The Effects of Resistance Exercise Training on Insulin Resistance Development in Female Rodents with Type 1 Diabetes

Mitchell James Sammut, University of Western Ontario, Kinesiology

Supervisor: Melling, C.W. J., The University of Western Ontario

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology

© Mitchell James Sammut 2023

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Part of the Biochemistry Commons, Exercise Physiology Commons, Exercise Science Commons, and the Molecular Biology Commons

Recommended Citation


This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlsadmin@uwo.ca.
Abstract

The etiology of insulin resistance (IR) development in type 1 diabetes mellitus (T1DM) remains unclear; however, impaired skeletal muscle metabolism may play a role. While IR development has been established in male T1DM rodents, female rodents have yet to be examined in this context. Resistance exercise training (RT) has been shown to improve IR and is associated with a lower risk of hypoglycemia onset in T1DM compared to aerobic exercise. Additionally, the molecular mechanisms mediating RT-induced improvements in insulin sensitivity remain unclear. Therefore, the purpose of this study was to investigate the effects of RT on IR development in female T1DM rodents. Forty Sprague-Dawley eight-week-old female rats were divided into four groups: control sedentary (CS; n=10), control trained (CT; n=10), T1DM sedentary (DS; n=10), T1DM trained (DT; n=10). Multiple low-dose Streptozotocin injections (20 mg/kg each day for seven consecutive days) were used to induce T1DM. Blood glucose levels were maintained in normal range (4-9 mmol/L) with intensive insulin therapy (one implanted insulin pellet; 2 IU/day). CT and DT underwent weighted ladder climbing 5 days/week for six weeks. Intravenous glucose tolerance tests (IVGTT) were conducted on all animals following the six-week period. Results demonstrate that DS animals exhibited significantly increased weekly blood glucose measures compared to all groups including DT (p<0.0001), despite similar insulin dosage levels. This was concomitant with a significant increase in insulin-adjusted area under the curve following IVGTT in DS (p<0.05), indicative of a reduction in insulin sensitivity. Both DT and DS exhibited greater serum insulin concentrations compared to CT and CS (p<0.05). DS animals also exhibited significantly greater glycogen content in white gastrocnemius muscle compared to all groups (p<0.05) whereas DT and DS animals exhibited greater p-Akt:Akt ratio in white vastus lateralis and citrate synthase activity in red vastus lateralis muscle compared to CS and CT (p<0.05). These
results indicate that female rodents with T1DM develop poor glycemic control and IR which can be attenuated with RT, possibly related to differences in intramyocellular glycogen content. This data supports the negative role of elevated muscle glycogen content on insulin sensitivity in T1DM and the potential role of RT in ameliorating these metabolic changes.

**Key Words**

Type 1 diabetes mellitus, insulin resistance, resistance exercise training, skeletal muscle, metabolism, mitochondrial capacity, females
Type 1 diabetes mellitus (T1DM) is a disease which develops when the body’s immune system attacks the pancreas resulting in the loss of insulin production in the body. Insulin is an important hormone produced by the pancreas which allows sugar (glucose) to be used as energy by different tissues in the body. As a result, people with T1DM have unregulated blood glucose levels which can cause a variety of negative health effects such as heart disease. The current treatment for T1DM involves the use of injected insulin which works to replace the lost hormone in the body. Unfortunately, some patients with T1DM develop a resistance to insulin whereby the blood glucose-utilizing effects of injected insulin does not work as efficiently anymore in important tissues such as skeletal muscle, known as insulin resistance (IR). Our understanding of why and how IR develops in people with T1DM is relatively unclear because limited research has focused on this issue, especially in females. Exercise has been shown to improve IR in different diseases, however certain forms of exercise such as aerobic exercise can cause a large and potentially dangerous decrease in blood glucose levels in people with T1DM. Resistance exercise on the other hand has been shown to result in more stable blood glucose levels during exercise in people with T1DM. However, it is not known if resistance exercise training is able to improve IR in females with T1DM. This study aimed to determine if resistance exercise training is effective in improving IR in female rodents with T1DM and how IR may develop in muscle tissue. We found that six weeks of resistance exercise training prevents IR from developing in female rodents with T1DM. We also found that IR in female rodents with T1DM was associated with alterations in the storage form of glucose (glycogen) in muscle tissue which is also prevented with resistance exercise training. This suggests that improper storage of glucose in muscle may contribute to the
development of IR and that resistance exercise training is an effective exercise intervention to improve IR and muscle glycogen storage in female rodents with T1DM.
Co-Authorship Statement

Dr. Jamie Melling of Western University, London, Ontario, Canada was involved in project organization and development, interpretation of findings, and thesis revision. David McBey was involved in animal care and harvesting of tissues. Amit Sayal was involved in tissue preparation and analysis.
Dedication

I would like to dedicate this thesis to my family and friends who have supported me through this process. I would not have been able to do any of it without you and your unwavering support and encouragement.
Acknowledgments

To my supervisor Dr. Jamie Melling, I am grateful for your invaluable mentorship, expertise, advice, patience, humour, and support since my fourth-year undergraduate thesis project, throughout my master’s, and beyond. Your enthusiasm for discovery and the advancement of scientific knowledge inspires and motivates me to continue in my academic career and pursuit of my research interests. I look forward to the new and exciting research projects ahead!

To my fellow graduate students, undergraduate thesis/summer students in the lab, and officemates: Silar Gardy, David McBey, Amit Sayal, Julianna Mereu, Theres Tijo, Stephanie Rizza, Alyssa Honkoop, and Andrew D’Souza. Thank you for your help, support, great conversations, and many laughs. For those of you continuing in academia, I hope to cross paths again at conferences and meetings in the future. I wish you all the best in your future endeavours!

To my friends: Thank you for your friendship, laughs, great times, and support throughout my entire masters. I couldn’t have done it without you!

To my family: I will never be able to thank you enough for your immense and unconditional love, encouragement, and support in every endeavour I have and will pursue. I would not be where I am today without you. Love you and thank you, from the bottom of my heart.
Table of Contents

Abstract ............................................................................................................................... ii

Summary for Lay Audience ............................................................................................... iv

Co-Authorship Statement ................................................................................................. vi

Dedication ......................................................................................................................... vii

Acknowledgements .......................................................................................................... viii

Table of Contents ............................................................................................................... ix

List of Abbreviations ....................................................................................................... xiii

List of Figures .................................................................................................................. xvi

List of Appendices .......................................................................................................... xvii

Chapter 1 .......................................................................................................................... 1

1.1 Overview of Type 1 Diabetes Mellitus ...................................................................... 1

1.2 Insulin Resistance .................................................................................................... 5

1.2.1. Definition of Insulin Resistance ........................................................................ 5

1.2.2. Assessment of Insulin Resistance .................................................................... 6

1.2.3. Insulin Resistance in Type 1 Diabetes Mellitus ............................................... 6

1.3 Skeletal Muscle ....................................................................................................... 8

1.3.1. Skeletal Muscle Insulin Signalling and Glucose Metabolism ....................... 8
1.3.2. Skeletal Muscle Insulin Resistance .....................................................9

1.3.3. Skeletal Muscle Insulin Resistance in T1DM.................................10

1.4 Resistance Exercise..................................................................................14

1.4.1. Benefits & Risks of Exercise in T1DM.............................................14

1.4.2. Resistance Exercise in T1DM..........................................................15

1.4.3. Resistance Exercise and Insulin Resistance ....................................15

1.4.4. Resistance Exercise and Insulin Resistance in T1DM.................16

1.5 Sex Differences in Insulin Sensitivity in Diabetes ..............................17

1.6 Rationale ...............................................................................................18

1.7 Purpose and Hypothesis.........................................................................19

Bibliography ..................................................................................................20

Chapter 2 ....................................................................................................29

2.1 Background...........................................................................................29

2.2 Materials and Methods.........................................................................31

2.2.1. Ethics Approval..................................................................................31

2.2.2. Animals ..........................................................................................31

2.2.3. Experimental Groups .....................................................................32

2.2.4. Experimental Procedures.............................................................32
2.2.4.1. T1DM Induction and Insulin Pellet Implantation ............32

2.2.4.2. Exercise Training ..............................................................32

2.2.5. Experimental Measures ..........................................................33

2.2.5.1. Body Weights and Blood Glucose .........................................33

2.2.5.2. Intravenous Glucose Tolerance Test ....................................33

2.2.5.3. Blood and Tissue collection ..................................................34

2.2.5.4. Muscle Glycogen Content ......................................................34

2.2.5.5. β-oxidation Activity .................................................................35

2.2.5.6. Citrate Synthase Activity .........................................................35

2.2.5.7. Western Blot ........................................................................36

2.2.5.8. Insulin and Estradiol Quantification .....................................37

2.3 Data Analysis .....................................................................................37

2.4 Results ...............................................................................................38

2.4.1. Animal Characteristics ...............................................................38

2.4.2. Carrying Capacity ........................................................................40

2.4.3 Serum Insulin and Estradiol Concentration ..................................40

2.4.4. Intravenous Glucose Tolerance Test ..........................................42

2.4.5. Correlation between Area Under the Curve and Estradiol ..........44
2.4.6. Muscle Glycogen Content, β-oxidation activity, and Citrate Synthase Activity .................................................................................................................. 44

2.4.7. Muscle Protein Content ........................................................................ 46

2.5 Discussion ..................................................................................................... 49

2.6 Conclusions .................................................................................................. 54

Bibliography ........................................................................................................... 56

Appendices ............................................................................................................. 63

Curriculum Vitae ................................................................................................... 80
List of Abbreviations

T1DM - Type 1 diabetes mellitus

T2DM – Type 2 diabetes mellitus

IR – Insulin resistance

HLA – Human leukocyte antigen

CVD – Cardiovascular disease

ICA – Islet cell antigen

IAA – Antibodies to insulin

GAD65 – Glutamic acid decarboxylase isoform 65

IA-2 – Insulinoma antigen 2/islet tyrosine phosphatase 2

ZnT8 – Zinc transporter isoform 8

TNF-α – Tumour necrosis factor alpha

IFN-γ – Interferon gamma

FFA – Free fatty acid

DKA – diabetic ketoacidosis

HBA1c – Glycated hemoglobin

OGTT – Oral glucose tolerance test

DCTT – Diabetes control and complications trial
IIT – Intensive insulin therapy
CIT – Conventional insulin therapy
AGE – Advanced glycation end product
HEC – Hyperinsulinemic euglycemic clamp
GIR – Glucose infusion rate
IVGTT – Intravenous glucose tolerance test
AUC – Area under the curve
BMI – Body mass index
INSR – Insulin receptor
IRS – Insulin receptor substrate
PI3K – Phosphoinositide3-kinase
Akt – Protein kinase B
GLUT4 – Glucose transporter type 4
G6P – Glucose-6-phosphate
HK – Hexokinase
TAG – Triacylglycerol
IMCL – intramyocellular lipid
DAG – Diacylglycerol
PKCθ – Protein kinase C θ
CD36 – Lipid transporter
$^{31}$P-MRS - Phosphorus magnetic resonance spectroscopy
PCr – Phosphocreatine
ROS – Reactive oxygen species
RT – Resistance Training

ERα – Estrogen receptor alpha

CS – Control sedentary

CT – Control trained

DS – T1DM sedentary

DT – T1DM trained

STZ – Streptozotocin

BSA – Bovine serum albumin

ELISA – Enzyme-linked immunosorbent assay

ANOVA – Analysis of variance

SCHAD – short-chain β-hydroxyacyl-CoA dehydrogenase

OXPHOS – Oxidative phosphorylation

GS – Glycogen Synthase

DNL – Denovo lipogenesis
List of Figures

Chapter 1 ......................................................................................................................................................1

Figure 1.1. Insulin Signalling Cascade in Skeletal Muscle Cells ...............................................9

Figure 1.2. Potential Mechanisms of Skeletal Muscle Insulin Resistance in T1DM
.............................................................................................................................................................13

Figure 1.3. Study Purpose and Hypothesis .........................................................................................19

Chapter 2 ......................................................................................................................................................29

Figure 2.1. Weekly blood glucose (a) and body mass (b) measures.................................39

Figure 2.2. Maximal carrying capacity for animals undergoing RT..............................40

Figure 2.3. Serum estradiol (a) and serum insulin (b) concentrations .......................41

Figure 2.4. IVGTT blood glucose (a) and insulin-adjusted IVGTT AUC (b) ......43

Figure 2.5. Linear regression between insulin-adjusted IVGTT AUC and serum estradiol
.............................................................................................................................................................44

Figure 2.6. Red gastrocnemius glycogen content (a), white gastrocnemius glycogen
content (b), soleus SCHAD activity (c), red vastus lateralis citrate synthase activity (d)
.................................................................................................................................................................................................45

Figure 2.7. Red vastus lateralis p-Akt:Akt ratio (a), white vastus lateralis p-Akt:Akt ratio
(b), red vastus lateralis OXPHOS protein content (c), representative western blots images
(d).....................................................................................................................................................................................46

Figure 2.8. Graphic Summary of Study Results ..............................................................55
List of Appendices

**Appendix A:** Multiple Low-dose Streptozotocin Protocol (rat) ...........................................63

**Appendix B:** Insulin Pellet Implantation (rat) .................................................................66

**Appendix C:** Intravenous Glucose Tolerance Test .....................................................67

**Appendix D:** Glycogen Assay .........................................................................................70

**Appendix E:** β-oxidation, short-chain β-hydroxyacyl-CoA dehydrogenase (SCHAD) Assay ........................................................................................................73

**Appendix F:** Bradford Protein Assay ........................................................................75

**Appendix G:** Western Blot Protocol .........................................................................76
Chapter 1

1.1. Overview of Type 1 Diabetes Mellitus

Diabetes mellitus is a metabolic chronic disease characterized by an impaired ability to control circulating blood glucose levels. The two major classifications of diabetes mellitus include type 1 (T1DM) and type 2 diabetes (T2DM). While both forms of diabetes manifest as chronically elevated blood glucose levels (hyperglycemia), the underlying mechanisms drastically differ. T2DM is the most common form of diabetes constituting approximately 90% of diagnoses and involves impairments of insulin-action on tissues in the body (insulin resistance; IR) and hyperinsulinemia resulting from lifestyle (e.g., nutrition, physical inactivity), environmental (e.g., pesticides, air pollutants) and/or genetic factors (1, 2). Comparatively, T1DM is less common, constituting the remaining approximately 10% of diagnoses of diabetes, and involves the loss of endogenous insulin production by the pancreas. It is believed that a combination of genetic and/or environmental factors influence the onset of T1DM (3). It has been reported that approximately 50% of the risk factors for the development of T1DM are hereditary in nature and governed by multiple human leukocyte Antigen (HLA) and non-HLA genes (3, 4). Despite the strong genetic component of T1DM development, the majority of individuals with high-risk genes do not go on to develop T1DM which suggests an important role of environmental factors such as viral infection (e.g., enterovirus) and nutritional deficiency (e.g., Vitamin D deficiency) in the triggering of disease onset (3). The prevalence of diabetes is predicted to rise significantly, with T2DM likely to account for the majority of the projected global increase to 10.9% of the population by 2045 (5). Similarly, the global prevalence of T1DM is also projected to increase by 60-107% by the year 2040 (6). In Canada, diabetes is predicted to affect 12% of the population by 2031, with T1DM accounting for 5-10% of diabetes prevalence (7). While the exact reasons for this increase are
unknown, it is theorised that a variety of lifestyle and environmental factors including obesity, dietary intake, viral infection (e.g., COVID-19), early life and maternal factors, micronutrient deficiency (e.g., Vitamin D deficiency), and pollutants are playing a role in mediating the observed increase (8). This increase in T1DM prevalence presents a significant economic burden and is projected to cost the Canadian health care system nearly five billion dollars by 2031 (7). T1DM is also associated with several prominent health complications affecting the Canadian population such as cardiovascular disease (CVD) (9), COVID-19-related complications (10), disability (11), and frailty (12) which will undoubtedly increase public health burden, especially as the population ages. Therefore, therapeutic strategies for the management of T1DM are of great importance as more of the population becomes affected by this disease.

The etiology of T1DM onset is unclear; however, it is believed that environmental factors can trigger an autoimmune-mediated destruction of the insulin-producing pancreatic beta cells in genetically predisposed individuals (13). This is evident by the presence of several circulating autoantibodies against pancreatic islets in at-risk individuals and those with diagnosed T1DM (13). Such antibodies include islet cell cytoplasmic antibodies (ICA), antibodies to insulin (IAA), glutamic acid decarboxylase isoform 65 (GAD65), insulinoma antigen 2/islet tyrosine phosphatase 2 (IA-2) and zinc transporter isoform 8 (ZnT8) (13). The invasion of beta cells by immune cells and the subsequent release of various cytokines such as IL-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ lead to apoptosis of the cell (14). The destruction of insulin-producing pancreatic beta cells is also accompanied by the dysfunction of nearby glucagon-producing alpha cells (15). Insulin and glucagon are the primary hormones responsible for glucose uptake in response to high blood glucose (hyperglycemia) and the increase in circulating glucose in response to low blood glucose (hypoglycemia), respectively (16).
In individuals without T1DM, blood glucose levels are tightly regulated and maintained between a narrow range of 4.0-8.0 mmol/L (17). Without circulating insulin, glucose from the blood is unable to be taken up by cells in the body for energy, resulting in hyperglycemia. In patients with T1DM, the release of glucagon is unregulated which exacerbates hyperglycemia due to insulin-deficiency (16). Moreover, the loss of glucose utilization and insulin-mediated inhibition of lipolysis results in increased circulating free fatty acids (FFA) (18). FFA’s are converted to ketone bodies by the liver, which serve as an alternative fuel source for cells (18). Increased production and output of ketone bodies into circulation increases the acidity of the blood which can be life-threatening (13). This is referred to as diabetic ketoacidosis (DKA) and is a common symptom present during the diagnosis of T1DM, especially in pediatric patients (19). Since its discovery in 1921, insulin has been used to ameliorate many of the metabolic abnormalities present in T1DM and has drastically increased patients life expectancy (20, 21). Unfortunately, exogenous insulin treatment remains suboptimal and does not mimic physiologic insulin release and kinetics (22). Consequently, the development of significant complications including micro- and macro-vascular disease persist in patients with T1DM (23).

Although T1DM has traditionally been considered a disease of juvenile-onset, it has been shown that up to 50% of diagnoses occur in adults (24). Common symptoms of undiagnosed T1DM include excessive urination (polyuria), excessive thirst (polydipsia), unintended weight loss, and DKA (24). The diagnosis of T1DM is based on a fasting blood glucose concentration >7.0 mmol/L and a non-fasting blood glucose concentration >11.1 mmol/L, in addition to the aforementioned symptoms. Hemoglobin is a protein found in red blood cells that binds and transports oxygen from the lungs to cells throughout the body (25). Glycated hemoglobin (HbA1c) can be used as a marker of glycemic control over several months and represents a percentage of total hemoglobin (25).
HbA1c can also be used in the diagnosis of T1DM at a concentration >48 mmol/mol (6.5%) (24); however, this method of diagnosis is less sensitive when compared to fasting or postprandial blood glucose measurements (24, 26). C-peptide, a by-product of insulin production, can also serve as a diagnostic tool for T1DM as it can reflect endogenous insulin deficiency (24, 27). Lastly, an abnormal postprandial glucose tolerance using an oral glucose tolerance test (OGTT) may also be used for the diagnosis of T1DM (24, 26). While the aforementioned diagnostic tools may aid in the classification of T1DM at diagnosis, they do not necessarily differentiate between the two forms of diabetes (24). As such, specific biomarkers of T1DM are used to confirm diagnosis of this disease (24). For example, 90% of newly diagnosed children and adolescents with T1DM present with measurable antibodies against beta cells (24, 28). Autoantibodies are routinely measured and used in T1DM preclinical risk assessment and diagnosis (24). T1DM has three distinct preclinical stages where stage 1 reflects the beginning of beta cell autoimmunity, defined as the presence of two or more islet auto-antibodies despite normoglycemia (29). Stage 2 is characterized by the presence of two or more islet auto-antibodies and the manifestation of glucose intolerance and/or dysglycemia without symptoms of T1DM (29). Stage 3 represents the complete manifestation of T1DM and is defined as the presence of two or more islet auto-antibodies accompanied with glucose intolerance and/or dysglycemia with overt symptoms of T1DM (e.g., DKA, polyuria, polydipsia) (29).

From 1982-1993, the Diabetes Control and Complications Trial (DCCT) was conducted to compare intensive insulin therapy (IIT) versus conventional insulin therapy (CIT) on long-term diabetes-related complications (30). IIT involves the use of an insulin pump or three or more daily insulin injections guided by frequent self-monitoring of blood glucose whereas CIT involves two daily injections of insulin with less frequent self-monitoring of blood glucose (30, 31). The
rationale for this trial was based on the hypothesis that chronic hyperglycemia was a major contributing factor in the pathogenesis of T1DM-related vascular complications and that tight glycemic control using IIT may improve mortality and morbidity in these patients (31, 32). Poor glycemic control and hyperglycemia can result in the buildup of advanced glycation end products (AGEs; glycated proteins and lipids), which damage cells in the body and contribute to T1DM-related complications (32–34). Indeed, results from the DCCT show that adequate glycemic control with IIT reduces the development and progression of complications such as retinopathy, nephropathy, and neuropathy and increases mortality compared to CIT in T1DM patients (31, 35, 36). Although IIT reduces hyperglycemia, it can be associated with various negative health effects in a significant proportion of patients such as dyslipidemia, hypertension, and obesity which are associated with complications such as CVD (37). Therefore, while IIT is the current standard of care for patients with T1DM, it does not necessarily mitigate the development of CVD in many patients.

1.2 Insulin Resistance

1.2.1 Definition of Insulin Resistance

The concept of insulin resistance was first described by Himsworth in 1936 after the observation that concurrent administration of glucose and insulin either resulted in a reduction in blood glucose in “insulin-sensitive” patients with diabetes or an increase in blood glucose in “insulin-insensitive” patients with diabetes (38). Today, IR refers to the attenuation of insulin action on cells in the body. Specifically, impaired peripheral tissue (e.g., skeletal muscle and adipose) glucose uptake, impaired suppression of adipose lipolysis, and impaired suppression of hepatic glucose output in response to circulating insulin (39, 40).
1.2.2 Assessment of Insulin Resistance

The gold-standard method for the assessment and diagnosis of IR is the hyperinsulinemic-euglycemic clamp (HEC) which involves a constant infusion of intravenous insulin with simultaneous infusion of glucose to maintain euglycemia (stable blood glucose levels). A greater glucose infusion rate (GIR) is indicative of greater insulin sensitivity as tissues can effectively uptake glucose from circulation for a given amount of infused insulin, thereby requiring a greater infusion of glucose into the blood to maintain euglycemia. In contrast, a lower GIR is indicative of IR as tissues are less sensitive to a given amount of infused insulin and take up glucose at a slower rate, thereby requiring less glucose infusion to maintain euglycemia (41). Although the HEC is considered the most direct assessment of IR, it can be costly, time-consuming, and laborious (41, 42) which may limit its clinical utility. Alternatively, administering a glucose challenge either orally with an OGTT or intravenously with an intravenous glucose tolerance test (IVGTT) are often used to indirectly assess IR by following the rise and fall in blood glucose in response to a bolus of glucose (41). The area under the curve (AUC) of blood glucose during an OGTT and IVGTT is indicative of insulin sensitivity whereby a lower and greater AUC reflect greater and lower glucose clearance (i.e., greater, and lower insulin sensitivity), respectively.

1.2.3 Insulin Resistance in Type 1 Diabetes Mellitus

While IR is typically associated with the pathogenesis of T2DM, significant whole-body IR has been established in approximately one-third of patients with T1DM (43, 44). The presence of IR in T1DM is referred to as “double diabetes” as both the loss of endogenous insulin production and resistance to insulin are present (45). This is concerning as a subset of patients with T1DM develop resistance to the current standard of care which is exogenous insulin treatment. Of clinical concern is the increased risk of micro and macro-vascular complications such as retinopathy and CVD that
IR and markers of IR pose in patients with T1DM (44, 46–48). Indeed, IR has been shown to be a better predictor of CVD compared to glycemia in T1DM (46). Considering that CVD is one of the leading causes of mortality in T1DM, the importance of mitigating IR in this population cannot be understated (49, 50).

Recently, it has become clear that IR development in T1DM is distinct from that observed in other metabolic disease states (51). Specifically, T1DM patients who develop IR are phenotypically different from individuals with T2DM and metabolic syndrome. Indeed, it has been shown that IR is present in those with T1DM who are lean and of normal body mass index (BMI) compared to healthy controls (43). IR is also observed in otherwise healthy and normal weight adolescents and young adults with T1DM (52–54). IR in these individuals is associated with reduced cardiovascular function such as left ventricular hypertrophy, diastolic dysfunction, and poor cardiopulmonary fitness including reduced peak oxygen consumption and oxygen uptake (53, 54). Additionally, hyperglycemia cannot completely explain IR development in this population as IR persists in many patients with T1DM despite adequate glycemia maintained with IIT (43). In support of this, findings from Bergman and colleagues (2012) report that IR in T1DM is not attributable to common predictive factors such as BMI, plasma lipids, visceral adiposity, or acute glucotoxicity and glycemic control (55). Moreover, it was also shown that IR in T1DM is tissue-specific, developing primarily in the liver and skeletal muscle of patients (55).
1.3 Skeletal Muscle

1.3.1 Skeletal Muscle Insulin Signalling and Glucose Metabolism

By mass, skeletal muscle tissue represents the largest depot in the body for glucose disposal and FFA metabolism in the postprandial state and is responsible for ~80% of glucose uptake under euglycemic hyperinsulinemic conditions (56). As such, skeletal muscle is an important metabolic tissue in the regulation of whole-body insulin sensitivity and substrate metabolism. In skeletal muscle cells, the initiation of the insulin signalling cascade involves the binding of circulating insulin to the extracellular domain of the tyrosine kinase insulin receptor (INSR) on the outside of the sarcolemma (57). The binding of insulin to the INSR results in auto-phosphorylation (kinase phosphorylation of itself) of tyrosine residues on the intracellular domain of the INSR, subsequently activating signalling proteins, including insulin receptor substrate (IRS) (Fig. 1.1) (57, 58). Activation of IRS recruits phosphoinositide 3-kinase (PI3K) and leads to the activation of protein kinase B (Akt), eventually resulting in the translocation of glucose transporter type 4 (GLUT4)-containing intracellular vesicles to the sarcolemma (57, 58). GLUT4 vesicles fuse with the sarcolemma which increases the abundance of GLUT4 and the uptake of glucose from circulation into the muscle cell. Once inside the muscle cell, glucose is phosphorylated by hexokinase to glucose-6-phosphate (G6P), “trapping” glucose in the cell (59). The main fates of G6P inside the muscle cell are 1) metabolism for ATP production through glycolysis and oxidation in the mitochondria, or 2) storage as intramyocellular glycogen (59). The flux through each intracellular pathway depends on the energy demand of the cell such that during high energy demands (e.g., during exercise), metabolism of G6P will increase, whereas, during low energy demands (e.g., at rest), glycogen storage will increase (59).
The binding of insulin to the insulin receptor (INSR) results in autophosphorylation of the intracellular domain of the INSR which recruits insulin receptor substrate (IRS) and activates phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt). Activation of Akt eventually leads to the translocation of intracellular vesicles containing glucose transporter type 4 (GLUT4) which fuse to the plasma membrane and increase glucose uptake via increased GLUT4 abundance. Glucose is phosphorylated by hexokinase (HK) once inside the cell and is primarily metabolized through glycolysis and oxidized in the mitochondria to produce ATP or stored as glycogen. Created using BioRender.

1.3.2. Skeletal Muscle Insulin Resistance

As skeletal muscle is the largest insulin-sensitive tissue, it is implicated in the development of IR (56, 60). The exact mechanisms underlying the development of IR in skeletal muscle are multifaceted and still unclear. However, one of the primary prevailing theories is the muscle lipotoxicity model of IR (61). This theory implicates ectopic lipid accumulation in the attenuation of
the intracellular insulin signalling cascade in skeletal muscle. Lipids are readily stored in skeletal muscle in neutral forms such as triacylglycerol (TAG) where they are used for energy production by the mitochondria. The pathology of IR may begin when the influx and storage of lipids in the muscle cell, also known as intramyocellular lipid (IMCL), exceeds the cells’ ability to handle such a lipid burden, i.e., lipid loads exceed the oxidative abilities of the cell resulting in “metabolic overload” (62). The lipid influx may be a result of increased levels of circulating FFAs in the blood due to adipose tissue IR (impaired suppression of lipolysis), liver lipid “spill-over”, and/or excess caloric or dietary lipid consumption, among other reasons (61, 63). With such a lipid influx, lipid intermediates, including diacylglycerol (DAG) and ceramides may begin to accumulate inside the cell (62, 63). These lipid intermediates, such as DAG, exhibit an insulin-desensitizing effect inside muscle cells by activating signalling proteins, such as protein kinase C θ (PKCθ), leading to the inhibitory phosphorylation of insulin signalling proteins, such as IRS, thus attenuating the insulin signalling cascade (63, 64). Additionally, insulin-desensitizing lipid intermediates may result in inflammatory signalling, oxidative stress, mitochondrial dysfunction, and downregulation of genes involved in glucose metabolism that further interfere with intracellular insulin signalling in muscle (61).

1.3.3. Skeletal Muscle Insulin Resistance in T1DM

Skeletal muscle IR has been established in patients exhibiting whole-body insulin sensitivity impairments (55, 65, 66). The etiology of skeletal muscle IR development in T1DM is unknown and has received relatively limited examination compared to other metabolic diseases such as T2DM and obesity. The muscle lipo-toxicity model of IR may apply in the setting of T1DM as our laboratory and others have observed elevated levels of skeletal muscle lipid content, including elevated DAG and lipid-transporter CD36 content in rodent models of T1DM with IR (67–70).
The source of excess lipid likely differs between T1DM and other metabolic disease states because insulin-insensitive patients with T1DM often do not exhibit excess adiposity (54, 55). Alternatively, increased IMCL in T1DM may arise from de novo lipid synthesis in muscle cells from excess glucose (70, 71). While findings of elevated muscle lipid are consistent in rodent models of T1DM, this observation is less consistent in patients with studies showing both elevated (51, 72) and non-elevated (54, 73, 74) IMCL content in T1DM individuals with IR. Therefore, it is likely that the muscle lipo-toxicity model of IR does not completely explain the mechanisms behind IR development in T1DM. Therefore, impairments involving both lipid and glucose metabolism in skeletal muscle may be involved (Fig. 1.2). It has been shown that 30-40% of the glucose during an OGTT is oxidized within skeletal muscle versus the 15% stored as intramyocellular glycogen (75). Thus, it is plausible that intracellular substrate oxidation within skeletal muscle is an important determinant of insulin sensitivity and glucose uptake. Indeed, it has been shown that skeletal muscle oxidative capacity is a better predictor of insulin sensitivity than muscle lipid status (76). Our laboratory has previously shown impairments in skeletal muscle oxidative capacity in a rodent model of T1DM with IR (69) which is supported by studies in humans showing impairments in mitochondrial function and oxidative capacity in the skeletal muscle of patients with T1DM despite normal glycemia through insulin treatment. Using in-vivo methods such as $^{31}$P-MRS, individuals with T1DM demonstrate impairments in skeletal muscle phosphocreatine (PCr) recovery which is an indirect measure of oxidative capacity (77). Insulin-stimulated oxidative ATP production assessed with $^1$H/$^{31}$P-MRS in skeletal muscle is impaired in individuals with T1DM who exhibited IR (73). Cree-Greene and colleagues (2015) also reported delayed skeletal muscle ADP recovery in insulin-resistant youth with T1DM using $^{31}$P-MRS (78). Moreover, in-vitro assessments using muscle biopsies from young adults with T1DM have found
intrinsic impairments in mitochondrial respiration and elevated reactive oxygen species (ROS) production (79, 80). Elevated mitochondrial ROS within muscle cells, have been shown to further impair insulin sensitivity through oxidative stress (81), suggesting that impairments in the oxidative ability of skeletal muscle may relate to IR development in T1DM. These data may also suggest that the downregulation of distal intracellular metabolism (e.g., glucose metabolism pathways) may be a superior marker of skeletal muscle IR compared to insulin signalling. For example, despite differences in muscle DAG content, our laboratory has observed no differences in DAG-mediated IRS1 phosphorylation (a marker of IRS1 inhibition) between insulin-resistant sedentary and insulin-sensitive exercise-trained rodents with T1DM (69, 70). Additionally, we and others have shown an “uncoupling” of Akt phosphorylation (a marker of activated insulin signalling) and insulin sensitivity (70, 82). Rather we have demonstrated muscle citrate synthase activity (a marker of oxidative capacity) to be a better indicator of IR in our pre-clinical model of T1DM (69).

Since IR can develop in patients with T1DM despite IIT, hyperglycemia may not play a primary role in IR development in this population (46, 83). A novel theory recently proposed by Gregory and colleagues (2020) has implicated peripheral hyperinsulinemia as a potential mechanism mediating the development of IR in T1DM (84). Physiologic insulin release from the pancreas involves insulin passing through the portal vein of the liver, where ~80% of insulin is cleared from circulation (85). This is known as the “first-pass effect,” such that the resulting concentration of circulating insulin seen at peripheral tissues is substantially less than what was released from the pancreas (83). In contrast, with subcutaneous injections of exogenous insulin in patients with T1DM, the first-pass effect is lost, and peripheral tissues are exposed to levels of circulating insulin ~2.5 times greater than that of non-T1DM individuals (84). Indeed, studies investigating
experimental peripheral hyperinsulinemia comparable to levels observed in T1DM have induced IR in healthy humans (86–88). Further, elicited hyperinsulinemia resulted in altered intramyocellular glucose metabolism between oxidative and non-oxidative pathways (86–88). It has also recently been shown that IR in patients with T1DM is more closely associated with hyperinsulinemia than hyperglycemia (83).

Figure 1.2. Potential Mechanisms of Skeletal Muscle Insulin Resistance in T1DM.

Hyperinsulinemia, hyperglycemia and impaired glucose and lipid metabolism such as excess glucose influx, increased intramyocellular lipid (IMCL), mitochondrial dysfunction, reactive oxygen species (ROS) emission, and oxidative stress may contribute to reduced insulin-stimulated glucose uptake in the skeletal muscle of patients with T1DM. Created using BioRender.
1.4. Resistance Exercise

1.4.1. Benefits & Risks of Exercise in T1DM

Exercise is an important therapeutic tool for individuals with T1DM and improves muscle strength, physical fitness, glycemic control, insulin requirements, blood lipid profile, blood pressure, micro- and macro-vascular health, insulin resistance, and mortality in this population (89, 90). Of importance, exercise improves cardiac function and health resulting in the prevention of CVD development in patients with T1DM (91–93). Due to the known health-promoting effects of exercise in this population, Diabetes Canada recommends exercise training for treating and managing T1DM (94).

One of the largest barriers to engaging in physical exercise in patients with T1DM is the fear of hypoglycemia (95, 96). As such, more individuals with T1DM do not meet physical activity recommendations compared to their non-T1DM counterparts (95). During prolonged moderate intensity exercise, blood glucose levels decline due to increases in insulin-independent glucose uptake by contracting skeletal muscles for energy production. In individuals without T1DM, glucose counter-regulation during exercise involves a neurohormonal response to regulate the fall in blood glucose levels. Specifically, during exercise there is a reduction in circulating insulin and an increase in glucagon to stimulate hepatic glycogen breakdown (glycogenolysis) and glucose production through gluconeogenesis (97). Patients with T1DM are challenged to reduce circulating exogenous insulin during exercise which in turn may suppresses glucagon release from the pancreas (97). The suppression of glucagon results in an uncoupling between skeletal muscle glucose uptake and the release of glucose into the bloodstream. The subsequent reduction in hepatic glycogenolysis and gluconeogenesis results in hypoglycemia. Hypoglycemia leads to
weakness and dizziness, resulting in unconsciousness, and can even be fatal if severe and unattended.

1.4.2. Resistance Exercise in T1DM

While exercise-induced hypoglycemia can occur with many forms of exercise in T1DM, it is primarily associated with continuous aerobic exercise at moderate intensity (96, 98). In contrast, anaerobic exercise, such as resistance exercise, generally involves more stabilized glycemia during exercise and may even result in a transient increase in blood glucose (96, 98–100). As such, it has been suggested that performing resistance exercise may help offset hypoglycemia risk in exercising individuals with T1DM (96, 98). Additionally, an important aspect of resistance exercise is the improvement of skeletal muscle mass and health. Indeed, impairments in skeletal muscle health have been observed in T1DM animal models and humans such as mitochondrial dysfunction, pathological fiber-type shifts, reduced myofiber size, and muscle weakness (79, 80, 101–103). These impairments have been shown to be improved with regular resistance exercise (104–106). Therefore, resistance exercise training (RT) may be a safe and effective method of exercise that may reduce hypoglycemia risk, and improve skeletal muscle health and IR, thereby reducing CVD risk in T1DM.

1.4.3. Resistance Exercise and Insulin Resistance

RT is an effective method of exercise to increase insulin sensitivity in young and old adults (107). For metabolic diseases exhibiting IR, including T2DM, engaging in RT has been shown to improve whole-body glucose homeostasis and insulin sensitivity (108–110). In T2DM, the glycemic and insulin-sensitizing effects of RT have also been found to be comparable to or even greater than those conferred by aerobic exercise training (108, 111). While the exact mechanisms governing
RT-induced increases in glycemic control and insulin sensitivity are not well understood, it is plausible that increased muscle mass has a mediating role (111–113). Hypertrophied muscle fibers theoretically provide a larger “sink” for glucose disposal and storage, thus increasing insulin-stimulated glucose uptake (111, 113). However, the mechanistic relationship between RT-induced muscle hypertrophy and insulin sensitivity remains unclear (113). For example, numerous studies have shown improvements in insulin sensitivity despite little to no observed changes in lean body mass with RT (111, 113). This suggests that RT may also improve the intrinsic metabolic capacity of skeletal muscle independent of changes in muscle mass (111–113). Indeed, RT has been shown to increase skeletal muscle mitochondrial content and oxidation of glycolytic and fatty-acid-derived energy substrates in adults with T2DM (112, 114). Additionally, six weeks of strength training significantly increased insulin-stimulated glucose uptake along with GLUT4, insulin receptor, and Akt content, as well as glycogen synthase content and activity in the skeletal muscle of older adults with T2DM, independent of muscle mass changes (110, 111). These findings suggest that changes in skeletal muscle insulin signalling, GLUT4 translocation, and substrate oxidation may mediate RT-induced changes in insulin sensitivity.

1.4.4. Resistance Exercise and Insulin Resistance in T1DM

Relative to T2DM, limited research has been conducted investigating the use of RT for improving IR in individuals with T1DM. Acute resistance exercise has been shown to improve 24-hour glycemic control and insulin dose requirements in adults with T1DM (115, 116). Further, studies assessing RT in adults and children with T1DM have shown significant improvements in glycemic parameters, including fasting blood glucose and HbA1c (115, 117–119). To date, no human studies have assessed the effects of RT on more direct measures of insulin sensitivity in T1DM or examined the molecular mechanisms governing these effects. In a pre-clinical rodent model of
T1DM, our laboratory has shown significant improvements in insulin sensitivity assessed by IVGTT after six weeks of RT (120). An improvement in insulin sensitivity in these animals was accompanied by elevated skeletal muscle GLUT4 protein content which may reflect a molecular adaptation mediating this effect (120). Due to the limited literature investigating the effects of RT on IR development in T1DM and the molecular mechanisms involved, more work in this area is warranted.

1.5. Sex Differences in Insulin Sensitivity in Diabetes

It has been shown that otherwise healthy females exhibit enhanced insulin sensitivity compared to males (121). Sex differences in skeletal muscle metabolism may help explain this discrepancy. Specifically, females exhibit a greater proportion of oxidative type I myofibers, greater myofiber capillarization, greater hexokinase content, greater ability to store and oxidize intramuscular lipid, and greater metabolic flexibility, all of which can contribute to enhanced insulin action and glucose uptake in skeletal muscle (121, 122). Further, the effects of circulating 17-β estradiol on skeletal muscle estrogen receptor (ER)α may provide an additional mechanism for sex differences in insulin sensitivity, evidenced by the increased development of IR in post-menopausal women (123). Therefore, it appears that females exhibit protection against the development of IR but do not differ from males once glucose intolerance manifests (121). It has been shown that female patients with T1DM exhibit greater IR compared to male patients with T1DM which in turn may elevate the risk of CVD in female patients with T1DM (65). In contrast to these reports, our laboratory has recently demonstrated a progressive worsening of glycemic control in male T1DM rodents which did not improve in response to additional exogenous insulin administration (124). Conversely, no such effect was observed in female rodents with T1DM (124). The discrepancy in
these findings may relate to differences in age and estradiol action as the mean age of female participants with T1DM (in the study of Millstein and colleagues; 2018) was 44 years.

1.6. Rationale

While IR has been thought to be characteristic of T2DM, it is now clear that patients with T1DM develop severe whole-body and tissue-specific IR despite adequate glycemic management with IIT. Concerning this, the presence of IR in T1DM represents a major risk factor for developing CVD and related complications in this population (44, 46). Our laboratory has demonstrated IR development in our male rodent model of T1DM compared to non-T1DM control rodents (68–70, 120). Moreover, IR is associated with impaired skeletal muscle lipid metabolism and oxidative capacity (68–70). Importantly, aerobic, resistance, and combined exercise training (resistance and aerobic training) improved insulin sensitivity and skeletal muscle metabolism in our male T1DM rodents with IR (68–70, 120). While aerobic exercise alone is sufficient to improve IR in T1DM, clinical and experimental data have reported significant reductions in blood glucose during this form of exercise (96, 124, 125). Considering that the fear of hypoglycemia is the largest barrier to exercise in patients with T1DM (95, 96), it is important to determine alternative exercise regimens which lower this risk. Resistance exercise is associated with a reduction in hypoglycemia onset in patients with T1DM and therefore may be a safer option for exercise in this population (96, 100). Previous work from our laboratory has suggested that sex differences in sensitivity to exogenous insulin treatment may exist between males and females with T1DM (124). However, literature investigating IR in females with T1DM is limited and consequently our understanding of insulin sensitivity and skeletal muscle metabolism in this population is poor. Additionally, cellular mechanisms in skeletal muscle governing IR and RT-induced improvements in insulin sensitivity in T1DM are unclear.
1.7. Purpose and Hypothesis

The purpose of this study was to investigate the effects of six weeks of RT on IR development in female rodents with T1DM. It was hypothesized that IR development in female rodents with T1DM would be concomitant with impairments in skeletal muscle metabolism and mitochondrial capacity (Fig. 1.3). Secondly, it was hypothesized that RT would improve IR in female rodents with T1DM concomitant with improvements in skeletal muscle metabolism and mitochondrial capacity.

![Figure 1.3. Study Purpose and Hypothesis.](image)

Female rodents with T1DM will exhibit IR and impaired skeletal muscle metabolism and signalling which will be rescued with 6 weeks of RT. Created using BioRender.
References


7. **Association CD.** Diabetes charter backgrounder-Ontario [Internet]. Canada: CDA; 2015 [cited 2015 Nov 23]. [date unknown].


2.1 Background

Type 1 diabetes mellitus (T1DM) is a chronic metabolic disease characterized by the loss of endogenous insulin production resulting from autoimmune-mediated pancreatic beta cell destruction. The significant reduction in systemic circulation of insulin results in hyperglycemia which can lead to the accumulation of advanced glycation end products within bodily tissues and the development of complications such as nephropathy, retinopathy, and cardiovascular disease (CVD) (1). The current standard of care for patients with T1DM consists of exogenous insulin administration to normalize glycemia; specifically, intensive insulin therapy (IIT), which involves the maintenance of blood glucose as close to the normal range as possible (4-9mmol/L) (2). While IIT helps individuals with T1DM avoid prolonged hyperglycemia, insulin resistance (IR) is a prominent feature in approximately one-third of patients treated with IIT (3–5). Termed “double diabetes,” the presence of IR in T1DM significantly increases the risk of diabetes-related complications such as CVD (5, 6).

While the etiology of IR in T1DM is currently unknown, it has been shown to be distinct from that of other dysfunctional metabolic states such as metabolic syndrome and type 2 diabetes mellitus (T2DM) (7). Indeed, IR is present in otherwise healthy and normal-weight adolescents and young adults with T1DM and is not explained by common predictive factors such as body mass index, plasma lipids, and visceral adiposity (8–10).

Skeletal muscle is responsible for ~80% of postprandial glucose uptake, making this tissue an important determinant of whole-body insulin sensitivity (11). Unlike in otherwise healthy individuals, the peripheral administration of insulin bypasses the natural flow of insulin through
the liver. That is, skeletal muscle becomes the “first-pass” tissue for insulin and is the primary tissue for managing dysglycemia in T1DM (12, 13). Previous research has reported impairments in skeletal muscle metabolism in humans with T1DM including mitochondrial dysfunction and muscle fiber type alterations (14–21). Specifically, mitochondrial impairments in skeletal muscle have been shown to relate to IR in patients with T1DM (19, 20). Considering the important “first-pass” role of skeletal muscle in T1DM, impairments in substrate oxidation in this tissue likely lead to the impairment of glycemic control and insulin sensitivity in T1DM (13). Importantly, it has been shown that ~30-40% of glucose is oxidized by skeletal muscle following an oral glucose tolerance test (22) and that skeletal muscle oxidative capacity is a superior predictor of insulin sensitivity compared to intramyocellular lipid status (23). Indeed, our laboratory has shown that sedentary T1DM rodents with IR exhibit reduced skeletal muscle oxidative capacity (24). Therefore, skeletal muscle oxidation of glucose may be an important determinant of insulin sensitivity in T1DM; however, the exact cellular mechanisms governing IR in T1DM remain unclear.

Exercise training has been shown to significantly increase insulin sensitivity and improve glucose homeostasis in patients with T1DM and animal models (24–31). Unfortunately, individuals with T1DM are largely inactive due to the fear of hypoglycemia, which is one of the largest barriers to exercise in this population, especially for those with IR (32–34). Aerobic exercise is associated with hypoglycemia in T1DM, characterized by a significant drop in blood glucose below 4 mmol/L (33, 35–37). Conversely, resistance exercise has been shown to be associated with a lower risk of exercise-induced hypoglycemia in patients with T1DM (33, 38, 39). Therefore, resistance exercise may be a safe and effective exercise intervention to improve IR in patients with T1DM; however, its examination in this context is limited.
Most work investigating IR in T1DM from a mechanistic standpoint has been conducted solely in males, while very limited work has been conducted in females (40). The investigation of females with T1DM is important as it has been shown that sex differences exist in the metabolic effects of exercise (41, 42). Indeed, it has been shown that female adults with T1DM exhibit differential alterations of mitochondria including lower oxidative capacity, and greater skeletal muscle IR compared to their male counterparts (15, 17, 43, 44). Moreover, no study has assessed the effects of resistance exercise training (RT) on IR development in female rodents with T1DM.

The purpose of this study was to examine the effects of six weeks of RT on IR development in female rodents with T1DM. It was hypothesized that IR in female rodents with T1DM would be concomitant with impairments in skeletal muscle mitochondrial capacity, and that six weeks of RT would ameliorate IR and improve the capacity of mitochondria in skeletal muscle.

2.2 Materials and Methods

2.2.1. Ethics Approval

The protocols utilized in this study were approved and monitored by the Canadian Council on Animal Care of Western University (London, Ontario, Canada).

2.2.2. Animals

Forty female Sprague Dawley rats were obtained from Charles River Laboratories (St. Constant, Que., Canada) at eight weeks of age. All rats were caged in pairs and housed in a room with a 12-hour light-dark cycle, at a temperature of 20.5°C, and relative humidity of 40%. Rats were provided access to standard rat chow and water ad libitum over the course of the study.
2.2.3. Experimental Groups

Rats were randomly assigned into one of four groups: control (non-T1DM) sedentary (CS; n=10), control resistance trained (CT; n=10), T1DM sedentary (DS; n=10), T1DM resistance trained (DT; n=10).

2.2.4. Experimental Procedures

2.2.4.1. T1DM Induction and Insulin Pellet Implantation

Upon arrival, all rats underwent an acclimatization process for one week. Following this, the two T1DM groups (DS and DT) underwent seven consecutive days of intraperitoneal low-dose injections of streptozotocin (STZ; Sigma-Aldrich) to induce T1DM (Appendix A). Over the seven days, 20 mg/kg of STZ dissolved in citrate buffer (0.1M, pH 4.5) was injected within fifteen minutes of solution preparation. Diabetes was confirmed following the seven days of STZ injections by two non-fasting blood glucose measurements of ~11 mmol/L or greater (45). After confirmation of diabetes induction, one insulin pellet (2 IU insulin/day) was surgically implanted subcutaneously into the abdominal region (Appendix B). The insulin dose was modified over the course of the study via the removal of a portion of the insulin pellet if required for appropriate blood glucose maintenance. Blood glucose was intended to be maintained between 4-9 mmol/L for each T1DM rat throughout the study.

2.2.4.2. Exercise Training

RT consisted of vertical ladder climbing with a weight-loaded bag secured to the proximal portion of the tail. Prior to week one of RT, CT and DT animals underwent two separate familiarization sessions consisting of 10 consecutive ladder climbs at 5, 15, 20, and 35% of their body weight (2-3 climbs at each weight). This allowed rats to become acquainted with the
process of ladder climbing before the commencement of training. Rats were allowed to rest in a dark box at the top of the ladder for ~1-2 minutes in between climbs. An initial maximal carrying capacity was determined during the first day of week one of training for each rat. This was determined by beginning each rat at a carrying load equal to 75% of their body weight and progressively adding 10-30 g until failure was reached (defined as an unwillingness to climb despite hind limb stimulation via touch and air bursts). Following the determination of maximal carrying capacity, training periods consisted of rats performing four consecutive climbs at 50, 75, 90 and 100% of their maximal carrying capacity, followed by as many climbs as possible until failure at 100% maximal carrying capacity. A new maximal carrying capacity was established every four days by starting with each rat’s previous maximal capacity and gradually adding 10-30 g until failure, following the progressive overload principle. CT and DT rats performed RT five days/week for six weeks.

2.2.5. Experimental Measures

2.2.5.1. Body Weights and Blood Glucose

The body weight of each rat was measured and recorded once per week over the course of the study. Weekly non-fasting blood glucose was also measured from a small blood droplet (~50 µL) obtained from the saphenous vein. The Freestyle Lite Blood Glucose Monitoring System (Abbot Diabetes Care, INC.) was used to analyse blood glucose values which were recorded in millimoles per litre (mmol/L).

2.2.5.2. Intravenous Glucose Tolerance Test

Intravenous glucose tolerance tests (IVGTTs) were performed for all rats following the 6-week training period (Appendix C). Following a 4-12 hour fast, a baseline blood glucose measurement
was obtained. Shorter fasting periods were used for DS and DT (~4 hours) compared to non-T1DM control (~12 hours) animals to avoid hypoglycemic episodes. A sterile dextrose solution (10%; 5 g dextrose in 50 ml ddH₂O) was injected (2 ml/kg) into the tail vein of the conscious rat. Blood glucose was then measured in 10-minute intervals up to 40 minutes post-injection. The area under the curve (AUC) for the IVGTT was determined 40 minutes post-dextrose injection.

2.2.5.3. Blood and Tissue Collection

Rats were sacrificed immediately following IVGTT during the final week of the study. Sacrifice was performed via anaesthetization with isoflurane, followed by cardiac exsanguination. Approximately three blood samples of 500 µl were collected from each rat. Blood samples were centrifuged for 30 minutes at 3,000 rpm, and serum was then transferred to 1.5 ml Eppendorf tubes. The lower limbs were dissected, and the vastus lateralis (red and white), gastrocnemius (red and white), and soleus muscles were removed and immediately frozen in isopentane cooled to -70°C by liquid nitrogen. Tissues and serum were then stored at -80°C until later analysis.

2.2.5.4. Muscle Glycogen Content

Red and white gastrocnemius muscle (~20 mg) was homogenized in 30% KOH saturated with Na₂SO₄ and boiled for 30 minutes (Appendix C). Glycogen in muscle samples was precipitated with 95% ethanol and samples were left to rest on ice for an additional 30 minutes. Samples were then centrifuged for 20-30 minutes at 3000 rpm. After centrifugation, the supernatant was discarded, and glycogen pellets were immediately resuspended in 3 ml of ddH₂O. Glycogen pellets were placed on ice until a homogenous solution resulted, and then split into three 1ml glass tubes for triplicate analysis. 1 ml of 5% phenol and 5 ml of sulfuric acid (96-98%) were subsequently added to each tube and allowed to stand for 5 minutes at room temperature and then incubated at
25-30°C for 10 minutes. The colour reaction of samples was analyzed using a spectrophotometer at a wavelength of 490 nm.

2.2.5.5. β-oxidation activity

Approximately 30 mg of soleus muscle was excised and placed in a 1.5 ml Eppendorf tube. Sample buffer (5 mM potassium dihydrogen orthophosphate (K2HPO4), 1 mM EDTA, 0.1 mM DTT; pH of 7.4) was added to soleus samples for a 1:10 weight-volume (w/v) ratio of tissue to buffer (Appendix D). Submerged tissue samples were homogenized with three, 1-3 second pulses by a basic mechanical homogenizer (IKA Laboratories). Homogenized soleus samples were added to an Eppendorf tube with assay buffer (1M Tris-HCl, pH 7.0, 0.5 M EDTA, pH 8.0, 10% Triton X-100) and 5 mM NADH. Sample Eppendorf’s were incubated for 4 minutes at 30°C to permeabilize mitochondria. Following incubation, the sample mixture was transferred to a cuvette, and the reaction was initiated by adding 5mM acetoacetyl CoA. The sample cuvette was vortexed, and the reaction was read for 2 minutes in 30-second intervals on a NanoDrop2000 C Spectrophotometer (Waltham, MA, USA) at 340 nm.

2.2.5.6. Citrate Synthase activity

Citrate synthase activity was measured in the red vastus lateralis muscle using an assay kit (#ab239712, Abcam). Approximately 10 mg of red vastus lateralis muscle was excised and placed in a 1.5 ml Eppendorf tube. Citrate synthase assay buffer was added to red vastus samples for a 1:10 weight-volume (w/v) ratio of tissue to buffer. Submerged tissue samples were homogenized with three, 1-3 second pulses by a basic mechanical homogenizer (IKA Laboratories). On a 96-well plate, 5 µl of sample was added to wells with reaction buffer, and the rate of absorbance change was read using a microplate reader at 412 nm at 13-second intervals over 6.5 minutes.
2.2.5.7. Western Blot

Red and white vastus lateralis muscle (20 mg) was submerged 1:20 (w/v) in lysis buffer (15 mM Tris pH=7.0, 600 mM NaCl and 0.1 mM EDTA). The submerged tissue was homogenized with three, 1-3 second pulses using a basic mechanical homogenizer (IKA Laboratories). Tissue homogenate was transferred into Eppendorf tubes (1.5 ml) and agitated on a shaker for 2 hours on ice. Samples were then centrifuged at 12,000 rpm for 20 minutes at 4°C. Sample supernatant was extracted and transferred to a different Eppendorf tube (1.5 ml) and stored at -80°C. Total sample protein concentration and loading volumes were determined using a Bradford protein assay (46) (Appendix E). In Eppendorf tubes, protein samples mixed with an equal volume of 2x Laemmli SDS-PAGE (4% SDS, 20% Glycerol, 10% β-mercaptoethanol, 0.015% bromophenol blue, 0.125M Tris, pH 6.8) were subsequently boiled at 90°C in a water bath for 5 minutes. 20 µg of the sample protein was then loaded into 12% polyacrylamide gels and ran at 75-150 V for 2 hours (Appendix F). Transfer of gel protein to nitrocellulose membranes (Bio-Rad) was conducted at 70 V for 90 minutes. Following completion of the transfer, membranes were stored at 4°C in TBS-T (Tris Buffer Saline, 0.1% Tween-20), overnight. Membranes were washed in fresh TBS-T for 5 minutes. Following washing, membranes were blocked with a 5% w/v solution of TBS-T and skim milk powder or bovine serum albumin (BSA) for 1-2 hours. Blocked membranes were then washed for 5 minutes in TBS-T. Washed membranes were incubated for 2 hours at room temperature with primary antibodies detecting: Akt (Cell Signalling; 4691), phosphorylated (ser473) Akt (Cell Signalling; 4060), and mitochondrial complexes (Abcam; ab110413). Following incubation, the primary antibody was removed, and membranes were subsequently washed in TBS-T for 10 minutes and repeated for three washes. Membranes were then incubated in a 5% w/v solution of TBS-T and skim milk powder or BSA, and secondary antibody (#170-6515 Goat anti-rabbit IgG
HRP conjugate, #170-6516 Goat anti-mouse IgG HRP Conjugate; BioRad, 57 Hercules, CA, USA) at a 1:20000 dilution for 2 hours at room temperature. After incubation, membranes were washed for 10 minutes in TBS-T and repeated for three washes. Membranes were then treated with Bio-Rad chemiluminescence substrate, and the images were subsequently captured using the BioRad Chemidoc MP System. Western blot image quantification was conducted using Image J.

2.2.5.8 Insulin and Estradiol Quantification

Insulin and estradiol serum concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (ELISA; Rat Insulin ELISA Kit, ALPCO; 17-β Estradiol ELISA Kit, Abcam). Blanks, standards, and serum samples were added to a 96-well microplate precoated with anti-Insulin or anti-Estradiol IgG. Insulin or 17-β Estradiol-HRP conjugate was added to each well and plates were incubated for 2 hours at 25-37°C. Following incubation, plates were aspirated and washed three to six times with wash buffer. The substrate solution was then added to each well and further incubated for 15-30 minutes. Following substrate incubation, stop solution was added to each well, and plates were immediately read at 450 nM using a microplate reader. The absorbance values of samples were adjusted based on the absorbance of blanks. Hormone concentration was interpolated from a standard curve generated from the blank and standards.

2.3 Data Analysis

GraphPad Prism 8 (GraphPad Software, Inc.) was used to complete statistical data analysis. Weekly blood glucose and body mass measures were analysed using a three-way analysis of variance (ANOVA) with time, diabetes, and exercise training as factors. Maximal carrying capacity, serum estradiol and insulin concentrations, IVGTT AUC, protein content, glycogen content, β-oxidation, and citrate synthase activity were analyzed using a two-way ANOVA with
diabetes and exercise training as factors. Tukey’s multiple comparisons test was used for post-hoc analysis when significant differences were observed. Significance was accepted at an alpha value of 0.05. Regression analysis of IVGTT AUC and serum estradiol was completed using linear regression.

2.4 Results

2.4.1. Animal Characteristics

Weekly body mass (Fig. 2.1a) and non-fasting blood glucose (Fig. 2.1b) measures were analysed to examine the influence of diabetes, time, and training. One animal from the DS met its endpoint prematurely and did not contribute to the data. For blood glucose, main effects of diabetes and time were statistically significant (p<0.0001). There was a significant interaction between time and diabetes (p<0.0001), time and training (p<0.05), diabetes and training (p<0.05), and time, diabetes, and training (p<0.05). During week two of the study, DS and DT had significantly higher blood glucose levels compared to CT and CS (p<0.0001), while DS and DT blood glucose did not differ (p>0.05). During week 10, DS blood glucose was significantly greater than all groups (p<0.0001). For body mass, main effects of diabetes (p<0.05), time (p<0.0001), and training (p<0.0001) were statistically significant. There was a significant interaction between diabetes and training (p<0.0001).
Figure 2.1. Weekly blood glucose (a) and body mass (b) data. STZ denotes the start of streptozotocin injections to induce T1DM in DS and DT animals. INSULIN denotes the start of insulin treatment for DS and DT animals. RT denotes the start of the resistance training regimen for CT and DT animals. a. Mean weekly non-fasting blood glucose measures (mmol/L). Data is presented as mean ± SD. # Denotes a significant difference between T1DM and control animals. * Denotes a significant difference between DS and all other groups. b. Mean weekly body mass measures (g). Data is presented as mean ± SD. No differences were observed between groups within any timepoint.
2.4.2. Carrying Capacity

Carrying capacities of CT and DT (Fig. 2.2) were recorded and analysed to ensure similar loads were carried by each group. A main effect of training day was statistically significant (p<0.0001). No significant differences (p>0.05) were observed in carrying capacity loads between CT and DT over the course of the study.

![Maximal Carrying Capacity Progression](image)

**Figure 2.2.** Maximal carrying capacity (g) for animals undergoing RT. Data is presented as mean ± SD. No differences were observed between groups.

2.4.3. Serum Insulin and Estradiol Concentration

Serum samples were taken immediately after the final IVGTT during the animal sacrifice. No differences in serum estradiol were observed between groups (p>0.05) (Fig. 2.3a). Serum insulin exhibited a main effect of diabetes (p<0.0001) (Fig. 2.3b). DS (p<0.0001) and DT (p<0.05) serum insulin was significantly greater than CS and CT animals. DS and DT insulin did not differ (p>0.05).
Figure 2.3. Serum estradiol (a) and serum insulin (b) concentrations. a. Mean serum estradiol (ng/L). Data is presented as mean ± SD. No differences were observed between groups. b. Mean serum insulin (ng/ml). Data is presented as mean ± SD. # Denotes a significant difference between T1DM and control rodents.
2.4.4. Intravenous Glucose Tolerance Test

Blood glucose measures during IVGTT (Fig. 2.4a) were used to determine AUC. For insulin-adjusted IVGTT AUC (Fig. 2.4b), there was a main effect of diabetes (p<0.0001) and training (p<0.05), and a significant interaction between diabetes and training (p<0.05). DS exhibited significantly greater insulin-adjusted IVGTT AUC compared to all groups (p<0.05).
Figure 2.4. IVGTT blood glucose (a) and insulin-adjusted IVGTT AUC (b). a. Mean blood glucose measures 40 minutes post-dextrose (mmol/L). Data is presented as mean ± SD. b. Mean insulin (ng/ml)-adjusted IVGTT AUC (mM*minutes). Data is presented as mean ± SD. * Denotes a significant difference between DS and all other groups.
2.4.5. Correlation between Area Under the Curve and Estradiol

Correlational analysis revealed a limited explanation of the variance in insulin-adjusted IVGTT AUC by serum estradiol ($R^2 = 0.1187$) when values from all groups were pooled. This result persisted when data was segregated by group (graph not shown; CT; $R^2 = 0.0.1056$, CS; $R^2 = 0.0355$, DT; $R^2 = 0.0044$, DS; $R^2 = 0.0075$).

![Graph](image)

**Figure 2.5.** Linear regression between insulin (ng/ml)-adjusted IVGTT AUC (mM*minutes) and serum estradiol (ng/L). Data is presented as mean ± SD.

2.4.6. Muscle Glycogen Content, β-oxidation activity, and Citrate Synthase Activity

Red gastrocnemius glycogen content (Fig. 2.6a) did not differ between groups ($p>0.05$). Glycogen content in the white gastrocnemius (Fig. 2.6b) exhibited a main effect of diabetes ($p<0.05$) and a significant interaction between diabetes and training ($p<0.05$). DS animals exhibited significantly greater glycogen in white gastrocnemius muscle compared to all groups ($p<0.05$). Soleus β-oxidation, short-chain β-hydroxyacyl-CoA dehydrogenase (SCHAD) activity (Fig. 2.6c) did not
differ between groups (p>0.05). Red vastus lateralis citrate synthase activity (Fig. 2.6d) exhibited a main effect of diabetes (p<0.05) and training (p<0.05).

Figure 2.6. Red gastrocnemius glycogen content (a), white gastrocnemius glycogen content (b), soleus SCHAD activity (c), red vastus lateralis citrate synthase activity (d). a. Mean red gastrocnemius glycogen content (g/100g tissue). Data is presented as mean ± SD. No differences were observed between groups. b. Mean white gastrocnemius glycogen content (g/100g tissue). Data is presented as mean ± SD. * Denotes a significant difference between DS and all other groups. c. Mean soleus SCHAD activity (µmol·g wet wt⁻¹·min⁻¹). Data is presented as mean ± SD. No differences were observed between groups. d. Mean red vastus lateralis citrate synthase activity (nmol/min/µg). Data is presented as mean ± SD. # Denotes a significant difference between T1DM and control animals.
2.4.7. Muscle Protein Content

In the red vastus lateralis, the ratio of p-Akt(ser473) to total Akt (Fig. 2.7a) did not significantly differ between groups (p>0.05). In the white vastus lateralis muscle, the ratio of p-Akt(ser473) to total Akt (Fig. 2.7b) exhibited a main effect of diabetes (p<0.05). Red vastus lateralis oxidative phosphorylation (OXPHOS) protein content of complexes I-V (Fig. 2.7c) did not significantly differ between groups (p>0.05). Protein content was normalized to ponceau staining of membranes.
C)

**COMPLEX I**

![Graph showing Complex I activity comparison between Sedentary and Trained groups.](image)

- T1D: P=0.1591
- RT: P=0.4847
- T1DxRT: P=0.2016

**COMPLEX II**

![Graph showing Complex II activity comparison between Sedentary and Trained groups.](image)

- T1D: P=0.5051
- RT: P=0.1822
- T1DxRT: P=0.8066

**COMPLEX III**

![Graph showing Complex III activity comparison between Sedentary and Trained groups.](image)

- T1D: P=0.1282
- RT: P=0.5840
- T1DxRT: P=0.9715

**COMPLEX IV**

![Graph showing Complex IV activity comparison between Sedentary and Trained groups.](image)

- T1D: P=0.4876
- RT: P=0.7921
- T1DxRT: P=0.5542

**COMPLEX V**

![Graph showing Complex V activity comparison between Sedentary and Trained groups.](image)

- T1D: P=0.9126
- RT: P=0.3453
- T1DxRT: P=0.3473
**Figure 2.7.** Red vastus lateralis p-Akt:Akt ratio (a), white vastus lateralis p-Akt:Akt ratio (b), red vastus lateralis OXPHOS protein content (c), representative western blots images (d). a. Mean red vastus lateralis p-Akt:Akt ratio. Data is presented as mean ± SD. No differences were observed between groups. b. Mean white vastus lateralis p-Akt:Akt. Data is presented as mean ± SD. # Denotes a significant difference between T1DM and control animals. c. Mean red vastus lateralis OXPHOS protein content (AU/Ponceau). Data is presented as mean ± SD. No differences were observed between groups.
2.5 Discussion

While IR is typically characteristic of other metabolic disease states such as T2DM, obesity, and metabolic syndrome, vast literature now exists establishing the presence of IR in patients with T1DM treated with IIT (3–6, 8–10, 19, 20). However, the exact mechanisms through which IR develops in the context of T1DM remain unclear. Previous research has implicated skeletal muscle as an important tissue in the management of glycemic control and insulin sensitivity in T1DM and has identified impairments in skeletal muscle metabolism in individuals with T1DM (14, 15, 17–20). As such, deficits in skeletal muscle metabolism and signalling likely play a substantial role in the development of IR in T1DM. The understanding of IR development in females with T1DM is limited and sex differences in IR may exist within the context of T1DM. While resistance exercise is associated with a lower risk of hypoglycemia onset compared to aerobic exercise in T1DM, it is unclear whether it is sufficient to improve IR in females with T1DM.

The primary finding of the current study is that similar to their male counterparts, female rodents with T1DM develop IR. By the end of the study (week 10), DS animals exhibited non-fasting blood glucose measures significantly greater than all groups. Additionally, IVGTT AUC was significantly greater in DS animals compared to all groups, indicative of a reduction in insulin sensitivity. To our knowledge, this would be the first evidence that insulin-treated female rodents with well-controlled T1DM develop IR. In line with our hypothesis, DT animals exhibited reduced blood glucose levels and lower IVGTT AUC compared to DS which indicates that six weeks of RT in female rodents with T1DM was able to prevent IR development. These findings are in line with literature reporting RT-induced improvements in insulin sensitivity in individuals with T2DM and in both males and females (47–53).
Our analyses of skeletal muscle tissue revealed distinct alterations in intramyocellular glycogen storage in sedentary and trained rodents with T1DM. Specifically, DS animals exhibited an elevation in post-IVGTT glycogen content within white gastrocnemius muscle. This may have been a result of the elevated levels of serum insulin observed in T1DM animals in this study as insulin plays an important role in promoting intramuscular glycogen synthesis (54). Indeed, patients with T1DM exhibit insulin levels ~2.5 times greater than non-T1DM individuals with similar glycemia (12). Subcutaneous insulin delivery bypasses the liver and results in elevated levels of systemic insulin in T1DM. Termed “peripheral hyperinsulinemia,” elevated levels of systemic insulin has been implicated in the development of IR in this population (12, 55).

Regardless of training status, T1DM rodents exhibited elevated Akt activation in the white vastus lateralis muscle during the IVGTT. Insulin-stimulated Akt activation has been shown to play an important role in glucose uptake and incorporation into glycogen in muscle cells (56). Despite elevated serum insulin and Akt activation in both T1DM groups, only DS animals exhibited increased intramyocellular glycogen content. These findings may provide mechanistic insights into IR development in T1DM: 1) IR in skeletal muscle in T1DM may develop downstream of Akt activation and 2) Elevated intramyocellular glycogen content may play a negative role on insulin sensitivity. In support of the latter, it has been shown that high muscle glycogen content significantly reduces insulin-stimulated glucose uptake in white gastrocnemius muscle (57). However, this reduction in insulin sensitivity in white muscle with high glycogen content was accompanied by reduced insulin-stimulated Akt activation (57). The assimilation into glycogen is a major pathway for insulin-stimulated glucose disposal in skeletal muscle and is tightly regulated to avoid glycogen over-accumulation. Glycogen content is inversely related to glycogen synthase (GS) activity such that elevated glycogen levels further inhibit synthesis via a reduction in GS
activity (58–62). In humans without diabetes, chronic hyperglycemia via glucose infusion has been shown to increase intramyocellular glycogen content, reduce insulin-stimulated GS activity, and reduce insulin sensitivity (63). The mechanisms leading to elevated glycogen content in white gastrocnemius muscle in DS animals are unclear; however, it is plausible that hyperinsulinemia led to an increase in fast-twitch muscle glucose uptake, which resulted in elevated glycogen content. It has been shown that glycolytic fast-twitch muscle fibers exhibit a greater capacity to store glycogen compared to oxidative slow-twitch muscle fibers (64), which in turn may explain why elevated glycogen content was not observed in red gastrocnemius muscle. In support of this theory, we have previously reported elevated glycogen content in type IIa fibers with IIT in male T1DM rodents (65). While few studies have directly compared muscular glycogen content in humans with and without T1DM, they did not observe differences between patients with T1DM treated with IIT and non-T1DM individuals (66, 67). However, these studies used non-invasive magnetic resonance spectroscopy and did not distinguish between glycogen in red versus white muscle tissue. This suggests that alterations in muscle glycogen storage with T1DM may be fiber specific.

In the current study, the increase in intramyocellular glycogen content observed in DS animals was not found in DT animals suggesting that RT prevented this metabolic abnormality. Following training, it is plausible that more glucose entering the skeletal muscle of DT animals would be diverted towards anabolic and biosynthetic pathways to support RT-induced muscle hypertrophy. This in turn would reduce glycogenesis flux and prevent elevated muscle glycogen content. Moreover, it has been shown that fast-twitch muscle fibers preferentially hypertrophy with high-intensity RT in females (68, 69), which would support our observations of reduced fast-twitch but not slow-twitch muscle glycogen content in DT compared to DS animals. Using metabolomic and
isotope tracer analysis, it has recently been shown that intracellular glucose and glycolytic intermediates are present at higher concentrations in the pentose phosphate, serine synthesis, and hexosamine pathways and are incorporated into non-essential amino acids in hypertrophying muscle both in-vitro and in-vivo (70). Furthermore, additional work is warranted to better understand the mechanisms governing RT-induced improvements in IR and the relationship between muscle glycogen content and anabolic pathways in T1DM.

In the current investigation, citrate synthase activity was elevated in both DS and DT animals despite no differences in mitochondrial protein content compared to non-T1DM control rodents. This finding contrasts our hypothesis and previous findings that citrate synthase activity is reduced in male T1DM rodents (24) as well as other reports of impaired skeletal muscle mitochondrial function in male and female patients with T1DM (14, 15, 17–20, 44). An explanation for this discrepancy may be that T1DM rodents exhibited a compensatory increase in muscle oxidative capacity to manage the increased intracellular glucose load during the IVGTT. Indeed, experimental chronic hyperinsulinemia in healthy humans has been shown to significantly reduce insulin-stimulated glucose utilization while enhancing oxidative glucose disposal in skeletal muscle (71). It has previously been shown that young women with T1DM do not exhibit impairments in in-vivo muscle mitochondrial oxidative capacity compared to age-matched women without T1DM (72). In combination with our findings, these data may suggest that impairments in mitochondrial oxidative capacity in skeletal is not implicated in the development of IR in females with T1DM. However, the mechanistic relationship between skeletal muscle mitochondrial capacity and IR development in female patients with T1DM remains unclear and requires further investigation.
Additionally, we observed a reduction in citrate synthase activity with RT in T1DM and non-T1DM control rodents which may appear counterintuitive to our previous findings of improved skeletal muscle oxidative capacity following aerobic and combined (aerobic and resistance) exercise training in male T1DM rodents (24). However, our findings are in line with multiple investigations reporting reductions in citrate synthase activity with RT (73). A potential explanation for this phenomenon is a “dilution” of the mitochondrial pool with RT-induced muscle fiber hypertrophy, i.e., mitochondrial biogenesis with RT occurs at a much slower rate compared to increases in muscle cell volume resulting in a reduction in citrate synthase activity (73). Further work is needed to understand the morphological changes in skeletal muscle in T1DM as a result of RT and its impact on the oxidative state of the muscle.

Our laboratory has previously shown increases in insulin-desensitizing lipid intermediates, such as diacylglycerol (74), in skeletal muscle after hyperinsulinemic-euglycemic clamp in male T1DM rodents using pre- and post-clamp tissue analysis (29). This suggests that excess glucose is converted to lipid in skeletal muscle in T1DM through de-novo lipogenesis (DNL) (75). β-oxidation activity was assessed in the current study to investigate the ability of skeletal muscle to oxidize lipid formed through DNL during IVGTT, as this may impact insulin sensitivity (76). No differences in soleus β-oxidation activity were observed between groups suggesting that improvements in IR were unrelated to differences in lipid oxidation. This may have been the result of IVGTT, as it is theorised that glucose and lipid “compete” for oxidation in skeletal muscle, such that lipid oxidation may be downregulated during periods of hyperglycemia (i.e., high glucose availability) (77, 78).

Since estradiol is associated with changes in insulin sensitivity and glucose and lipid metabolism (41, 79), we measured circulating serum levels of 17-β estradiol in all groups. We did not observe
a significant difference in serum estradiol between groups and did not observe a strong explanation of variance in IVGTT AUC by estradiol which suggests that circulating estradiol in these animals did not significantly influence glucose tolerance. Previous work has suggested that a subset of the female population with T1DM exhibit fluctuations in insulin sensitivity through the menstrual cycle while other female patients do not (80–83). Our findings indicate a weak relationship between estradiol serum concentration and insulin sensitivity assessed by IVGTT in our female rodents with T1DM. Indeed, we have previously shown that estradiol concentration over the menstrual cycle likely does not play an important role in glycemic control during exercise in our female rodent model of T1DM (36).

2.6 Conclusions

The results of the current study demonstrate that female rodents with T1DM develop IR and that six weeks of RT prevents the decline in insulin sensitivity. In T1DM rodents, intramyocellular glycogen content in white gastrocnemius muscle is elevated but is prevented with RT independent of changes in circulating insulin levels, Akt signalling, and muscle oxidative capacity associated with T1DM. This suggests that alterations in muscle glycogen storage may have a negative effect on insulin sensitivity in T1DM. While the role of RT in the mitigation of increased muscle glycogen storage is unclear, increased anabolic pathway flux to support muscle hypertrophy is a plausible mechanism. Taken together, these findings support the therapeutic role of RT as an effective intervention to improve IR in females with T1DM.
Figure 2.8. Graphic summary of study results. Female rodents with T1DM exhibit hyperglycemia, IR, and hyperinsulinemia concomitant with increased protein kinase B (Akt) phosphorylation (ser473), increased citrate synthase (CS) activity, and increased intramyocellular glycogen content after an intravenous glucose tolerance test (IVGTT). RT prevents hyperglycemia, IR, and improper glycogen storage associated with T1DM. Created using BioRender.
References


44. Derella CC, Thomas J, Harris RA. Women Have Greater Endothelin-B Receptor Function and Lower Mitochondrial Capacity Compared to Men With Type 1 Diabetes. *The Journal of clinical endocrinology and metabolism*, dgad189. 2023


Appendices

Appendix A. Multiple Low-dose Streptoztocin Protocol (rat)

**PURPOSE:**
To induce Type I diabetes in rats

**MATERIALS:**
Gloves
Lab Coat
Streptozotocin (STZ)
5X Stock Citric Acid/Citrate Buffer
- Anhydrous Citric Acid
- Sodium Citrate Dihydrate
- MilliQ Deionized Water
13M HCl
3 Falcon Tube
Sterile Filter

**EQUIPMENT:**
Biological Safety Cabinet
Weigh Scale
pH Meter

**PROCEDURE:**
*Preparing 5X Citric Acid/Citrate Buffer*

1. For a pH 4.6 buffer at 765 mM (5X stock solution), in a beaker, Add
   i. 13.8g Anhydrous Citric Acid (Sigma) or 15.1g Citric Acid Monohydrate
   ii. 23.8g Sodium Citrate Dihydrate (Sigma)
   Mix into
   iii. 175mL of MilliQ water

   The pH should be at 4.6, Add HCl or NaOH to adjust (do not over-shoot pH)

2. Once the proper pH is obtained, add MilliQ water until you are close to the 200 ml mark (pH will move slightly). If satisfied with the pH, adjust volume in a 250 ml graduated cylinder and filter in a 0.2μm filter.

3. Store at room temperature. This is your 5X stock solution.

*Making up Streptozotocin (STZ) for Injection*

**NOTE** Animals should be pre-weighed prior to making up STZ to ensure accurate amounts of STZ to be prepared.
1. Using pre-made buffer, put 1 mL of buffer in a 50 mL Falcon Tube and add 4 mL of distilled water filtered through a 0.2μm syringe filter. Check the pH. This gives you a working concentration of 153 mM.

2. The desired pH is between 4.5-4.7. Under the fume hood, add 1 drop at a time of concentrated HCl to the buffer, checking pH in between until desired pH is reached.

3. Once pH is reached, add 1 mL distilled water (sterile filtered through a 0.2μm syringe filter as before). If pH is below 4.5, restart.

4. Weigh out an appropriate amount of STZ for the number of animals (see calculations below) that will be injected in a 15 minute time frame.

Ex. Rats will be injected at 20mg/kg, so for 10 animals at an ideal weight of 200g (avg. weight of rats to be injected), you will require a minimum of 40mg.

\[
20\text{mg/kg} \times 0.2\text{kg} = 4\text{mg per animal}
\]

The amount of STZ weighed out should be more than the minimum as some solution will be lost in filtering. (4mg (per animal) \times 12 \text{rats} = 48\text{mg total} (0.048g)

5. Dissolve the STZ into buffer (keeping in mind a comfortable injection volume). Shake to dissolve powder (approx. 1 min). Sterile filter using a 0.2μm syringe filter.

Ex. 48mg STZ ÷ 3 mL buffer = 16mg/mL solution

\[
4\text{mg} \div 16\text{mg/mL solution} = 0.25\text{mL}
\]

6. STZ is time dependent and must be used within 15 minutes

*Injecting and Follow-Up of the Animals*

1. Promptly inject each rat with the solution (intraperitoneal) at a dosage rate of 20mg/mL (in this example, 0.25mL). Do not use anymore STZ solution more than 15 minutes after it has been dissolved in the sodium citrate buffer.

2. Dispose of any container having come into contact with the STZ (in either powder or dissolved form) into a biohazardous waste receptacle. Dispose of needles into a sharps container.

3. Return injected rats to their cage. Record the date of STZ injection and add a biohazard label to the cage (leave biohazard label on cage for at least 3 days following the last injection).

4. Repeat this procedure the following day.
5. Check blood glucose daily. Diabetes is achieved with two non-fasting blood glucose readings of >18 mmol. Diabetes should be achieved after 5-8 injections (i.p. 20mg/kg).

REFERENCES:


Appendix B. Insulin Pellet Implantation (rat)

**PURPOSE:**
Exogenous insulin administration in diabetic rats.

**PROCEDURE:**

1. Anesthetize the animal with isoflurane machine set to 4-5% with an O2 rate of 1 L/min. Once animal is unconscious, remove it from the chamber and place in the nose cone. Reduce isoflurane to 3% to maintain the plane of anesthesia.

2. Shave a small area on the abdomen where the pellet will be implanted.

3. Apply 10% providone-iodine solution to the skin, followed by 70% ethanol, to disinfect the site of insertion.

4. Make a small subcutaneous incision.

5. Using forceps, briefly immerse the pellet in 10% providone-iodine solution, rinse with saline and insert into subcutaneous region.

6. Use 1 pellet for the first 350g of body weight.

7. Pinch the skin closed after the last pellet is inserted. Place a drop of 10 % providone-iodine solution over the opening.

8. Close the incision by suturing.

9. Place the animal under a heat lamp and monitor until it recovers from anesthesia.

10. Record on the cage card that insulin pellets have been implanted.

**REFERENCES:**

http://www.linshincanada.com

https://ir.lib.uwo.ca/etd/4800
Appendix C. Intravenous Glucose Tolerance Test

MATERIALS:
15 ml Falcon tube
Dextrose
Distilled water
0.2 um syringe filter
5 ml vacutainer
Emla Cream
3 ml syringe
27 G needles
Green drape
Microcentrifuge tubes Vaseline
30 G needles
Gauze squares

EQUIPMENT:
Mettler balance
Biological safety cabinet
Heat lamp with 175 w bulb
Glucometer (Freestyle mini) Timer

Procedure:
Fasting Rats:

1. At 21 on the night before the IVGTT is to be performed, transfer the rats to a clean cage.

2. Transfer the rat's tube to the new cage, ensuring that there is no food inside of it.

3. Place a fresh lid on the cage, with no food on it. Put a water bottle on the lid.

4. When leaving the animal quarters, return the light switch to the auto position.
Glucose preparation:

1. On the morning of the IVGTT, dispense 50ml distilled water into a clean 50ml conical Falcon tube.

2. Weigh out 5 g of dextrose and add it to the tube containing the sterile water. Dissolve by vortexing to result in a 10% glucose solution.

3. In a biological safety cabinet, sterilize the glucose solution using a 0.2 um syringe filter.

4. Transfer the sterile glucose solution to a 5 ml vacutainer.

Intravenous Glucose Tolerance test (IVGTT):

1. Place the cage containing the rat under a heat lamp. Remove the tube from the cage, and provide water for the rat. Warm the rat in this manner for 10 minutes.

2. Remove the rat from the cage and scrub the tail using soap and water.

3. Measure and record the rat's weight.

4. Obtain a baseline blood glucose reading and collect a baseline serum sample.

5. Return the rat to the cage and warm for an additional 20 minutes.

6. Place the rat's tail in a container of warm tap water for 30-60 seconds.

7. One person should dry the tail and restrain the rat by wrapping it in a green drape so the tail is exposed. Occlude the tail vein by applying pressure.

8. Using a 3 ml syringe and a 27 G needle, a second person will draw up the 10% glucose solution at a dose of 2 ml/kg (=1 g/kg). Locate the tail vein and draw back on the plunger to ensure that the needle is within the vein.

9. The restrainer will now release occlusion while the glucose solution is injected. Draw back the plunger a couple of times during the injection to ensure that the needle remains located within the vein.

10. Start the timer and return the rat to its cage. Continue warming it for the duration of the IVGTT.
ll. Check and record the blood glucose values for the rat at 5, 10, 20, 30-, 40-minutes post glucose challenge.

12. Upon completion of the IVGTT, remove the rat from under the heat lamp and return the tube to the cage. Place food on the wire lid of the cage and ensure that the rat has access to water.

13. Note on the cage card that an IVGTT has been performed.

Reference:

Hall, Katherine, "THE EFFECT OF AEROBIC AND RESISTANCE EXERCISE ON INSULIN SENSITIVITY AND GLYCEMIC CONTROL IN TYPE 1 DIABETIC RATS" (2011). Digitized Theses. 3531. https://ir.lib.uwo.ca/digitizedtheses/3531
Appendix D. Glycogen Assay

**MATERIALS:**
Tissue samples
Fume hood
3 x 100mm glass test tubes
Potassium hydroxide (KOH) pellets
Sodium sulfate (Na₂SO₄)
95% ethanol
Glycogen powder
Phenol crystals
96-98 % Sulfuric Acid (H₂SO₄)
490nm spectrophotometer

Standard Curve:

Prepare standard curve with stock glycogen solution (1mg/mL).

<table>
<thead>
<tr>
<th>Standard</th>
<th>[Glycogen] (mg/ml)</th>
<th>Volume (μl)</th>
<th>Water (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000</td>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>0.005</td>
<td>5</td>
<td>995</td>
</tr>
<tr>
<td>3</td>
<td>0.010</td>
<td>10</td>
<td>990</td>
</tr>
<tr>
<td>4</td>
<td>0.050</td>
<td>50</td>
<td>950</td>
</tr>
<tr>
<td>5</td>
<td>0.100</td>
<td>100</td>
<td>900</td>
</tr>
<tr>
<td>6</td>
<td>0.200</td>
<td>200</td>
<td>800</td>
</tr>
<tr>
<td>7</td>
<td>0.300</td>
<td>300</td>
<td>700</td>
</tr>
<tr>
<td>8</td>
<td>0.500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>9</td>
<td>1.000</td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

*Colour Reaction:*

Add 1 ml of 5% phenol.

Rapidly add 5 ml of 96-98 % H₂SO₄.

Let samples stand for 10 min.

Vortex and place in water bath (25 to 30°C) for 10 to 20 minutes.

Read tubes at 490 nm.

**Procedure:**

1. Cut and weigh approximately 20mg of tissue samples and place in glass test tube.
2. Saturate 30% KOH stock solution with sodium sulfate (Na₂SO₄)
3. Add 0.5 ml of 30 % KOH saturated with Na₂S₀₄. Make sure that tissue is completely submerged.

4. Put caps on tubes and immerse in boiling water bath until homogenous solution is obtained (30 minutes).

5. Place tubes on ice.

6. Precipitate glycogen with 1mL of 95 % ethanol for 30 minutes (on ice).

7. Spin tubes at 840 x g (3 000 rpm on Sorval) for 20 to 30 minutes.

8. Remove supernatants and immediately dissolve precipitates in 3mL ddH₂O. Do not allow precipitated to dry.

9. Pipette 1 ml glycogen solution into 3 separate glass test tubes (3 x 100mm).
- 3 separate tubes of glycogen solution are necessary for analysis in triplicates.

10. Add 1 ml of 5 % phenol.

11. Rapidly add 5 ml of 96-98 % H₂SO₄.

12. Let samples stand for 10 min.

13. Vortex and place in water bath (25 to 30°C) for 10 to 20 minutes.

14. Read tubes at 490 nm.

Solutions:

<table>
<thead>
<tr>
<th>Glycogen Stock Solution (1mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100mL</td>
</tr>
<tr>
<td>100mg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>30% Potassium Hydroxide Stock Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>300g</td>
</tr>
<tr>
<td>1L</td>
</tr>
</tbody>
</table>

Reaction is exothermic; therefore, it might be necessary to put on ice.
**SPEC. PROCEDURE:**

1. Turn on ~20 minutes before needed (TRANS)
2. Set desired λ & insert appropriate filter
3. Insert a blank tube (dH₂O)
4. With spec. on “TRANS”, set to 0.000 using “zero” button & while pressing “zero set”
5. Release “zero set”, but adjust value to 100.00 with spec set to “TRANS” using “100% T/OA” adjustments
6. Set spec from “TRANS” to “ABS” using LHS button
7. Check that “ABS” reading is 0.00; if not adjust with “100%T/OA”
8. Check that a true a zero reading has been reached by reading the abs values of 2 other blank tubes
9. Read 3 reagent tubes (blank) and record zero on the middle value using “100%T/OA”
10. Read all standard samples

**Reference:**

Appendix E. β-oxidation, short-chain β-hydroxyacyl-CoA dehydrogenase (SCHAD) Assay

Part A: Solutions

1. **Lysis buffer:**
   a. Measure: 5 mM potassium dihydrogen orthophosphate (K$_2$HPO$_4$), 1 mM EDTA, and 0.1 mM DTT.
   b. Adjust to a pH of 7.4.

2. **SCHAD Buffer (50mM Tris-HCl, 1mM EDTA):**
   a. Make stock 0.5M EDTA (pH 8.0):
      i. Measure 146.12g; add to 400 ml of ddH2O.
      ii. Add very concentrated NaOH dropwise until pH 8.0 (colour reaction: white to translucent).
      iii. Bring to 500 ml ddH2O.
   b. Make 1M Tris-HCl (pH 7.0):
      i. Measure 157.6g; add to 1L ddH2O.
      ii. Adjust pH to 7.0.
   c. Make *working* buffer (~250 sample runs):
      i. 100 mM ddH2O
      ii. 10 mM *stock* Tris-HCl buffer (pH 7.0)
      iii. 400ul *stock* 0.5 EDTA buffer (pH 8.0)
      iv. Check solution pH (7.0)
      v. Bring solution to 200 mL with ddH2O.

3. **5mM acetoacetyl CoA (stored at -20°C).**
   a. 5 mg vial reconstituted in 1.03 mL ddH2O.

4. **10% Triton X-100.**
   a. 5 mL Triton X-100 diluted in 45 mL ddH2O (stored at room temperature).

5. **5 mM NADH (stored at 4°C).**
   a. Reconstitute 3.54mg of sample in 1 mL ddH2O.
   b. Make fresh *daily* and keep on ice.

6. **Assay buffer (stored at 30°C, made fresh daily):**
   a. 1 mL Triton X-100
   b. 49 mL SCHAD buffer

Part B: Muscle homogenization

1. Place approximately 30 mg of muscle tissue and place into a 1.5 mL Eppendorf tube on ice.
2. Submerge sample in lysis buffer for a 1:10 weight-volume ratio.
3. Keep buffer-muscle mixture on ice while subjecting to three, 1-3 second pulses by a mechanical homogenizer.

Part C: Procedure

1. Turn on spectrophotometer and water bath at least 20 minutes prior start. Assay carried out at 30°C.
2. Prepare assay buffer fresh daily and keep in water bath at 30°C.
3. “Zero” prior sampling by placing blank cuvettes with only assay buffer.
4. Prepare quartz cuvette (340 nm wavelength cannot penetrate disposable plastic cuvettes), pipetting repeatedly slowly:
   a. 800 uL assay buffer
   b. 10 uL NADH
   c. 35 uL sample
5. Mixture should be incubated for 4 minutes at 30°C to allow for mitochondrial permeability in water bath.
6. Start measurement routine following incubation period. Wait an additional 1 minute following the 4-minute incubation period (a total of 5 minutes pre-assay incubation).
7. Initiate reaction by adding 10 uL of acetoacetyl CoA.
8. Mix sample cuvette using pipette.
9. Read for 2 minutes, every 30 seconds.
10. Calculate:
    a. Determine the specific activity (SA):
        i. \( \text{SA} = \frac{\Delta \text{Abs}}{\text{volume assayed (mL)}} / \frac{6.3 \times 10^{-3}}{\text{nmol} \cdot \text{min}^{-1}} \)
    b. Total amount of protein available in the homogenate
        i. \( = \frac{[\text{protein}]}{\text{mg/mL}} \times \frac{\text{mass of wet wt. of tissue}}{0.025} \)
    c. Total number of units = SA * total amount of protein
    d. Units/wet weight = total # of units / total mass of tissue homogenized.

References


Appendix F. Bradford Protein Assay

Procedure:
1. Dilute 1 part dye reagent with 4 parts ddH2O (Bio Rad 500-0006). One microplate requires 25 mL reagent.
2. Filter the diluted solution through Whatman 1 filter paper (store solution at room temperature up to two weeks).
3. Add the indicated amounts of BSA (1 mg/mL) or unknown protein sample, water, and reagent respectively into a 96-well microplate.

<table>
<thead>
<tr>
<th>Protein (ug)</th>
<th>Water (uL)</th>
<th>BSA (uL)</th>
<th>Unknown sample (uL)</th>
<th>Reagent (uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>1</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>2</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>3</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>4</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>6</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Unknown</td>
<td>9.5</td>
<td></td>
<td>0.5</td>
<td>200</td>
</tr>
</tbody>
</table>

4. Shake and incubate at room temperature for a couple of minutes.
5. Read absorbance at 595 nm.

References


Appendix G. Western Blotting Protocol.

Sample preparation:
1. Place homogenized samples on ice.
2. Load the amount of protein in each well previously determined by a protein loading curve.
3. The volume of homogenate needed is determined from a Bradford protein quantification assay (See Bradford Assay protocol).
4. Label a new set of Eppendorf tubes with the appropriate sample names.
5. Dilute the volume of sample in sample buffer (1:1 ratio) and vortex.
6. Boil sample and buffer mixture for up to 5 minutes at 90°C.
7. Prior loading samples into gel allow samples to return to room temperature. It may be necessary to vortex or quickly centrifuge samples before loading.

Preparation of gels:
1. Clean short glass plates with 70% ethanol before use and then prepare gel cassette.
2. Prepare separating gel according to chart relative to the number of gels and percent acrylamide to be used (12% separating gel recipe was used for all blots in this study).

<table>
<thead>
<tr>
<th>12 % Gel</th>
<th>2 gels</th>
<th>4 gels</th>
<th>6 gels</th>
<th>8 gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddH20</td>
<td>6.663 mL</td>
<td>13.325 mL</td>
<td>19.988 mL</td>
<td>26.65 mL</td>
</tr>
<tr>
<td>Acrylamide solution</td>
<td>16 mL</td>
<td>24 mL</td>
<td>32 mL</td>
<td></td>
</tr>
<tr>
<td>Separating gel buffer:</td>
<td>10 ml.</td>
<td>15 mL</td>
<td>20 mL</td>
<td></td>
</tr>
<tr>
<td>SDS solution</td>
<td>200</td>
<td>400</td>
<td>600</td>
<td>800</td>
</tr>
<tr>
<td>10% APS solution</td>
<td>125</td>
<td>250</td>
<td>375</td>
<td>500</td>
</tr>
<tr>
<td>TEMED</td>
<td>12.5</td>
<td>25</td>
<td>37.5</td>
<td>50</td>
</tr>
<tr>
<td>Total Volume</td>
<td>20 ml.</td>
<td>40</td>
<td>60 mL</td>
<td></td>
</tr>
</tbody>
</table>

3. After 10 minutes of mixing, pour separating gel using Pasteur pipette while trying to eliminate bubbles.
4. Immediately overlay the gel with water saturate isobutanol to ensure a continuous charge from separating to stacking gel.
5. Wait approximately 45-60 minutes for gel to polymerize and rinse off overlay solution with ddH20 and dry clean with filter paper when stacking gel (4%) is ready to pour.
6. Prepare and pour stacking gel (4%) according to the chart below:

<table>
<thead>
<tr>
<th>% Gel</th>
<th>2 gels</th>
<th>4 gels</th>
<th>6 gels</th>
<th>8 gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddH₂O</td>
<td>5.992 mL</td>
<td>11.983 mL</td>
<td>17.975 mL</td>
<td>23.967 mL</td>
</tr>
<tr>
<td>Acrylamide solution</td>
<td>1.333 mL</td>
<td>2.667 mL</td>
<td>5.333 mL</td>
<td></td>
</tr>
<tr>
<td>Stacking gel buffer</td>
<td>2.5 mL</td>
<td>7.5 mL</td>
<td>10 mL</td>
<td></td>
</tr>
<tr>
<td>SDS solution</td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>400</td>
</tr>
<tr>
<td>10% APS solution</td>
<td>62.5</td>
<td>125</td>
<td>187.5 gL</td>
<td>250</td>
</tr>
<tr>
<td>TEMED</td>
<td>12.5</td>
<td>25</td>
<td>37.5</td>
<td>50</td>
</tr>
<tr>
<td>Total Volume</td>
<td>10 mL</td>
<td>20 mL</td>
<td>30 mL</td>
<td>40 mL</td>
</tr>
</tbody>
</table>

7. Place the correct sized comb between the glass plates, ensuring no air bubbles are trapped in the wells continue to pour stacking gel mixture on the ends of the comb.

8. Prepare IL of lx running buffer per 2 gels and store in the refrigerator.

9. Once the stacking gel has polymerized (30 minutes), gentle remove comb and fill wells with lx running buffer.

10. Load correct amount of sample and ladder using micropipette with loading tip.

11. Once loading is complete place gels in running unit.

12. Fill running unit with cold lx running buffer (chamber inside the cassette and the outside).

13. Run gels at 70V until through the stacking portion of the gel (—30 minutes) and then 125-130V until sample dye has reached the front of the glass.

14. During the running period prepare transfer buffer and keep in refrigerator.

Transfer of Gels to Nitrocellulose:

1. Cut filter paper and nitrocellulose to appropriate size (short plate size).

2. Soak filter paper, nitrocellulose, and Brillo pads in cold transfer buffer for 20 minutes.

3. Once running period is complete, assemble the transfer apparatus ("sandwich") as shown below, making sure to remove all air bubbles between gel and nitrocellulose paper (keep sandwich completely submerged in transfer buffer at all times).

4. Place "sandwich" into transfer holding tank making sure the black, negative side is facing the black transfer unit. Fill tank with cold transfer buffer and add ice pack into the unit to keep transfer period cold throughout.

5. Connect to power supply and run at 70V for 1.5 hours.
Blocking:

1. Prepare 1x TBS per 2 blots.
2. After transfer, gently remove gel and place in small container with 5% blocking solution (optional, rinse gel once with 1x ITBS for 5 minutes before blocking). Incubate up to 2 hours on shake at room temperature.
3. After blocking prepare primary antibody (minimum 20ml of solution).
4. Wash blots 1x in TTBS for 5 minutes.
5. Incubate blots in primary antibody solution overnight at 40°C or for two hours at room temperature.
6. Once finished, the primary antibody solution can be stored in the refrigerator for use within a week or stored in the freezer for long term storage.
7. Wash blots 3x in TTBS for minutes each.
8. Prepare secondary antibody (confirm HRP, not AP) solution (1:5000-2)
9. Incubate blots for 1 hour on shaker at room temperature,
10. Wash blots 3x in TTBS for 10 minutes each. Keep in 1x TBS for long-term storage.

Western Blotting Solutions:

<table>
<thead>
<tr>
<th>Name</th>
<th>Components, concentrations, pH</th>
<th>Example amounts</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acrylamide solution</td>
<td>Acrylamide – 30% (w/v)</td>
<td>Acrylamide – 150 g</td>
<td>4°C</td>
</tr>
<tr>
<td></td>
<td>Bis-acrylamide – 0.8% (w/v)</td>
<td>Bis-acrylamide – 4 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total V - 500 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Separating gel buffer</td>
<td>Tris – 1.5 M</td>
<td>Tris – 90.9 g</td>
<td>4°C</td>
</tr>
<tr>
<td></td>
<td>SDS – 0.4 % (w/v)</td>
<td>SDS – 2 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH – 8.8</td>
<td>Total V - 500 mL</td>
<td></td>
</tr>
<tr>
<td>3. SDS solution</td>
<td>SDS – 10% (w/v)</td>
<td>SDS – 10 g</td>
<td>Room temperature</td>
</tr>
<tr>
<td></td>
<td>Total V - 100 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. 2X Laemmli SDS-PAGE sample buffer</td>
<td>Tris - 0.125 M</td>
<td>Tris – 7.57 g</td>
<td>Room temperature, fumehood</td>
</tr>
<tr>
<td></td>
<td>Glycerol – 20% (v/v)</td>
<td>Glycerol – 100 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SDS – 4%</td>
<td>SDS – 20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-mercaptoethanol – 10% (v/v)</td>
<td>β-mercaptoethanol – 50 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bromophenol blue – 0.015% (w/v)</td>
<td>Bromophenol blue – 0.075 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH – 6.8</td>
<td>Total V – 500 mL</td>
<td></td>
</tr>
<tr>
<td>5. Stacking gel buffer</td>
<td>Tris – 0.5 M</td>
<td>Tris – 30.3 g</td>
<td>4°C</td>
</tr>
<tr>
<td></td>
<td>SDS – 0.4% (w/v)</td>
<td>SDS – 2 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH – 6.8</td>
<td>Total V – 500 mL</td>
<td></td>
</tr>
<tr>
<td>10X running buffer</td>
<td>Tris – 0.25 M</td>
<td>Tris – 60.6 g</td>
<td>4°C</td>
</tr>
<tr>
<td></td>
<td>Glycine – 1.92 M</td>
<td>Glycine – 288 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SDS – 1% (w/v)</td>
<td>SDS – 20 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total V – 2 L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### References

Gardy, Silar, "Increased Lipid Metabolism in Response to Repetitive Aerobic Exercise During Proestrus in Type 1 Diabetes Mellitus Rats" (2022). Electronic Thesis and Dissertation Repository. 8828. [https://ir.lib.uwo.ca/etd/8828](https://ir.lib.uwo.ca/etd/8828)
Curriculum Vitae

EDUCATION
University of Western Ontario | Master’s of Science – Exercise Biochemistry & Metabolism, Kinesiology
2021 – 2023

University of Western Ontario | Bachelor of Science (Western Scholars), Honors Specialization Kinesiology
2017 – 2021

AWARDS, HONORS & SCHOLARSHIPS
• 3M Educational Scholarship – 2017
• The Western Scholarship of Excellence – 2017
• University of Western Ontario Dean’s Honor List – 2018-2021
• UWO In-Course Scholarship Year III - 2019
• NSERC Undergraduate Student Research Award (USRA) – 2020
• Western Scholars – 2020
• UWO In-Course Scholarship Year IV - 2020
• Undergraduate Summer Research Internship (USRI) Award – 2021
• Ontario Graduate Scholarship (OGS) – 2021-2022
• Dr. Michael Yuhasz Gold Medal – 2021
• Western Graduate Research Scholarship (WGRS) – 2021-2022
• UWO Faculty Association (UWOFA) Scholarship – 2021
• Arthur and Sonia Labatt Ontario Graduate Scholarship – 2022
• Canada Graduate Scholarship, Master’s (NSERC CGS-M) – 2022-2023
• Ontario Graduate Scholarship (OGS) – 2022 (Declined for CGS-M)
• York University 14th Muscle Health Awareness Day Research Poster Award - 2023

PUBLICATIONS
Journal Articles
1. Authors: Jordan C. Larocque; Silar Gardy; Mitchell Sammut; David P. McBey, C.W James Melling
   Title: Sexual Dimorphism in Response to Repetitive Bouts of Acute Aerobic Exercise in Rodents with Type 1 Diabetes Mellitus
   Journal: PlosOne
   Citation: Larocque, J. C., Gardy, S., Sammut, M., McBey, D. P., & Melling, C. W. J. (2022). Sexual dimorphism in response to repetitive bouts of acute aerobic exercise in rodents with type 1 diabetes mellitus. PloS one, 17(9), e0273701. https://doi.org/10.1371/journal.pone.0273701
2. Authors: Michelle Dotzert, Matthew McDonald, T. Dylan Olver, Mitchell Sammut, C.W. James Melling
   Title: The Influence of Combined Aerobic and Resistance Exercise Training versus Standard Intensive Insulin Therapy on Insulin Resistance Development in a Rodent Model of Type 1 Diabetes
**Journal:** Journal of diabetes and its complications  

**PRESENTATION & CONFERENCE EXPERIENCE**

1. *American Diabetes Association 83rd Scientific Sessions*  
   San Diego, California | June 2023  
   **Title of Presentation:** The Effects of Resistance Exercise Training on Insulin Resistance Development in Female Rodents with Type 1 Diabetes

2. *York University 14th Muscle Health Awareness Day*  
   Toronto, ON | May 2023  
   **Title of Presentation:** The Effects of Resistance Exercise Training on Insulin Resistance Development in Female Rodents with Type 1 Diabetes

3. *London Health Sciences Research Day*  
   London, ON | May 2022  
   **Title of Presentation:** The Influence of Combined Aerobic and Resistance Exercise Training versus Standard Intensive Insulin Therapy on Insulin Resistance Development in a Rodent Model of Type 1 Diabetes

4. *2022 International Biochemistry of Exercise Conference (IBEC)*  
   Toronto, ON | May 2022  
   **Title of Presentation:** The Influence of Combined Aerobic and Resistance Exercise Training versus Standard Intensive Insulin Therapy on Insulin Resistance Development in a Rodent Model of Type 1 Diabetes

5. *Undergraduate Summer Research Internship (USRI)*  
   London, ON | August 2021  
   **Title of Presentation:** The Effects of Estrogen in the Glucoregulatory Response to Exercise in Type 1 Diabetes

**RESEARCH EXPERIENCE**

1. *USRI, Exercise Biochemistry Lab – Western University | Undergraduate Research Summer Student*  
   London, ON | May – Aug 2021

2. *4th Year Thesis, Exercise Biochemistry Lab – Western University | 4th Year Thesis Student*
London, ON | September 2020 – April 2021

3. *NSERC USRA, Hoffman Lab* – St. Joseph’s Hospital | Undergraduate Research Summer Student  
   London, ON | May – August 2020

4. *Gait and Brain Lab – Parkwood Institute* | St. Joseph's Health Care London | Undergraduate Research Assistant Volunteer 
   London, ON | Apr – Dec 2019

**TEACHING & MENTORING EXPERIENCE**

1. *Teaching Assistant (TA)* – Western University, Sep 2021-April 2022, Sep 2022-April 2023  
   **Courses:** KIN 2992, KIN 3360B

2. *Undergraduate Mentor* – Western University, May 2022-Present  
   **Supervised:** 4th year thesis students, summer research students