanding of these adaptations in recent years [4], the mechanisms linking exercise to its health benefits remain poorly understood [5]. Myokines, signalling molecules that engage in interorgan crosstalk, appear to contribute to these adaptations and drive subsequent health benefits [6]. As skeletal muscle adaptations vary according to the mode, intensity, frequency, and duration of exercise [3], the magnitude of exercise-induced myokine release and their impact on metabolic health may also depend on the stimulus applied to the muscles.

Irisin, an exercise-induced adipo-myokine, is primarily secreted by skeletal muscle. Its release is regulated by peroxisome proliferator-activated receptor (PPAR) γ coactivator-1α (PGC-1α), which stimulates its precursor, fibronectin type III domain-containing protein 5 (FNDC5) [7]. Irisin plays an integral role in energy metabolism through various processes, including: the browning of adipose tissue and the subsequent increase in thermogenesis [7,8]; the stimulation of skeletal muscle mitochondrial biogenesis and oxidative metabolism; and the increase in glucose and fatty acid uptake in myocytes and adipocytes [9-12]. Initial reports revealed that circulating irisin increases following chronic aerobic training in both human and animal models [7]. However, further studies on the effect of chronic exercise on irisin, including both aerobic and resistance exercise training,

produced contradictory results [13-15]. A single acute bout of exercise, though, appears to be sufficient to trigger irisin release [16,17]; but, the extent to which irisin is released may depend on exercise intensity [18]. For instance, when comparing high-intensity exercise to low- or moderate-intensity exercise, acute-based studies generally report an enhanced irisin response with higher exercise intensities in healthy adult participants across age, sex, and fitness levels [9,19-21]. Conversely, in a meta-analysis of 16 trials examining irisin after acute moderate-intensity continuous training (MICT) or high-intensity interval training (HIIT) in healthy adults, Cosio et al. (2022) reported inconclusive results on the acute effect of HIIT on irisin concentrations, perhaps due to the limited amount of available studies investigating HIIT [22]. As such, the impact of exercise intensity on the change in irisin during an acute bout of exercise is understudied, especially in clinical populations. As the presence of chronic conditions like obesity and T2DM generally alters baseline irisin concentrations [23-25], these metabolic disorders may also cause a varied response to exercise-induced irisin. In a group of adults living with obesity, an acute bout of MICT had an equal impact on irisin release compared to HIIT during exercise and a greater impact after 50 minutes of rest post-exercise [26]. Furthermore, Norheim et al. (2014) compared the acute release of irisin in healthy adults to those who were both pre-diabetic and overweight and observed a diminished response in the pre-diabetic group [14]. No study has yet to assess the acute impact of exercise intensity on irisin according to obesity status and its association with insulin sensitivity.

Understanding the effect of acute exercise intensity on irisin release in obesity, and its relationship with insulin sensitivity, is essential given the therapeutic potential of irisin in the treatment of metabolic disorders. Therefore, the primary objective of this study was

to investigate the impact of HIIT and MICT on the acute release of irisin in individuals living with overweight/obesity and those with a healthy weight. Further, we aimed to explore whether irisin release during an acute bout of exercise was associated with greater insulin sensitivity among those of differing obesity status. We hypothesized that increased exercise intensity will generate a greater release of irisin in healthy weight individuals and that this change will be associated with greater insulin sensitivity.

3.3 Materials and Methods

Study Design and Participants

A total of 52 participants took part in this randomized crossover design trial: the Irisin and Metabolic Exercise Training Study (iMET Study; NCT03514238, clinicaltrials.gov). Participants were recruited on a voluntary basis from the community using social media platforms, newspaper/newsletters, family physician clinics, and community events. Participant inclusion criteria were: aged between 19-50 years old and had a body mass index (BMI) between 18.5-24.9 kg/m^2 (non-OW/OB) or above 28.0 kg/m^2 (OW/OB). Individuals with a BMI slightly below the conventional 30 kg/m^2 were included within the OB group considering that both overweight and obesity are associated with impaired cardiometabolic health outcomes and chronic conditions, such as T2DM [27,28]. Exclusion criteria for the participants involved: impaired glucose metabolism, including pre-diabetes or a previous diagnosis of type 2 diabetes; injuries or chronic conditions that could prevent exercise participation or impede performance; diabetes-related medications, or medications that impact heart rate; or a significant weight change over the last six months (≥10 kg). Additionally, participants with a BMI ≥28.0 kg/m^2 were excluded if they were considered physically active (≥150 minutes of moderate-to-vigorous physical activity

(MVPA) per week in 10-minute bouts) as measured by accelerometers. Finally, participants with a final glucose measurement above ≥11.1 mmol/L (200 mg/dL) at the end of a 75g oral glucose tolerance test (OGTT) (120 minutes) were excluded from the remainder of the study, as these values are indicative of glucose intolerance, which may impact the primary outcome measure, irisin [29,30]. For safety considerations, Canadian Society for Exercise Physiology (CSEP) Physical Activity Readiness Questionnaires (PAR-Q+) and Get Active Questionnaires [31] were administered. Adequate representation of males and females was ensured in the current study design, with comparable proportions of sex in both obesity status groups. Recruitment began in April 2018 and the trial was completed in August 2019 upon collection of all data of the number of participants required to meet our sample size calculation.

Overview of Protocol

An overview of the experimental design of the study is presented in Figure 7. Participants presented to the CELLAB for the initial baseline assessment. One week later, participants were randomized into one of the three conditions of acute MICT, HIIT, or a control session. The conditions were each separated by a minimum of 24 hours to allow plasma irisin to return to baseline concentrations. A third party used SPSS statistics version 22.0 (SPSS, Chicago, USA) to generate the random allocation sequence for the study with a 1:1:1 allocation ratio. Participants and research staff were blinded to interventions through concealment with envelopes until interventions were assigned. The study was approved by the University of New Brunswick Research Ethics Board (UNB-2017-105), and all protocols adhered to the general principles of the Declaration of Helsinki. Each participant provided written informed consent for their participation.

Outcome Measure: Change in Plasma Irisin Concentrations and Protein Expression

Blood samples were collected intravenously from the antecubital vein by a registered nurse before (0 minutes), during (14 and 28 minutes), and after (35 minutes) each acute condition. Blood was drawn into 3 mL Vacutainer collection tubes coated with an anticoagulant, ethylenediaminetetraacetic acid (EDTA), prior to being pipetted into 1.5 mL microcentrifuge tubes in duplicate in a biosafety cabinet (Cat# 1323TS, Thermo Fisher Scientific, Waltham, USA). Each microcentrifuge tube was then centrifuged at 1600g for 15 minutes (4°C) and the plasma was collected and stored at -80°C until further analysis.

Plasma irisin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) and protein expression was assessed with western blotting. Immunoassay was performed using a commercially available ELISA (Cat# DY9420-05, R&D Systems, Minneapolis, USA) and ancillary reagent kit (Cat# DY008B, R&D Systems, Minneapolis, USA) to allow the measurement of plasma irisin concentrations for each individual participant. Optical density was determined using an EPOCH 2 Microplate Spectrophotometer with Gen5 software (BioTek, Winooski, USA). Optical imperfections were corrected by subtracting the readings at 540nm from the readings at 450nm. Standard curves were generated with a four-parameter logistic curve-fitting method; irisin concentrations were then extrapolated from the optical density measurements (MyAssays, MyAssays Ltd., Brighton, UK). The first assay was performed in duplicate – with a coefficient of variation below 2.0% – while the subsequent assays were performed in singlets as previously performed in our laboratory [32].

Sub-samples were randomly selected within each insulin sensitivity categorization of the non-OW/OB and OW/OB groups for irisin protein expression analysis by gel

electrophoresis with western blot (n = 12). This measurement was included to provide further validation of the presence of irisin within our sample of participants to reinforce the certainty of our results in light of criticism surrounding irisin measurement and detection, as discussed in the limitations section of the manuscript. First, plasma samples were diluted 25 times with Type 1 water. Then, 4x Laemmli sample buffer (Cat# 1610747, Bio-Rad, Mississauga, Canada) was prepared (9:1) by adding dithiothreitol (DTT) into the diluted samples in a 1:3 buffer to plasma sample ratio, which were boiled at 99°C for 5 minutes and subsequently cooled on ice. Protein loading and transfer was performed using two methods: 1) 15 µl of diluted plasma samples and Precision Plus Protein All Blue Standards (Cat# 1610373, Bio-Rad, Mississauga, Canada) were loaded to 4-20% Mini-PROTEAN TGX stain-free gels (Cat# 4568096, Bio-Rad, Mississauga, Canada) and proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Cat# 1620177, Bio-Rad, Mississauga, Canada) using a Trans-Blot Turbo Transfer System (Cat# 1704150, Bio-Rad, Mississauga, Canada), and 2) 15 µl of diluted plasma samples and Precision Plus Protein Standards Kaleidoscope™ ladder (Cat# 1610375, Bio-Rad, Mississauga, Canada) were loaded to 12% acrylamide resolving gel and proteins were transferred to nitrocellulose membranes (Bio-Rad, Cat# 1620112) at 90V for 90min at 4°C using transfer buffer (Cat# 10026938, Bio-Rad, Mississauga, Canada). 1X Tris Buffer Saline Tween 20 (TBST) was used as the washing buffer (10X-TBS Cat# 1706435, Bio-Rad, Mississauga, Canada), diluted with Type 1 water and mixed well with Tween 20 (total concentration 0.05%) (Cat# 1610781, Bio-Rad, Mississauga, Canada). Membranes were then blocked with EveryBlot Blocking Buffer (Cat# 12010020, Bio-Rad, Mississauga, Canada) for 10 minutes at 60 rpm at room temperature and incubated overnight at 4°C with a rabbit anti-irisin polyclonal

antibody (dilution 1:2000 with blocking buffer) (Cat# AG-25B-0027B, AdipoGen, RRID:AB_2490459, San Diego, USA). Blots were washed 3 times with 1X TBST for 5 minutes each at 150 rpm. HRP-linked anti-rabbit IgG (Cat# 7074, Cell Signaling Technology, RRID:AB_2099233, Danvers, USA) was used as a secondary antibody and was incubated for 2 hours at room temperature (dilution 1:15000 with blocking buffer). Blots were washed again 3 times with 1X TBST for 5 minutes each at 150 rpm. Bands were then visualized with Clarity Max ECL Western Blotting Substrate (Cat# 1705062, Bio-Rad, Mississauga, Canada) via chemiluminescence. Densitometric analysis was performed using Image Lab software version 6.1 (Bio-Rad, Mississauga, Canada).

Outcome Measure: Insulin Sensitivity

Insulin sensitivity was estimated from the insulin and glucose values obtained from an OGTT performed during the initial baseline testing visit, following a 12-hour fasting period. Blood draws were obtained immediately prior to consuming the standard 75g oral glucose load (Cat# 401009P, Thermo Fisher Scientific, Waltham, USA), and at 30, 60, and 120 minutes. A registered nurse collected the samples intravenously from the antecubital vein with tubes coated with lithium heparin. Once blood samples were centrifuged (15 minutes, 2000g, 4°C), plasma was collected, aliquoted, and then stored at -80°C until further analysis was performed. Insulin concentrations were quantified by ELISA (Cat# 90095, Crystal Chem, Elk Grove Village, USA) and glucose was assessed by a glucose assay kit (Cat# 88873, Crystal Chem, Elk Grove Village, USA) according to the manufacturer's protocols.

The Matsuda index uses the insulin and glucose concentrations derived from the OGTT to provide an approximation of whole-body insulin sensitivity, combining both

hepatic and peripheral tissue sensitivity to insulin [33]. The four-point insulin sensitivity index is calculated as follows: 10,000/√([fasting glucose x fasting insulin] x [mean glucose x mean insulin]). In addition to ELISA, a glucometer was used during the testing procedure to obtain real-time glucose measurements from blood drawn intravenously (OneTouch Ultra2, LifeScan, Malvern, USA) to determine study eligibility.

Exposure Variable: Acute Bouts of Exercise and Control (Rest)

Each participant performed, in a random order, three acute testing sessions: 1) MICT, 2) HIIT, and 3) a control session. Exercise sessions were completed on a Velotron cycle ergometer (SRAM, Chicago, USA). Due to the potential effect of food and exercise on irisin concentrations in the acute phase [16,34], participants were asked to avoid eating food or drinking caffeine two hours prior to exercise, and to refrain from exercising within 24 hours of the session.

The MICT exercise session involved 35 minutes of cycling at 50-55% heart rate reserve (HRR). During HIIT, after every five minutes of cycling at 50-55% HRR, intensity was increased to 85-90% HRR for two-minute intervals for a total of 35 minutes. During the control session, participants remained seated in a reclined position for 35 minutes. Blood samples were drawn at the beginning of the session (pre-exercise), during the exercise, and immediately after (post-exercise), corresponding to T0, 14, 28, and 35. For HIIT, samples were obtained at the end of each interval, reflecting peak intensity of the interval.

To calculate the target HR range for each exercise session, the following formula was used: target HR = (HRR = (HR_{max} - HR_{rest}) x % + HR_{rest}). HR_{max} was taken from the peak HR value obtained during the cardiorespiratory fitness test and HR_{rest} was taken

during the baseline assessment session (average measurement of two readings after remaining seated for 5 and 10 minutes). The workload (watts (W)) needed to reach the target HR range (MICT: 50-55%, HIIT: 85-90%) for the MICT and HIIT sessions was also determined by the cardiorespiratory fitness test. The wattage used during the stage of the cardiorespiratory fitness test in which the participants reached the HR needed for the target HR was selected as the workload to be used for the MICT and HIIT sessions. Participants were instructed to maintain a cadence of 80-100 rpm. If the participants did not meet the target HR while in this rpm range, the workload was adjusted accordingly.

Exploratory Variables

Participants self-reported their age, sex, and race to our research staff. Anthropometric measures, body composition, cardiorespiratory fitness, metabolic profile, and physical activity levels were analyzed for exploratory purposes and to describe the sample.

Anthropometric Measures and Body Composition

BMI was calculated from height and body weight measurements (BMI = kg/m^2). Height and weight were recorded using a stadiometer and calibrated column scale to the nearest 0.5 cm and 0.1 kg, respectively (SECA707, SECA, Mt. Pleasant, USA) [31]. Waist circumference was measured at the upper lateral border of the iliac crest at the end of a normal expiration [31]. The average of two measurements was recorded, to the nearest 0.5 cm, with an anthropometric measuring tape. Participant height, weight, and waist circumference were performed in accordance with the CSEP protocols [31]. The BODPOD was used to estimate participant body mass, body fat, and fat free mass via air displacement plethysmography (COSMED, Concord, USA). The BODPOD involves highly reliable and

valid measurements with minimal error, reported as 3.5% and 0.8% for body fat percentage and fat free mass, respectively, in our laboratory [35].

Lipid Profile

Finger-prick capillary whole blood samples were collected and analyzed using the Cholestech LDX Analyzer (Abbott, Abbott Park, USA). Samples were collected in a LDX lithium heparin 40 µl capillary tube and dispensed into a cassette reader. Total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, non-HDL cholesterol and LDL/HDL cholesterol were recorded in mmol/L. Blood samples were obtained while in a fasted state during the baseline assessment session. The Cholestech LDX Analyzer has been found to be a suitable method to rapidly assess lipids when compared to laboratory methods [36].

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed using a modified Balke-Ware maximal oxygen consumption (VO_{2max}) test protocol on the cycle ergometer [37]. After a five-minute warm-up stage at 50W, the workload increased by 25W each minute until volitional fatigue. Participants maintained a consistent cadence of 80-90 rpm throughout the duration of the test. Gas exchange was continuously recorded on the TrueOne 2400 Metabolic Cart (ParvoMedics, Salt Lake City, USA) to measure VO_{2peak}, which was identified as the highest oxygen consumption reached over a 5-second average during the last 30 seconds of the test. HR measurements were recorded with the Zephyr BioHarness 3.0 and analyzed with OmniSense Software (Medtronic, Dublin, Ireland). The cardiorespiratory fitness test also required that participants refrained from exercise, smoking, eating, and drinking (including caffeine and alcohol) for 12 hours before the test. However, for safety concerns,

participants were instructed to eat a light snack to break their 12-hour fast prior to the maximal exercise test.

Physical Activity Levels

Participant physical activity levels were directly assessed through tri-axial accelerometry. An ActiGraph GT3X accelerometer (ActiGraph, Pensacola, USA) was worn on their right hip for seven consecutive days during waking hours. To be included in the analysis, a minimum of four valid days – with at least 10 hours of wear time – was required. Values were imputed to a seven day sum and average, to reflect a full week of activity. Data was analyzed using the ActiLife Software version 5 (ActiGraph, Pensacola, USA). Physical activity intensity was quantified by activity counts, categorized into validated age-specific cut-points by Freedson et al. (1998) for sedentary, light, moderate, and vigorous activity [38].

Statistical Analysis

Descriptive statistics were performed stratified by obesity status (non-OW/OB: BMI 18.5-24.9 kg/m^2 vs. OW/OB: BMI ≥28.0 kg/m^2) and insulin sensitivity (low-Si [<50^{th} percentile] vs. high-Si [>50^{th} percentile]) to present baseline characteristics. Continuous and categorical variables are presented as mean ± standard deviation or median (25^{th}-75^{th} percentiles) and n (%), with group comparisons performed using independent samples t-tests. Pearson correlations were performed to examine the association between insulin sensitivity and irisin concentrations, as well as with anthropometric, fitness, and metabolic variables. Total irisin area under the curve (AUC) was measured using the trapezium method for each individual and each condition, both unadjusted and adjusted for baseline values (Le Floch et al., 1990). A one-way ANOVA was performed to compare AUC

conditions and obesity status. Additionally, to determine the acute effect of exercise on irisin concentrations, and if differences were due to obesity status, repeated measures ANOVAs were performed for each condition. For these tests, the assumption of normality was met, Greenhouse-Geisser sphericity corrections were applied, and post-hoc analyses corrected for multiple comparisons were conducted. A power analysis calculation was performed to quantify the sample size required for a repeated measures ANOVA. Assuming a power of 0.80 with an effect size of 0.30 and an alpha level of 0.05, a total sample size of 18 participants was required for each obesity status group. However, as exercise interventions typically observe a 30 to 40% dropout rate, this proportion of dropouts was accounted for in this calculation by recruiting 8 additional participants per group. Due to sample size considerations, the main results of our analysis using the ELISA (n = 52) were for systemic irisin concentrations, while the main results of our analysis using western blot (n = 12) were for irisin protein expression and served as additional validation of our data. The significance level was accepted at $p < 0.05$, and analyses were performed using IBM SPSS statistics version 22.0 (SPSS, Chicago, USA).

3.4 Results

Participant Flow

As demonstrated in Figure 6, initially, 178 participants were screened over the phone after expressing interest in the study. After applying the exclusion criteria, 42 participants were excluded, and 69 declined due to personal reasons. Therefore, 67 participants were eligible and completed the first visit at the Cardiometabolic Exercise and Lifestyle Laboratory (CELLAB) at the University of New Brunswick (female n = 41; male n = 26). From this point on, 10 individuals were considered dropouts, as they wished to

terminate their participation in the study due to personal reasons or discomfort with intravenous blood draws (female n = 8; male n = 2). Further, 2 were excluded due to high physical activity levels (female n = 0; male n = 2), and 1 was excluded due to a 2-hour glucose value ≥11.1 mmol/L (200 mg/dL) in the OGTT (female n = 1; male n = 0). Although 54 participants completed the study (female n = 32; male n = 22), 2 participants were excluded due to missing data of the primary outcome measure (female n = 2; male n = 0). As such, 52 participants were included in the final study sample (female n = 30; male n = 22).

General Characteristics

Tables 3.1 and 3.2 demonstrate baseline characteristics of the sample according to obesity status and insulin sensitivity. OW/OB individuals were older (OW/OB: 37.8 ± 8.1 vs. non-OW/OB: 30.0 ± 8.7 years, $p < 0.01$), had a higher waist circumference and body fat percentage (all $p < 0.001$), a lower cardiorespiratory fitness ($p < 0.001$), and took fewer steps each day ($p < 0.01$) than non-OW/OB individuals. Non-OW/OB individuals displayed a significantly healthier metabolic profile than OW/OB in terms of triglycerides, HDL cholesterol, LDL/HDL cholesterol, and insulin sensitivity (all $p < 0.05$). When grouped by low- and high-Si, age remained significantly different between non-OW/OB and OW/OB individuals ($p < 0.05$). No significant differences in body composition, fitness, or metabolic profiles were observed between low- and high-Si groups in non-OW/OB nor OW/OB individuals (all $p > 0.05$), with the exception of fasting glucose in OW/OB (low-Si: 5.3 ± 0.4 vs. high-Si: 4.9 ± 0.4 mmol/L, $p = 0.03$).

Association between Insulin Sensitivity and Body Composition, Fitness, and Irisin Release

Table 5 shows that insulin sensitivity was negatively associated with BMI (r = -0.40, p = 0.003), weight (r = -0.39, p = 0.005), waist circumference (r = -0.41, p = 0.003), body fat percentage (r = -0.30, p = 0.029) and fat mass (r = -0.37, p = 0.008). Insulin sensitivity was positively associated with the amount of time spent each week in MVPA (r = 0.36, p = 0.008) and the number of steps taken each day (r = 0.53, p < 0.001). Insulin sensitivity was also positively correlated with irisin concentrations during MICT at baseline and after 28 minutes of exercise, and after 35 minutes of rest in the control condition (all p < 0.05). When stratifying non-OW/OB and OW/OB individuals into low- and high-Si groups (Table 6), only non-OW/OB individuals with high-Si displayed a significant association between irisin concentrations and insulin sensitivity in all conditions (p < 0.05). During MICT, a significant dose-response association was observed between irisin concentrations and insulin sensitivity in the non-OW/OB high-Si group: T0 (r = 0.57, p = 0.051), T14 (r = 0.68, p = 0.016), T28 (r = 0.74, p = 0.006), and T35 (r = 0.77, p = 0.004). Additionally, a similar but non-significant (except for T28) trend was observed during HIIT for the association between insulin sensitivity and irisin concentrations at baseline – T0 (r = 0.54, p = 0.073), and after T14 (r = 0.56, p = 0.058), T28 (r = 0.60, p = 0.041), and T35 (r = 0.42, p = 0.171) minutes in the non-OW/OB high-Si group. At rest, non-OW/OB high-Si individuals displayed a strong correlation between irisin concentrations and insulin sensitivity (all p < 0.05). In non-OW/OB low-Si individuals and all OW/OB individuals (of either low- or high-Si), irisin concentrations were not associated with insulin sensitivity in any of the experimental conditions (all p > 0.05).

Impact of Time and Obesity Status on Irisin Release and Changes According to Insulin Sensitivity

Changes in plasma irisin concentration over time during MICT, HIIT, and control according to obesity status are shown in Figure 8. At baseline, irisin concentrations were significantly lower in OW/OB individuals compared to non-OW/OB ($p < 0.01$). Results from repeated measures ANOVAs demonstrated an effect of time on the acute release of irisin concentrations during both MICT ($F(3) = 6.263$, $p = 0.003$) and HIIT ($F(3) = 11.015$, $p < 0.001$) exercise sessions. Post-hoc analyses corrected for multiple comparisons revealed significant differences between irisin at T0 and T14 for both MICT and HIIT sessions, as well as between T0 and T28, between T14 and T35, and between T28 and T35 during HIIT sessions (all significant at $p < 0.05$). Furthermore, there was an effect of obesity status observed in all experimental conditions with non-OW/OB individuals displaying significantly higher irisin concentrations than OW/OB (all $p < 0.05$). A significantly greater total AUC was observed in non-OW/OB compared to OW/OB individuals in the control ($p < 0.001$), MICT ($p = 0.001$), and HIIT conditions ($p = 0.002$). However, when adjusting for baseline differences, no significant differences were observed between obesity group or condition (all $p > 0.05$).

In terms of irisin protein expression, non-OW/OB individuals with low-Si had a 1.58-fold (Figure 9; $p < 0.01$) increase in irisin expression between pre- and post-exercise during HIIT. Non-OW/OB with high-Si had a 2.03-fold ($p < 0.05$) increase during HIIT – with peak expression observed at T14. During MICT, irisin protein expression was raised in non-OW/OB individuals by 1.26-fold in high-Si and 1.73-fold in low-Si individuals at T28 (all $p < 0.05$). OW/OB individuals only significantly increased irisin expression during

HIIT, such that OW/OB with low-Si and OW/OB with high-Si had a 1.24 and 1.54 -fold increase in irisin at T35, respectively (Figure 10; all $p < 0.05$) [2].

3.5 Discussion

The purpose of this study was two-fold: 1) to assess the impact of acute HIIT and MICT exercise according to obesity status; and 2) to explore whether the acute release of irisin was associated with insulin sensitivity. First, we report that individuals with overweight/obesity have lower circulating irisin concentration at baseline as compared to those with a healthy weight. Furthermore, irisin concentration is affected by acute exercise with a decrease at the onset of exercise followed by an increase over time, regardless of obesity status. However, our data suggest an increase in irisin protein expression with acute exercise, with a greater response in healthy weight individuals. Second, irisin release during acute exercise is associated with greater insulin sensitivity, but mostly in those with a healthy weight. Together, these data demonstrate that overweight/obesity appears to blunt the irisin response to acute exercise and the relationship between irisin and insulin sensitivity. Moreover, from a clinical standpoint, our data highlight that individuals with overweight/obesity may need to perform more intense exercise to stimulate irisin, as significant increases in irisin protein expression were only seen during HIIT exercise and not MICT. Therefore, these findings could have clinical implications for obesity management and treatment by dictating the optimal exercise prescription for irisin release. This is not trivial, considering the therapeutic potential of irisin in the treatment of obesity

[2] Whole-blot images of plasma irisin around 24 kDa provided in supplementary files, representing the unprocessed and uncropped blots of Figures 9 and 10.

and prevention of T2DM. Altogether, the results of this randomized crossover trial provide further insight into the acute impact of exercise on irisin release according to obesity status and its relation to insulin sensitivity.

The presence of chronic conditions that alter glucose metabolism can impact basal rates of irisin concentrations [23-25]. For instance, we report here that individuals with overweight/obesity present with lower baseline irisin concentrations and expression compared to their healthy weight counterparts. Furthermore, we observed a significant inverse association between BMI and circulating irisin concentration at baseline in each condition (r = -0.36 to -0.29, all $p < 0.05$). These findings are similar to those of both Makiel et al. (2023) and Ebert et al. (2016) who observed negative associations between circulating irisin concentrations and body fat in humans [25,39], and Khalafi et al. (2020) who reported reduced plasma irisin concentrations as BMI increased in rats [40]. On the contrary, other studies reported positive associations between irisin and measures of adiposity, including BMI [41-43]. Some postulate that elevated irisin concentrations in obesity may reflect a compensatory response to obesity-related glucose metabolism dysfunction – known as 'irisin resistance' – in order to maximize the anti-hyperglycemic effects of the adipo-myokine [43-45]. However, the group of individuals with overweight/obesity in our sample did not have impaired glucose metabolism (as reflected by a Matsuda Index ≥2.5 [46]), which may perhaps explain the lack of elevated irisin in this group. If they did have impaired glucose metabolism, baseline irisin levels would likely have been higher [47]. As previously suggested [44], when individuals with obesity progress to T2DM, irisin secretion may begin to decline. In our sample of participants with overweight/obesity, it is possible that the secretory failure of irisin is caused by chronic inflammation, which is well known

to affect those with excess adiposity [48]. Chronic inflammation is associated with increased macrophage infiltration into adipocytes, which may be responsible for the secretion of both pro- and anti-inflammatory cytokines [49]; the abundance and secretion of some anti-inflammatory adipokines is reduced in obesity [50,51], which may be the case for irisin.

Studies by our group [16,52,53] and others [22] clearly establish that acute exercise triggers the release of irisin in healthy adults and animals. While some studies show no acute effect of exercise on irisin in individuals with obesity [9,54], the present data demonstrate that acute exercise (MICT and HIIT) raises both irisin concentration and protein expression in all participants, regardless of obesity status. However, our data does show that the response may be blunted in individuals with overweight/obesity, as healthy weight individuals have a greater response. This finding is supported by a meta-analysis from our group that revealed that those with a higher BMI had a 7.7% (95% CI: −14.8% to −0.6%) lower irisin concentration following an acute bout of exercise compared to those with a normal weight [16]. It is important to consider that the magnitude of this response appears to be modified by the intensity of exercise performed, especially in individuals with overweight/obesity. We show here that the increase in irisin protein expression measured by western blotting is significantly greater during HIIT exercise than during MICT in healthy weight individuals; however, in individuals with overweight/obesity, significant increases in irisin protein expression are only observed during HIIT exercise and not during MICT. This may be clinically relevant, as it suggests that individuals with overweight/obesity may need to perform higher intensities of exercise to release irisin, which helps to guide weight and/or glucose management in this population. These data

align with previous literature suggesting that a greater exercise intensity leads to enhanced irisin release [9,19-21].

Other biological and training-related factors may have impacted our findings. For instance, some data suggest that baseline irisin may differ according to age [9,55]; however, evidence for a varied acute exercise response due to aging is scarce. Some suggest that a difference in basal irisin may not translate to a difference in acute response [9,32,56]. For instance, Huh et al. (2014) reported that the change in irisin release during acute aerobic exercise was not associated with age, despite differences in baseline irisin concentrations [9]. Data from our group also demonstrated that age was not associated with a different irisin response to an acute bout of circuit training [32]. Additionally, while some report differences in irisin release between males and females, the effect of sex is unclear [57]. In accordance with these findings, the current analysis observed no sex differences in the acute irisin response in neither obesity status group ($p < 0.05$; results not shown). Furthermore, we [16] and others [20] have shown that irisin release may be impacted by training-related factors. Although cardiorespiratory fitness has been shown to be the best predictor of post-exercise irisin concentrations [16], we observed no association between cardiorespiratory fitness and the change in irisin during exercise (MICT: $r = -0.08$; $p = 0.570$; HIIT: $r = -0.04$; $p = 0.766$; data not shown). Furthermore, irisin release did not appear to be affected by the volume of exercise performed, as others have reported [20], as no association was present between the average watts of each session and the change in irisin (MICT: $r = 0.054$, $p = 0.702$; HIIT: $r = -0.041$, $p = 0.776$; data not shown). As such, we believe that our data reflect the effect of training only and that the volume of exercise between conditions did not impact irisin release.

From a clinical standpoint, the physiological relevance of irisin is highlighted by its therapeutic potential in the prevention and treatment of T2DM. For instance, irisin stimulation enhances insulin sensitivity, resulting in increased glucose uptake in both human and animal models through several mechanisms, including enhanced mitochondrial function, reduced inflammation, and attenuated obesity [58]. The present analysis adds to the literature by confirming a link between acute exercise-induced irisin release and greater insulin sensitivity. When stratifying by obesity status, only the higher insulin sensitivity group in those with a healthy weight displayed significant positive associations between irisin release and insulin sensitivity during acute HIIT and MICT. In individuals with overweight/obesity, no significant relationship was observed between irisin release and insulin sensitivity. These findings suggest that individuals with overweight/obesity, as well as healthy weight individuals with weaker insulin sensitivity, have a blunted response to exercise-induced irisin release, which may be explained by impairments in glucose uptake. During exercise, glucose uptake – which is primarily attributed to GLUT4 translocation [59] – drastically increases as a result of irisin treatment, as reported in several *in vitro* experiments [9,12]. In differentiated L6 muscle cells (a murine model), recombinant irisin treatment induced the translocation of GLUT4 to the plasma membrane, and subsequent glucose uptake, through the activation of the AMPK and p38 MAPK pathways [12]. Importantly, AMPK activation has been shown to be impaired in individuals with obesity and insulin resistance [60,61] and in obese and diabetic animal models [62,63]. Consequently, a blunted AMPK response, and downstream impairment in GLUT4 expression and glucose uptake, may explain the diminished irisin response observed in our sample of those with overweight/obesity and normal weight individuals with weaker insulin sensitivity.

Nevertheless, GLUT4 expression can be increased through exercise, even in the presence of metabolic dysfunction. For instance, although individuals with T2DM present with reduced GLUT4 expression [64,65] and lower baseline irisin levels [24], exercise may enhance glucose metabolism through a change in these molecules. In fact, data shows that acute HIIT can increase GLUT4 protein content by as much as 369% in individuals with T2DM [66]; however, no data on irisin were presented in this study. Therefore, further studies should address the mechanistic link between irisin release, GLUT4 expression, and AMPK activation during exercise in order to further elucidate the antihyperglycemic and anti-obesity effects of this adipo-myokine.

Limitations & Strengths

The current study has some limitations that should be acknowledged. First, the current analysis assessed irisin during and only immediately after acute exercise. Previous studies have shown that irisin release may continue to rise in the resting state following exercise [26]; therefore, it is possible that the true peak irisin concentrations were not captured. This may also account for the lack of difference between the exercise bouts and the control session. Second, although participants were asked to consume the same food before each condition, nutritional information was not recorded in our study, which could have potentially interfered with irisin concentrations [34]. However, others have demonstrated that there is no postprandial influence on circulating irisin concentrations [55,67], and participants refrained from food intake and caffeine consumption for two hours prior to exercise. Finally, concerns with the detection and measurement of irisin are well-known [68,69], including the validity of commercially available ELISA kits and the specificity of irisin antibodies. Some of these concerns were addressed by the current study, as: 1) the

use of the western blotting technique, which reinforced the certainty of our results, allowed the detection of irisin at a known molecular weight of 24 kDa; and 2) the antibody we used for western blotting has been previously validated by Jedrychowski et al. (2015), who went on to confirm the presence of irisin in human plasma using tandem mass spectrometry [70]. Investigating irisin protein expression via western blotting using human blood plasma strengthens our study and adds to the limited amount of literature that has analyzed irisin with human plasma or serum with immunoblotting [7,55,70-73]. Furthermore, another strength of our study is the use of the Matsuda index as a measure of insulin sensitivity, as it is strongly correlated with the gold standard measure of insulin sensitivity, the euglycemic insulin clamp ($r = 0.73, p < 0.0001$) [33]. Finally, the present study is strengthened by a robust research design, with an experimental control condition where each participant acted as their own control.

Conclusion

Due to its physiological role in metabolism, exercise-induced irisin release has the potential to both prevent and treat chronic conditions, like obesity and T2DM, and their related cardiometabolic consequences. From an acute perspective, we demonstrate that irisin release is associated with greater insulin sensitivity, especially in healthy weight individuals. In comparison, individuals with overweight/obesity (who have lower levels of circulating irisin concentrations at baseline) appear to have a blunted response to exercise-induced irisin release. Increasing the intensity of exercise may lead to a substantially improved irisin protein expression response in healthy weight individuals and may result in a significant irisin protein expression response in those with overweight/obesity. Consequently, individuals with overweight/obesity appear to need a greater stimulus of

exercise in order to induce irisin release, especially in regard to protein expression. The findings presented here suggest that exercise prescription should involve a greater workload in this population in order to reap the many health benefits associated with exercise-induced irisin release. Further investigation into the effect of HIIT on irisin release in individuals with overweight/obesity is warranted.

3.6 References

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3.7 Tables and Figures

Table 5. Descriptive characteristics of the sample according to obesity status.

	Non-OW/OB Group (n = 26)	OW/OB Group (n = 26)	*p*
Age (years)	30.0 ± 8.7 28.5 (22.8-36.3)	37.8 ± 8.1 37.5 (32.3-46.5)	**0.001**
Female (%)	18 (69.2)	16 (61.5)	0.583
Weight (kg)	67.7 ± 9.6 67.2 (59.1-76.1)	101.8 ± 18.5 99.3 (89.4-112.6)	**<0.001**
Body Mass Index (kg/m^2)	22.2 ± 1.5 22.2 (21.3-23.5)	33.9 ± 6.5 32.2 (29.8-34.2)	**<0.001**
Waist Circumference (cm)	81.4 ± 7.2 81.3 (76.6-86.4)	112.9 ± 14.4 110.5 (102.7-117.3)	**<0.001**
Body Fat (%)	20.8 ± 8.6 19.7 (12.1-29.9)	41.2 ± 10.6 42.0 (32.8-50.4)	**<0.001**
Fat Mass (kg)	13.8 ± 5.7 13.2 (8.5-18.2)	42.5 ± 16.7 37.7 (30.9-46.6)	**<0.001**
Fat Free Mass (kg)	53.8 ± 10.7 48.8 (45.5-63.1)	59.1 ± 12.6 56.9 (49.7-69.2)	0.106
Relative VO_{2max} (ml/kg/min)	38.5 ± 8.7 37.9 (31.5-46.6)	24.7 ± 5.8 25.0 (19.5-29.1)	**<0.001**
MVPA 10-min bouts (mins/week)	144.3 ± 136.7 105.3 (40.5-198.4)	47.8 ± 40.6 46.5 (7.8-90.9)	**0.002**
Steps (per day)	8628.3 ± 2909.2 7548.4 (6509.9-9791.1)	6349.7 ± 1759.8 6099.5 (4846.2-7853.1)	**0.001**
Triglycerides (mmol/L)	0.9 ± 0.4 0.8 (0.6-1.2)	1.3 ± 0.7 1.1 (0.9-1.5)	**0.030**
HDL Cholesterol (mmol/L)	1.6 ± 0.4 1.6 (1.3-2.0)	1.2 ± 0.3 1.2 (0.9-1.5)	**0.001**
LDL Cholesterol (mmol/L)	2.7 ± 0.7 2.6 (2.3-3.3)	3.0 ± 0.7 2.9 (2.4-3.7)	0.239
LDL/HDL Cholesterol (mmol/L)	1.9 ± 0.7 1.7 (1.4-2.6)	2.7 ± 1.4 2.3 (1.9-3.0)	**0.015**
Fasting Glucose (mmol/L)	4.9 ± 0.5 4.9 (4.6-5.3)	5.1 ± 0.4 5.1 (4.8-5.4)	0.241
OGTT-0 Glucose (mmol/L)	5.5 ± 1.9 5.5 (4.4-6.3)	5.2 ± 1.2 5.3 (4.5-5.9)	0.460
OGTT-30 Glucose (mmol/L)	9.2 ± 2.9 9.2 (6.9-11.0)	8.3 ± 2.2 7.5 (6.7-9.8)	0.241
OGTT-60 Glucose (mmol/L)	7.7 ± 2.7 7.5 (5.7-9.2)	8.3 ± 2.0 8.2 (6.3-10.1)	0.408
OGTT-120 Glucose (mmol/L)	5.9 ± 2.0 5.7 (4.2-6.9)	6.3 ± 1.7 6.3 (4.8-7.6)	0.357
Insulin Sensitivity (MI)	39.1 ± 26.2 36.5 (17.4-56.3)	21.3 ± 24.1 15.4 (8.5-20.3)	**0.014**

Note: Continuous data are presented as mean ± standard deviation and median (25^{th}-75^{th} percentile), while categorical variables are presented as n (%). Non-OW/OB: non-overweight/obese group; OW/OB: overweight/obese group; VO_{2max}: maximal oxygen consumption; MVPA: moderate-to-vigorous physical activity; HDL: high-density lipoprotein; LDL: low-density lipoprotein; OGTT: oral glucose tolerance test; MI: Matsuda index. Significant change represented by $p < 0.05$.

Table 6. Baseline characteristics according to obesity status, grouped by insulin sensitivity.

	Non-OW/OB Group (n = 26)			OW/OB Group (n = 26)		
	Low Si (*<50th percentile)* (n = 13) Si = 18.1 ± 7.9	High Si (*≥50th percentile)* (n=13) Si = 60.0 ± 20.5	*p*	Low Si (*<50th percentile)* (n=13) Si = 8.9 ± 3.7	High Si (*≥50th percentile)* (n=13) Si = 33.6 ± 29.4	*p*
Age (years)	25.0 ± 7.2 23.0 (21.5-25.0)	34.9 ± 7.4 34.0 (31.0-39.5)	**0.002**	34.6 ± 8.3 33.0 (27.0-41.0)	41.0 ± 6.7 38.0 (35.0-49.0)	**0.041**
Female (%)	6 (46.2)	8 (61.5)	0.452	8 (61.5)	8 (61.5)	0.500
Weight (kg)	70.6 ± 10.4 69.6 (62.0-80.5)	64.8 ± 8.1 66.5 (56.4-70.3)	0.128	104.5 ± 18.1 99.6 (89.0-117.2)	99.2 ± 19.3 99.1 (83.6-111.9)	0.471
Body Mass Index (kg/m^2)	22.6 ± 1.4 22.4 (21.6-23.7)	21.8 ± 1.6 22.0 (20.7-22.7)	0.241	35.6 ± 7.3 32.6 (31.7-37.9)	32.3 ± 5.5 31.0 (28.5-33.8)	0.206
Waist Circumference (cm)	82.3 ± 7.3 83.0 (77.3-87.6)	80.5 ± 7.3 80.5 (74.6-85.2)	0.531	116.7 ± 16.2 113.8 (104.8-124.1)	109.0 ± 11.7 109.0 (98.0-111.9)	0.173
Body Fat (%)	19.6 ± 9.3 16.4 (11.8-27.9)	21.9 ± 8.1 21.9 (14.5-30.1)	0.499	42.6 ± 11.3 43.9 (31.7-50.8)	39.8 ± 10.1 38.7 (34.4-46.7)	0.517
Fat Mass (kg)	13.5 ± 5.9 12.3 (8.0-17.7)	14.2 ± 5.8 14.5 (8.5-19.1)	0.763	45.0 ± 17.8 42.0 (33.0-55.9)	39.9 ± 15.8 33.0 (29.4-47.0)	0.206
Fat Free Mass (kg)	13.5 ± 5.9 12.3 (8.0-17.7)	50.7 ± 8.4 47.9 (45.3-60.3)	0.143	58.7 ± 10.8 57.4 (50.4-69.9)	59.6 ± 14.7 56.4 (47.1-67.5)	0.448

Relative VO_{2max} (ml/kg/min)	39.0 ± 7.8 39.6 (32.2-46.3)	38.0 ± 9.8 36.0 (29.5-46.6)	0.765	23.9 ± 6.0 24.5 (18.0-26.7)	25.5 ± 5.6 27.0 (20.0-29.7)	0.479
MVPA 10-min bouts(mins/week)	127.8 ± 159.0 72.0 (38.6-150.8)	160.8 ± 114.3 144.7 (59.2-262.8)	0.549	37.5 ± 40.3 21.5 (0.0-74.0)	58.1 ± 39.7 58.5 (21.4-91.1)	0.203
Steps (per day)	7766.8 ± 3249.82 6972.0 (5973.5-8137.9)	9489.9 ± 2337.3 9440.4 (7264.4-12007.2)	0.134	5733.0 ± 1837.1 5837.0 (3849.9-7538.1)	6966.3 ± 1501.1 6159.6 (5865.9-8497.5)	0.073
Triglycerides (mmol/L)	1.1 ± 0.5 1.1 (0.7-1.2)	0.8 ± 0.3 0.8 (0.6-0.9)	0.135	1.5 ± 0.8 1.3 (1.0-1.7)	1.1 ± 0.5 0.9 (0.7-1.3)	0.142
HDL Cholesterol (mmol/L)	1.6 ± 0.5 1.8 (1.0-2.0)	1.6 ± 0.3 1.5 (1.4-1.8)	0.755	1.2 ± 0.3 1.1 (0.9-1.5)	1.3 ± 0.4 1.3 (1.0-1.5)	0.608
LDL Cholesterol (mmol/L)	2.8 ± 0.6 2.6 (2.4-3.3)	2.7 ± 0.8 2.6 (1.9-3.4)	0.800	3.0 ± 0.8 3.0 (2.5-3.5)	2.9 ± 0.7 2.9 (2.2-3.7)	0.717
LDL/HDL (mmol/L)	2.1 ± 0.8 1.7 (1.5-2.7)	1.7 ± 0.6 1.6 (1.1-2.4)	0.302	2.8 ± 1.4 2.4 (1.9-3.0)	2.7 ± 1.4 2.2 (1.8-3.1)	0.836
Fasting Glucose (mmol/L)	5.0 ± 0.6 4.9 (4.5-5.3)	4.9 ± 0.5 4.9 (4.6-5.2)	0.592	5.3 ± 0.4 5.2 (5.0-5.6)	4.9 ± 0.4 5.1 (4.6-5.2)	**0.032**

Note: Low- and high-Si are categorized as below or above the 50th percentile of insulin sensitivity based on the Matsuda index. Continuous data are presented as mean ± standard deviation and median (25th-75th percentile), while categorical variables are presented as n (%). Non-OW/OB: non-overweight/obese group; OW/OB: overweight/obese group; Si: insulin sensitivity; VO_{2max}: maximal oxygen consumption; MVPA: moderate-to-vigorous physical activity; HDL: high-density lipoprotein; LDL: low-density lipoprotein. Significant change represented by $p < 0.05$.

Table 7. Associations between insulin sensitivity and anthropometric measures, physical activity, and irisin in the whole sample.

Insulin Sensitivity		Insulin Sensitivity	
Age (years)	0.18 (0.204)	Irisin Control T0	0.15 (0.304)
Sex	-0.06 (0.678)	Irisin Control T14	0.12 (0.381)
Weight (kg)	**-0.39 (0.005)**	Irisin Control T28	0.16 (0.272)
Body Mass Index (kg/m^2)	**-0.40 (0.003)**	Irisin Control T35	**0.30 (0.032)**
Waist Circumference (cm)	**-0.41 (0.003)**	Irisin MICT T0	**0.46 (0.001)**
Body Fat (%)	**-0.30 (0.029)**	Irisin MICT T14	0.26 (0.065)
Fat Mass (kg)	**-0.37 (0.008)**	Irisin MICT T28	**0.31 (0.028)**
Fat Free Mass (kg)	-0.12 (0.375)	Irisin MICT T35	0.16 (0.252)
Relative VO_{2max} (ml/kg/min)	0.22 (0.112)	Irisin HIIT T0	0.13 (0.356)
Relative VO_{2max} (ml/FFM/min)	0.04 (0.755)	Irisin HIIT T14	0.25 (0.069)
MVPA 10-min bouts (mins/week)	**0.36 (0.008)**	Irisin HIIT T28	0.22 (0.118)
Steps (per day)	**0.53 (<0.001)**	Irisin HIIT T35	0.19 (0.169)

Note: Data presented as *r* (*p* value). VO_{2max}: maximal oxygen consumption; MVPA: moderate-to-vigorous physical activity; MICT: moderate-intensity continuous training; HIIT: high-intensity interval training. Significant change represented by $p < 0.05$.

Table 8. Associations between insulin sensitivity and irisin according to obesity status and insulin sensitivity categorization.

Insulin Sensitivity							
Non-OW/OB – Low Si *<50th percentile*		Non-OW/OB – High Si *>50th percentile*		OW/OB – Low Si *<50th percentile*		OW/OB – High Si *>50th percentile*	
Irisin CON T0	-0.10 (0.761)	Irisin CON T0	**0.71 (0.010)**	Irisin CON T0	0.05 (0.891)	Irisin CON T0	-0.14 (0.655)
Irisin CON T14	0.06 (0.863)	Irisin CON T14	**0.69 (0.014)**	Irisin CON T14	-0.12 (0.736)	Irisin CON T14	-0.04 (0.887)
Irisin CON T28	0.04 (0.894)	Irisin CON T28	**0.72 (0.009)**	Irisin CON T28	-0.23 (0.505)	Irisin CON T28	-0.27 (0.379)
Irisin CON T35	0.12 (0.704)	Irisin CON T35	**0.58 (0.050)**	Irisin CON T35	0.03 (0.940)	Irisin CON T35	0.01 (0.970)
Irisin MICT T0	0.18 (0.587)	Irisin MICT T0	**0.57 (0.051)**	Irisin MICT T0	0.23 (0.488)	Irisin MICT T0	0.30 (0.324)
Irisin MICT T14	0.26 (0.407)	Irisin MICT T14	**0.68 (0.016)**	Irisin MICT T14	0.15 (0.654)	Irisin MICT T14	0.03 (0.921)
Irisin MICT T28	0.26 (0.411)	Irisin MICT T28	**0.74 (0.006)**	Irisin MICT T28	0.14 (0.674)	Irisin MICT T28	0.04 (0.901)
Irisin MICT T35	0.09 (0.784)	Irisin MICT T35	**0.77 (0.004)**	Irisin MICT T35	-0.26 (0.440)	Irisin MICT T35	0.05 (0.878)
Irisin HIIT T0	-0.09 (0.779)	Irisin HIIT T0	0.54 (0.073)	Irisin HIIT T0	-0.13 (0.696)	Irisin HIIT T0	-0.02 (0.947)
Irisin HIIT T14	0.22 (0.498)	Irisin HIIT T14	0.56 (0.058)	Irisin HIIT T14	0.08 (0.806)	Irisin HIIT T14	0.12 (0.694)
Irisin HIIT T28	0.24 (0.448)	Irisin HIIT T28	**0.60 (0.041)**	Irisin HIIT T28	0.17 (0.623)	Irisin HIIT T28	-0.12 (0.701)
Irisin HIIT T35	0.03 (0.927)	Irisin HIIT T35	0.42 (0.171)	Irisin HIIT T35	0.23 (0.498)	Irisin HIIT T35	-0.07 (0.829)

Note: Low- and high-Si are categorized as below or above the 50th percentile of insulin sensitivity based on the Matsuda index. Data presented as age adjusted correlations: *r* (*p* value). Non-OW/OB: non-overweight/obese group; OW/OB: overweight/obese group; VO_{2max}: maximal oxygen consumption; MVPA: moderate-to-vigorous physical activity; CON: control; MICT: moderate-intensity continuous training; HIIT: high-intensity interval training. Significant change represented by $p < 0.05$.

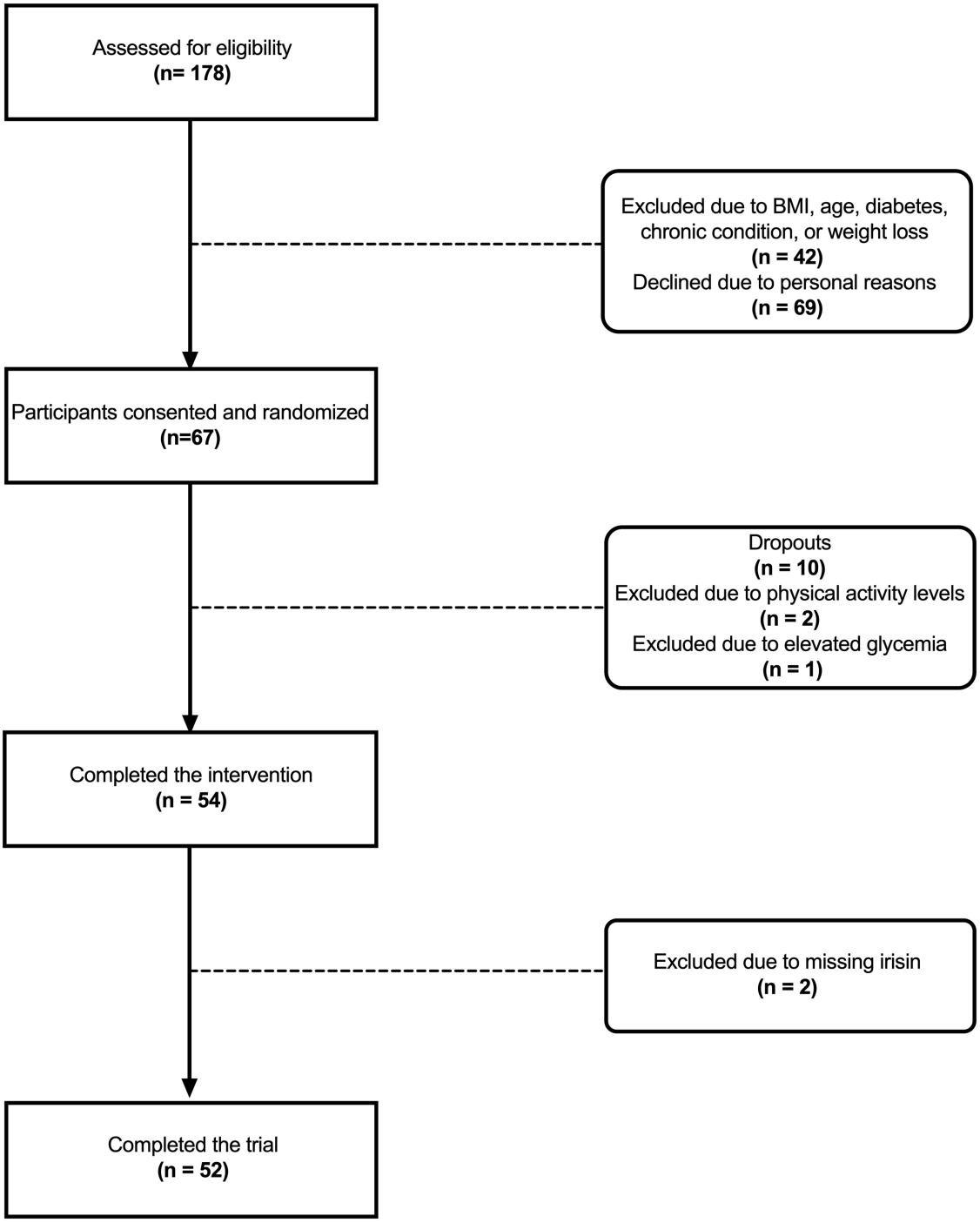


Figure 6. CONSORT flow diagram describing the flow of participants from the recruitment process to the final sample size.

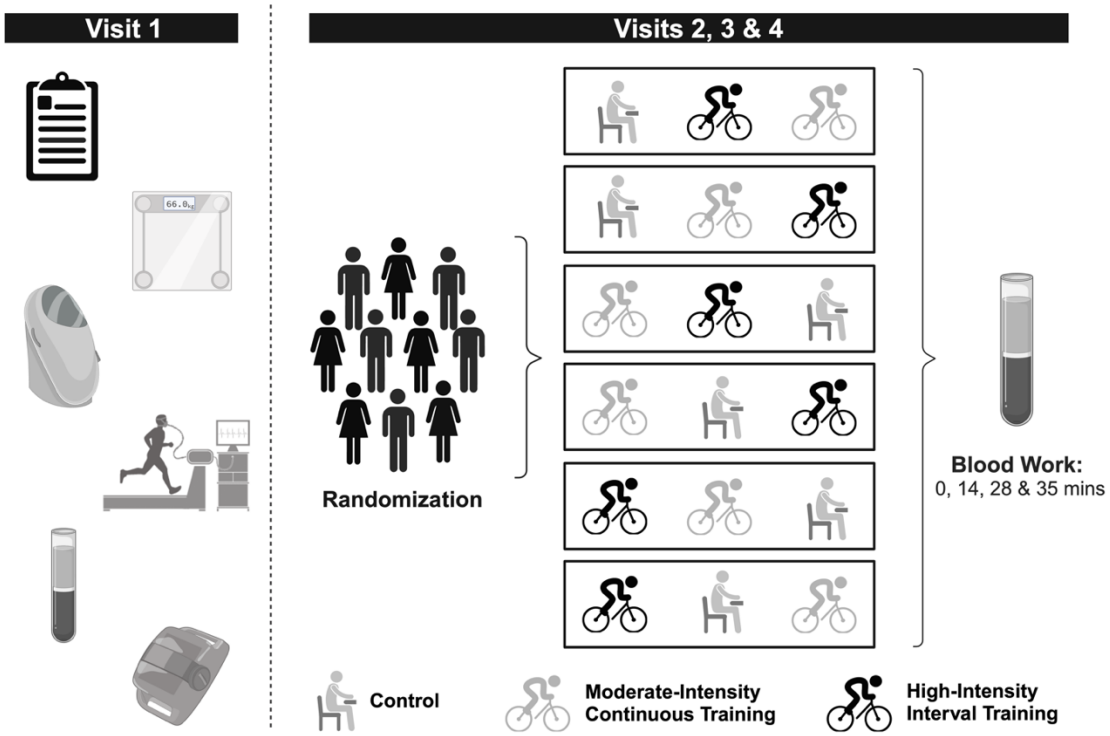
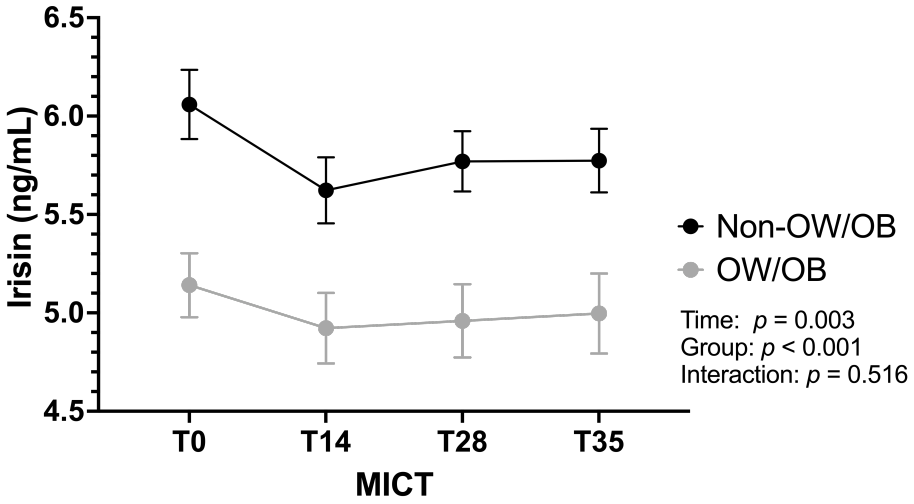


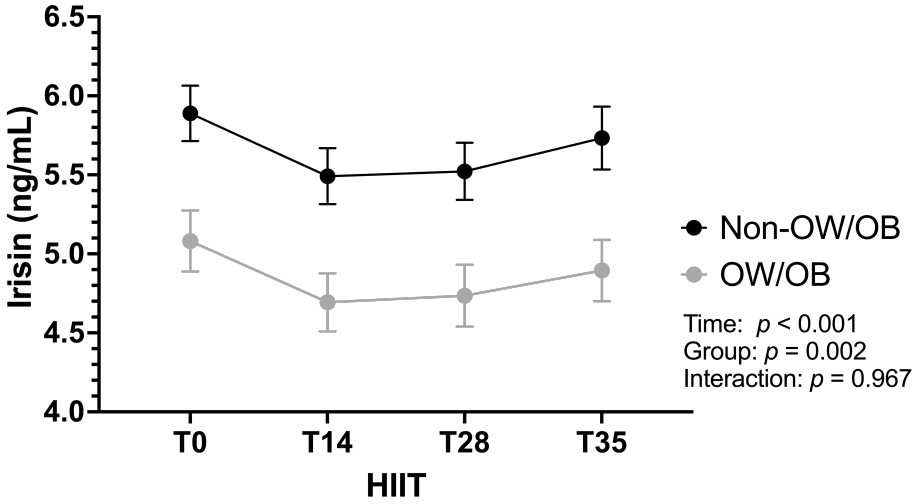
Figure 7. Experimental design of the iMET Study.

Figure 8. Irisin concentrations during acute bouts of: (A) moderate-intensity continuous training (MICT), (B) high-intensity interval training (HIIT), and (C) rest – control (CON) according to group. N = 52.

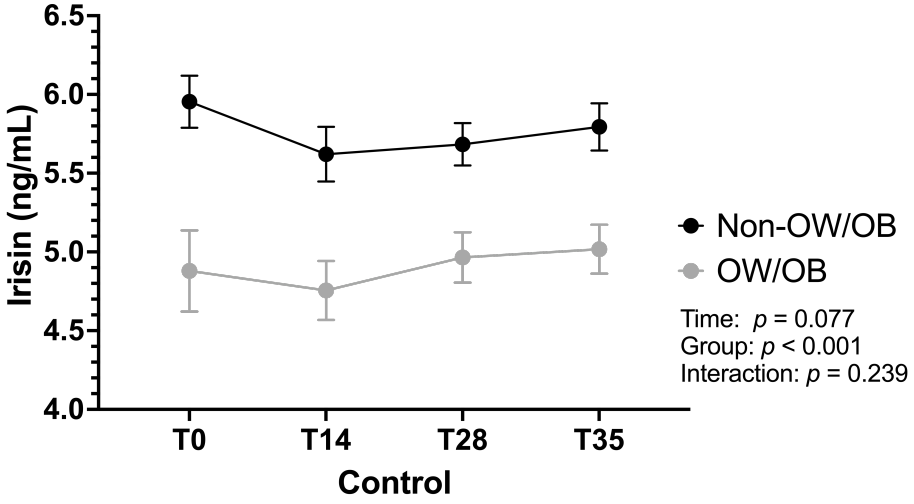
A


6.5
6.0
5.5
5.0
4.5
Irisin (ng/mL)
T0
T14
T28
T35
MICT
Non-OW/OB
OW/OB
Time: p = 0.003
Group: p < 0.001
Interaction: p = 0.516

B


6.5
6.0
5.5
5.0
4.5
4.0
Irisin (ng/mL)
T0
T14
T28
T35
HIIT
Non-OW/OB
OW/OB
Time: p < 0.001
Group: p = 0.002
Interaction: p = 0.967

C


6.5
6.0
5.5
5.0
4.5
4.0
Irisin (ng/mL)
T0
T14
T28
T35
Control
Non-OW/OB
OW/OB
Time: p = 0.077
Group: p < 0.001
Interaction: p = 0.239

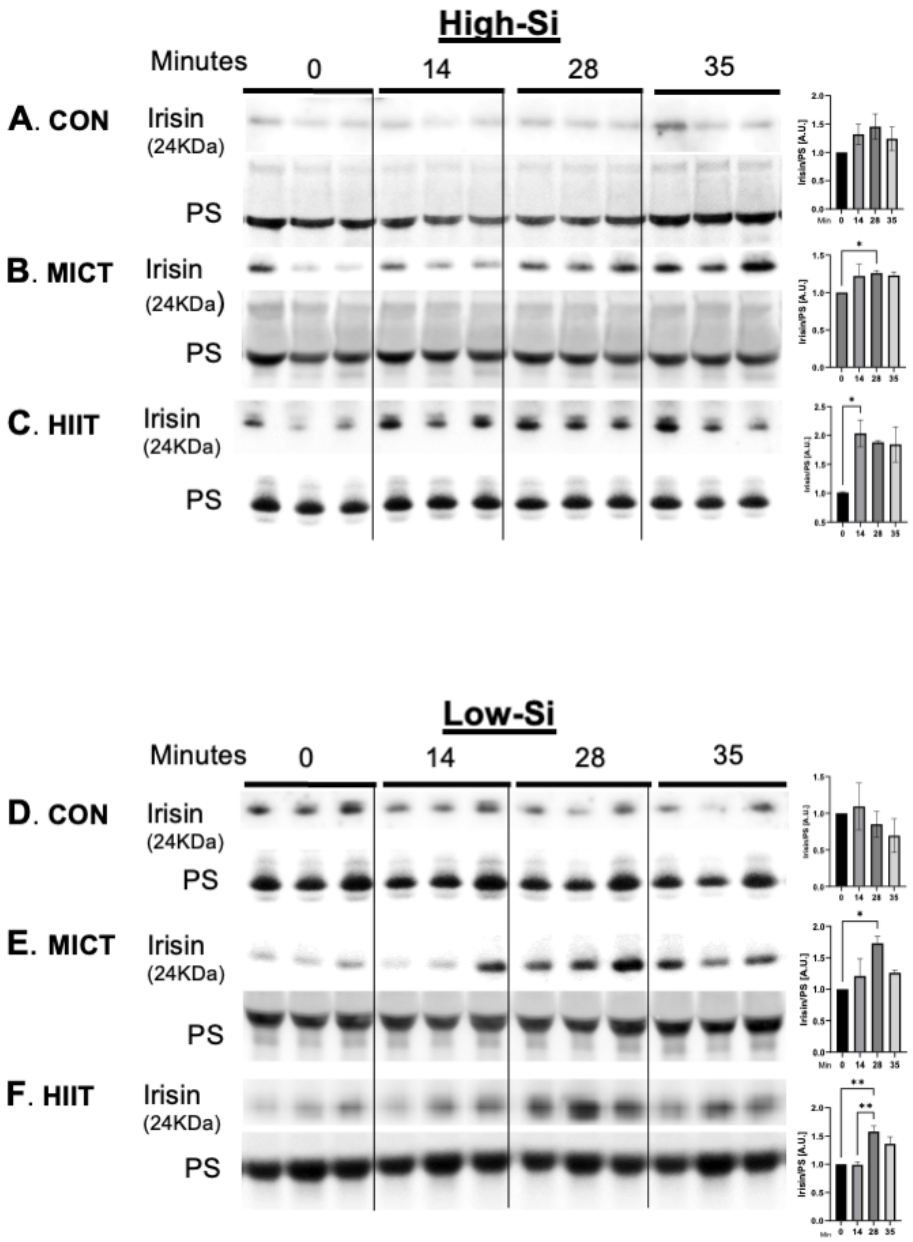


Figure 9. Human plasma irisin (24kDa) protein expression seems to be significant in non-overweight/obese (non-OW/OB) individuals with varying insulin sensitivities and exercise conditions (n = 3).

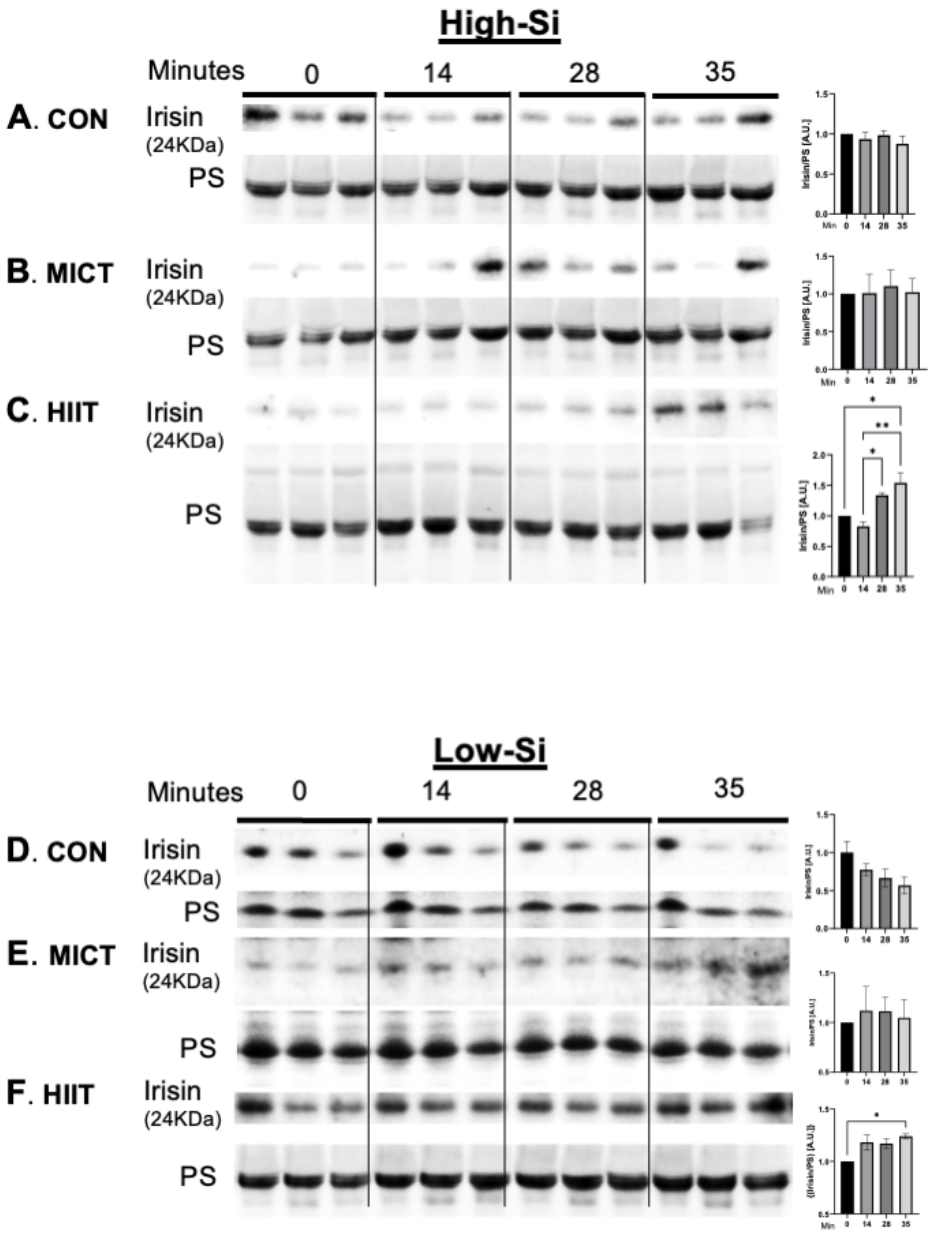


Figure 10. Human plasma irisin (24kDa) protein expression seems to be significant in individuals with overweight/obesity (OW/OB) with varying insulin sensitivities and exercise conditions (n = 3).

SUPPLEMENTARY FILES

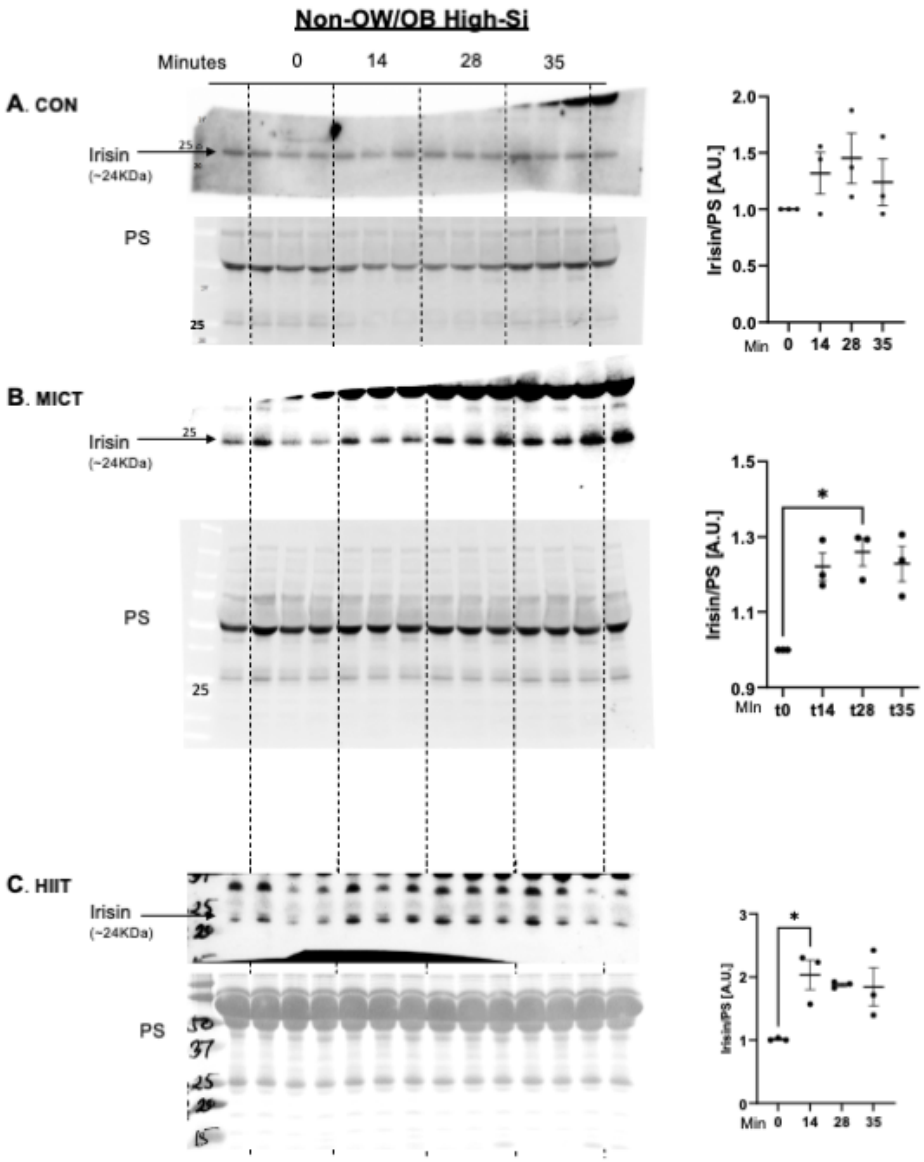


Figure 11. Human plasma irisin (24kDa) protein expression seems to be significant in non-overweight/obese (non-OW/OB) individuals with high insulin sensitivity and varying exercise conditions (n = 3).

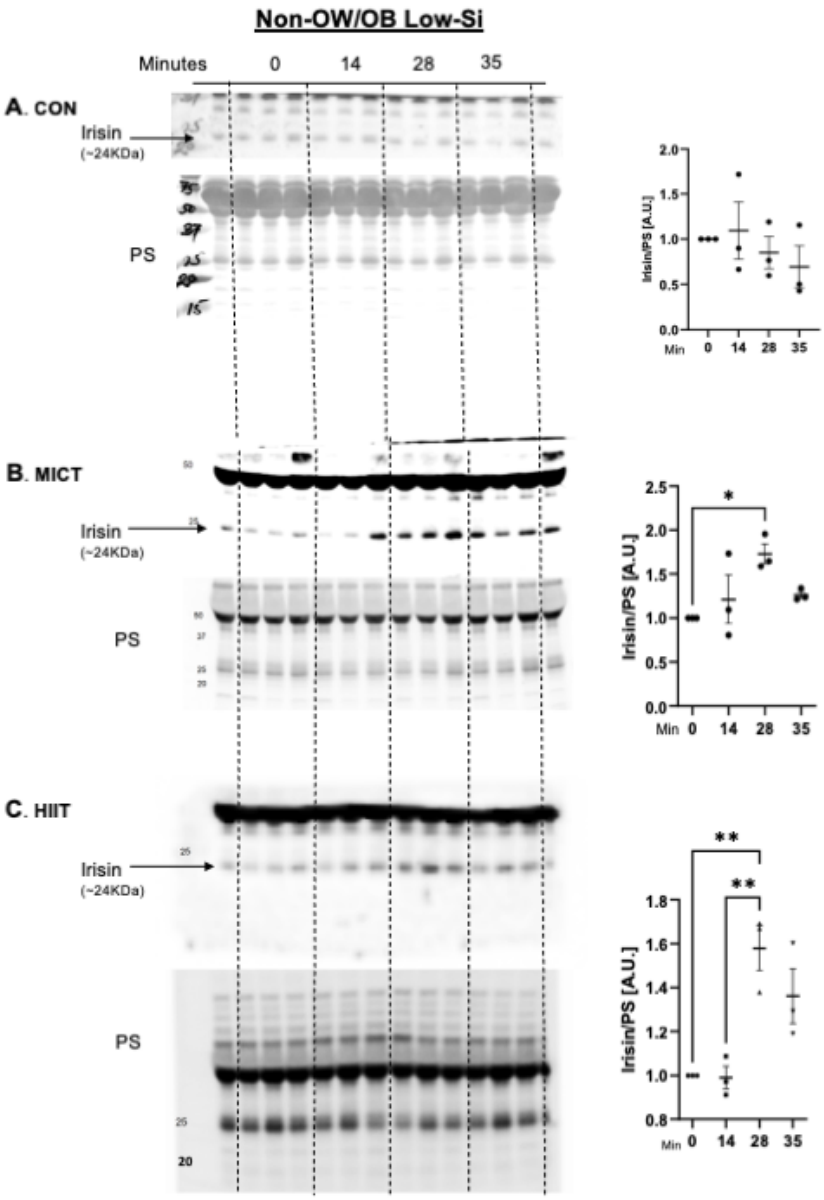


Figure 12. Human plasma irisin (24kDa) protein expression seems to be significant in non-overweight/obese (non-OW/OB) individuals with low insulin sensitivity and varying exercise conditions (n = 3).

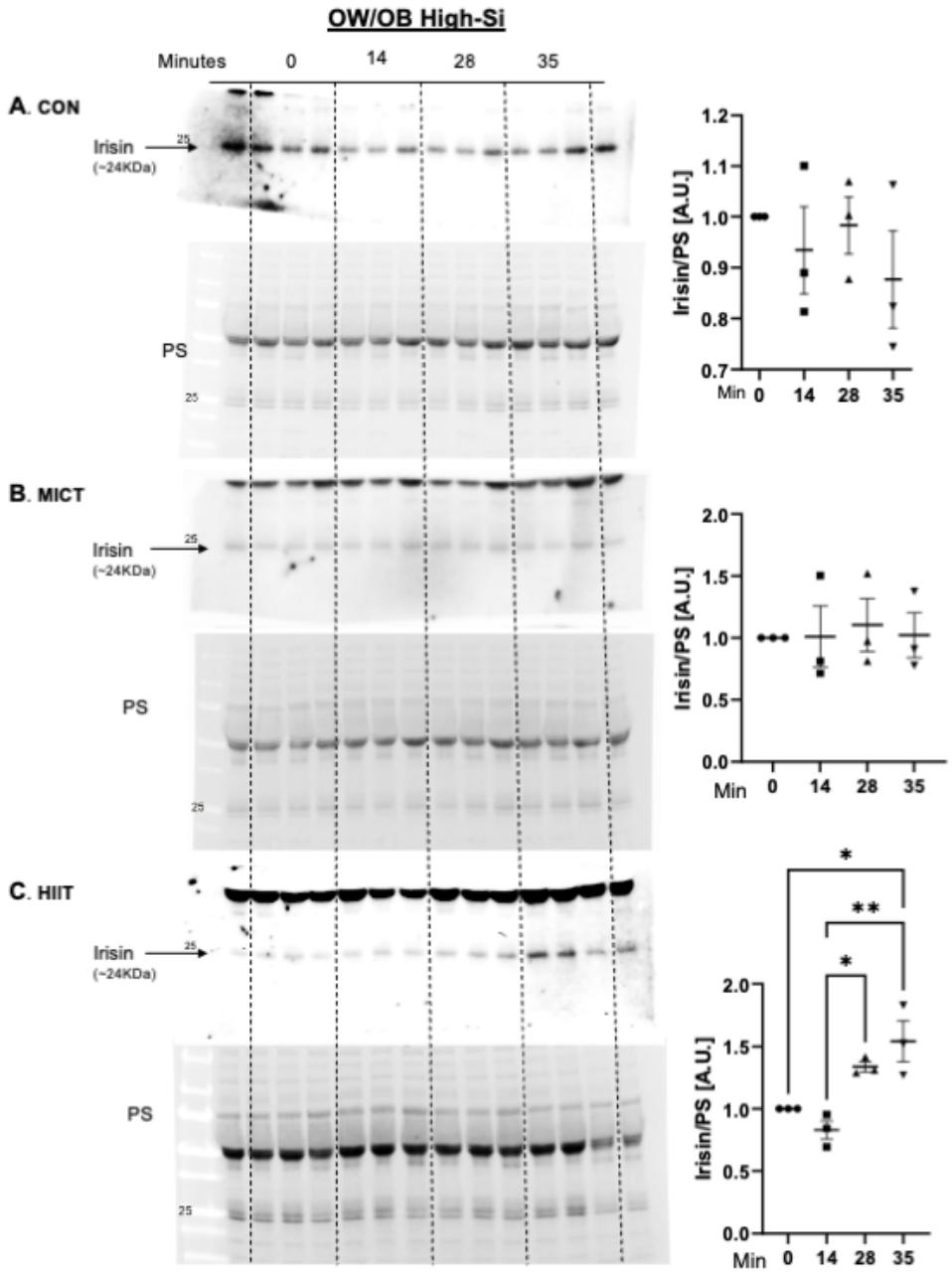


Figure 13. Human plasma irisin (24kDa) protein expression seems to be significant in individuals with overweight/obesity (OW/OB) with high insulin sensitivity and varying exercise conditions (n = 3).

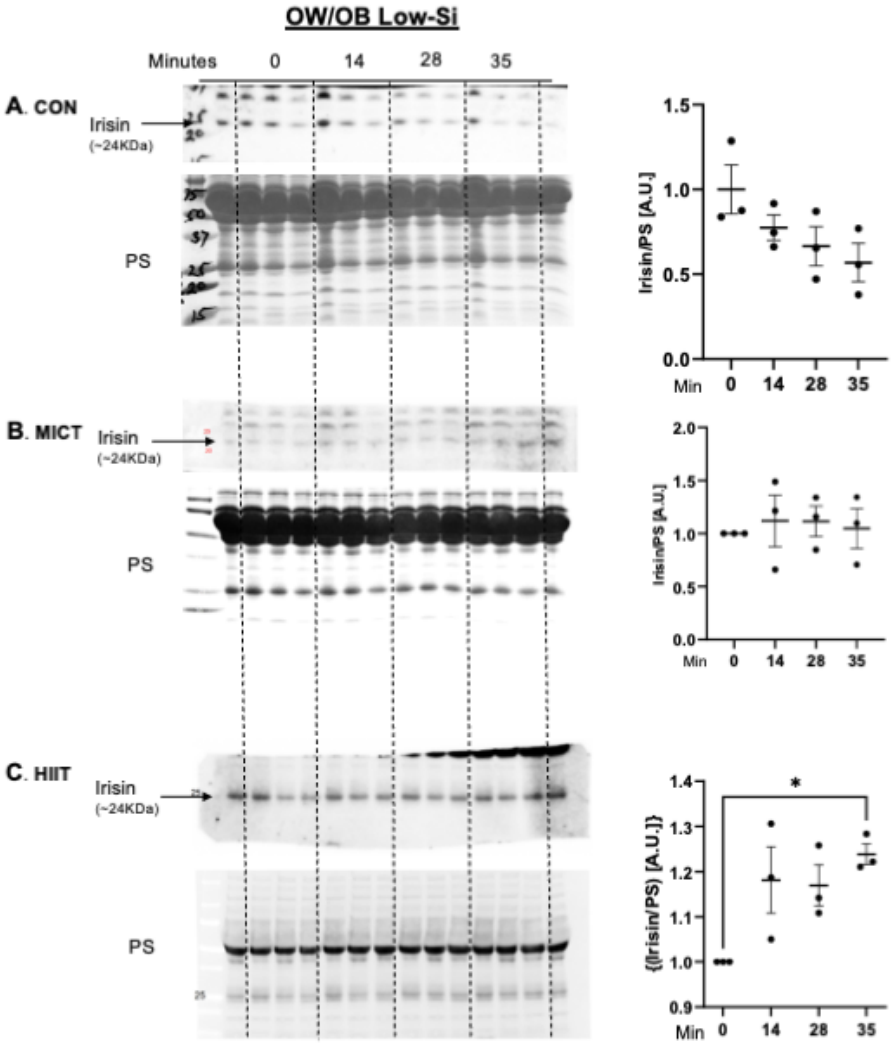


Figure 14. Human plasma irisin (24kDa) protein expression seems to be significant in individuals with overweight/obesity (OW/OB) with low insulin sensitivity and varying exercise conditions (n = 3).

Acknowledgements: The authors are grateful to the volunteers for their participation in this project and to the CELLAB students and staff who were involved in data collection and analysis. The authors would also like to give special recognition to Benjamin H. Colpitts, Martine Girard and Spencer Smith for their contribution to this study.

B.V.R. was supported by a Canadian Institutes of Health Research Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award and a University of New Brunswick Dr. William S. Lewis Doctoral Fellowship. A.M.T. was supported by University of New Brunswick Graduate Research Scholarships, while L.P. was supported by a New Brunswick Health Research Foundation (ResearchNB) Summer Studentship.

Competing interests: The authors declare there are no competing interests.

Author contributions: B.V.R. and M.S. conceived and designed research; B.V.R. performed experiments; B.V.R. and Y.P. analyzed data; B.V.R., Y.P., A.M.T., L.E.P., and M.S. interpreted results of experiments; B.V.R., Y.P., A.M.T., and L.E.P. prepared figures; B.V.R. and M.S. drafted the manuscript; B.V.R., Y.P., A.M.T., L.E.P., and M.S. edited, revised, and approved the final version of the manuscript.

Funding: This research was supported by an Establishment Grant from the New Brunswick Health Research Foundation (ResearchNB), an Operating Grant from Diabetes Action Canada, and an Infrastructure Grant from the Canada Foundation for Innovation.

Data availability: All relevant data are reported within the manuscript.

Supplementary material: Supplementary data are available with the article.

4.0 PROJECT III

Insights on the Impact of Exercise Intensity on Irisin Concentration and its Predictive Role in Glycemic Response in Adults with Prediabetes and Type 2 Diabetes: The INTENSITY Study

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4.1 Abstract

BACKGROUND: Exercise is a cornerstone in the management of type 2 diabetes (T2D), yet individual cardiometabolic responses vary considerably. Irisin, an exercise-induced myokine, might explain some of this heterogeneity.

PURPOSE: This study aimed to investigate the impact of increased exercise intensity on circulating irisin concentrations and their association with individual glycemic response over 28 weeks of aerobic training (AT) in individuals with prediabetes or T2D.

METHODS: This secondary analysis of the INTENSITY trial (NCT03787836) included 38 individuals with prediabetes or T2D (58.4 ± 9.1 years; 44.7% female). After an initial 16 weeks of moderate-intensity supervised AT (4.5 METs; Phase 1), participants were randomized to either increased-intensity (6.0 METs; n=20) or maintained-intensity groups (4.5 METs; n=18) (Phase 2) for an additional 12 weeks of AT. Circulating irisin concentrations (ELISA) and glycemia (HbA_{1c}) were measured at baseline, 16, and 28 weeks. Glycemic responder status (high vs. low) was defined by a change in HbA_{1c} above or below the minimal clinically important difference (-0.3%).

RESULTS: Circulating irisin concentrations did not significantly change over time across either intervention phase, with no group x time interaction (all $p > 0.05$). A pre-existing difference in circulating irisin was detected between groups at baseline, with the maintained group exhibiting significantly lower concentrations compared to the increased intensity group (β = -0.54 ng/mL, 95% CI [-0.97, -0.11], $p = 0.01$). Individual increases in irisin in Phase 1 significantly predicted greater HbA_{1c} reductions (β= –0.33%, 95% CI [-0.62, -0.05], $p = 0.02$), and were associated with 3.8 times increased odds of being a

glycemic high responder (OR = 3.8, 95% CI [1.1, 13.7], $p = 0.04$), but this association was absent in Phase 2.

CONCLUSIONS: Our findings indicate that even minor individual increases in irisin were linked to early, clinically meaningful glycemic improvements. These subtle increases (~8%) predicted a 3.8-fold increased likelihood of achieving a high glycemic response, highlighting irisin's potential as a critical early biomarker in T2D prevention and management.

Keywords: Irisin; Myokines; Exercise; Diabetes; Aerobic Training; Individual Response; Diabetes Management.

4.2 Introduction

Exercise is a cornerstone in the management of type 2 diabetes (T2D) and its associated cardiometabolic consequences.[1] Engaging in regular physical activity enhances β-cell function,[2] insulin sensitivity,[3] and overall glycemia,[4] and is associated with a decreased risk of both cardiovascular and all-cause mortality in this population.[5] Despite these well-known benefits, individuals exhibit considerable variability in their exercise response,[6-8] with some even responding adversely.[9] In fact, evidence indicates that up to 20% of individuals with T2D might not experience the favourable improvements in glycemia and muscle mitochondrial function that are typically observed after exercise training.[10,11]

Given the substantial variability in individual exercise responses and their impact on cardiometabolic outcomes, researchers have sought to identify factors underlying these differences. Generally, this heterogeneity is thought to stem from a combination of intrinsic and extrinsic biological factors (e.g. age, sex, genetics, sleep patterns, health status, etc.).[12,13] Beyond these broader influences, glycemic exercise response variability is further affected by a complex range of determinants. For instance, baseline clinical markers, including HbA_{1c}, BMI, and β-cell function can affect exercise response in older adults with prediabetes.[14] Moreover, in individuals with T2D, specific exercise prescription parameters (e.g. frequency, intensity, duration, and mode), physical inactivity, and exercise timing in relation to meals and certain medications may also contribute to heterogeneity.[13] Additionally, sub-analyses of the HART-D trial in adults with T2D highlight the contribution of skeletal muscle metabolism to individual response heterogeneity, including unique gene expression patterns[10] and key metabolic regulators, such as peroxisome

proliferator-activated receptor-γ coactivator-1α (PGC-1α).[15] Moreover, emerging evidence suggests that molecular transducers of exercise, including proteomic factors,[12] may contribute to interindividual response heterogeneity, positioning myokines, including irisin, as potential mediators of exercise-induced metabolic adaptations.

Irisin, an exercise-induced myokine increasingly recognized for its pivotal role in glycemic regulation,[16,17] is released into the circulation via an overexpression of PGC-1α, which drives the expression of its precursor: fibronectin type III domain-containing protein 5 (FNDC5).[18] Its contributions to glucose homeostasis, including the promotion of hepatic glycogenesis and inhibition of gluconeogenesis,[19,20] increased expression and translocation of glucose transporter 4 (GLUT4),[21] and suppression of pro-inflammatory cytokines,[22,23] collectively highlight irisin's therapeutic relevance for the prevention and management of T2D. However, its full therapeutic potential may be compromised due to the reduced systemic availability of irisin in those with T2D, as evidenced by the diminished circulating concentrations generally reported in this population.[24,25] Despite data supporting exercise-induced increases in irisin following aerobic and/or resistance training in individuals with T2D,[26,27] findings remain inconsistent.[28-30] These contradictory results likely reflect differences in study populations and exercise protocols, with varying exercise intensities playing a key role.[31] Crucially, studies have shown that higher intensities of aerobic training (e.g. continuous vigorous or high-intensity interval training (HIIT)) may enhance circulating irisin more effectively than moderate intensities in both humans and animal models of T2D.[27,32-34] Yet, these studies are limited by smaller sample sizes or single-sex cohorts. Thus, a comprehensive understanding of the impact of exercise intensity on

chronic irisin adaptations in T2D, and their association with individual glycemic response heterogeneity, warrants further investigation.

As such, this study aims to: 1) evaluate the impact of increasing exercise intensity on circulating irisin concentrations in individuals with prediabetes or T2D throughout a 28-week aerobic training intervention, and determine if these changes differ between maintained and increased intensity groups; and 2) investigate whether changes in irisin are associated with improvements in glycemia, and explore the individual variability in this relationship across exercise intensity and glycemic responder categorization.

4.3 Materials and Methods

Participants

This secondary analysis utilizes data from the Improving Individual Glycemic Response with Exercise Intensity (INTENSITY) trial (NCT03787836): a two-phase, double-blind, randomized quasi-experimental trial of 28 weeks of aerobic training. The primary purpose of the INTENSITY trial was to investigate the impact of increasing exercise intensity on glycemia in individuals living with T2D and prediabetes.[35] The present analysis examines changes in circulating irisin levels in response to exercise and their association with glycemic outcomes.

Participants for the INTENSITY trial were recruited between April 2019 and November 2020 in Fredericton, New Brunswick and surrounding areas via public advertisements, social media, and newsletters distributed to local universities and medical diabetes clinics. Written informed consent was obtained from all participants prior to study participation, and all experimental procedures were approved by the University of New Brunswick Research Ethics Board (UNB-2018-168). Participants met the following

inclusion criteria: ≥19 years old; current physician-diagnosed prediabetes or T2D (confirmed by a duplicate measured average glycated hemoglobin (HbA_{1c}) of ≥ 5.7%); and not currently partaking in any regular physical activity, or averaging ≥10,000 steps/day over a seven-day period (PiezoRxD pedometer: StepsCount Inc., Deep River, ON, Canada). Exclusion criteria for the main trial were: low iron/anemia; red blood cell altering conditions; current cardiovascular disease impeding safe participation in aerobic exercise training; or medication impacting accurate heart rate monitoring for exercise intensity prescription. For this secondary analysis, participants were additionally excluded if they were missing any primary or secondary outcome data (n = 38).

Study Design and Protocol Overview

The INTENSITY trial, comprehensively outlined by Hrubeniuk et al. (2021),[35] consisted of two distinct intervention phases. Throughout the 28-week supervised aerobic training intervention, participants attended six in-person assessments: two baseline (prior to phase 1), two midpoint (for follow-up and randomization), and two post-intervention (following phase 2).

Phase 1: Participants underwent 16 weeks of supervised aerobic training at an absolute intensity of 4.5 metabolic equivalents (METs) on a treadmill. This phase included a 4-week progressive lead-in, increasing weekly exercise time to 150 minutes (week 1: 80 mins, week 2: 100 mins, week 3: 120 mins, week 4: 135 mins), followed by 150 mins/week for the remaining 12 weeks. Participants freely adjusted speed and/or grade within ± 2.5% of the prescribed intensity (re-evaluated monthly to account for improvements in cardiorespiratory fitness). Weekly session frequency (>1 session/week) was determined by the participant.

Phase 2: All participants were randomized (blocked by HbA_{1c} responder status) into either a maintained-intensity (4.5 METs) or increased-intensity group (6.0 METs) for an additional 12 weeks of supervised aerobic training (150 mins/week). Phase 2 training metrics (frequency, speed, grade), supervision, and monthly re-evaluations followed identical methods as Phase 1.

Outcome Measure: Change in Plasma Irisin Concentrations

Blood samples were drawn intravenously by a registered nurse during each of the first of the baseline, midpoint (16 weeks), and post-intervention assessments (28 weeks). Blood was collected in 3 mL ethylenediaminetetraacetic acid (EDTA)-coated Vacutainer tubes, then transferred into 1.5 mL microcentrifuge tubes in duplicate in a biosafety cabinet (Cat# 1323TS, Thermo Fisher Scientific, Waltham, USA). The tubes were centrifuged at 2500g (15 minutes, 4°C), after which plasma was separated and stored at -80°C for future analysis.

Plasma irisin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cat# DY9420-05; ancillary reagent kit: Cat# DY008B, R&D Systems, Minneapolis, USA) following the manufacturer's protocol (lot #: P348921; linear range = 2.5-8.0 ng/mL). Optical density was measured with an EPOCH 2 Microplate Spectrophotometer using Gen5 software (BioTek, Winooski, USA), with corrections subtracting readings at 540nm from those at 450nm. Standard curves were generated using a four-parameter logistic curve-fitting method, and irisin concentrations were determined through the extrapolation of the optical density measurements (MyAssays, MyAssays Ltd., Brighton, UK). The initial assay was performed in duplicate

with a coefficient of variation (CV) below 2.0%, and subsequent assays were performed in singlets, as previously established in our laboratory.[36-38]

Outcome Measure: Change in Glycemia

Glycemia, measured by HbA_{1c}, was assessed using a DCA Vantage Analyzer (Siemens Healthineers, Erlangen, Germany) at baseline, 16 and 28 weeks. A 1 µl whole blood sample was collected via single-use lancet (Safe-T Pro Plus, Accu-Chek, Roche Diagnostics, Basel, Switzerland). The DCA Vantage Analyzer has demonstrated accurate results comparable to traditional laboratory-based HbA_{1c} measurements.[39-41] Reliability was enhanced through the collection of two HbA_{1c} measurements at each intervention time point (<7 days apart), with the mean value used in all analyses. The CV% for the HbA_{1c} measurements in the INTENSITY trial was 0.06% (95% CI [0.05%, 0.07%]).[42]

Exploratory Variables

At baseline, research staff recorded participant age, sex, family history of diabetes, diabetes duration, and medications were recorded by the research staff. At each assessment, alterations to T2D medication prescriptions (dosage and number) were self-reported, alongside evaluations of anthropometric measures, body composition, cardiorespiratory fitness, and metabolic profile for exploratory purposes and sample description.

Anthropometric Measures, Body Composition and Metabolic Profile

All anthropometric measures were taken in accordance with Canadian Society for Exercise Physiology (CSEP) protocols.[43] BMI was calculated from height and body weight (BMI = kg/m^2) using a stadiometer and calibrated column scale to the nearest 0.5 cm and 0.1 kg, respectively (SECA707, SECA, Mt. Pleasant, USA). Waist circumference was assessed with a measuring tape at the upper lateral border of the iliac crest at the end of a

normal expiration, recorded twice to the nearest 0.5 cm. Body mass, fat mass, and fat-free mass were estimated with the BOD POD via air displacement plethysmography (COSMED, Concord, USA) following a 12-hour overnight fast. Our laboratory has reported highly reliable and valid results with the BOD POD (CV%: 3.5% for body fat percentage and 0.8% for fat-free mass).[44] Total energy intake and macronutrients were self-reported using a 24-hour dietary recall questionnaire at baseline and post-intervention, analyzed via Food Processor Version 10.10 (Esha Research Inc., Salem, OR, USA). Fasting blood lipids (total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, TC to HDL-cholesterol ratio (TC/HDL), and triglycerides) were obtained at each assessment (CardioChek® PA Analyzer, PTS Diagnostics, Inc., Indianapolis, IN, USA).

Cardiorespiratory Fitness

A modified Balke and Ware treadmill test protocol[45] assessed cardiorespiratory fitness using a TrueOne 2400 Metabolic Cart (ParvoMedics, Salt Lake City, USA). Participants walked at a constant pace of at 3.4 miles per hour (mph) throughout the treadmill test. The grade started at 0% for 2 minutes, then increased to 5%, and then by 1% each minute until a 15% incline was reached. If maximal capacity was not reached, the grade was maintained while speed increased by 0.5 mph each minute until volitional fatigue. A Polar FT1 heart rate monitor (Polar, Kempele, Finland) continuously recorded heart rate throughout the test. Peak oxygen consumption (VO_{2peak}) was reported as the highest VO_2 measured over a 15-second average during the final minute.

Every four weeks, a submaximal aerobic test was integrated into the warm-up of a regular training session to re-evaluate cardiorespiratory fitness, accounting for

physiological adaptations and ensuring appropriate training intensity prescription. During this 5-minute warm-up, treadmill speed and grade gradually increased until they reached their corresponding phase and intervention category's MET value (4.5 METs or 6.0 METs) to identify target heart rate for the upcoming four weeks of training.

Statistical Analysis

Data normality was visually inspected and assessed using Shapiro-Wilk tests. Continuous and categorical data are presented as mean ± standard deviation and n (%), respectively. Independent samples t-tests and chi-square tests assessed intervention group differences at baseline (maintained vs. increased intensity) and differences in exercise-induced changes in irisin between high and low glycemic responders. Participants were categorized into glycemic responder groups based on HbA_{1c} changes relative to the minimal clinically important difference (MCID) after Phase 1 of the intervention (high responder: ≤-0.3%; low responder: ≥-0.3%).[46] Pearson correlations examined associations between irisin concentrations and glycemia, anthropometric, fitness, and metabolic variables both at baseline and for changes in response to the intervention.

Linear mixed-effects modelling (LMM) was used to analyze repeated measures, accounting for both within and between-group differences. A multi-model analytical framework was employed. Phase-specific LMMs examined changes within the two distinct intervention periods (Phase 1: pre-intervention to mid-point; Phase 2: mid-point to post-intervention) to capture distinct within-subject change trajectories for each phase, individual heterogeneity in these phase-specific changes, and random between-subject variability. This phase-specific approach evaluated the effect of each intervention phase on circulating irisin and various exploratory variables, as well as the effect of exercise

intensity during Phase 2. Overall intervention LMMs provided a comprehensive assessment of changes across each time point throughout the 28-week intervention, allowing for post-hoc comparisons. For objective 2, a phase-specific LMM investigated whether irisin changes were associated with glycemic improvements. In this model, irisin was person-mean centered to isolate within-individual variation in irisin concentrations, allowing individual differences in phase-specific associations with HbA_{1c}. All models included adjustments for potential covariates and incorporated random intercepts, while phase-specific LMMs also included random slopes for time. Models were constructed using an iterative approach guided by the evaluation of fixed effects based on overall model fit, assumption checks, and statistical significance. Finally, binary logistic regression assessed whether phase-specific changes in irisin predicted glycemic responder categorization, including adjustments for covariates. Analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corporation, Armonk, NY, USA) with graphs generated via GraphPad Prism version 10.1 (GraphPad Software, LLC., San Diego, CA, USA). The significance level for all analyses was accepted at $p \leq 0.05$.

4.4 Results

General Characteristics

A total of 38 participants (44.7% female) with a mean age of 58.4 ± 9.1 years who completed the intervention were included in this secondary analysis. Of these participants, 18 were randomized to the maintained-intensity group and 20 to the increased-intensity group. There were no significant baseline differences between the intervention groups in any of the descriptive measures (all $p > 0.05$; Table 9).

Effects of the Intervention on Body Composition

During Phase 1, significant phase-specific improvements were observed, including reductions in fat mass (β = -1.82 kg, 95% CI [-2.7, -0.9], $p < 0.01$) and increases in fat-free mass (β= 0.75 kg, 95% CI [0.3; 1.2], $p < 0.01$). Waist circumference significantly reduced in response to both intervention phases individually (Phase 1: β = -1.73 cm, 95% CI [-2.8, -0.6], $p < 0.01$; Phase 2: β = -2.69 cm, 95% CI [-4.3, -1.1], $p < 0.01$). A significant group x time interaction indicated that the increased-intensity group experienced greater reductions than the maintained-intensity group in Phase 2 (β = 2.05, $p < 0.05$, 95% CI [0.30, 4.08]). Additionally, VO_{2peak} significantly increased in both phases (Phase 1: β = 1.18 mL/kg/min, 95% CI [0.3, 2.1], $p = 0.01$; Phase 2: β = 3.27 mL/kg/min, 95% CI [1.0, 5.5], $p = 0.01$).

While individual phase-specific changes in body weight and BMI were not significant, their cumulative effect across the 28-week intervention revealed a significant overall time effect (both $p < 0.05$). Specifically, post-intervention measurements were significantly lower than at baseline pre-intervention (weight: Δ -1.57 kg, 95% CI [0.1, 3.1], $p = 0.04$; BMI: Δ -0.53 kg/m^2, 95% CI [0.0, 1.0], $p = 0.03$). Significant overall time effects were also detected in all body composition variables (all $p < 0.05$), with post-hoc tests indicating cumulative reductions in waist circumference from pre- to post-intervention (Δ -1.61 cm, 95% CI [-3.2, -0.0], $p = 0.04$) and fat mass (Δ -2.33 kg, 95% CI [-3.9, -0.8], $p < 0.01$), as well as Phase 1 increases in fat-free mass from pre-intervention to mid-point (Δ +0.75 kg, 95% CI [0.1, 1.4], $p = 0.01$). A significant VO_{2peak} group x time interaction effect was detected ($p < 0.01$), with post-hoc results demonstrating greater improvements in the

increased-intensity group compared to the maintained-intensity group during Phase 2 (Δ +2.1 mL/kg/min, 95% CI [0.5, 3.7], $p < 0.01$).

Effects of the Intervention on Metabolic Profile Markers

Neither phase-specific or cumulative intervention LMMs revealed significant effects for any of the lipid profile markers, fasting insulin, or fasting glucose (all $p > 0.05$). While a significant group x time interaction was found ($p = 0.03$), the cumulative model includes time points measured prior to group randomization. Follow-up post-hoc comparisons were not significant (all $p > 0.05$). A trend towards a reduction in HbA_{1c} was identified in both Phase 1 (β = -0.23, 95% CI [-0.5, 0.0], $p = 0.08$) and Phase 2 (β = -0.32, 95% CI [-0.7, 0.0], $p = 0.06$), and no group x time interaction was revealed following randomization ($p > 0.05$).

Effects of the Intervention on Circulating Irisin Concentrations and the Impact of Intensity

According to the phase-specific LMM, irisin concentrations were not significantly altered during Phase 1 (β = 0.13 ng/mL, 95% CI [-0.12, 0.38]) or during Phase 2 (β = 0.17 ng/mL, 95% CI [-0.17, 0.51]) (both $p > 0.05$). No differences were observed between intervention groups following randomization ($p >0.05$). However, a significant main effect of group indicated pre-existing differences in baseline irisin concentrations, with lower circulating levels in the maintained-intensity than the increased-intensity group (β = -0.54 ng/mL, 95% CI [-0.97, -0.11], $p = 0.01$). The cumulative intervention LMM reported consistent findings, showing no significant effect of time or a group x time interaction (both $p > 0.05$), but a significant group effect when including pre-randomization irisin concentrations ($F(1, 36.0) = 5.92$, $p = 0.02$; Figure 15). Model results remained consistent

in both unadjusted and adjusted for age, sex, BMI, and diabetes medication, and no significant effects were demonstrated in any of the covariates. Figure 16 exhibits changes in irisin throughout the intervention.

Irisin Concentration Changes and Glycemic Response

Figure 17 illustrates changes in irisin according to glycemic response categorization. During intervention Phase 1, nearly half of the participants (47%, N = 18/38) were identified as high responders, having achieved a change in glycemia of ≤-0.3%. This group had a significantly greater change in irisin concentrations compared to those categorized as low responders (-0.1 ± 0.6 vs. 0.4 ± 0.7 ng/mL, $p = 0.03$). Although irisin concentrations were not significantly different between high and low glycemic responders during Phase 2 (0.0 ± 0.7 vs. 0.1 ± 0.6 ng/mL, $p = 0.73$), an additional six participants became high responders (16%, N = 6/38).

Associations Between Irisin and Metabolic Outcomes

At baseline, irisin concentrations were not significantly associated with anthropometric, body composition, fitness, or dietary intake (all $p > 0.05$). Inverse associations were, however, observed with baseline lipid profile markers, including triglycerides ($r = -0.47$, $p < 0.01$), TC ($r = -0.32$, $p = 0.05$), LDL-cholesterol ($r = -0.35$, $p = 0.04$), and TC/HDL ratio ($r = -0.43$, $p = 0.01$). Exercise-induced changes in irisin were significantly correlated with Phase 1 changes in triglycerides ($r = -0.57$, $p < 0.01$) and TC/HDL ($r = -0.40$, $p = 0.01$). During Phase 2, the increased intensity group exhibited significant negative associations between changes in irisin and triglycerides ($r = -0.77$, $p < 0.01$), TC/HDL ($r = -0.68$, $p < 0.01$), TC ($r = -0.50$, $p = 0.03$), and fasting insulin ($r = -0.51$, $p = 0.02$). However, in the maintained intensity group, changes in irisin were only

correlated with HDL-cholesterol (r = 0.71, p < 0.01) (Table 10). As shown in Figure 18, changes in irisin were negatively associated with HbA_{1c} changes during Phase 1 (r = -0.40, p = 0.01), but not Phase 2 (r = -0.12, p = 0.46). A non-significant inverse trend emerged in high responders (Phase 1: r = -0.42, p = 0.08; Phase 2: r = -0.65, p = 0.06).

Irisin as a Predictor of Glycemic Response

A phase-specific LMM investigated whether changes in irisin concentrations predicted glycemic response. In Phase 1, increases in irisin significantly predicted greater reductions in HbA_{1c} (β = –0.33%, 95% CI [-0.62, -0.05], p = 0.02). This relationship was no longer significant in Phase 2 after randomization into maintained- vs. increased-intensity groups (β = 0.12%, 95% CI [-0.40, 0.65], p = 0.64). No significant main effects of group or interaction effects were observed in HbA_{1c}. After further adjustments for age, sex, BMI, diabetes duration, and baseline diabetes medication use, irisin remained a significant predictor of glycemia in Phase 1 (β = –0.35%, 95% CI [-0.65, -0.05], p = 0.02). No other covariate had a significant effect on HbA_{1c} (all p > 0.05).

Binary logistic regressions revealed that the change in irisin during Phase 1 significantly predicted glycemic responder categorization, with each 1 ng/mL increase in irisin concentration associated with 3.8 times increased odds of being a glycemic high responder (OR: 3.8, 95% CI [1.1, 13.7]; p = 0.04). Adjustments were made for age, sex, BMI, and diabetes medications, none of which were significant predictors (all p > 0.05). Phase 2 irisin changes did not significantly predict glycemic responder categorization (all p > 0.05).

4.5 Discussion

This study investigated the role of circulating irisin in exercise-induced metabolic adaptations during a 28-week aerobic training intervention in adults with prediabetes or T2D. Our findings indicated that while circulating irisin concentrations did not significantly change overall or in response to increased exercise intensity, minor individual increases were associated with early, clinically meaningful glycemic improvements, as evidenced by a 0.33% reduction in HbA_{1c} during the first 16 weeks. Specifically, each 1 ng/mL increase in irisin was associated with a 3.8-fold increased likelihood of being a high responder. These irisin changes were also associated with favourable effects on lipid profile markers across both intervention phases. Collectively, these data suggest a potential link between irisin and key metabolic adaptations, particularly during the early stages of aerobic training, supporting the role of this myokine as a potential early biomarker for optimizing personalized exercise prescription strategies in individuals with impaired glycemia.

Impact of Aerobic Training Intensity on Circulating Irisin

Increasing exercise intensity did not lead to significantly greater circulating irisin concentrations in individuals with prediabetes or T2D, a finding that provides insight into skeletal muscle molecular adaptations in response to chronic aerobic training. While this finding contrasts with suggestions that higher exercise intensity has a greater effect on circulating irisin than lower intensities in individuals with T2D,[27,32-34] our results align with other studies reporting no discernible increase in irisin following high-intensity aerobic training modalities.[47,48] For instance, Ghodrati et al. (2023) reported no increases in irisin after 12 weeks of high-intensity continuous aerobic training paired with resistance training, in a cohort similar to the current study.[47] Considering the fact that AMPK and p38 MAPK are intensity-dependent upstream regulators of PGC-1α,[49] a key regulator of irisin

production, a greater irisin response would be expected with higher training intensities. However, chronic hyperglycemia, insulin resistance, and systemic inflammation are known to impair these signaling pathways,[50,51] potentially blunting the irisin response to chronic exercise in individuals with T2D, as may have occurred in our study.

Nevertheless, the chronic exercise-induced irisin response remains inconsistent across trials, especially in the context of T2D, which may be driven by a complex interplay of factors. For instance, discrepant findings may be attributable to protocol differences, even within the same exercise mode. Studies reporting chronic increases in circulating irisin in this population were often conducted with HIIT,[27,34] suggesting a potential effect of aerobic training protocols, rather than just intensity. Yet, a direct comparison of high-intensity aerobic exercise protocols demonstrated that only the continuous protocol increased irisin, and HIIT did not.[33] Notably, increases in circulating irisin in T2D were primarily identified in male cohorts, but studies that didn't observe adaptations predominantly involved female participants.[28,29,47,52] Thus, while sex differences may contribute to reported differences in exercise-induced irisin adaptations, our analysis, which had a near-equal sex distribution (55% male), did not identify significant sex-related effects. Furthermore, T2D-related complications and disease durations may also contribute to inconsistent findings. A meta-analysis by Hou et al. (2023) suggested that circulating irisin may be lower in individuals with T2D-related complications than in those without.[53] This observation is further supported by additional findings from the INTENSITY study by Thomson et al. (2025), which showed that exercise-induced irisin responses varied according to T2D duration,[38] with individuals who had T2D for longer durations exhibiting greater response than those with shorter durations, specifically in the increased-intensity

group. Together, these observations emphasize the complexity of the factors driving the substantial heterogeneity in irisin's exercise response.

Not only are inconsistent findings attributable to variations in exercise protocols and overall population characteristics, but individual differences in exercise responses may also play a large role. Comprehensive statistical modeling in our study allowed us to go beyond average group effects and elucidate individual differences in chronic exercise-induced irisin response. Indeed, our analysis highlighted considerable individual variability in exercise response to irisin, despite the absence of significant average changes. Moreover, our analysis highlighted that some individuals experienced meaningful changes in irisin that were linked to a favourable cardiometabolic response, including in glycemia.

Irisin as a Predictor of Early Glycemic Adaptations

This study proposes a link between circulating irisin dynamics in response to aerobic training and early glycemic adaptations in individuals with impaired glycemia. Our findings indicate that even subtle irisin improvements predict meaningful glycemic benefits, evidenced by reductions in HbA_{1c} of 0.33% with each 1 ng/mL increase in circulating concentrations. While seemingly modest, this degree of change is not trivial, given its clinical implications. Crucially, HbA_{1c} is a well-established predictor of diabetes-related complications,[54,55] with evidence from the landmark UK Prospective Diabetes Study demonstrating that each 1% reduction in HbA_{1c} was associated with a 37% decreased risk of microvascular complications.[55] They identified HbA_{1c} as a continuous marker of complications, implying that even our detected 0.3% HbA_{1c} reduction contributes to this risk reduction. Moreover, this reduction is meaningful clinically, achieving a common MCID threshold used in clinical trials.[46,56] Together, these results emphasize the potential

clinical significance of minor irisin improvements and the importance of identifying exercise interventions capable of eliciting chronic adaptations to optimize therapeutic outcomes in T2D.

The observed inverse relationship between irisin and HbA_{1c} contributes to the expanding body of evidence supporting irisin's role in glucose metabolism,[16,17] This finding is consistent with previous research establishing irisin's association with various glucose metabolism markers, such as fasting glucose, insulin, HOMA-IR, and HbA_{1c} in individuals with T2D.[57-59] Interestingly, despite this strong association observed after the first 16 weeks of aerobic training, irisin no longer predicted glycemic changes in the second phase of the intervention. This could reflect an early, initial adaptation to aerobic training, a period that contributes to a legacy effect – a concept theorizing that early glycemic improvements can have sustained reductions in the risk of diabetes complications, even if HbA_{1c} deteriorates over time.[60] It is also plausible that the shorter duration of training between phases (3 vs 4 months) may have affected the ability to detect changes in HbA_{1c} due to red blood cell turnover.[61] Additionally, the increased complexity in design in Phase 2 following randomization may have introduced even greater heterogeneity in individual responses, potentially obscuring a sustained relationship between irisin and HbA_{1c}. Although the significant association was only observed among glycemic responders in Phase 1, these findings reinforce irisin's potential as an early biomarker of favourable metabolic adaptation and its clinical utility in informing personalized exercise prescription.

Intervention Effects on Body Composition, Fitness, and Metabolic Profile

Exploratory analyses revealed that the 28-week aerobic training intervention elicited significant improvements in body composition and cardiorespiratory fitness,

including reductions in fat mass and waist circumference, as well as increases in VO_{2peak}. Notably, our findings demonstrated moderate to strong relationships between changes in circulating irisin and several metabolic profile markers, further highlighting irisin's physiological relevance in T2D. Specifically, changes in circulating irisin were inversely associated with changes in triglycerides, TC, TC/HDL ratio, and fasting insulin, and positively associated with HDL-cholesterol. These findings point to irisin's contribution to exercise-induced lipid metabolism regulation, consistent with its proposed role in enhancing lipid utilization via AMPK signaling.[62] Furthermore, while our baseline associations between irisin and lipid profiles align with Stankovic et al. (2025), our study further exhibits the correlation between exercise-induced changes in irisin and favourable lipid alterations, particularly with increased exercise intensity.[63] Altogether, these findings position irisin as a key myokine involved in beneficial cardiometabolic adaptations to exercise, influencing both lipid and glycemic regulation in individuals with prediabetes or T2D.

Strengths and Limitations

Several limitations of the study must be acknowledged. First, the relatively small sample size may have limited statistical power, particularly in Phase 2, where there was a disproportionate amount of high vs. low responders. Second, while concerns exist regarding commercial irisin ELISA accuracy and reliability,[64,65] the kit used demonstrated good between-day reliability and intra-individual precision in direct comparisons, *(Rioux et al. (2025), unpublished data)* with concentrations consistent with mass spectrometry.[66] Third, although some diabetes medication use (e.g. metformin) might influence irisin,[67] our analyses controlled for baseline medication use. Fourth, although diet may influence

irisin concentrations,[68] others have reported no such postprandial influence on circulating irisin;[69,70] moreover, our analyses revealed no significant changes in dietary intake or macronutrient patterns. Finally, the INTENSITY study prescribed exercise with absolute intensities. However, although intensity readjustment was performed every four weeks of the intervention with a submaximal test, and heart rate data confirmed training was performed at appropriate relative intensities[42], the difference between the two group was small, which could explain some of our results. Nevertheless, our study is strengthened by rigorous statistical analyses using a multi-model LMM framework with both phase-specific and cumulative intervention models, robustly accounting for fixed and random effects, distinguishing individual variability in irisin and glycemic responses. Finally, the robust longitudinal study design, including blinded glycemic response categorization, subsequent randomization into intensity groups, high adherence, and supervised training, collectively enhance the strength and overall validity of our findings.

Conclusion

This secondary analysis of the INTENSITY study provides critical insight into the complex metabolic response to exercise in individuals with prediabetes or T2D. This study suggests that early, subtle increases in irisin of only 8% were associated with clinically meaningful glycemic reduction of 0.33% in HbA_{1c}, and increased the likelihood being a glycemic high responder by almost four-fold. Furthermore, the increased-intensity group did experience broad cardiometabolic benefits, including enhanced cardiorespiratory fitness, reductions in waist circumference and better lipid profile. Ultimately, these insights further elucidate irisin's role in both glucose and lipid metabolism, informing personalized exercise prescription strategies in individuals with prediabetes or T2D.

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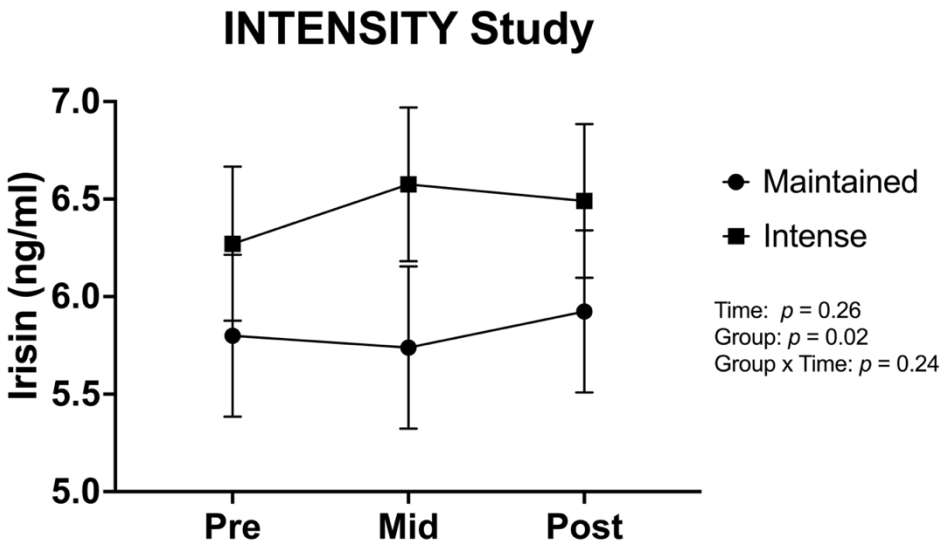
4.7 Tables and Figures

Table 9. Descriptive characteristics of the sample at baseline.

	Whole Sample (n = 38)	**Maintained** (n = 18)	**Intense** (n = 20)	***p***
Age (years)	58.4 ± 9.1	57.8 ± 8.9	58.9 ± 9.6	0.74
Sex – Female (%)	17 (44.7%)	10 (55.6)	7 (35.0)	0.20
Ethnicity – Caucasian (%)	35 (92.1%)	16 (88.9)	19 (95.0)	0.21
Systolic BP (mmHg)	132.9 ± 11.2	132.3 ± 12.4	133.5 ± 10.4	0.75
Diastolic BP (mmHg)	83.3 ± 5.7	83.6 ± 5.7	83.1 ± 5.9	0.79
Weight (kg)	95.1 ± 18.4	95.0 ± 19.7	95.2 ± 17.7	0.98
Body Mass Index (kg/m^2)	32.4 ± 6.0	32.7 ± 6.4	32.1 ± 5.7	0.74
Waist Circumference (cm)	112.4 ± 13.0	112.8 ± 14.4	112.0 ± 12.0	0.85
Body Fat (%)	40.3 ± 8.9	42.3 ± 9.4	38.3 ± 8.2	0.17
Fat Mass (kg)	39.0 ± 12.7	41.0 ± 13.9	37.1 ± 11.5	0.37
Fat Free Mass (kg)	56.0 ± 11.4	54.1 ± 11.3	57.9 ± 11.5	0.32
Diabetes Duration (months)	102.9 ± 84.2	84.9 ± 73.3	118.2 ± 91.5	0.24
Diabetes Medications (#)	1.5 ± 1.1	1.6 ± 1.3	1.4 ± 1.0	0.49
HbA1c (%)	6.9 ± 0.9	6.9 ± 0.9	7.0 ± 0.9	0.81
Total Cholesterol (mmol/L)	4.3 ± 1.0	4.4 ± 1.0	4.3 ± 1.0	0.66
HDL Cholesterol (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.2	0.33
LDL Cholesterol (mmol/L)	2.2 ± 0.9	2.2 ± 0.8	2.1 ± 1.1	0.73
TC/HDL Cholesterol (mmol/L)	3.5 ± 1.0	3.5 ± 1.1	3.5 ± 1.0	0.95
Triglycerides (mmol/L)	2.1 ± 1.4	2.0 ± 1.5	2.2 ± 1.3	0.56
Plasma Glucose (mmol/L)	6.4 ± 2.8	5.8 ± 2.1	7.0 ± 3.3	0.18
Plasma Insulin (pmol/L)	51.8 ± 44.0	45.2 ± 30.4	57.8 ± 53.6	0.39
Relative VO_{2peak} (mL/kg/min)	25.2 ± 5.5	25.7 ± 6.3	24.7 ± 4.7	0.58
Daily Caloric Intake (kcals)	2084.1 ± 740.1	1937.1±524.2	2216.4±884.3	0.24

Continuous data are presented as mean ± standard deviation. BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TC: total cholesterol; VO_{2peak}: peak oxygen consumption. Significant change (bolded values) indicated by $p < 0.05$.

A



B

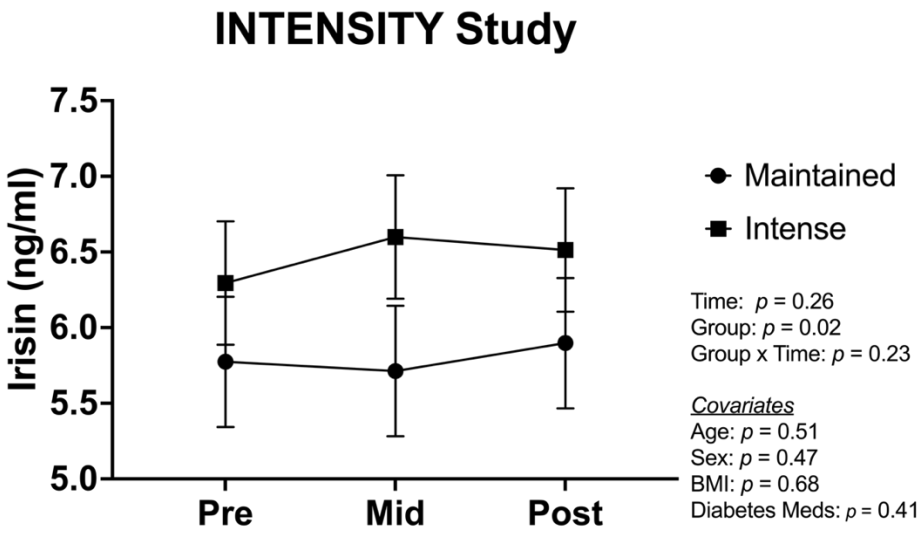


Figure 15. Circulating irisin concentrations by intensity group across the intervention.

*Estimated marginal means with 95% confidence intervals derived from categorical linear mixed-effects modeling, with estimates **A)** unadjusted and **B)** adjusted for covariates. Significant pre-existing difference detected between groups at baseline prior to randomization ($p < 0.05$), warranting the visual representation of distinct group trajectories from pre-intervention to mid-point, despite identical dosage of moderate-intensity exercise during phase 1 (crucially, no significant group x time interaction was observed).*

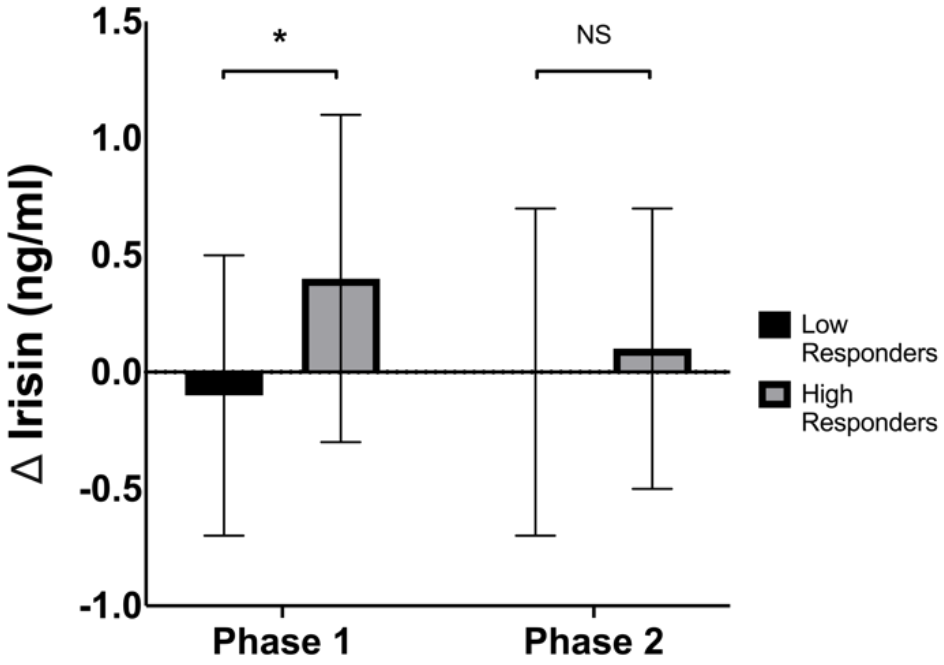


Figure 16. Change in irisin according to glycemic response categorization in each phase of the intervention.

**Indicates statistically significant differences between low and high responders ($p < 0.05$).*

Table 10. Associations between changes in irisin and changes in body composition and metabolic profile according to intervention group.

	Δ Irisin Phase 1	**Δ Irisin Phase 2**	
	Whole Sample (n = 38)	**Maintained** (n = 18)	**Intense** (n = 20)
Δ Weight (kg)	-0.23 (0.17)	0.02 (0.94)	-0.37 (0.11)
Δ BMI (kg/m^2)	-0.22 (0.19)	0.03 (0.92)	-0.37 (0.11)
Δ WC (cm)	0.00 (0.99)	0.20 (0.44)	-0.28 (0.23)
Δ BF (%)	-0.21 (0.22)	0.19 (0.48)	-0.14 (0.55)
Δ FM (kg)	-0.18 (0.28)	0.07 (0.80)	-0.25 (0.30)
Δ FFM (kg)	-0.01 (0.96)	-0.05 (0.84)	-0.36 (0.12)
Δ Relative VO_{2peak} †	0.14 (0.39)	0.15 (0.59)	0.34 (0.17)
Metabolic Profile ‡			
Δ TC	-0.26 (0.12)	0.20 (0.44)	**-0.50 (0.03)**
Δ HDL-C	0.19 (0.25)	**0.71 (<0.01)**	0.24 (0.32)
Δ LDL-C	-0.08 (0.64)	0.10 (0.70)	-0.22 (0.36)
Δ TC/HDL	**-0.40 (0.01)**	-0.35 (0.15)	**-0.68 (<0.01)**
Δ TG	**-0.57 (<0.01)**	-0.12 (0.63)	**-0.77 (<0.01)**
Δ Plasma Glucose	-0.39 (0.02)	0.31 (0.23)	-0.35 (0.14)
Δ Plasma Insulin	-0.07 (0.66)	0.03 (0.92)	**-0.51 (0.02)**
Δ HbA_{1c} (%)	-0.19 (0.26)	0.09 (0.73)	0.12 (0.62)
Δ P1 HbA_{1c} (%)	**-0.40 (0.01)**	-----	-----
Δ P2 HbA_{1c} (%)	-----	-0.04 (0.88)	-0.19 (0.42)

Data presented as *r* (*p* value). BMI: body mass index; WC: waist circumference; BF: body fat; FM: fat mass; FFM: fat-free mass; VO_{2peak}: peak oxygen consumption; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglycerides; HbA_{1c}: glycated hemoglobin; P1: phase 1; P2: phase 2. † Measured in mL/kg/min. ‡ All metabolic profile variables measured in mmol/L, except for insulin in pmol/L. Phase 1 (Δ pre-intervention to mid-point); phase 2 (Δ mid-point to post-intervention). Significant change (bolded values) indicated by $p < 0.05$.

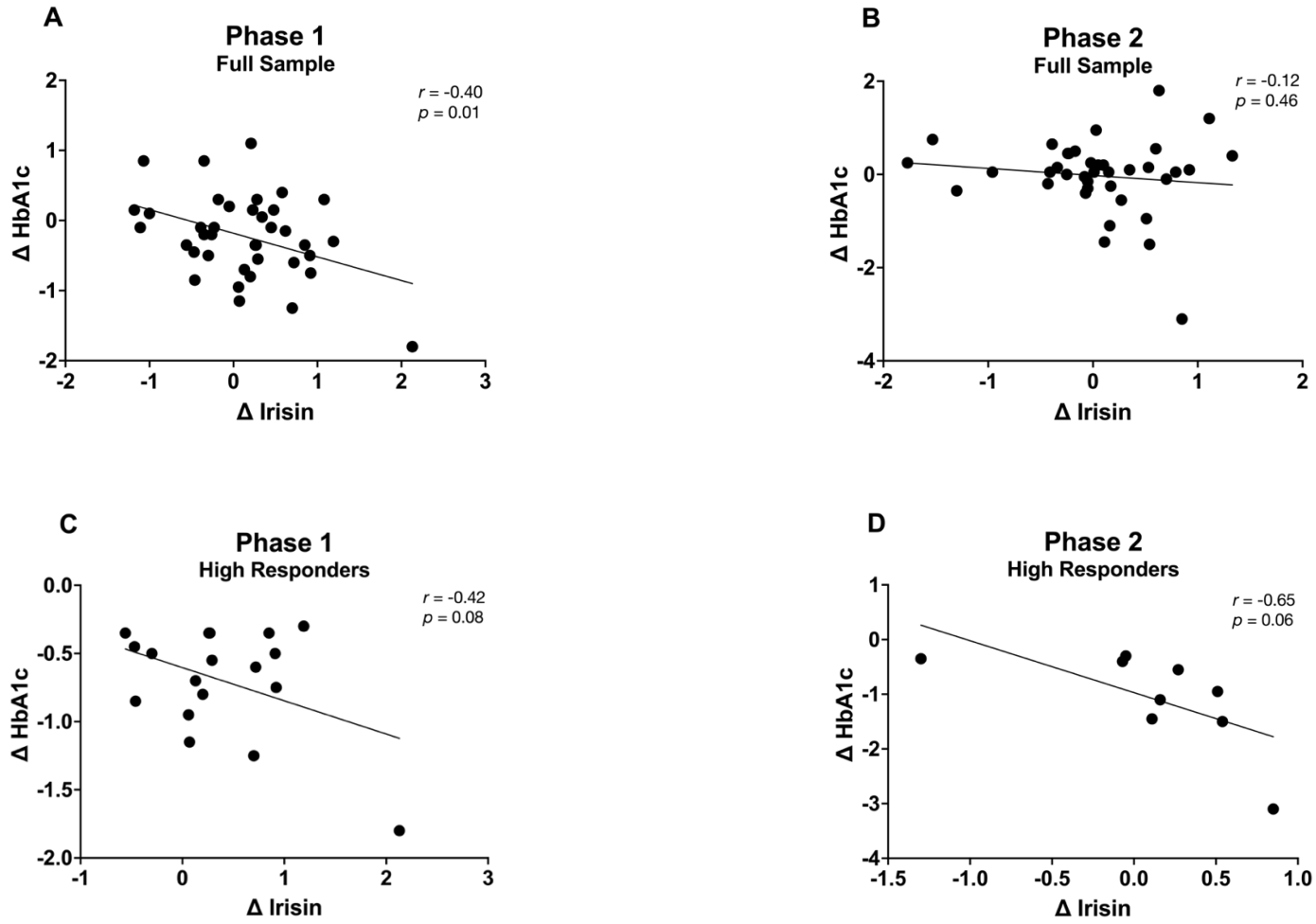


Figure 17. Associations between the change in irisin and glycemia, A) during Phase 1 (pre-intervention to mid-point), B) Phase 2 (mid-point to post-intervention), C) in high responders during Phase 1, and D) in high responders during Phase 2.

Acknowledgements: The authors are grateful to the volunteers for their participation in this project and to the CELLAB students and staff who were involved in data collection and analysis.

B.V.R. was supported by a Canadian Institutes of Health Research Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award and a University of New Brunswick Dr. William S. Lewis Doctoral Fellowship.

Competing interests: The authors declare there are no competing interests.

Author contributions: B.V.R., T.H., N.M.J., M.S. conceived and designed research; B.V.R. and Y.P. performed experiments; B.V.R. and Q.R.E. analyzed data; B.V.R., Y.P., A.M.T., Q.R.E., T.H., N.M.J., S.C., and M.S. interpreted results of experiments; B.V.R. and Y.P. prepared figures; B.V.R. and M.S. drafted the manuscript; B.V.R., T.H., A.M.T., Q.R.E., N.M.J, S.C. and M.S. edited, revised, and approved the final version of the manuscript.

Funding: The INTENSITY study was supported by the New Brunswick Health Research Foundation (Research New Brunswick), Diabetes Action Canada, and the Heart and Stroke Foundation of New Brunswick.

Data availability: All relevant data are reported within the manuscript.

Supplementary material*:* N/A.

5.0 DISCUSSION

5.1 Summary and Impact of Findings

This dissertation examined the exercise-induced irisin response across diverse metabolic health statuses and exercise intensities. Through a series of investigations, this work aimed to achieve three objectives: 1) comprehensively evaluate the analytical performance of commercially available irisin ELISA kits to ensure reliable and precise quantification of circulating irisin in human plasma; 2) assess acute irisin responses across different exercise intensities in individuals with and without overweight/obesity, while exploring its association with insulin sensitivity; and 3) investigate the impact of increasing exercise intensity on chronic circulating irisin adaptations in individuals with prediabetes or T2D and assess irisin's predictive role in achieving individual and clinically meaningful glycemic improvements.

To directly address critical measurement issues in irisin quantification, the first objective of this dissertation was to rigorously compare the quantitative performance of two commercially available ELISA kits: Phoenix Pharmaceuticals and R&D Systems. A key finding of this comprehensive statistical analysis was the identification of substantial analytical discrepancies between these kits. Notably, notwithstanding the more widespread use of the Phoenix Pharmaceuticals kit,[1] R&D Systems exhibited superior between-day reliability and within-subject precision in our samples, accompanied by a stronger alignment with mass spectrometry-derived concentrations.[2]

Such inconsistencies are well-documented in irisin research, as evidenced by significant variability in ELISA-derived concentrations not only between kits from different manufacturers,[3-7] but also between versions from the same manufacturer[7-9] and

even different lots.[10] Despite these known limitations, mass spectrometry (the gold standard[11]) remains largely inaccessible, which has led to the reliance on ELISA kits in exercise studies[1] as a more affordable alternative. These analytical discrepancies became apparent during preliminary work for this dissertation, as initial attempts to quantify irisin using a Phoenix Pharmaceuticals kit revealed considerable variability in baseline irisin concentrations across measurement days, thereby threatening the reliability and validity of subsequent investigations. This reliance on potentially compromised quantification methods, in addition to large inconsistencies in concentrations reported across kits,[5-7,10] emphasizes the clear need for data delineating these analytical challenges. By demonstrating the superior reliability and precision of the R&D Systems irisin ELISA kit, this work directly contributes to resolving inconsistencies across irisin studies that have previously hindered the characterization of irisin's true response to exercise. Moreover, these findings pave the way for greater comparability and interpretability in future exercise trials. With this methodological foundation established, our confidence in the data obtained justified the selection of the R&D Systems ELISA kit for Projects II and III.

To advance the current understanding of irisin's exercise response from an acute perspective, the second objective of this dissertation was to investigate the impact of exercise intensity on irisin responses in individuals with and without overweight/obesity, while exploring its relation to insulin sensitivity. Consistent with previous literature,[12-14] we observed that individuals with overweight/obesity presented with significantly lower baseline irisin concentrations compared to those of a healthy weight. These findings provide crucial insight into the influence of metabolic health on irisin regulation, both at baseline and in response to acute exercise. Notably, however, the acute irisin response to

exercise was blunted in our cohort of individuals with overweight/obesity. Specifically, irisin concentrations were only significantly elevated in response to an acute bout of HIIT in this population, and not MICT. In contrast, the healthy weight group exhibited significant increases with both HIIT and MICT, but a more pronounced response to increased intensity.

These findings reinforce the integral role of exercise intensity as a determinant of irisin responses. Crucially, they also demonstrate that obesity may compromise this acute response, an observation implying altered underlying regulatory mechanisms. Moreover, further stratification by insulin sensitivity demonstrated that individuals with metabolic dysregulation, characterized by lower insulin sensitivity, may also experience blunted irisin responses, highlighting its potential influence on metabolic health outcomes. The diminished responses observed in our cohort may be mechanistically linked to underlying impairments in glucose uptake. Irisin upregulates GLUT4 translocation via AMPK[15-17]; however, this AMPK activation is frequently blunted in individuals with obesity and insulin resistance.[18,19] These mechanistic intricacies may, therefore, differentiate the magnitude of irisin's increases after a single acute bout of exercise between individuals with differing metabolic health statuses and other individual characteristics,[20-22] which may clarify some of the observed variation in the literature.

Together, these findings offer clinically relevant considerations for personalized exercise prescription: while exercise effectively stimulates irisin responses in healthy weight individuals, it may be necessary to prescribe greater exercise intensities to elicit comparable responses in individuals with overweight/obesity. Furthermore, these data reinforce irisin's association with greater insulin sensitivity and support its role in glucose

metabolism.[1] Moreover, to provide additional confidence in the ELISA data obtained in this analysis, western blotting was also employed to confirm the presence of irisin at its known molecular weight. Also, changes in irisin concentrations did not appear to be affected by differences between conditions, suggesting that the observed responses were primarily driven by intensity rather than total work. Altogether, this dissertation contributes to elucidating irisin's acute responses to exercise, a fundamental aspect that is crucial for a comprehensive understanding of irisin's physiological role in exercise metabolism.

Building on the complexities of irisin's acute, intensity-dependent responses, the third objective of this dissertation was to assess the impact of increasing exercise intensity on chronic irisin adaptations in individuals with prediabetes or T2D. We observed that circulating irisin concentrations remained unchanged across the entire 28-week aerobic training intervention. However, despite this absence of a significant group-level change, our individual-level analyses revealed that minor increases in irisin in the first 16 weeks of training, specifically as low as ~8%, were associated with a nearly four-fold increase in the likelihood of being a high glycemic responder. This pivotal observation from the first phase of the trial may establish irisin as an early biomarker linked to clinically meaningful cardiometabolic benefits in individuals with prediabetes or T2D.

Contrary to our hypothesis that higher training intensities would elicit greater adaptations, irisin concentrations did not change throughout the intervention, with no discernible impact from increasing exercise intensity at the group level. This was surprising, considering that irisin's upstream regulators (AMPK/PGC-1α) are intensity-dependent,[23] and that previous research has reported that chronic exercise can increase circulating irisin concentrations in T2D populations, with significantly different responses

according to exercise intensity.[24-27] However, the current investigation provides human data from a more diverse cohort that addresses limitations from previous studies, including: analyses exclusively in men;[24,25] in small sample sizes;[26] and in animal models,[27] which limit generalizability to human physiology. Our findings, therefore, offer vital insights into the nuanced nature of irisin changes in response to chronic exercise in this clinical population, suggesting that factors beyond intensity alone may influence long-term adaptations.

Nevertheless, several factors may account for this unexpected outcome. First, although participants were randomized into groups of varying absolute intensities (maintained-intensity: 4.5 METs; increased-intensity: 6.0 METs), Hrubeniuk et al. (2024) indicated that participants were training within the intended relative intensities in the main trial.[28] Yet, the subtle distinction in overall physiological demand may not have been sufficient to show a discernible difference in chronic irisin adaptations between groups. Recognizing that exercise volume also significantly contributes to exercise adaptations, participant groups were matched for total exercise time per week, allowing for a focused examination of the distinct impact of varying exercise intensities. Furthermore, as a secondary analysis, the original study design may not have been optimally structured to detect intensity-specific chronic irisin adaptations in previously inactive participants. The initial physiological adaptations experienced by previously inactive participants during the first 4 months of aerobic exercise (prior to randomization) may have obscured any subtle intensity-dependent irisin distinctions that might have emerged had they been randomized to their intensity groups from the outset. However, our findings align with other studies that also reported a lack of chronic irisin increase in response to aerobic training

interventions in T2D.[29,30] This indicates that additional factors beyond the scope of this dissertation are likely involved in the complex heterogeneity of irisin responses to chronic exercise. Therefore, further investigations are warranted to identify the contribution of these determinants in individuals with T2D.

5.2 Implications for Personalized Exercise Prescription

By comprehensively delineating the complexities of irisin's exercise response across diverse metabolic contexts, this dissertation provides strong support for the importance of personalized exercise prescription strategies to optimize irisin's therapeutic potential in individuals with obesity and/or T2D. Our investigations reveal that MU individuals may exhibit distinct irisin physiology, as evidenced by the lower circulating concentrations observed in individuals with overweight/obesity and a significant inverse association with HbA_{1c} in T2D. This inherent physiological difference is further supported by altered exercise-induced responses, as detailed in the iMET study. Specifically, while both MICT and HIIT may effectively stimulate irisin responses in healthy weight individuals, higher exercise intensities may be necessary to elicit significant acute responses in individuals with overweight/obesity. Moreover, data from the INTENSITY trial revealed considerable individual variability in chronic irisin adaptations, particularly in its association with glycemic benefits, which further establishes the critical relevance of individual response heterogeneity in guiding personalized strategies.

Overall, the variability in irisin exercise responses reported throughout this dissertation highlights the value of shifting from a 'one size fits all' approach towards more individualized, targeted exercise prescription for MU individuals. While exercise intensity may influence irisin's exercise-induced responses, its optimal application may differ

significantly between individuals and across varying metabolic states. The clinical relevance of this individual-level insight becomes apparent from the fact that over half of the participants in the chronic exercise trial failed to reach the threshold for a beneficial glycemic response. These compelling findings powerfully reinforce the necessity of investigating individual response heterogeneity to clarify the complex, inconsistent irisin responses documented across chronic exercise studies.

5.3 Conclusions

This dissertation advances our understanding of its complex role in exercise metabolism, delineating its intricate relationship with metabolic health status and exercise intensity. Through a series of investigations, this work offers novel insights by establishing robust quantification methods that address critical analytical discrepancies in irisin research, elucidating how acute intensity-dependent responses differ according to metabolic health status, and revealing significant individual heterogeneity in chronic adaptations and its potential as an early biomarker for glycemic improvement. To the best of our knowledge, this is the first comprehensive examination of intensity-dependent irisin responses from both acute and chronic exercise perspectives across a range of metabolic health statuses. These findings accentuate the significance of the independent regulation between FNDC5 expression and irisin's subsequent release into the circulation,[31] offering a vital framework for interpreting the differential impact on irisin physiology and circulating levels resulting from distinct acute and chronic exercise stimuli. Together, the insights gained from these works have significant clinical implications, as they inform the development of personalized exercise strategies to maximize irisin's associated health benefits. In addition to the substantial insights, this research also identifies avenues for

future investigations, including the need for larger studies to characterize irisin's complex response across diverse populations and exercise parameters, especially with longitudinal approaches. Nevertheless, this dissertation provides a foundation for future research aimed at optimizing irisin's role in precision exercise medicine for improved health in clinical populations.

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Conference Presentations:

1. *Impact of Exercise Intensity on Acute Irisin Release and Insulin Sensitivity Across Obesity Status*. 67th Annual Meeting of the American College of Sports Medicine, Boston, MA, USA. May 2024. (Thematic poster/oral presentation)
2. *Elastic band resistance training and diabetes education improves quality of life in older adults living with type 2 diabetes regardless of obesity status: Results from the Band-Frail Study*. Research NB – Health Research Week, Saint John, NB. October 2023. (Poster presentation)
3. Influence of Type 2 Diabetes Mellitus Duration on the Effectiveness of a Lifestyle Intervention: Results from the Intensity Study. 28th Annual Congress European College of Sports Science, Paris, France. July 2023. (Poster presentation)
4. A Cross-Over Examination Of Exercise Intensity And Its Impact On The Acute Release Of Irisin In Individuals Of Different Obesity Status. Atlantic Provinces

Exercise Scientists and Socioculturalists (APES+) Conference 2023, Moncton, NB. March 2023. (Oral presentation)

5. Potential effects of a pre-bariatric surgery exercise intervention on irisin response and its association with physical capacity. Canadian Society for Exercise Physiology Annual Conference, Fredericton, NB. November 2022. (Poster presentation)
6. Outdoor Time and Metabolically Healthy Obesity in Children: Results from the Canadian Health Measures Survey. 65th Annual Meeting of the American College of Sports Medicine, Minneapolis, MN, USA. May 2018. (Oral presentation)
7. Outdoor Time and Metabolically Healthy Obesity in Children: Results from the Canadian Health Measures Survey. Atlantic Provinces Exercise Scientists and Socioculturalists (APES+) Conference, Halifax, NS. March 2018. (Oral presentation)
8. Association Between Fundamental Movement Skills And Health Indicators in Children Aged Between 9 and 12 Years Old. New Brunswick Health Research Foundation - 9th Annual Health Research Conference, Moncton, NB. November 2017. (Oral presentation)
9. Association Between Physical Activity Intensity and Physical Capacity Among Individuals Awaiting Bariatric Surgery. 5th Canadian Obesity Summit - Canadian Obesity Network, Banff Springs, AB. April 2017. (Poster presentation)
10. Association Between Handgrip Muscle Strength and Cardio-Metabolic Z-Score in Children Aged Between 6 and 19 Years of Age: Results from the Canadian Health

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11. Association Between Handgrip Muscle Strength and Cardio-Metabolic Z-Score in Children Aged Between 6 and 19 Years of Age: Results from the Canadian Health Measures Survey. Atlantic Provinces Exercise Scientists and Socioculturalists (APES+) Conference, Charlottetown, PEI. March 2017. (Oral presentation)
12. 3-Minute Thesis: The Impact of Resistance Training on the Acute Secretion of Irisin in Overweight and Obese Younger and Older Adults. University of New Brunswick - Kinesiology Research Day, Fredericton, NB. March 2017. (Oral presentation)
13. Association Between Physical Activity Intensity and Physical Capacity Among Individuals Awaiting Bariatric Surgery. New Brunswick Health Research Foundation - 8th Annual Health Research Conference, Saint John, NB. October 2016. (Poster + Oral presentation)
14. Association Between Physical Activity Intensity and Physical Capacity Among Individuals Awaiting Bariatric Surgery. University of New Brunswick – Kinesiology Research Day, Fredericton, NB. April 2016. (Oral presentation)

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10/23	Population Health Presentation Category Finalist – Health Research Week 2023 ResearchNB
04/23	1st Place PhD Students Category Presentation Award – APES+ Conference – $300 Atlantic Provinces Exercise Scientists and Socioculturalists+
09/18-08/21	President's Doctoral Tuition Award – $20,500 University of New Brunswick
09/18-08/21	CIHR Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award (with UNB/NBIF Top-Ups) – $135,000 Canadian Institutes of Health Research
09/18-08/21	Dr. William S. Lewis Doctoral Fellowship – $100,000 University of New Brunswick
05/18-08/18	NBHRF & CIHR – Strategy for Patient-Oriented Research – Maritime SPOR SUPPORT Unit (NBHRF & CIHR-SPOR-MSSU) Studentship – $35,000 New Brunswick Health Research Foundation (ResearchNB)
05/17-05/18	CIHR Frederick Banting and Charles Best Canada Graduate Scholarship Master's Award (with UNB/NBIF Top-Ups) – $27,500 Canadian Institutes of Health Research

03/18	NBHRF Travel Grant – $1,500
	New Brunswick Health Research Foundation (ResearchNB)
03/18	Best Submitted Abstract – APES+ Conference – $50
	Atlantic Provinces Exercise Scientists and Socioculturalists+
10/17	Nomination: Vanier Canada Graduate Scholarship – CIHR
	University of New Brunswick
03/17	1st Place Masters Level Poster Award & Student Travel Award –$500
	5th Canadian Obesity Summit, Canadian Obesity Network
03/17	Rising Star Award – $250
	New Brunswick Health Research Foundation (ResearchNB)
11/16	Oral Presentation Award – 1st Place Master's Students Category – 8th
	Annual New Brunswick Health Research Conference – $250
	New Brunswick Health Research Foundation (ResearchNB)
2016-*Present*	Sir Howard Douglas Scholar
	University of New Brunswick
09/16-08/17	STEM Scholarship – $4,000
	New Brunswick Innovation Foundation
05/16-08/16	NBHRF Summer Studentship Award – $6,592
	New Brunswick Health Research Foundation (ResearchNB)