THE ACUTE IMPACT OF A SINGLE DOSE OF RESVERATROL ON INSULIN SENSITIVITY, WHOLE BODY FAT OXIDATION, AND INTRACELLULAR SIGNALING IN SKELETAL MUSCLE AND ADIPOSE TISSUE IN OVERWEIGHT AND OBESE MEN

by

Cameron B. Williams

A thesis submitted to the School of Kinesiology and Health Studies
In conformity with the requirements for the degree of Master of Science.

Queen’s University
Kingston, Ontario, Canada
(June, 2013)

Copyright © Cameron B. Williams, 2013
Abstract

Resveratrol (RSV) is a natural compound that improves mitochondrial function and metabolic health in animal models. Thus far, RSV’s effects on metabolic outcomes in humans are controversial, and RSV’s acute mechanism has not yet been confirmed in vivo. This study aimed to evaluate the effect of an acute dose of RSV on insulin sensitivity and fatty acid oxidation, and to determine RSV’s mechanism of action in human skeletal muscle and adipose tissue. Overweight males (n=8; BMI, 30.5±3.6; VO₂peak, 34.0±7.3 ml/kg) reported to the lab on 2 occasions and were provided a breakfast supplemented with 0.3g of RSV or a placebo pill. Experiments were performed in random order using a double blind crossover design. Gas exchange measures, blood samples, and skeletal muscle and adipose tissue biopsies were obtained before and 2 hours after the supplement meal. RSV acutely improved insulin sensitivity, but had no effect on fatty acid oxidation. Additionally, RSV supplementation had no effect on the intracellular signaling of key proteins proposed to mediate its effects in skeletal muscle and adipose tissue. Taken together, these results suggest a single dose of RSV can acutely enhance insulin sensitivity, but its mechanism of action is not conserved across species, and its intracellular signaling pathway is different in humans than previously thought. Due to its insulin sensitizing effect, RSV retains its clinical value, but further research is required to determine its most useful application for human metabolic health.
Co-Authorship

This thesis presents the work of Cameron Williams in collaboration with Dr. Brendon Gurd.

The impact of a single dose of resveratrol on insulin sensitivity, whole body fat oxidation, and intracellular signaling in skeletal muscle and adipose tissue in overweight and obese men is presented according to the guidelines for the Journal of Physiology. Cameron Williams was responsible for reviewing relevant literature to identify the research question, conducting the intervention, performing the data analyses, and drafting the manuscript. All aspects were a collaborative effort between Cameron Williams and Dr. Brendon Gurd. In addition, Dr. Craig Simpson provided medical supervision and contributed to study design. Finally Brittany Edgett was instrumental in blood collection.

Chapter 2 is a reproduction of Skeletal muscle SIRT1 and the genetics of metabolic health: therapeutic activation by pharmaceuticals and exercise originally published August, 2012 in The Application of Clinical Genetics. Cameron Williams was responsible for reviewing the relevant literature and drafting the manuscript. The paper was co-authored by Dr. Brendon Gurd. It has been reproduced herein with the express permission of both the authors (C.B. Williams and B.J. Gurd) and the approval of the editorial board of The Application of Clinical Genetics and Dove Medical Press Ltd. Please see Appendix D for written permission.
Acknowledgements

I would like to begin by thanking my supervisor, Dr. Brendon Gurd. Choosing to pursue a Master’s degree under his tutelage was an important and rewarding academic decision. He has invested a lot of time and money into me, and continually provided the support and guidance I have needed to maintain forward progress in my academic and scientific career. So thank you Brendon, it means a lot.

I would also like to thank my lab mates that have helped, supported, and enhanced every aspect of my grad school experience. I couldn’t ask for a better group of people to work with. A lot of laughs and a lot of memories! Brit, Tina, Trish, Marysa, Jasmin, Colin, Mel, and Jason. Thanks guys!

I would also like to thank Dr. Michael Tschakovsky for his continued mentorship from my undergrad into my graduate studies. It was his offer in my 4th year that first introduced me to research and was likely the catalyst for my accepting a graduate school position. Thanks Dr. T.

Last, but definitely not least, I would like to thank my parents for all the love, help, and support they have given me through my entire academic career and more importantly my life. I’ve got a couple years left so hopefully you aren’t ready to throw in the towel just yet!

I have truly enjoyed my time in the Gurd Lab and won’t soon forget the past two years. Thank you to everyone who was part of that!
Table of Contents

Abstract ................................................................................................................................. ii
Co-Authorship ...................................................................................................................... iii
Acknowledgements ........................................................................................................ iv
List of Figures ..................................................................................................................... viii
List of Tables ....................................................................................................................... ix
List of Abbreviations ........................................................................................................ x

Chapter 1 Introduction ....................................................................................................... 1
  1.1 General Introduction .................................................................................................... 1
  1.2 Metabolic Function: Importance of Mitochondria ..................................................... 2
  1.3 Resveratrol: A Promising Candidate As An Exercise Mimetic ............................... 3
  1.4 Thesis Objectives and Experimental Approach ...................................................... 4
  1.5 Thesis Organization ................................................................................................. 5
  1.6 References ................................................................................................................ 5

Chapter 2 Literature Review: SIRT1 and Metabolic Health ............................................. 9
  2.1 Abstract ..................................................................................................................... 9
  2.2 Introduction ............................................................................................................... 10
  2.3 Skeletal Muscle Mitochondria: Mechanisms of Metabolic Disease ...................... 12
  2.4 Genetic Control of Skeletal Muscle Mitochondrial Content: The SIRT1/PGC-1α Axis ............................................................................................................ 16
      2.4.1 Physiological Control of SIRT1 Activity ............................................................ 18
      2.4.2 Pharmacological Activation of SIRT1 ............................................................... 20
  2.5 Targeting SIRT1 activation as therapeutic intervention ......................................... 21
      2.5.1 Health Benefits Associated with Pharmaceutical Activation of SIRT1 ......... 21
      2.5.2 Health Benefits of SIRT1-PGC1α Axis Activation During Exercise ............ 24
  2.6 Conclusion and Future Direction ........................................................................... 26
  2.7 References ................................................................................................................ 27

Chapter 3 Literature Review: Resveratrol ........................................................................ 39
  3.1 Overview .................................................................................................................. 39
  3.2 Resveratrol .............................................................................................................. 39
      3.2.1 Resveratrol’s Role in Metabolic Health ............................................................. 40
      3.2.2 Insulin Sensitivity ............................................................................................ 41
3.2.3 Fatty Acid Oxidation ........................................................................................................ 42
3.2.4 Further Investigation Required ....................................................................................... 43
3.3 Resveratrol’s Mechanism of Action in Skeletal Muscle .................................................. 43
  3.3.1 Calcium/Calmodulin Dependent Pathway ..................................................................... 44
  3.3.2 SIRT1 Dependent Pathway ............................................................................................ 44
  3.3.3 Resveratrol’s Mechanism in Humans ......................................................................... 46
  3.3.4 Insulin Sensitivity ........................................................................................................ 47
  3.3.5 Fatty Acid Oxidation .................................................................................................... 48
3.4 Adipose Tissue and Metabolic Health .............................................................................. 49
3.5 Resveratrol’s Mechanism of Action in Adipose Tissue ..................................................... 51
  3.5.1 Adipose Tissue Mitochondrial Biogenesis ................................................................... 52
3.6 Thesis Purpose .................................................................................................................. 54
3.7 Thesis Hypotheses ............................................................................................................. 54
3.8 References ......................................................................................................................... 55

Chapter 4 A Single Dose of Resveratrol Acutely Improves Insulin Sensitivity, But Has No Effect On Whole Body Fat Oxidation or SIRT1 Activation in Skeletal Muscle and Adipose Tissue in Overweight and Obese Men ........................................................................................................ 60
4.1 Abstract ............................................................................................................................ 60
4.2 Introduction ...................................................................................................................... 61
4.3 Methods ............................................................................................................................ 64
  4.3.1 Participants .................................................................................................................. 64
  4.3.2 Experimental Protocol ............................................................................................... 65
  4.3.3 Baseline Testing ......................................................................................................... 65
  4.3.4 Supplement Intervention Protocol ............................................................................. 65
  4.3.5 Physiological Measurements ..................................................................................... 65
  4.3.6 Fasting Glucose, Insulin, HOMA-IR and Estimates of Substrate Utilization .......... 67
  4.3.7 Western Blot Analysis ............................................................................................... 68
  4.3.8 Statistical Analysis .................................................................................................... 69
4.4 Results .............................................................................................................................. 72
  4.4.1 HOMA-IR, Insulin and Glucose .................................................................................. 72
  4.4.2 Fatty Acid Oxidation .................................................................................................. 72
  4.4.3 Skeletal Muscle Signaling .......................................................................................... 73
## Table of Contents

4.4.4 Adipose Tissue Signaling .................................................................................. 73  
4.5 Discussion ............................................................................................................... 78  
  4.5.1 Resveratrol and Insulin Sensitivity ................................................................. 78  
  4.5.2 Whole Body Fatty Acid Oxidation ................................................................. 80  
  4.5.3 Resveratrol and SIRT1 activation in skeletal muscle ........................................ 81  
  4.5.4 Cellular signaling in Adipose Tissue ............................................................... 83  
4.6 Limitations ............................................................................................................. 84  
4.7 Summary ............................................................................................................... 85  
4.8 References ............................................................................................................. 86  
Chapter 5 General Discussion ..................................................................................... 93  
  5.1 Summary of Key Findings .................................................................................... 93  
  5.2 Study Strengths .................................................................................................... 93  
  5.3 Study Implications ............................................................................................... 94  
  5.4 Study Limitations .................................................................................................. 95  
  5.5 Future Research ................................................................................................... 96  
  5.6 M.Sc. Research Experience .................................................................................. 97  
  5.7 Conclusions ......................................................................................................... 98  
  5.8 References .......................................................................................................... 98  
Appendix A Research Ethics Board - Letter of Informed Consent .......................... 100  
Appendix B Muscle Biopsy Information Sheet ......................................................... 110  
Appendix C Research Ethics Board Approval ............................................................. 115  
Appendix D Dove Medical Press Permission to Reproduce Publication ................. 118
List of Figures

Figure 1. Pathways involved in the SIRT1-PGC-1α axis. ................................................................. 15
Figure 2. Proposed mechanism of resveratrol action in skeletal muscle. ................................. 46
Figure 3. Proposed mechanism of resveratrol action in adipose tissue................................. 53
Figure 4. Experimental Visit Timing. .......................................................................................... 67
Figure 5. Impact of an acute dose of resveratrol (RSV) on 2 hour insulin sensitivity............ 74
Figure 6. Whole body fat oxidation is unchanged by resveratrol (RSV)................................. 75
Figure 7. Resveratrol alters p38 MAPK phosphorylation but does not alter other intramuscular signaling pathways. .......................................................................................................................... 76
Figure 8. Adipose tissue signaling is unaltered by resveratrol.................................................. 77
List of Tables

Table 1. Subject characteristic summary. ................................................................. 71
List of Abbreviations

ACC – acetyl-CoA carboxylase

AICAR – 5-aminooimidazole-4-carboxamid ribonucleotide

AMP – adenosine monophosphate

AMPK – AMP-activated protein kinase

ANOVA – analysis of variance

AROS – active regulator of SIRT1

ATP – adenosine triphosphate

BMI – body mass index

cAMP – cyclic 3’5’-adenosine monophosphate

CamKII – calcium/calmodulin-dependent protein kinase II

CamKKβ - calcium/calmodulin-dependent protein kinase kinase-β

CHO – carbohydrate

cm – centimeter

CPT-I – carnitine palmitoyltransferase

CV – confidence variable

DAG – diacylglycerol

DBC-1 – deleted in breast cancer-1

DNA – deoxyribonucleic acid

EDTA – ethylene diamine tetra-acetic acid

EGTA – ethylene glycol tera-acetic acid

ELISA – enzyme-linked immunoabsorbent assay

Epac1 – exchange protein activated by cAMP-1
g – grams
GAPDH – glyceraldehyde 3-phosphate dehydrogenase
GLUT4 – glucose transporter type 4
h – hour
HOMA-IR – homeostatic model assessment – insulin resistance
kcal – kilocalories
kg – kilogram
L – liter
LCFA – long chain fatty acid
LKB1 – liver kinase B1
mg – milligram
min – minute
mL – milliliters
mRNA – messenger ribonucleic acid
NaCl – sodium chloride
NAD+ – nicotinamide adenine dinucleotide
NAMPT – nicotinamide phosphoribosyltransferase
p38 MAPK – p38 mitogen activated protein kinase
PDE – phosphodiesterase
PGC-1α – peroxisome proliferator-activated receptor γ coactivator-1α
PI3K – phosphoinositide 3-kinase
PL – placebo
PLC – phospholipase-C
PPARγ – peroxisome proliferator-activated receptor-γ
RER – respiratory exchange ratio
ROS – reactive oxygen species
RPM – revolutions per minute
RSV – Resveratrol
SD – standard deviation
SDS-PAGE – sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec – second
SIRT1 – silent mating type information regulation 2 homologue 1
SUMO – small ubiquitin-like modifers
T2D – type II diabetes
µIU – micro international units
µL – microliter
UPC1 – uncoupling protein 1
VCO₂ – volume of carbon dioxide
VO₂ – volume of oxygen
W – watts
yrs – years
Chapter 1

Introduction

1.1 General Introduction

The incidence of overweight and obesity in Canada has risen drastically over the last 30 years, with recent estimates suggesting 13.2 million Canadians are overweight and another 4.6 million are obese (Lau et al. 2007; Statistics Canada, 2011). Overweight and obesity are strong risk factors for a multitude of diseases, including cardiovascular disease, diabetes, hypertension, dyslipidemia, certain cancers, and premature mortality (Kopelman, 2000). Not all obese individuals develop metabolic syndrome, approximately 10-25% of obese people are metabolically healthy (Bluher, 2010), however, obesity remains a significant risk factor for metabolic dysfunction and reports have demonstrated the prevalence of metabolic syndrome is directly related to the degree of obesity (Weiss et al. 2004). In addition to the health implications, this excess weight represents a substantial economic burden, costing Canada an estimated $4.6 billion annually in the management and treatment of obesity and obesity related disease (Public Health Agency of Canada, 2011). More importantly, many of the associated health risks with overweight and obesity stem from the impaired physiological function of metabolically active tissue, specifically, skeletal muscle and adipose tissue (Holloway, 2009; Kelley et al. 1999; Kelley et al. 2002). Accordingly, the investigation and understanding of the mechanisms responsible for the physiological impairment in both skeletal muscle and adipose tissue are important for tailoring interventions aimed at ameliorating this metabolic dysfunction.
1.2 Metabolic Function: Importance of Mitochondria

Mitochondria are the principle energy source for the cell, converting nutrients into energy (adenosine triphosphate; ATP) through cellular respiration. Healthy skeletal muscle is characterized by a large number of mitochondria, sensitivity to insulin, and efficient fatty acid oxidation, while physiologically impaired skeletal muscle, often seen with obesity, has observable decrements in mitochondrial content and function (Menshikova et al. 2007). These maladaptive changes are associated with insulin insensitivity, impaired fatty acid oxidation, and the production of reactive oxygen species, all of which are causally linked to a number of metabolic diseases (Menshikova et al. 2007; Kelley et al. 2002; Kim et al. 2000). A genetic basis for the link between metabolic dysfunction and mitochondrial content is illustrated through observed down regulation of oxidative and mitochondrial gene expression associated with metabolic disease (Mootha et al. 2003). This genetic control of mitochondrial biogenesis is an important determinant of skeletal muscle health (see Chapter 2 for details) and warrants investigation of the signaling pathways in the myocyte responsible for mitochondrial gene expression. Further, the exploration of factors that influence these pathways is important in order to understand how interventions aimed at improving metabolic health work.

Adipose tissue’s primary function is the storage of triglycerides during energy surplus and the release of fatty acids during energy deficits. Additionally, adipose tissue acts as an endocrine organ, producing an array of molecules implicated in the metabolic control of fat cells as well as cells found in the brain, liver, muscle, and pancreas (Frigolet Vazquez-Vela et al. 2008). Adipose tissue dysfunction is associated with insulin insensitivity, tissue specific lipotoxicity, hyperlipidemia, and low-grade systemic inflammation, all of which are indicative of impairments in both its endocrine and energetic functions (Frigolet Vazquez-Vela et al. 2008; Weisberg et al. 2003). Again, observed down regulation of mitochondrial biogenesis in white adipose tissue with metabolic disease suggests a genetic basis for this physiological dysfunction.
(Bogacka et al. 2005; Cadoudal et al. 2008; Laye et al. 2009; Sutherland et al. 2009; Wilson-Fritch et al. 2004). This evidence justifies the investigation of pathways involved in adipose tissue mitochondrial biogenesis and the identification of factors that influence these pathways.

The risks to systemic health associated with maladaptive changes in metabolic function are considerable; however, both skeletal muscle and adipose tissue possess the ability to adapt to external stressors in a metabolically positive way, thus these risks are preventable and potentially reversible (Holloszy and Coyle, 1984; Smorlesi et al. 2012). Endurance exercise introduces a systemic metabolic stress capable of provoking metabolic remodeling of both myocytes and adipocytes (Holloszy, 2008; Laye et al. 2009). These training based adaptations involve changes in protein content and enzyme activity, altering substrate utilization, and improving energy homeostasis (Wan et al. 2012b; Narkar et al. 2008). Skeletal muscle specific adaptations are largely attributable to increased mitochondrial biogenesis (Holloszy, 2008), while many of the benefits seen in adipose tissue stem from the upregulation of key enzymes in the lipolytic and glyceroneogenic pathways (Wan et al. 2012b; Wan et al. 2012a). This evidence suggests that targeting these adaptive tissues should be a priority of current health interventions.

1.3 Resveratrol: A Promising Candidate As An Exercise Mimetic

Given the failure of the majority of individuals to achieve adequate daily physical activity (Haskell et al. 2007; King et al. 2000; Lau et al. 2007), the identification of active compounds that can mimic the benefits of endurance exercise on skeletal muscle and adipose tissue is clinically relevant. Resveratrol (RSV; 3,5,4’-trihydroxystilbene), a naturally occurring polyphenol most commonly found in red grape skin, is a potential exercise mimetic for humans. A number of different models characterizing RSV’s mechanism of action in skeletal muscle have been proposed based on work in animals and cells (detailed in chapters 2 and 3), although none of these have been confirmed in humans. Further, of the few studies that have explored RSV’s
mechanism of action in adipose tissue, none have used an in vivo human model. Current understandings of RSV’s effects in human skeletal muscle and adipose tissue are very limited; consequently, intervention studies have begun in humans, without a clear understanding of mechanisms. RSV’s ability to mimic some of the benefits of exercise (without actually training) suggests that it may hold significant clinical value, thus understanding the mechanisms involved is an important step in the research process.

1.4 Thesis Objectives and Experimental Approach

As outlined above, animal, cellular, and preliminary human trials have positioned RSV as a potential exercise mimetic with possible clinical applications. From the available literature it is evident that our current understanding of RSV’s effect on metabolic outcomes and its mechanism of action in both skeletal muscle and adipose tissue is incomplete. Thus, the purpose of this thesis was to 1) to determine if the mechanism(s) of RSV action, previously demonstrated in cellular and animal models, are conserved in human skeletal muscle and adipose tissue, and 2) to investigate the acute effects of RSV on insulin sensitivity and whole body fatty acid oxidation. To achieve this, participants ingested a placebo or RSV supplement on separate visits to the lab in a randomized double blind crossover fashion. A meal challenge was provided to assess RSV’s effect on glucose tolerance and insulin sensitivity. Gas exchange measures and a plasma blood sample, along with skeletal muscle and subcutaneous adipose biopsies were obtained pre-supplement and two hours post-supplement in order to examine RSV’s effect on whole body fatty acid oxidation, insulin sensitivity, and intracellular signaling in skeletal muscle and adipose tissue.
1.5 Thesis Organization

In Chapter 2 the reader will find a detailed review of the literature surrounding the induction of mitochondrial biogenesis in skeletal muscle, establishing SIRT1 and the SIRT1-PGC-1α axis as a valuable target for interventions aimed at ameliorating metabolic health. The published manuscript found in Chapter 2 has been reproduced with permission of Dove Medical Press and both authors – myself and co-author Dr. Brendon Gurd. Chapter 3 focuses on the literature surrounding the specific mechanisms by which RSV acts in skeletal muscle and adipose tissue, as well as a review of our current understanding of RSV’s effect on metabolic health in humans. As Chapter 2 was focused solely on skeletal muscle, in Chapter 3 the reader will find the pertinent background literature positioning adipose tissue as a relevant target for RSV mediated adaptations. Chapter 4 contains the manuscript detailing the study of RSV’s mechanism of action in human skeletal muscle and adipose tissue. Chapter 5 provides a discussion on the key findings of this research, the limitations, and future directions. Finally, several appendices are attached to the end of this document to provide more complete examples of items referred to throughout this thesis.

1.6 References


Chapter 2

Literature Review: SIRT1 and Metabolic Health

Skeletal muscle SIRT1 and the genetics of metabolic health: Therapeutic activation by pharmaceuticals and exercise

2.1 Abstract

SIRT1 (silent mating type information regulation 2 homologue 1) is implicated in the control of skeletal muscle mitochondrial content and function through deacetylation of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and participation in the SIRT1/PGC-1α axis. The SIRT1-PGC-1α axis control of skeletal muscle mitochondrial biogenesis is an important therapeutic target for obesity and obesity related metabolic dysfunction, as skeletal muscle mitochondrial dysfunction is implicated in the pathogenesis of multiple metabolic diseases. This review will establish the importance of the SIRT1-PGC-1α axis in the control of skeletal muscle mitochondrial biogenesis, and explore possible pharmacological and physiological interventions designed to activate SIRT1 and the SIRT1-PGC-1α axis in order to prevent and/or treat obesity and obesity related metabolic disease. The current evidence supports a role for therapeutic activation of SIRT1 and the SIRT1-PGC-1α axis by both pharmaceuticals and exercise in the treatment and prevention of metabolic disease. Future research should be directed toward the feasibility of pharmaceutical activation of SIRT1 in humans and refining exercise prescriptions for optimal SIRT1 activation.

Key Words SIRT1, PGC-1α, Resveratrol, Obesity, Metabolic Disease, Exercise
2.2 Introduction

Decreases in mitochondrial content and function accompanying the development of overweight and obesity represent an underlying mechanism of several metabolic disorders including insulin resistance, dyslipidemia, type II diabetes, hypertension, and cardiovascular disease (Lau et al. 2007; Kopelman, 2000). In 2005, an estimated 23.2% of the worldwide adult population were overweight and 9.8% were obese (Kelly et al. 2008). Current projections predict that 58.7% of the global adult population will be overweight or obese by 2030 (Wang et al. 2008), an estimate that represents potentially a massive economic burden for healthcare systems worldwide. In the United States, these rates would increase obesity related costs to 16% ($860 billion USD) of total healthcare expenditure (Wang et al. 2008). Type II diabetes alone is already costing $376 billion USD each year globally, a figure that is projected to grow 30% in the next 20 years (Zhang et al. 2010; Shaw et al. 2010). Given the economic and health costs associated with overweight and obesity, and their associated diseases, there is an urgent need for effective preventative and therapeutic interventions targeting weight gain and metabolic disease.

Skeletal muscle accounts for approximately 40% of body mass (Kamei et al. 2004) and plays a critical role in the maintenance of metabolic health. Healthy skeletal muscle has a highly oxidative phenotype, readily oxidizes lipids, is sensitive to insulin, and efficiently stores glucose (Muioio and Newgard 2006; Dyson, 2010). Consistent with the importance of mitochondria in healthy muscle, impairments in skeletal muscle function that contribute to obesity associated disease (decreased fatty acid oxidation, impaired insulin sensitivity, etc.) are causally linked to decreases in mitochondrial content and function (Kelley et al. 2002; Kim et al. 2000; Ritov et al. 2005; Menshikova et al. 2007; Mootha et al. 2003; Patti et al. 2003). In fact, decreases in mitochondrial content are apparent in early stage overweight and obesity and appear to contribute
to both weight gain *per se*, and to the metabolic dysfunction that underlies the development of metabolic disease (Holloway *et al*. 2009; Kelley *et al*. 2002).

Genetic control of skeletal muscle mitochondrial content is regulated via a complex network of signaling pathways, transcriptional factors and transcription co-factors. PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1-α) is a transcription factor co-activator that has been implicated as a key regulator of mitochondrial gene expression and mitochondrial biogenesis (Lin *et al*. 2002). The central role of PGC-1α in the regulation of mitochondrial content has positioned this protein as an important target in therapies aimed at preventing and/or treating disease (Wu *et al*. 1999; Handschin and Spiegelman 2006). SIRT1 (silent mating type information regulation 2 homologue 1) interacts with PGC-1α in skeletal muscle increasing PGC-1α’s transcriptional activity through deacetylation (Gerhart-Hines *et al*. 2007). Given its apparent ability to activate PGC-1α and increase mitochondrial content in skeletal muscle (Gerhart-Hines *et al*. 2007), SIRT1 has itself been implicated as a key player in metabolic health (Canto and Auwerx 2009; Guarente, 2006). It should be noted that SIRT1 is not solely responsible for the control of PGC-1α acetylation status, and evidence of normal mitochondrial biogenesis in mice lacking SIRT1 deacetylase activity suggests an inherent redundancy in the PGC-1α deacetylation pathway (Philp *et al*. 2011). Despite this redundancy, the bulk of evidence suggests an important role of SIRT1 in skeletal muscle adaptations; thus, there is considerable interest in interventions designed to target SIRT1 and the SIRT1/PGC-1α axis as a means of increasing mitochondrial content, skeletal muscle function, and metabolic health.

Within this context, this review will first examine the role of skeletal muscle mitochondria in the development of obesity and metabolic disorders and the importance of the SIRT1/PGC-1α axis in the determination of skeletal muscle mitochondrial content. Once the importance of the SIRT1/PGC-1α axis to metabolic health has been established, we will examine
potential interventions, pharmacological and otherwise, designed to activate SIRT1 in an attempt to prevent and/or treat obesity and obesity associated metabolic disorder.

### 2.3 Skeletal Muscle Mitochondria: Mechanisms of Metabolic Disease

Although not all individuals who are overweight or obese develop metabolic disorders, in general, there appears to be a progression from overweight, to obese, to metabolically diseased. Consistent with this idea, being overweight as a young adult is highly predictive of obesity later in life (Herman et al. 2009), and adult obesity is strongly associated with increased risk for many diseases, including insulin resistance, type II diabetes, and cardiovascular disease (Lau et al. 2007; Kopelman, 2000; Kim et al. 2000; Boyko et al. 2000). Changes in mitochondrial content and function are implicated in the development of several metabolic diseases. While the importance of mitochondrial dysfunction is controversial (Holloway, 2009) there is significant evidence that mitochondrial content is altered in metabolic dysfunction. For example, the decline in skeletal muscle mitochondria associated with altered metabolic health is eloquently demonstrated by observations that mitochondrial oxidative capacity is lower in obese than in lean adults, and further decreased in type II diabetics (i.e. lean > obese > T2D) (Kelley et al. 2002). Further, a positive correlation has been observed between mitochondrial oxidative capacity and insulin sensitivity in lean and obese young adults (Hickey et al. 1995; Simoneau et al. 1995) and in sedentary and exercise trained older adults (Houmard et al. 1991). While it is not clear whether mitochondrial dysfunction (defined as either decreases in individual mitochondrial oxidative capacity and/or decreased mitochondrial content) precedes initial weight gain, there is substantial evidence that mitochondrial function is impaired in both obesity (Kim et al. 2000; Simoneau et al. 1995; Simoneau et al. 1999; Kelley et al. 1999) and type II diabetes (Asmann et al. 2006; Mootha et al. 2003). At present, there is still debate regarding the exact mechanism(s) linking mitochondrial dysfunction to metabolic disease; however, the current literature suggests that
either impaired fatty acid oxidation and/or increased oxidative stress resulting from altered mitochondrial function are central in the aetiology of metabolic disease.

Fatty acid oxidative capacity is correlated with insulin sensitivity in obese individuals (Simoneau et al. 1999) and impairments in lipid oxidation, accompanied by a concurrent upregulation of fatty acid transport (Bonen et al. 2004), appears to underlie the accumulations of intramuscular fat associated with obesity and metabolic disease (Kim et al. 2000). Impaired lipid oxidation in obese skeletal muscle appears to be related to dysfunctional mitochondria lacking appropriate oxidative enzyme activity, a decreased mitochondrial content within skeletal muscle, or a combination of both (Kelley et al. 2002; Kim et al. 2000; Thyfault et al. 2004; Ritov et al. 2005; Holloway et al. 2009; Holloway, 2009). As individuals progress from obesity to metabolic disease there is a decrease in the content or quality of mitochondria that results in an accumulation of intramuscular fat that is intimately related to decreases in metabolic health.

The accumulation of intramuscular fat is implicated in the development of a number of comorbidities, particularly insulin insensitivity and type II diabetes (Petersen and Shulman 2002; Muoio and Newgard 2006; Holland et al. 2007; Goodpaster et al. 2000; Pan et al. 1997). Intramuscular lipid accumulation and the associated increases in lipid species, particularly diacylglycerol (DAG) (Timmers et al. 2008) and ceramides (Summers, 2006; Holland et al. 2007), have been linked to the disruption of the insulin receptor cascade (Holloway, 2009). Decreased insulin action (insulin insensitivity, and the eventual development of insulin resistance and type II diabetes) is independently associated with the progression of hypertension, hyperlipidemia, and is known to be atherogenic (Defronzo and Ferrannini 1991; Petersen and Shulman 2002).

In addition to a decreased capacity to oxidize fat, impaired mitochondrial function also contributes to chronic increases in oxidative stress via increased lipid peroxidation. Decreased fatty acid usage results in slowed intramuscular lipid turn over and a resulting maladaptive
peroxidation of intramuscular lipids and reactive lipid species accumulation (Goodpaster et al. 2001; Samocha-Bonet et al. 2010). Lowered lipid turnover rates, coupled with dysfunctional mitochondria prone to reactive oxygen species (ROS) production, facilitate the peroxidation of lipids and the production of lipotoxic lipid species in the intramuscular lipid pool (Schrauwen and Hesselink 2004). These lipotoxic lipid species further interfere with mitochondrial function and intracellular signaling through the disruption of protein and DNA structure, potentiating lipid peroxidation in a damaging cycle that contributes to the development of metabolic disease (Schrauwen and Hesselink 2004). (See Schrauwen and Hesselink (2004) for detailed review).

The importance of skeletal muscle mitochondria for overall metabolic health is illustrated through the association of mitochondrial dysfunction and metabolic disease. The accumulation of damaging reactive lipid species within the muscle is hypothesized to result from impaired mitochondrial function, and the ensuing oxidative stress is implicated in the pathophysiology of a host of metabolic diseases. Specifically, oxidative stress is suggested to contribute to the development of obesity and several other diseases associated with metabolic syndrome, including coronary artery disease, hypertension and type II diabetes. We refer the reader to the following references for more detailed information on the role of oxidative stress in obesity and metabolic disease (Furukawa et al. 2004; Roberts and Sindhru 2009). The activation of PGC-1α has been implicated in the control of anti-oxidant expression and the prevention of mitochondrial oxidative damage in mice (Wenz et al. 2009), reinforcing the importance of targeting the SIRT1-PGC-1α axis to improve metabolic health. Improving skeletal muscle mitochondrial content may reverse this lipid accumulation and potentially ameliorate the associated metabolic dysfunction. Thus, skeletal muscle mitochondrial content, through its role in the determination of intramuscular fat content, is intimately related to systemic metabolic health.
Figure 1. Pathways involved in the SIRT1-PGC-1α axis.

Model of pathways leading to activation of the silent mating type information regulation 2 homolog 1 (SIRT1)/peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) axis in skeletal muscle: SIRT1 deacetylase activity is directly influenced through posttranslational modification and indirectly through exercise, caloric restriction, and pharmaceutical activation.

Notes: Exercise-induced activation of adenosine monophosphate-activated protein kinase increases nicotinamide adenine dinucleotide, a necessary substrate in the SIRT1 deacetylase reaction, and directly phosphorylates cytosolic PGC-1α prior to nuclear translocation. Caloric restriction activates adenosine monophosphate-activated protein kinase and increases nicotinamide adenine dinucleotide, while pharmaceuticals (resveratrol/SRT1720) activate adenosine monophosphate-activated protein kinase, induce SIRT1 posttranslational modification, and may augment SIRT1 deacetylase activity through direct interaction (dashed line). SIRT1 activates PGC-1α through deacetylation, increasing PGC-1α-mediated autoexpression and transcription of mitochondrial genes.

Abbreviations: Ac, acetyl; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; NAD+, nicotinamide adenine dinucleotide; P, phosphate; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; PTM, posttranslational modification; SIRT1, silent mating type information regulation 2 homolog 1.
2.4 Genetic Control of Skeletal Muscle Mitochondrial Content: The SIRT1/PGC-1α Axis

Plasticity is a defining characteristic of skeletal muscle and thus there is great potential for the remodeling of metabolic function towards a healthy phenotype in all populations. For example, exercise training can significantly increase mitochondrial oxidative activity and fat oxidation, decrease intramuscular lipid accumulation and oxidative stress and improve insulin sensitivity, all parameters of healthy skeletal muscle (Bruce et al. 2006; Goodpaster et al. 2000; Gibala et al. 2006; Ferrara et al. 2008). These adaptations are largely due to increases in mitochondrial content and function resulting from the upregulation of a genetic program of mitochondrial protein controlled through the SIRT1/PGC-1α axis.

PGC-1α drives the transformation of skeletal muscle towards an oxidative phenotype via interaction with, and activation of a plethora of transcription factors involved in the induction of mitochondrial genes encoded within both nuclear and mitochondrial DNA (Wu et al. 1999; Lin et al. 2002; Puigserver and Spiegelman 2003; Scarpulla, 2008; Lira et al. 2010). While the regulation of the transcriptional activity of PGC-1α is complex (Holloszy, 2008; Gibala, 2009), induction of PGC-1α mediated transcription is accomplished through acute activation of PGC-1α (Wright et al. 2007; Perry et al. 2010) and chronic upregulation of PGC-1α protein content via an autoregulatory loop (Hanschien et al. 2003). Importantly, the acute activation of PGC-1α, accomplished via post-translational modification, appears to be an essential first step in the induction of PGC-1α mediated transcription of mitochondrial genes (Canto and Auwerx 2009). PGC-1α is post-translationally modified by phosphorylation via p38 mitogen activated protein kinase (MAPK) and AMP-activated protein kinase (AMPK) (Puigserver et al. 2001; Jaeger et al. 2007; Wright et al. 2007). In addition, PGC-1α acetylation status is also implicated in PGC-1α
transcriptional activity and mitochondrial biogenesis (Rodgers et al. 2005; Gerhart-Hines et al. 2007). For example, acetylation levels of PGC-1α are inversely correlated with skeletal muscle oxidative capacity in murine muscle (Canto et al. 2009), while genetic mutation of acetylation sites on PGC-1α (mimicking the deacetylated state) markedly increase PGC-1α transcriptional activity and mitochondrial biogenesis in skeletal muscle cells (Canto et al. 2009).

Through the deacetylation of acetylated-lysine residues, the sirtuins (a family of evolutionarily conserved deactylases) modify protein activity and have been implicated in a variety of cellular processes including the stress response and cellular energy control in mammals (Dali-Youcef et al. 2007; Yang and Sauve 2006; Guarente, 2006). While 7 sirtuins have been identified in humans, several of which are implicated in metabolic control, including the mitochondrial sirtuins SIRT3, 4, and 5 (Flick and Luscher 2012), the current review will focus on SIRT1 in skeletal muscle and metabolic disease. SIRT1 directly interacts with PGC-1α in mouse hepatocytes (Rodgers et al. 2005) and is responsible for deacetylation of PGC-1α in 293T cells (Rodgers et al. 2005) and PC12 neuronal cells (Nemoto et al. 2005). In addition, SIRT1 interacts with PGC-1α in C2C12 skeletal muscle cells (Gerhart-Hines et al. 2007) and SIRT1 mediated deacetylation of PGC-1α appears to play an important, but perhaps non-essential (Philp et al. 2011), role in the induction of PGC-1α mediated transcription (Canto et al. 2009). In support of this evidence from cells, SIRT1 is implicated in caloric restriction, exercise, and AICAR induced upregulation of mitochondrial content and function in skeletal muscle of mice (Gerhart-Hines et al. 2007; Canto et al. 2010; Canto et al. 2009). Further, in transgenic animal models, overexpression of SIRT1 consistently improves insulin sensitivity (Banks et al. 2008; Bordone et al. 2007) and exerts a protective effect against metabolic disorders commonly associated with high fat feeding (Pfluger et al. 2008). This evidence implicates the SIRT1/PGC-1α axis in skeletal muscle in the upregulation of genes implicated in mitochondrial content (Gurd et al. 2011; Lagouge et al. 2006) as well as fatty acid oxidation and energy expenditure (Gerhart-Hines
et al. 2007) and illustrates the importance of this axis for any intervention designed to reverse skeletal muscle dysfunction. As such, targeting the SIRT1/PGC-1α axis may be a practical treatment model for obesity and metabolic disorder.

2.4.1 Physiological Control of SIRT1 Activity

In vivo, SIRT1 activity is regulated by substrate availability, post-translational modification, and the formation of both inhibiting and activating complexes (Zschoernig and Mahlknecht 2008). Activation of SIRT1 through one, or several, of these mechanisms is expected to upregulate mitochondrial gene expression via the SIRT1/PGC-1α axis, resulting in improved mitochondrial and metabolic function.

Substrate availability is implicated in SIRT1 activation, primarily through changes in cellular redox potential. NAD⁺ is a substrate in SIRT1 mediated deacetylation, which suggests changes in [NAD⁺] can affect SIRT1 activity (Rodgers et al. 2005; Sauve et al. 2006). Consistent with this hypothesis, SIRT1 activity (measured as a decrease in PGC-1α acetylation status) and [NAD⁺] are increased concomitantly during nutrient deprivation in hepatocytes (Rodgers et al. 2005) and C2C12 skeletal muscle cells (Gerhart-Hines et al. 2007; Rodgers et al. 2005) and following nutrient deprivation (Canto et al. 2010) and exercise (Canto et al. 2009; Canto et al. 2010) in intact mouse muscle. Recent reports suggest that AMPK, an intracellular energy sensor (Hardie, 2007), mediates increases in [NAD⁺] during periods of nutrient restriction, and thus plays a key role in regulating SIRT1 activity and transcriptional programs controlled through the SIRT1/PGC-1α axis (Canto et al. 2009; Canto et al. 2010). Additionally, nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in the NAD⁺salvage pathway (Revollo et al. 2004; Yang et al. 2007), is implicated in the control of intramuscular [NAD⁺] and thus SIRT1 activity. NAMPT is upregulated following interventions known to activate SIRT1 in skeletal muscle, specifically, exercise training in humans (Costford et al. 2010) and acute exercise
in mice (Canto et al. 2010). These results comprise the main evidence supporting the hypothesis that SIRT1 activity is increased in response to altered energy status through increased substrate availability via AMPK, NAMPT, or both. Interestingly, in both cell (Anderson et al. 2003) and animal models (Chabi et al. 2009; Gurd et al. 2009; Ferrara et al. 2008) SIRT1 activity does not always correspond to changes in [NAD^+]. These results suggest that SIRT1 activity is under more complex control than can be explained through alterations in substrate availability alone.

Several recent lines of evidence have implicated post-translational modifications of SIRT1 and the formation of inhibitory (Kim et al. 2008; Escande et al. 2010) and activating complexes in the control of SIRT1 deacetylase activity (Kim et al. 2007; Nin et al. 2012). A number of phosphorylation sites have been identified on SIRT1 (Gerhart-Hines et al. 2011; Sasaki et al. 2008) and phosphorylation of these sites consistently increases SIRT1 deacetylase activity (Gerhart-Hines et al. 2011; Sasaki et al. 2008; Nasrin et al. 2009). In addition to phosphorylation, SIRT1 activity is influenced by sumoylation (Yang et al. 2007), methylation (Liu et al. 2011), and nitrosylation (Kornberg et al. 2010). Sumoylation by the SUMO family of enzymes increases SIRT1 catalytic activity (Yang et al. 2007), while it is unclear whether methylation directly affects SIRT1 deacetylase activity (Liu et al. 2011). Transnitrosylation of SIRT1 has also been demonstrated, resulting in increased acetylation of SIRT1 targets, suggesting an inhibitory effect on SIRT1 deacetylase activity with nitrosylation (Kornberg et al. 2010). In addition to post-translational modifications, SIRT1 deacetylase activity can be modified through the formation of regulatory complexes. Specifically, SIRT1 deacetylase activity is inhibited when forming a complex with DBC-1 (deleted in breast cancer-1) (Nin et al. 2012) and activated when forming a complex with AROS (active regulator of SIRT1) (Kim et al. 2007). These types of SIRT1 activity regulations (both post-translational modifications and regulatory complex formation) have not been thoroughly explored in skeletal muscle, making it an important direction for future research.
The physiological pathways controlling SIRT1 activity, outlined above, can be activated by both exercise and caloric restriction. The exact mechanism by which caloric restriction impacts SIRT1 activity remains unclear, although it is hypothesized that caloric restriction initiates a disturbance to metabolic homeostasis, activating AMPK and subsequently increasing cellular [NAD$^+$], resulting in an increased activation of SIRT1 (Canto et al. 2009; Canto and Auwerx 2009; Canto et al. 2010; Gerhart-Hines et al. 2007; Rodgers et al. 2005). Caloric restriction may also increase SIRT1 activity through activation of NAMPT, increasing flux through the NAD$^+$ salvage pathway (Anderson et al. 2003). Despite the uncertainty surrounding the mechanism of caloric restriction mediated SIRT1 activation, it is apparent that caloric restriction increases SIRT1 deacetylation activity and can play a role in the activation of the SIRT1-PGC-1α axis, increasing fatty acid oxidation in skeletal muscle (Canto et al. 2009), and providing protective metabolic effects against an energy imbalance (Canto and Auwerx 2009). We refer the reader to the following references for a more detailed review on the role of SIRT1 in caloric restriction (Canto and Auwerx 2009; Canto and Auwerx 2009).

2.4.2 Pharmacological Activation of SIRT1

In addition to physiological activation, SIRT1 can also be pharmacologically activated through interactions with a number of natural and synthetic molecules. Resveratrol, a naturally occurring polyphenol found in grape skins, has been implicated in the activation of SIRT1 (Feige et al. 2008; Lagouge et al. 2006; Smith et al. 2009; Howitz et al. 2003). While resveratrol administration can increase SIRT1 activity by as much as 8-fold (Howitz et al. 2003), the exact mechanism of activation remains elusive. Although initially believed that resveratrol interacts with and activates SIRT1 directly, more recent investigations have demonstrated that this interaction may be an artifact of the fluorophore detection method (Borra et al. 2005; Pacholec et al. 2010), and in vivo resveratrol mediated activation of SIRT1 appears to result from activation...
of upstream targets (Lagouge et al. 2006; Chen et al. 2012; Park et al. 2012). The synthetic production of SIRT1 activators has also been successful; in particular, SRT1720 increases SIRT1 deacetylase activity more potently than resveratrol in both cell lines and within skeletal muscle of mice (Feige et al. 2008; Howitz et al. 2003; Milne et al. 2007). However, as with resveratrol, whether SRT1720 activates SIRT1 directly or indirectly remains unclear (Milne et al. 2007; Pacholec et al. 2010). Despite the uncertainty surrounding the mechanism of these molecules and their interaction with SIRT1, their ability to increase SIRT1 deacetylase activity is well established. The potential for activation of the SIRT1/PGC-1α axis through pharmacological means makes SIRT1 an intriguing target for future therapeutic intervention.

2.5 Targeting SIRT1 activation as therapeutic intervention

The remainder of this review will explore the potential benefits and limitations of targeting SIRT1, through pharmacological, and physiological interventions, for the purpose of activating the SIRT1/PGC-1α axis to stimulate mitochondrial biogenesis and improve skeletal muscle function and metabolic health.

2.5.1 Health Benefits Associated with Pharmaceutical Activation of SIRT1

The pharmacological activation of SIRT1 exerts its therapeutic effects on skeletal muscle through the SIRT1/PGC-1α axis. By increasing mitochondrial content and function through PGC-1α mediated transcription, improved skeletal muscle function may prevent or reverse obesity and metabolic disease. In skeletal muscle, resveratrol activates SIRT1 resulting in deacetylation of PGC-1α and the induction of a genetic profile associated with improved mitochondrial function and fatty acid metabolism (Lagouge et al. 2006). As mentioned previously, while SIRT1 is required for resveratrol mediated deacetylation of PGC-1α in mouse embryonic fibroblasts (Lagouge et al. 2006) there is some debate regarding resveratrol’s direct interaction with SIRT1.
(Pacholec et al. 2010; Dai et al. 2010), with some evidence suggesting that resveratrol’s effects may occur via an AMPK mediated mechanism (Lagouge et al. 2006; Chen et al. 2012; Park et al. 2012). Regardless, AMPK and SIRT1 are believed to function in concert in skeletal muscle (Canto et al. 2010) and this uncertainty only questions whether resveratrol acts directly on SIRT1, not whether resveratrol activates the SIRT1/PGC-1α axis.

Consistent with the above, activation of the SIRT1/PGC-1α axis following resveratrol treatment in mice increases mitochondrial and fatty acid gene expression and increases mitochondrial content in skeletal muscle (Lagouge et al. 2006). Accompanying these improvements in skeletal muscle function are improved insulin sensitivity in mouse models (Lagouge et al. 2006; Baur et al. 2006; Chen et al. 2012) and a resistance to the deleterious effects of aging and high fat diet on metabolic health (Lagouge et al. 2006; Baur et al. 2006; Pearson et al. 2008). At present there are relatively few studies examining metabolic impact of resveratrol in humans. However, a recent study in obese males indicates that 30 days of dietary resveratrol supplementation increased skeletal muscle SIRT1 and PGC-1α protein content, intrinsic mitochondrial function, and improved several markers of cardiovascular and metabolic health (Timmers et al. 2011). Two other studies have also reported beneficial effects of dietary resveratrol in humans including improved glucose tolerance in older adults (Crandall et al. 2012) and a reduction of the oxidative and inflammatory response to a high fat meal (Ghanim et al. 2011). While these studies provide promising initial findings indicating that targeting SIRT1 via resveratrol supplementation may prove therapeutic for obesity and obesity associated disease, randomized control studies are still needed to confirm these beneficial effects in a larger population. In addition there is little data regarding whether prolonged intake of resveratrol has any negative implications either within skeletal muscle, within other organs (liver for example) or on overall metabolic health in humans.
The use of the synthetic SIRT1 activator SRT1720 has been proposed to selectively activate SIRT1 with greater potency, efficacy and selectivity than resveratrol (Howitz et al. 2003; Feige et al. 2008; Milne et al. 2007). The chronic administration of SRT1720 in mice decreases acetylation of PGC-1α and a number of other SIRT1 targets (Feige et al. 2008) and increases oxidative capacity, lipid oxidation, insulin sensitivity, and provides a protective effect against diet-induced obesity (Feige et al. 2008; Milne et al. 2007; Smith et al. 2009). In addition to protective metabolic effects, mice on high fat diets given SRT1720 ran twice the distance in endurance trials and improved muscle function in a variety of functional tests when compared to controls fed the same diet (Feige et al. 2008). These skeletal muscle adaptations make SRT1720 an appealing molecule for therapeutic intervention. Similar to resveratrol, controversy exists regarding whether SRT1720 activates SIRT1 through direct interaction, or acts indirectly via activation of AMPK through alterations in cellular energy status (Pacholec et al. 2010). Thus the metabolic adaptations attributed directly to SRT1720-SIRT1 interaction versus an AMPK mediated SIRT1 activation are difficult to distinguish (Feige et al. 2008). Phase I and II clinical trials examining the safety and metabolic benefits of a drug closely related to SRT1720, SRT2104, are currently underway and represent an important look into the ability of SRT1720/2104 to increase mitochondrial content in skeletal muscle and improve metabolic function in obesity and metabolic disease.

At present, interventions with both resveratrol and SRT1720 in mice, and in preliminary human studies (resveratrol only) appear to activate the SIRT1/PGC-1α axis, improve mitochondrial content in skeletal muscle and improve metabolic health. These findings support the suggestion that these pharmaceuticals are promising potential agents for prevention and treatment of obesity and its related diseases and warrant further investigation.
2.5.2 Health Benefits of SIRT1-PGC1α Axis Activation During Exercise

Exercise, as a component of lifestyle intervention, is an alternative to pharmacological interventions aimed at improving skeletal muscle mitochondrial function and metabolic health (Haskell et al. 2007). An emerging line of evidence suggests that exercise targets SIRT1, and the resulting activation of the SIRT1/PGC-1α axis underlies many of the beneficial effects associated with exercise (Gurd, 2011; Benton et al. 2008).

An acute bout of exercise in rat and mouse muscle increases SIRT1 activity (Gurd, 2011) and deacetylation of PGC-1α immediately, and for several hours following exercise (Canto et al. 2010; Canto et al. 2009). Exercise training is also implicated in the activation of SIRT1 in animal models with both increases in SIRT1 activity (Gurd et al. 2009; Koltai et al. 2010; Chabi et al. 2009; Ferrara et al. 2008) and deacetylation of PGC-1α (Li et al. 2011) observed following chronic contractile activity and treadmill running. Activation of SIRT1 was also observed in human skeletal muscle after 2 and 6 weeks of exercise training (Gurd, 2011; Gurd et al. 2011). While skeletal muscle SIRT1 protein content is also elevated following acute exercise and exercise training in both rats (Ljubicic et al. 2009; Suwa et al. 2008) and humans (Guerra et al. 2010; Little et al. 2010) the implications of elevated SIRT1 content in muscle remain unclear (Gurd et al. 2012). It should also be noted that although PGC-1α deacetylation was normal in mice expressing SIRT1 lacking deacetylase activity (Philp et al. 2011), the majority of observations in intact animal and human skeletal muscle support the involvement of SIRT1 in mediating PGC-1α acetylation status. Also consistent with the contention that SIRT1 and the SIRT1/PGC-1α axis play a critical role in the control of skeletal muscle mitochondrial content are numerous animal studies reporting that increased SIRT1 activity is accompanied by increases in PGC-1α transcriptional activity (Gurd et al. 2009; Chabi et al. 2009; Ljubicic et al. 2009; Suwa et al. 2008; Dumke et al. 2009).
The activation of the SIRT/PGC-1α axis by exercise is accompanied by increases in markers of oxidative capacity and mitochondrial content in animals (Chabi et al. 2009; Gurd et al. 2009; Gurd et al. 2011) and humans (Little et al. 2010; Gurd et al. 2011). Further, markers of improved fatty acid oxidation and insulin sensitivity are also found in animals and humans accompanying activation of the SIRT1/PGC-1α axis by exercise (Suwa et al. 2008; Gurd et al. 2011; Little et al. 2010). In addition to mitochondrial adaptations, activation of the SIRT1/PGC-1α axis is linked to the attenuation of age related decline in skeletal muscle health in exercised rodents (Ferrara et al. 2008; Koltai et al. 2010; Ljubicic et al. 2009) via decreased oxidative stress and DNA damage, factors also implicated in the pathogenesis of many metabolic disorders associated with obesity (Furukawa et al. 2004). Consistent with these observations, exercise training improves oxidative capacity and fatty acid oxidation in skeletal muscle from obese adults (Menshikova et al. 2005; Menshikova et al. 2007; Goodpaster et al. 2003), improves insulin sensitivity in obesity and type II diabetes (Knowler et al. 2002; Laaksonen et al. 2005) and decreases both risk factors for, and symptoms of metabolic disease (Hawley and Gibala 2009; Tjonna et al. 2008). In summary, exercise appears to activate the SIRT1/PGC-1α axis and improve skeletal muscle mitochondrial function and metabolic health. These results highlight the preventative and therapeutic potential of exercise for obesity and obesity related disease.

As an alternative treatment in obesity and metabolic disease, exercise has several inherent advantages over pharmaceutical intervention. First, the improved metabolic function associated with exercise comes at minimal financial cost, while a pharmaceutical intervention carries a substantial financial commitment from both the individual and healthcare provider (Wang et al. 2008; Shaw et al. 2010). Second, in addition to improved skeletal muscle mitochondrial function and metabolic/cardiovascular health regular exercise is associated with a myriad of beneficial effects ranging from prevention and treatment of mental disorders (Wolff et al. 2011) and cancer (Pekmezi and Demark-Wahnefried 2011) to alleviating symptoms and improving quality of life in
many chronic diseases (Pedersen and Saltin 2006). Third, exercise is implicated in a systemic improvement of health with little to no risk of adverse side effects (Haskell et al. 2007; Warburton et al. 2006). Pharmaceuticals are often associated with undesirable side effects, and are inherently designed to be specific, eliminating the possibility of a systemic health improvement. Finally, there is evidence that exercise, as part of a lifestyle intervention, induces superior improvements compared to pharmaceutical intervention in subjects with metabolic disease (Knowler et al. 2002). In light of these arguments, it makes both health and financial sense that exercise becomes a first line tool in both the prevention and treatment of obesity and obesity related disease.

### 2.6 Conclusion and Future Direction

The current review has focused on the contribution of SIRT1 to skeletal muscle function and metabolic health. Future should not only continue to investigate SIRT1 function, but should also focus on other members of the Sirtuin family. The contribution of SIRT1 to overall metabolic health occurs, in part, through SIRT1’s influence on PGC-1α and skeletal muscle mitochondrial function. As skeletal muscle mitochondrial dysregulation is implicated in obesity and obesity related metabolic disease SIRT1 has become attractive target for therapeutic intervention. The activation of SIRT1, and consequently the SIRT1/PGC-1α axis results in upregulation of mitochondrial genes and improved skeletal muscle mitochondrial content and function. SIRT1 activity can be modified by pharmaceuticals and exercise, providing an array of options to pursue for implementing a therapeutic intervention. A clear need for further investigation of the feasibility of pharmaceutical intervention in humans is evident, as, for the most part, human trials are in their infancy or are non-existent. With exercise, exploration of the optimal dose and intensity will expand the possibility of tailored prescriptions targeting SIRT1 activity.
2.7 References


Chapter 3

Literature Review: Resveratrol

3.1 Overview

Chapter 2 has outlined the importance of the SIRT1-PGC-1α axis and its influence on mitochondrial biogenesis in skeletal muscle. This portion of the literature review highlights the proposed models of resveratrol’s mechanism of action in both skeletal muscle and adipose tissue and our current understanding of resveratrol’s effects on metabolic health in humans. Additionally, Chapter 3 highlights the relevant literature positioning adipose tissue as another potential target for resveratrol mediated effects. Chapter 3 concludes with the purpose of the current thesis and its hypotheses.

3.2 Resveratrol

The identification of active compounds capable of mimicking the benefits of exercise on metabolic function has become a clinically relevant goal due to the metabolic and health complications associated with overweight and obesity. A number of natural and synthetic agents that can be classified as exercise mimetics have been identified, yet very few of them are deemed suitable for human consumption. Resveratrol (3,5,4’-trihydroxystilbene; RSV), a naturally occurring polyphenol, first attracted scientific interest in the 1990s when it was linked to the cardioprotective effects of red wine (Siemann and Creasy, 1992). RSV was subsequently shown to possess powerful antitumorigenic effects in a number of animal derived cellular cancer models (Jang et al. 1997). In 2003, Howitz et al. identified RSV as a potent activator of SIRT1 that mimicked the effects of caloric restriction on SIRT1 activity and resulted in extended lifespan in
lower order eukaryotes (Howitz et al. 2003). Following these reports, RSV’s ability to activate SIRT1 and ameliorate metabolic health has been consistently demonstrated in animal models (Lagouge et al. 2006; Baur et al. 2006; Pearson et al. 2008; Dal-Pan et al. 2010), raising the question, can RSV exert these same effects in humans and could it be a potential pharmacological ally in the pursuit of improved metabolic health?

3.2.1 Resveratrol’s Role in Metabolic Health

Preclinical trials involving cellular and animal models have positioned RSV as a candidate for pharmacological improvements in metabolic health. In animals, RSV’s ability to activate SIRT1 and its associated downstream effectors mediates an increase in oxidative capacity and mitochondrial content within skeletal muscle (Lagouge et al. 2006; Park et al. 2012; Price et al. 2012) and may also activate mitochondrial biogenic pathways in adipose tissue (Park et al. 2012; Um et al. 2010). These RSV induced adaptations translate into increased aerobic fitness and exercise capacity (Lagouge et al. 2006), enhanced lipid oxidation (Lagouge et al. 2006), improved insulin sensitivity (Baur et al. 2006; Chen et al. 2012; Lagouge et al. 2006; Su et al. 2006), increased lipolytic activity (Szkudelska et al. 2009; Pedersen et al. 2008), delayed age-associated functional decline in oxidative stress protection and locomotor activity (Pearson et al. 2008; Baur et al. 2006; Park et al. 2012), and a metabolic profile resistant to weight gain (Lagouge et al. 2006; Dal-Pan et al. 2010). Tissue specific changes are also apparent, with evidence of increased expression of genes coding for troponins (involved with contractile activity), respiratory apparatus, oxidative enzymes, and ATPases in skeletal muscle (Lagouge et al. 2006; Pearson et al. 2008), and the down regulation of genes associated with proinflammatory pathways in adipose tissue (Pearson et al. 2008). While the litany of metabolic benefits outlined above is exciting, the extent to which RSV may induce similar adaptations in humans remains unclear.

40
In contrast to the relatively consistent reports in animal trials, human clinical trials with RSV have reported varying and conflicting effects of RSV on metabolic outcomes. To further complicate the interpretation of human trials, very little consistency exists with respect to the dosage and duration of RSV treatment, the populations used, or the outcomes measured; therefore, we will attempt to summarize the most relevant data to metabolic health to illustrate the current understanding of RSV action in human skeletal muscle and adipose tissue.

3.2.2 Insulin Sensitivity

Insulin sensitivity has been the primary outcome of a number of clinical trials, and represents an important risk factor for metabolic dysfunction. Some human trials agree with preclinical evidence from animals, suggesting RSV can improve insulin sensitivity. This insulin sensitizing effect has been observed in older males with type II diabetes following 4 weeks of 10mg/day RSV (Brasnyo et al. 2011), in obese males following 30 days of 150mg/day RSV (Timmers et al. 2011), and in older males following 1-2g/day RSV (Crandall et al. 2012). In contrast to these reports, no change in insulin sensitivity was observed in non-obese women following 12 weeks of 75mg/day RSV (Yoshino et al. 2012) or in obese men following 4 weeks of 1.5g/day RSV (Poulsen et al. 2013). An explanation for these conflicting results remains elusive. But owing to the varied dosages and timing of the final RSV supplement before testing in the chronic studies, whether the positive reports of improved insulin sensitivity are attributable to direct RSV influence through acute carryover effects, or an indirect RSV influence, mediated through metabolic adaptations, remains unclear. Future research aimed at isolating the acute effects of RSV on insulin sensitivity may help resolve these questions and advance the current understanding of RSV’s potential role in human metabolic health.
3.2.3 Fatty Acid Oxidation

Animal studies have consistently demonstrated RSV’s ability to attenuate weight gain (Lagouge et al. 2006; Dal-Pan et al. 2010) and improve whole body lipid metabolism (Lagouge et al. 2006). These improvements have been attributed to increased expression of genes associated with oxidative phosphorylation (Lagouge et al. 2006; Baur et al. 2006), increasing content of oxidative and mitochondrial proteins, and enhancing fatty acid oxidation (Lagouge et al. 2006). While there is one report of modestly improved intrinsic mitochondrial function and moderate increases in gene expression associated with mitochondrial biogenesis in a chronic human trial, these results did not translate into increased fatty acid oxidation or basal energy expenditure as previously seen in animals (Timmers et al. 2011). More recent studies have confirmed that rates of fatty acid oxidation and basal energy expenditure are unchanged with chronic RSV supplementation (Poulsen et al. 2013; Yoshino et al. 2012). Moreover, in contrast to Timmers et al. (2011), these studies have observed no effect of RSV on gene expression of pathways involved in mitochondrial biogenesis (Poulsen et al. 2013; Yoshino et al. 2012). These chronic adaptations, observed in animal models and some human trials, are proposed to derive from RSV’s influence on energy sensing networks (Park et al. 2012; Price et al. 2012; Canto et al. 2009), however, the acute effect of RSV on fatty acid oxidation remains undefined. Similar to the controversial results surrounding RSV’s insulin sensitizing effects in humans, whether the positive reports of increased fatty acid oxidation are the result of chronic adaptation or an acute carryover effect from the final dose of RSV is unclear. Consequently, further investigation aimed at isolating the acute effects of RSV to better understand the mechanism of RSV mediated fatty acid oxidation is warranted.
3.2.4 Further Investigation Required

The above reports highlight the significant controversy that exists with regards to RSV’s role in human metabolic health. Very little work was found that explored the mechanisms underlying the observed effects in human intervention studies, forcing speculation, and making interpretations of the conflicting reports very difficult. Understanding the mechanism of RSV action is an important step towards predicting and understanding RSV’s effects. While animal studies demonstrated impressive results, driving the desire to begin clinical trials, it seems that an important step was overlooked – an evaluation of RSV mechanism of action in human tissue and its acute effects on metabolic outcomes.

3.3 Resveratrol’s Mechanism of Action in Skeletal Muscle

The positive metabolic adaptations associated with RSV treatment in skeletal muscle are largely attributed to RSV mediated activation of SIRT1 and the SIRT1-PGC-1α axis (Howitz et al. 2003; Price et al. 2012; Hubbard et al. 2013; Park et al. 2012; Lagouge et al. 2006). Initial reports hypothesized a direct interaction between RSV and SIRT1, postulating that RSV increased the affinity of SIRT1 for its deacetylase targets via a conformational shift in tertiary structure (Borra et al. 2005; Howitz et al. 2003). More recently, the direct interaction between RSV and SIRT1 has been challenged, with evidence suggesting there were methodological limitations to the fluorophore detection method used in original examinations of RSV’s effects on SIRT1 (Pacholec et al. 2010). These authors demonstrated that the interaction of RSV with SIRT1 was augmented by the fluorophore-linked substrates used to visualize the reaction and suggested that RSV’s activation of SIRT1 was unlikely to occur in vivo (Pacholec et al. 2010). A recent report suggests that naturally occurring hydrophobic amino acids may mimic the role of the fluorophore-linked substrate in vivo and facilitate the direct interaction of RSV with SIRT1 (Hubbard et al. 2013). However, the original skepticism of RSV’s direct interaction with SIRT1
led to the search for RSV targets upstream of SIRT1, and the proposition of an indirect pathway responsible for RSV mediated activation of SIRT1 (Park et al. 2012). Meanwhile, some groups continued to draw a direct connection between RSV and SIRT1 (prior to the most recent evidence from Hubbard et al. (2013)) and proposed an alternative mechanism for RSV mediated metabolic benefits, in keeping with the idea of direct activation of SIRT1 by RSV (Price et al. 2012).

3.3.1 Calcium/Calmodulin Dependent Pathway

First proposed by Park et al. (2012), the calcium calmodulin dependent pathway suggests that RSV indirectly activates SIRT1 through inhibition of phosphodiesterase (PDE) activity, an upstream modulator of the cyclic 3’5’-adenosine monophosphate (cAMP) pathway (Path 1 in Figure 2). PDE facilitates the conversion of cAMP into the inactive 5’-adenosine monophosphate (AMP); therefore, the proposed inhibition of PDE by RSV would act to increase cAMP concentration and thus activation of its downstream targets, specifically exchange protein activated by cAMP-1 (Epac1) (de Rooij et al. 1998). Epac1 is then proposed to act through phospholipase-C (PLC) to activate calcium/calmodulin-dependent protein kinase II (CamKII) increasing intramyocellular Ca^{2+} concentration (Park et al. 2012). This increase in cytosolic Ca^{2+} concentration would then activate calcium/calmodulin-dependent protein kinase kinase-β (CamKKβ), resulting in the phosphorylation and activation of AMPK, increasing the concentration of NAD^{+} (a necessary substrate in the SIRT1 deacetylase reaction) and thus increasing SIRT1 activity and the activity of its downstream targets (Park et al. 2012).

3.3.2 SIRT1 Dependent Pathway

An alternative pathway involving the direct activation of SIRT1 by RSV has also been proposed (Path 2 in Figure 2). Price et al. (2013) postulated that RSV mediated activation of SIRT1 results in SIRT1 mediated deacetylation and activation of liver kinase B1 (LKB1) (Lan et al. 2008), which in turn phosphorylates AMPK, increasing intracellular NAD^{+} concentrations,
further activating SIRT1 deacetylase activity by means of a positive feedback loop (Price et al. 2012). Using a SIRT1 knock out mouse model, a moderate RSV dose was unable to increase AMPK phosphorylation and NAD$^+$ concentrations in skeletal muscle, while the same dose increased both AMPK phosphorylation and NAD$^+$ concentration in wild type mice (Price et al. 2012). While the authors acknowledge their uncertainty of how RSV originally activates SIRT1, new evidence of hydrophobic amino acids facilitating the direct allosteric activation of SIRT1 by RSV may provide some insight into this mechanism (Hubbard et al. 2013). Interestingly, a high dose of RSV was able to induce AMPK phosphorylation in the absence of SIRT1, suggesting a potential dose-dependent activation of alternative pathways (Price et al. 2012) and the possibility of multiple RSV targets.
Figure 2. Proposed mechanism of resveratrol action in skeletal muscle.
Schematic of the intracellular signaling responsible for RSV mediated activation of SIRT1 and mitochondrial biogenesis in skeletal muscle. Pathway 1 (pink) represents indirect RSV activation of SIRT1 through activation of the cAMP pathway (Park et al. 2012). Pathway 2 (blue) represents the proposed direct RSV activation of SIRT1 (Price et al. 2012). Arrows represent activation, while red circle represent inhibition.

3.3.3 Resveratrol’s Mechanism in Humans

RSV’s mechanism of action in human skeletal muscle remains poorly defined. Similar to the conflicting reports of RSV’s effects on metabolic outcomes, the available data on RSV mediated intracellular signaling are unclear, and no study has explored in vivo acute mechanisms of RSV action in humans. In keeping with cellular models, the use of human skeletal muscle cells has cast doubt on the conservation of RSV’s mechanism of action across species. Specifically, skeletal muscle cells harvested from human muscle biopsies treated with RSV demonstrated impaired glucose uptake, down regulated insulin signaling, and blunted activation of AMPK, a
key intermediate in both direct (Price et al. 2012) and indirect (Park et al. 2012) RSV mediated SIRT1 activation. While these results come from a physiologically removed model and may not reflect the impact of RSV on human skeletal muscle in vivo, clinical reports from long-term RSV trials provide equally contradictory evidence. For example, while Timmers et al. (2011) observed an increase in AMPK phosphorylation, and protein content of both SIRT1 and PGC-1α following 30 days of RSV supplementation, no effect of 4 weeks of RSV on phosphorylation of AMPK, total acetylated lysine or PGC-1α mRNA has also been reported (Poulsen et al. 2013). Additionally, no effect on SIRT1 or PGC-1α protein content, phosphorylation of AMPK, or the activation of a number of genetic pathways involved in mitochondrial biogenesis was observed following 12 weeks of RSV (Yoshino et al. 2012). Furthermore, while these reports of protein content and activation following chronic interventions may afford some insight into RSV’s effects in humans, they do not necessarily reflect a RSV mediated change in intramuscular signaling as this would be a transient effect as opposed to a chronic adaptation. Investigation of RSV’s effect on intracellular signaling would best be assessed with an acute dose, in an attempt to isolate RSV’s mechanism of action as opposed to signaling changes mediated by adaptation.

3.3.4 Insulin Sensitivity

The impact of RSV on intracellular pathways involved in insulin signaling in skeletal muscle are of particular interest as it is currently unclear if the purported insulin sensitizing effects of RSV are a result of acute changes in signaling, or chronic adaptations in muscular function. In type II diabetic patients, phosphorylation of AKT, a critical intermediate in the insulin-signaling pathway (Krook et al. 1998), was increased in platelets along with insulin sensitivity following 4 weeks of 10mg/day RSV treatment (Brasnyo et al. 2011). In contrast, the acute treatment of human skeletal muscle cells attenuated insulin-induced phosphorylation of AKT and its upstream regulator PI3-kinase (Skrobuk et al. 2012). Due to the difference in study
design (acute vs. chronic) and population (cellular model vs. *in vivo*), a comparison between these
two studies is difficult to make. However, these findings provide further confirmation of the
uncertainty surrounding RSV’s action in humans, and lend support for the need for acute
investigations of RSV’s mechanism in human skeletal muscle, a key organ for insulin function, to
isolate the effects of a single dose of RSV on intracellular signaling, as opposed to the effect of
chronic adaptation. Additionally, the activation of p38 mitogen activated protein kinase (MAPK)
has been demonstrated to enhance insulin sensitivity, possibly through the activation of GLUT4
at the plasma membrane (Sweeney *et al.* 1999). In cellular models the pharmacological inhibition
of p38 MAPK decreased glucose transport by up to 70%, independent of insulin signaling
pathway activation (Somwar *et al.* 2000) and GLUT4 translocation to the plasma membrane
(Sweeney *et al.* 1999). These findings suggest the activation of p38 MAPK may enhance GLUT4
catalytic activity through the dissociation of GLUT4 from an inhibitory complex or the removal
of an allosteric inhibitor (Furtado *et al.* 2002), independent of insulin signaling. Animal models
have demonstrated RSV’s ability to activate p38 MAPK in skeletal muscle (Menzies *et al.* 2013),
although this has not been confirmed in human trials, presenting a novel area for future research.

### 3.3.5 Fatty Acid Oxidation

Enhanced fatty acid oxidation observed in animal (Lagouge *et al.* 2006) and some human
(Timmers *et al.* 2011) trials is also of interest, due to the metabolic consequence of fat
accumulation in skeletal muscle (Holloway *et al.* 2009; Holland *et al.* 2007). The uptake of long
chain fatty acids (LCFA) into the mitochondria by carnitine palmitoyltransferase 1 (CPT-1) for
beta-oxidation has been proposed as the rate-limiting step in fatty acid oxidation (Roepstorff *et al.*
2005). Following a negative energy balance, the ratio of AMP/ATP is increased, resulting in the
activation of AMP-activated protein kinase (AMPK) by AMP. The phosphorylation and
activation of AMPK induces the phosphorylation and inhibition of AMPK’s downstream target,
acetyl-CoA carboxylase (ACC). This inhibition decreases the production of malonyl-CoA, relieving its inhibition of CPT-1, increasing flux of fatty acid into the mitochondria for oxidation (Roepstorff et al. 2005; Hue and Taegtmeyer, 2009). RSV’s proposed activation of AMPK (Park et al. 2012; Price et al. 2012) and its ability to mimic a negative energy balance (Timmers et al. 2011) suggests that it may increase fatty acid oxidation via the AMPK signaling cascade outlined above. Whether RSV acutely activates the same AMPK pathways in humans as observed in animals remains unclear, but an investigation into the acute effect of RSV on whole body fatty acid oxidation may provide some insight into these mechanisms.

Due to the conflicting and contradictory reports of metabolic adaptations in humans and the limited evidence surrounding potential intracellular pathway activation in human skeletal muscle presented above, it appears plausible that RSV’s mechanism of action may be different in humans than that previously seen in animals. Understanding the underlying mechanisms of RSV action is critical to understanding these conflicting results and maximizing possible applications for RSV in humans. An investigation into the acute in vivo effects of RSV on human skeletal muscle could provide insight into the signaling pathways involved in RSV’s effect, and provide some insight into whether the proposed mechanism of action is conserved in humans and whether we should expect the same effects as previously seen in animal studies. If these pathways differ in human skeletal muscle, then the predicted effects of RSV on mitochondrial biogenesis and metabolic adaptation may no longer be valid, and new avenues of research may be exposed.

3.4 Adipose Tissue and Metabolic Health

Adipose tissue carries out three important functions: the storage of triglycerides, the hydrolysis and release of fatty acids, and the production of a number of molecules implicated in systemic metabolic control (Frigolet Vazquez-Vela et al. 2008). Adipose tissue dysfunction results in the impairment of both its endocrine and energetic functions, leading to insulin
insensitivity, hyperlipidemia, tissue specific lipotoxicity, and low-grade systemic inflammation (Frigolet Vazquez-Vela et al. 2008; Weisberg et al. 2003). While the evidence for RSV mediated effects on adipose tissue is limited, the available literature suggests RSV may carry therapeutic potential in human adipocytes.

Lipolytic activity, responsible for the hydrolysis and release of fatty acids from adipose stores, is decreased in obese individuals (Langin et al. 2005), and may contribute to the maladaptive accumulation of adipose tissue associated with obesity. Increased fat mobilization and oxidation is a hallmark of caloric restriction and exercise in mammals, and appears to play a role in improved metabolic health (Picard et al. 2004; Timmers et al. 2011). Isolated human adipocytes from obese subjects demonstrate decreased SIRT1 content and activity when compared to lean controls (Pedersen et al. 2008) and caloric restriction serves to reverse this trend, increasing SIRT1 content in vivo and in vitro within the adipocyte (Pedersen et al. 2008). As a potent activator of SIRT1 in skeletal muscle, RSV may also play a role in activating SIRT1 within adipocytes and, as such, may help to prevent/reverse obesity related impairments in lipolytic function. In rodents, the activation of SIRT1 in adipose tissue represses genes involved in adipogenesis controlled by peroxisome proliferator-activated receptor-γ (PPARγ), increasing lipolytic activity and the mobilization of free fatty acids (Picard et al. 2004). Evidence from human adipocytes agrees with rodent data, with increased β-adrenergic stimulated lipolytic activity in adipose tissue following SIRT1 activation (Pedersen et al. 2008). Interestingly, the addition of RSV to adipocyte explants further increased epinephrine stimulated lipolysis, suggesting a synergistic effect of RSV on adipose tissue lipolytic activity (Pedersen et al. 2008). In contrast, following 30 days of RSV supplementation in humans, no statistically significant change was seen in adipose tissue lipolysis following a meal (Timmers et al. 2011). These results suggest that RSV’s influence on lipolysis may require an existing lipolytic signal, such as β-adrenergic stimulation, and acts to sensitize the adipocyte to pro-lipolytic signaling.
The regulation of adipose tissue mitochondrial biogenesis has become a focus of a growing number of studies, in part due to the proposed role of adipose tissue mitochondria in the regulation of whole body metabolic health (Wilson-Fritch et al. 2004; Sutherland et al. 2009; Bogacka et al. 2005; Choo et al. 2006). As mentioned above, adipocyte dysfunction is associated with a host of metabolic disorders, including insulin insensitivity, dyslipidemia, and the abnormal release of pro-inflammatory cytokines (Frigolet Vazquez-Vela et al. 2008). Evidence of diminished PGC-1α expression and down regulation of mitochondrial biogenesis in adipocytes of diabetic rats (Sutherland et al. 2009; Laye et al. 2009; Wilson-Fritch et al. 2004) and humans (Bogacka et al. 2005) provides a genetic link for adipocyte metabolic impairment.

Exercise is implicated in the activation of PGC-1α and the induction of mitochondrial biogenesis in rat adipose tissue (Sutherland et al. 2009; Laye et al. 2009), while insulin-sensitizing drugs are capable of the same activation in the adipose tissue of both rats (Wilson-Fritch et al. 2004) and humans (Bogacka et al. 2005). Additionally, the overexpression of PGC-1α in human adipocytes results in a phenotypical shift, increasing expression of uncoupling protein 1 (UCP1), mitochondrial proteins, and increased fatty acid oxidation (Tiraby et al. 2003). This coordinated upregulation of mitochondrial proteins and pathways involved in fatty acid oxidation improved measures of metabolic function, such as mitochondrial copy number, insulin sensitivity, and circulating lipid concentrations (Bogacka et al. 2005; Laye et al. 2009; Sutherland et al. 2009; Wilson-Fritch et al. 2004). While no study has explored RSV’s action on PGC-1α in human adipose tissue, the pathways outlined in skeletal muscle suggest there may be a role for RSV to play in the induction of mitochondrial biogenesis in adipose tissue.

### 3.5 Resveratrol’s Mechanism of Action in Adipose Tissue

Although RSV’s mechanism of action has been relatively well studied in murine skeletal muscle, very little evidence of the signaling pathways involved in RSV’s action in adipose tissue
is available. The pathways characterized in skeletal muscle suggest the induction of mitochondrial biogenesis may also be possible in adipose tissue, although this has yet to be studied. The following section will outline possible mechanisms of RSV action in human adipose tissue using the limited evidence available from the literature.

3.5.1 Adipose Tissue Mitochondrial Biogenesis

While the evidence for RSV mediated mitochondrial biogenesis in adipose tissue is less extensive than its effect on lipolysis, exercise induced mitochondrial adaptations in adipose tissue suggest an exercise mimetic such as RSV may play a similar role (see Figure 3). The activation of AMPK by RSV appears to be necessary for RSV’s influence on skeletal muscle mitochondrial biogenesis (Um et al. 2010). The activation of AMPK by RSV has also been observed in mouse adipose tissue, inducing the upreguation of both SIRT1 and PGC-1α protein content, both of which are involved in the initiation of mitochondrial biogenesis (Um et al. 2010). In contrast to the animal results, no effect on adipose tissue SIRT1 or PGC-1α protein content or activation pathways implicated in mitochondrial biogenesis was observed following 12 weeks of RSV in non-obese women (Yoshino et al. 2012). Analogous to the results seen in skeletal muscle, these conflicting reports demonstrate our incomplete understanding of RSV’s action in human adipose tissue. While Yoshino et al. (2012) negative findings suggest there may not be a role for RSV in human adipose tissue mitochondria biogenesis, owing to the chronic nature of the trial, it is possible that the effect of RSV on adipose tissue is transient rather than adaptive in nature. However, without investigation of RSV’s acute effects on the proposed pathways, these conclusions remain speculative.
Figure 3. Proposed mechanism of resveratrol action in adipose tissue.
Schematic of the intracellular signaling responsible for RSV mediated mitochondrial biogenesis and lipolysis in adipose tissue. Black arrows represent activation, while red circles represent inhibition. Figure adapted from Park et al (2012), Price et al (2012), and Pedersen et al (2008).
3.6 Thesis Purpose

Given the evidence provided, it is clear that our current understanding of RSV’s metabolic impact in humans is incomplete. Clinical trials have failed to observe consistent outcomes on a number of metabolic parameters, most notably insulin sensitivity and fatty acid oxidation. Strong evidence of RSV mediated activation of SIRT1 in animals has led to the assumption that RSV’s mechanism of action is conserved in humans, yet the controversial evidence from long-term RSV supplementation studies suggests otherwise. The transition from animal studies directly to chronic human interventions has ignored an important step in the process: the evaluation of RSV’s acute effects in skeletal muscle and adipose tissue and its mechanism of action in humans. In understanding the mechanisms responsible for RSV’s reported effects, we will be able to assess its potential applications, since those alluded to in animal studies may not be feasible. Therefore, the purpose of this thesis was two-fold: 1) to determine if the mechanism(s) of RSV action, previously demonstrated in cellular and animal models, are conserved in human skeletal muscle and adipose tissue, and 2) to investigate the acute effects of RSV on insulin sensitivity and whole body fatty acid oxidation.

3.7 Thesis Hypotheses

Despite the difficulty in formulating a hypothesis considering the controversial literature surround RSV’s effect on human metabolic outcomes and intracellular signaling pathways, based on the existing literature characterizing RSV’s acute effect I hypothesized that 1) an acute dose of RSV will activate the same pathways responsible for mitochondrial biogenesis in human skeletal muscle and adipose tissue, and 2) owing to the conserved activation of intracellular pathways, RSV would acutely improve insulin sensitivity and increase whole body fatty acid oxidation.
3.8 References


A single dose of resveratrol acutely improves insulin sensitivity, but has no effect on whole body fat oxidation or SIRT1 activation in skeletal muscle and adipose tissue in overweight and obese men

4.1 Abstract

Purpose: The purpose of this study was to investigate the acute effect of a single dose of resveratrol (RSV) on insulin sensitivity and whole body fatty acid oxidation, and to determine RSV mechanism of action in human skeletal muscle and adipose tissue.

Methods: 8 sedentary males (Age: 23.8 ± 2.4 yrs; BMI: 32.7 ± 7.1; WC: 106.4 ± 21.2 cm; VO_{2pk}: 34.0 ± 7.3 ml/kg/min) reported to the lab on 2 occasions following a standardized dinner and 12 h fast and provided a meal challenge supplemented with 0.3g of RSV or a placebo. Experiments were performed in random order using a double blind crossover design. Gas exchange measures, blood samples, and a muscle biopsy were obtained in the fasted state and again, with the addition of an adipose tissue biopsy, 2 hours post-absorptive.

Results: Insulin sensitivity was significantly increased in the RSV condition post-absorptively compared to placebo. No change in post-absorptive whole body fatty acid oxidation was observed between conditions. In skeletal muscle, the decrease in post-absorptive phosphorylation of p38-MAPK observed following placebo (-27 ± 11%) was reversed with RSV (+36 ± 19%). Phospho-AKT(Ser473) increased post-absorptively following both placebo (+82 ± 22%) and RSV (+73 ± 21%) in skeletal muscle, but no significant interaction was observed. No difference in the phosphorylation of AMPK, ACC, and AKT(Thr308), or the acetylation of p53 was observed in skeletal muscle between RSV and placebo. No effect of RSV on the phosphorylation of p38
MAPK, AMPK, ACC, AKT(Ser473), and AKT(Thr308), or the acetylation of p53 was observed in adipose tissue.

**Conclusions:** A single dose of RSV can acutely improve insulin sensitivity in overweight and obese men. RSV mechanism of action is not conserved across species and its intracellular signaling pathway is different in humans than previously thought.

**Keywords:** SIRT1, obesity, supplement, mitochondrial biogenesis

### 4.2 Introduction

Obesity is strongly associated with the development of metabolic dysfunction and disease (Kopelman, 2000). Importantly, a significant portion of the metabolic dysfunction associated with obesity can be attributed to deficiencies in both mitochondrial content and function (Menshikova et al. 2005; Holloway, 2009). In skeletal muscle, mitochondrial genes are down regulated in obese individuals compared to lean controls (Mootha et al. 2003) and functional impairments of existing mitochondria further contribute to metabolic dysfunction (Ritov et al. 2005; Kelley et al. 2002). Declines in mitochondrial content and function with obesity and metabolic diseases are also observed in adipose tissue (Bogacka et al. 2005; Wilson-Fritch et al. 2004; Laye et al. 2009) with these decrements contributing to overall adipocyte dysfunction (i.e. impaired lipid storage, increased fatty acid release, overproduction of inflammatory cytokines) (Frigolet Vazquez-Vela et al. 2008). While lifestyle interventions, most notably exercise, effectively reverse obesity induced mitochondrial dysfunction (Menshikova et al. 2005; Holloszy, 2008; Holloway, 2009; Menshikova et al. 2007; Sutherland et al. 2009), poor adherence rates (Carey and Kingwell, 2009; Wing, 1999) and the continued rise in the incidence of obesity are indicative of the need for the investigation of novel treatment modalities. As such, pharmacological interventions are an attractive option, in particular the use of naturally occurring compounds proposed to exert positive metabolic benefits. Resveratrol (RSV), a plant derived polyphenol, has garnered interest
due to its purported effects on energetic homeostasis and metabolic health, warranting further investigation into its possible applications and clinical relevance.

Experimental trials in cellular and animal models suggest RSV mimics the beneficial effects commonly observed with a negative energy balance, traditionally achieved through caloric restriction or exercise (Barger et al. 2008; Lagouge et al. 2006; Pearson et al. 2008). In animal models, in addition to protection against diet induced obesity and improved metabolic health (Lagouge et al. 2006; Dal-Pan et al. 2010; Baur et al. 2006; Pearson et al. 2008), RSV’s influence translates into improved insulin sensitivity (Su et al. 2006; Baur et al. 2006; Kang et al. 2012; Andersen et al. 2011; Marchal et al. 2012). In contrast to animal studies, the available human trials involving RSV have reported varying and conflicting results with respect to RSV’s influence on metabolic outcomes. RSV’s insulin sensitizing effect has been observed in some (Brasnyo et al. 2011; Timmers et al. 2011; Crandall et al. 2012), but not all (Yoshino et al. 2012; Poulsen et al. 2013), chronic RSV supplementation trials. Whether the insulin sensitizing effects of RSV when observed in humans are due to RSV mediated chronic adaptations in metabolic function or an acute carryover effect from the final dose of RSV prior to testing is unclear, leading to the question, is RSV’s most useful function a chronic therapeutic aid or a valuable adjunct to acute endogenous glucose metabolism?

Improved fatty acid oxidation, often a measure of systemic metabolic health, observed in animal models (Lagouge et al. 2006) and some human trials (Timmers et al. 2011) suggests RSV may play a role in augmenting substrate selection, while other reports suggest RSV has no effect on whole body fatty acid oxidation following chronic RSV supplementation in humans (Yoshino et al. 2012; Poulsen et al. 2013). The intracellular signaling mechanism proposed to mediate RSV’s action suggests the activation of AMPK (Park et al. 2012; Price et al. 2012; Lagouge et al. 2006) could increase fatty acid flux into the mitochondria, enhancing fatty acid oxidation (Roepstorff et al. 2005; Itani et al. 2003). However, the acute activation of these pathways by
RSV has not been evaluated in humans, consequently RSV action on the pathways responsible for fatty acid oxidation and whole body substrate selection remains poorly defined.

The metabolic improvements observed with RSV in animal trials can, in part, be attributed to the activation of intracellular signaling pathways leading to the upregulation of mitochondrial and oxidative genes (Lagouge et al. 2006; Baur et al. 2006; Um et al. 2010). Specifically, RSV mediated increases in mitochondrial content and function appear to occur via the activation of Sirtuin 1 (SIRT1) (Howitz et al. 2003; Hubbard et al. 2013; Price et al. 2012; Park et al. 2012; Pearson et al. 2008; Lagouge et al. 2006; Baur et al. 2006), an NAD$^+$ dependent deacetylase responsible for the deacetylation and activation of a number of factors critical to both energy metabolism and mitochondrial content and function (Rodgers et al. 2005; Gerhart-Hines et al. 2007; Williams and Gurd, 2012). Although it has been established that RSV’s action requires SIRT1 in cellular and animal models, the exact mechanism responsible for RSV mediated SIRT1 activation is unclear. Specifically, there is controversy regarding whether RSV acts directly on SIRT1 (Hubbard et al. 2013; Price et al. 2012), or whether it targets upstream protein resulting in indirect SIRT1 activation (Park et al. 2012). Regardless of the exact mechanism, it is accepted that the activation of SIRT1, by whichever means, is implicit in RSV’s effect on metabolic health in cellular and animal models.

While cellular and animal studies demonstrate a clear link between RSV mediated activation of SIRT1 and associated metabolic benefits, RSV’s mechanism of action in human skeletal muscle and adipose tissue has not been confirmed. At present, no study has examined acute changes in signaling induced by RSV in human skeletal muscle and adipose tissue, and information that can be gleaned from chronic interventions is limited due to inconsistent results. Specifically, while increases in SIRT1 skeletal muscle protein content, increased citrate synthase activity, and increased expression of a number of genes associated with oxidative phosphorylation were observed following 30 days of RSV supplementation (Timmers et al.
2011), other studies observed no change in the activation status of intermediates in the proposed RSV-SIRT1 pathway or a number of markers of mitochondrial biogenesis in skeletal muscle or adipose tissue (Poulsen et al. 2013; Yoshino et al. 2012). Although these chronic observations are not indicative of acute intracellular signaling, these controversial results from human trials suggest that the mechanism of RSV action proposed, based on cellular and animal work, may not translate to human tissue. Consequently there is a need for studies examining the acute effect of RSV on both systemic metabolic function and the activation of intracellular signaling pathways characterized in cellular and animal models in human tissue.

The purpose of this study is two-fold: 1) to determine if the mechanism(s) of RSV action, previously described in cellular and animal models, are conserved in human skeletal muscle, and 2) determine the acute effects of a single dose of RSV on insulin sensitivity and whole body fatty acid oxidation. We hypothesized that: 1) an acute dose of RSV will activate the same pathways responsible for mitochondrial biogenesis in human skeletal muscle and adipose tissue, and 2) owing to the conserved activation of intracellular pathways, RSV will acutely improve insulin sensitivity and increase whole body fatty acid oxidation.

4.3 Methods

4.3.1 Participants

Eight overweight/obese, sedentary males volunteered to participate in this study (participant characteristics are presented in Table 1). All participants reported less than 1 hour per week of structured exercise at enrollment (aerobic training, resistance training, etc.) and were overweight (waist circumference of greater than 94 cm) (Lean et al. 1995). All participants were instructed to maintain exercise and nutritional habits as best they could, but to avoid the use of nutritional supplements and foods high in naturally occurring resveratrol (grapes, some teas, and peanuts) for the duration of the study. Additionally participants were instructed to avoid exercise,
caffeine, drugs, and alcohol for a minimum of 24 h prior to each visit. The study was approved by the Health Sciences Human Research Ethics Board at Queen’s University (Appendix C), and verbal and written explanation of the experimental protocol and associated risks was provided to all participants prior to obtaining written informed consent (Appendix A and B).

4.3.2 Experimental Protocol

The experimental protocol consisted of (i) baseline testing, and (ii) a RSV and placebo supplement intervention assessing the impact of a RSV supplement and meal on a number of physiological parameters. The supplements were administered using a randomized cross over, double blind design, with each condition separated by a 7 day wash out period.

4.3.3 Baseline Testing

Participants reported for the first laboratory visit a minimum of 72 h prior to any intervention day. During this visit anthropometric measures were obtained and VO_{2}peak was assessed on a friction-braked cycle ergometer (Monark, Ergomedic 874E, Vansbro, Sweden) using an incremental ramp protocol. The VO_{2}peak ramp protocol consisted of a 5 min loadless warm-up followed by a step increase to 80 W for 1 min and subsequent increases in work rate of 24 W·min^{-1} to volitional exhaustion (determined by the inability of the participant to maintain a cadence of 60 RPM). Gas exchange was measured continuously using a metabolic cart (Moxus AEI Technologies, Pittsburgh, PA). Relative VO_{2}peak, absolute VO_{2}peak, and peak hear rate were calculated as the average of their respective values measured in the final 30 sec of the protocol.

4.3.4 Supplement Intervention Protocol

Participants reported to the laboratory on two subsequent occasions to participate in a placebo supplement intervention and a RSV supplement intervention in random order. On the
evening prior to each experimental visit, participants were provided a standardized dinner which included a Stouffer Sauté Sensation beef pot roast (380 kcal; 22 g carbohydrate (CHO), 5 g fat, 14 g protein), 500 mL of 2% milk (260 kcal; 12 g CHO, 5 g fat, 9 g protein) and a dole apple cinnamon fruit cup (160 kcal; 30 g CHO, 3.5 g fat, 2 g protein) before reporting to the lab having fasted overnight (≥ 12 h). Participants were asked to drive or walk slowly to the lab, and take the elevator in order to limit their physical activity. Upon arrival at the lab, participants rested for 10 minutes after which gas exchange measures were collected (Moxus AEI Technologies, Pittsburgh, PA) for 15 minutes while participants rested in seated position. Immediately following the gas exchange collection, a venous blood sample and a muscle biopsy were obtained while participants rested in the supine position (for details please see Physiological Measurements). Subsequently, participants were given a standardized breakfast of a plain bagel (190 kcal; 36 g CHO, 1 g fat, 7 g protein), 15 g of cream cheese (45 kcal; 1 g CHO, 4 g fat, 1 g protein), and 200 mL of orange juice (100 kcal; 23 g CHO, 0 g fat, 1 g protein), which they were given 10 min to eat. This meal was immediately followed by the ingestion of unlabeled identical capsules containing either 0.3 g of RSV supplement (resVida, DSM, Heerlen, Netherlands) or microcrystalline cellulose placebo supplement (PCCA Canada, London, ON) with 200 mL of water. RSV was capsulated in 0.15g capsules, thus an absolute dose was provided to each participant, as small alterations were not possible. Following ingestion of the supplement, participants were bed rested for 105 min and provided water ad libitum, at which time post-absorptive gas exchange values were recorded for 15 min. 120 min following the ingestion of the supplement, a post-absorptive blood sample, muscle biopsy, and adipose tissue biopsy were obtained in the same order as the fasted samples described above (See Figure 4 for experimental visit timing). The supplement order was randomized and given in a double blind fashion on consecutive visits, a minimum of 7 days apart.
4.3.5 Physiological Measurements

Resting blood samples were collected by venipuncture from an antecubital vein in sterile tubes (BD Vacutainer, Franklin Lakes, NJ) coated with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Plasma was separated by centrifugation at 3500 RPM for 10 minutes at 4°C. Samples were stored at -80°C until analysis. Muscle biopsy samples were obtained using the Bergstrom needle biopsy technique (Bergstrom, 1975) with the addition of manual suction from the vastus lateralis following local anaesthetization (2% lidocaine). Both muscle biopsies on each experimental visit were obtained from the same leg from separate incisions approximately 2-3 cm apart. Adipose tissue biopsies were also obtained using a Bergstrom needle with the addition of manual suction following local anaesthetization (2% lidocaine). Adipose tissue biopsies were obtained from the abdominal region with the incision made approximately 5 cm lateral to the umbilicus and the needle inserted laterally through this incision. Both muscle and adipose tissue were immediately blotted, snap-frozen in liquid nitrogen, and stored at -80°C until analysis.

4.3.6 Fasting Glucose, Insulin, HOMA-IR and Estimates of Substrate Utilization

Fasted blood glucose was determined via a hexokinase reaction assay performed at Kingston General Hospital (Kingston, Ontario). Fasted insulin levels were determined with a commercially available enzyme-linked immunoabsorbent assay (ELISA) kit (Alpco Diagnostics,
Salem, NH). All samples were run in duplicate with the CV being <10% for all values. Insulin sensitivity was estimated using homeostatic model assessment – insulin resistance (HOMA-IR) with the equation

\[
\text{HOMA-IR} = \frac{\text{fasting insulin (µIU/mL) x fasting blood glucose (mmol/L)}}{22.5}
\]

(Matthews et al. 1985)

While the use of the homeostatic model was originally developed for the assessment of insulin sensitivity in the fasted state, it has since been used as an index of insulin sensitivity in a number of non-steady state conditions, including the post-absorptive state following a meal challenge (Crandall et al. 2012) and immediately following exercise (Jamurtas et al. 2006; Magkos et al. 2008).

Respiratory exchange ratio (RER) was determined from recorded gas exchange values at fasted and post-absorptive time points. A 10 min average was taken from 3 to 13 min of recorded gas exchange values to minimize the impact of any adjustment period following putting on the mask and/or participant anticipation of the mask being removed. RER was used to estimate relative substrate contribution to total substrate oxidation assuming a linear relationship between an RER of 0.7 (100% fat, 0% CHO), and 1.0 (0% fat, 100% CHO) at rest. Specifically, the fat derived component for energy expenditure at each time point was calculated according to the following formula (Holloway et al. 2006): fat (g·min⁻¹) = 1.695 · O₂ production (l·min⁻¹) – 1.701 · CO₂ production (l·min⁻¹). To convert fatty acid oxidation rates to kcal·min⁻¹ resulting values were multiplied by 9 kcal·g⁻¹.

4.3.7 Western Blot Analysis

30-50 mg pieces of frozen skeletal muscle were homogenized in 2 mL of lysis buffer (210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 30 mM Heps, 20 mM EDTA) while 40-50 mg of pieces of frozen adipose tissue were homogenized in 275 µL of lysis buffer. Both types of tissue
were added to tubes filled with the appropriate amount of pre-chilled (4°C) lysis buffer. Samples were subsequently homogenized for 3 seconds at 20 000 RPM (muscle) or 15 000 RPM (adipose) (Polytron PT10/35 GT Benchtop Homogenizer, Kinematic, Luzern, Switzerland).

Protein concentrations were determined for all homogenates using a commercially available protein assay kit (Pierce, Rockford, IL). Samples were diluted to equivalent concentrations with a mixture of 4x Laemmli sample buffer and H2O and then denatured by heating to 95°C for 5 min. Proteins were separated by SDS-PAGE using 8% (phospho-ACC, ACC), 10% (phospho-AMPK, AMPKα, phosphor-AKT Ser473 and Thr308, pan AKT, acetyl-p53, GAPDH), and 15% (phospho-p38 MAPK, p38 MAPK) polyacrylamide gels and were subsequently transferred to a polyvinylidene difluoride membrane. For the detection of proteins, commercially available antibodies were used for GAPDH (Millipore, Billerica, MA) and phospho-p38 MAPK Thr180/Tyr182, p38-MAPK, phospho-AMPKα Thr172, AMPKα, phospho-ACC Ser79, ACC, acetyl-p53 Lys379, phospho-AKT Ser473, phospho-AKT Thr308, and pan AKT (Cell Signaling Technologies, Danvers, MA). Membranes were blocked with 5% BSA in TBS-T (0.1%) and immunoblotted with primary antibody. Proteins were visualized by chemiluminescence detection according to the manufacturer’s instructions (Millipore, Billerica, MA). Blots were imaged using the FluorChem Cell Biosciences imaging system (ProteinSimple, Santa Clara, CA) and quantified using AlphaView technology (ProteinSimple, Santa Clara, CA). Equal protein loading for all western blots were confirmed using ponceau staining for total protein. Equal protein loading for all skeletal muscle western blots was additionally confirmed using GAPDH as a loading control.

4.3.8 Statistical Analysis

A two-way, repeated measures analysis of variance (ANOVA) was used to compare the effect of condition (RSV and placebo) and time (fasted and post-absorptive) for blood measures,
gas exchange analysis, and all muscle biopsy derived data. A Bonferroni correction was used for post hoc pairwise comparison of means for main effects and significant interactions. As adipose tissue biopsies were obtained at a single time point, paired t-tests were used to compare the effect of placebo or RSV on protein content and phosphorylation or acetylation status. Data analysis was completed with GraphPad Prism v 5.01 (GraphPad Software Inc. La Jolla, CA). Statistical significance was accepted at p < 0.05.
Table 1. Subject characteristic summary.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>23.8 ± 2.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>182.5 ± 10.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>111.3 ± 37.5</td>
</tr>
<tr>
<td>BMI</td>
<td>32.7 ± 7.1</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>106.4 ± 21.2</td>
</tr>
<tr>
<td>VO$_2$ peak (ml·min$^{-1}$·kg$^{-1}$)</td>
<td>34.0 ± 7.3</td>
</tr>
</tbody>
</table>

N=8. Values are mean ± SD. yrs, years; cm, centimeters; kg, kilograms; BMI, body mass index.
4.4 Results

4.4.1 HOMA-IR, Insulin and Glucose

A significant interaction effect (p=0.02) was observed for HOMA-IR. Post hoc analysis demonstrated a significant increase post-absorptive from fasted with both placebo (1.7 ± 0.7 to 6.4 ± 1.3; p<0.01) and RSV (2.0 ± 1.0 to 4.3 ± 0.7; p<0.05; Figure 5A). A significant interaction effect (p<0.05) was observed for plasma insulin concentration. Post hoc analysis demonstrated a significant increase (p<0.05) post-absorptive from fasted with both placebo (7.2 ± 3.5 µIU/L to 27.6 ± 5.7 µIU/L) and RSV (7.8 ± 3.3 µIU/L to 19.0 ± 2.5 µIU/L; Figure 5B). There was no effect of time (fasted vs. post-absorptive) or condition (placebo vs. RSV) on plasma glucose concentrations (Figure 5C).

4.4.2 Fatty Acid Oxidation

Respiratory exchange ratio (RER) was unchanged post-absorptive in both placebo and RSV (Figure 6A). Estimated rates of fatty acid oxidation increased in a time dependent fashion approaching significance (main effect of time, p=0.08) from 0.87 ± 0.15 to 1.04 ± 0.22 kcal/min and 0.97 ± 0.19 to 1.11 ± 0.18 kcal/min with placebo and RSV respectively (Figure 6B). Post hoc analyses revealed no statistical difference from fasted with either placebo or RSV and no interaction effect was observed (p=0.90). Mean VO\textsubscript{2} increased in a time dependent fashion (p<0.01). Post hoc analysis revealed a significant (p<0.05) increase post-absorptive from fasted (306 ± 38 ml/min to 373 ± 39 ml/min) with placebo and no significant increase post-absorptive from fasted (340 ± 34 ml/min to 387 ± 44 ml/min) with RSV. Mean VCO\textsubscript{2} also increased in a time dependent fashion (p<0.01). Post hoc analysis revealed a significant (p<0.05) increase post-absorptive from fasted (248 ± 30 ml/min to 304 ± 30 ml/min) with placebo and no significant increase post-absorptive from fasted (274 ± 26 ml/min to 312 ± 35 ml/min) with RSV.
4.4.3 Skeletal Muscle Signaling

A significant effect of RSV was observed for phospho-p38 MAPK (interaction, p<0.05) with the decrease in phosphorylation observed following placebo (-27 ± 11%) reversed with RSV (+36 ± 19%) (Figure 7A). Post hoc analyses demonstrated no statistical difference from fasted in either condition. Phospho-AKT (Ser473) increased in a time dependent fashion (p<0.01) following both RSV (+73 ± 21%) and placebo (+82 ± 22%), but no significant interaction was observed (p=0.79) (Figure 7A). Post hoc analysis demonstrated a significant increase (p<0.01) from fasted with both placebo and RSV. There were no significant changes with time (fasted vs. post-absorptive) or condition (placebo vs. RSV) in the phosphorylation of AMPKα, ACC, and AKT (Thr308) or the acetylation status of p53 (Figure 7A).

4.4.4 Adipose Tissue Signaling

There was no effect of RSV on the phosphorylation status of p38 MAPK, AMPKα, ACC, AKT (Ser473), and AKT (Thr308), or the acetylation status of p53 in adipose tissue (Figure 8A) compared to placebo. Due to the limited size of the adipose tissue samples and low protein yield, western blot analysis of adipose tissue protein was limited to the above-mentioned proteins, and no further analysis was feasible.
Figure 5. Impact of an acute dose of resveratrol (RSV) on 2 hour insulin sensitivity.
HOMA-IR (A) was used as an index of insulin sensitivity while participants were fasted (open bars) or 2 hours post-absorptive (shaded bars). Plasma insulin (B) and glucose concentration (C) are also shown.
* Significantly different (p<0.05) from fasted state within same condition.
† Significant interaction (p<0.05) between RSV and placebo
Figure 6. Whole body fat oxidation is unchanged by resveratrol (RSV).
Resting respiratory exchange ratio (RER) (A) and fatty acid oxidation rate (B) was measured while participants were fasted (open bars) and 2 hours post-absorptive (shaded bars). No statistical differences were apparent.
Figure 7. Resveratrol alters p38 MAPK phosphorylation but does not alter other intramuscular signaling pathways.

The change in phosphorylated protein content (A) in the post-absorptive (post-ab) state (expressed relative to fasting) in the placebo (PL; open bars) and resveratrol (RSV; shaded bars) conditions. Representative blots for all protein (both phosphorylated and total) are also shown (B).

* Significantly different (p<0.05) from fasting.
† Significant interaction (p<0.05) between RSV and placebo response.
Figure 8. Adipose tissue signaling is unaltered by resveratrol.
Phosphorylated protein content (A) in the post-absorptive (post-ab) state following resveratrol (RSV) expressed relative to placebo (PL). Representative blots for all protein (both phosphorylated and total) are also shown (B)
4.5 Discussion

The present study examined the effect of a single dose of RSV (0.3g) on acute insulin sensitivity, whole body fatty acid oxidation, and intracellular pathways involved in both insulin signaling and the induction of mitochondrial biogenesis in skeletal muscle and adipose tissue in overweight/obese males. Our results demonstrate that a single dose of RSV: 1) lowers HOMA-IR, primarily due to reduced plasma insulin two hours after a meal, potentially via p38 MAPK mediated sensitization of skeletal muscle insulin signaling and glucose uptake; 2) has no effect on whole body fatty acid oxidation; and 3) does not appear to influence the activation status of proteins (AMPK, p53) implicated in the current understanding of RSV mediated activation of SIRT1, suggesting that neither SIRT1, nor the signaling pathways involved in the initiation of mitochondrial biogenesis were activated in either skeletal muscle or adipose tissue.

4.5.1 Resveratrol and Insulin Sensitivity

The most interesting, and potentially clinically relevant, result of the present study is the observation that an acute dose of RSV improved HOMA-IR two hours post meal challenge (Figure 5A). This effect, attributable to the combined result of lowered plasma insulin and unchanged plasma glucose concentrations suggests that RSV acutely improves late phase insulin sensitivity. Further, the similar post-absorptive phosphorylation of AKT, a critical intermediate in the insulin-signaling pathway (Krook et al. 1998), observed in both skeletal muscle (Figure 7) and adipose tissue (Figure 8), despite lower plasma insulin concentration with RSV, suggests that RSV may improve insulin sensitivity and glucose clearance in peripheral tissue. Based on the current results, the exact mechanism by which RSV enhances sensitivity of the insulin signaling
pathway is not clear, thus further analysis of intermediates upstream and downstream of AKT may help elucidate this mechanism. This is the first study to demonstrate improved late phase insulin sensitivity following a single dose of RSV in young overweight and obese men. Previous studies using longer durations of RSV supplementation have demonstrated insulin-sensitizing effects. For instance, insulin sensitivity was improved in older type 2 diabetic males (Brasnyo et al. 2011), obese males (Timmers et al. 2011), and in older men and women (Crandall et al. 2012) following 4-12 weeks of RSV supplementation. In contrast, no improvements in insulin sensitivity were observed in obese men (Poulsen et al. 2013) or in non-obese women (Yoshino et al. 2012) following RSV supplementation of 4 and 12 weeks respectively. While the conflicting results are difficult to explain, the mechanisms responsible for a chronic improvement in insulin sensitivity would not account for the observed acute insulin sensitizing effect of RSV. The insulin sensitizing effect of a chronic intervention is proposed to results from adaptation, while an acute response is likely attributable to transient changes in intracellular enzyme activation and signaling. Moreover, the current findings suggest that the positive results from chronic studies may be attributable to the acute carryover effect of the final RSV dose rather than a result of metabolic/mitochondrial remodeling. These findings suggest a potential novel clinical outcome, indicating that RSV may serve to acutely boost endogenous insulin action, possibly in addition to therapeutic/preventative benefit via the induction of sustained adaptations.

Accompanying enhanced late phase insulin sensitivity, we have also observed an increased phosphorylation of p38 MAPK in skeletal muscle with RSV (Figure 7). RSV’s effect on p38 MAPK activation may be explained by its proposed activation of the calcium/calmodulin pathway (Park et al. 2012), resulting in the activation of CamKII and its downstream effector, p38 MAPK (Wright et al. 2007). Both cellular (Sweeney et al. 1999) and animal (Somwar et al. 1999)
models have demonstrated improved insulin sensitivity following activation of p38 MAPK via phosphorylation. Further, the pharmacological inhibition of p38 MAPK decreases glucose transport by 50-70% (Sweeney et al. 1999), independent of GLUT4 translocation to the plasma membrane (Sweeney et al. 1999) or activation of upstream insulin signaling targets, such as AKT (Somwar et al. 2000). These results suggest that p38 MAPK may enhance GLUT4 catalytic activity through either the removal of an allosteric inhibitor or the dissociation of GLUT4 from an inhibitory complex (Furtado et al. 2002).

Although we are unable to confirm GLUT4 translocation or activation in the current study, our results agree with the proposed mechanism that upstream signaling (phosphorylation of AKT) implicated in GLUT4 translocation was unchanged from placebo with RSV; however, phosphorylation of p38 MAPK was increased as was late phase insulin sensitivity following the meal challenge. The activation of p38 MAPK with RSV was not apparent in adipose tissue, which suggests that skeletal muscle is a more likely site for acute improvements in insulin sensitivity following a dose of RSV. Alternatively, the similar activation of AKT with RSV, despite lowered insulin concentrations indicates a possible acute sensitization of the insulin-signaling pathway upstream of AKT in both skeletal muscle and adipose tissue. This result may be indicative of novel RSV targets in the insulin-signaling cascade and is worthy of further research.

4.5.2 Whole Body Fatty Acid Oxidation

In contrast to our hypothesis, the results from the current study demonstrate no effect of RSV on whole body fatty acid oxidation. Our results agree with previous reports observing no change in basal fatty acid oxidation or fat mass (Yoshino et al. 2012), no change in respiratory...
quotient (RQ) or rates of fatty acid oxidation (Poulsen et al. 2013), and a slight, albeit non-significant, increase in RQ and decrease in the rate fatty acid oxidation (Timmers et al. 2011) with chronic RSV treatment. In support of the unchanged contribution of fatty acid to total substrate oxidation, the activation of AMPK and its downstream target ACC, important regulators of fatty acid flux into the mitochondria for oxidation (Itani et al. 2003; Hue and Taegtmeyer, 2009), were unaffected by RSV in both skeletal muscle and adipose tissue. The activation of AMPK and its inhibition of ACC are critical steps in the inhibition of malonyl-CoA and ensuing increase in fatty acid flux into the mitochondria through carnitine palmitoyltransferase 1 (CPT-1) (Roepstorff et al. 2005) for oxidation. The lack of AMPK activation and ACC inhibition suggest RSV had no effect on substrate selection through this signaling pathway in skeletal muscle and adipose tissue under the conditions imposed in the current study.

4.5.3 Resveratrol and SIRT1 activation in skeletal muscle

In both muscle cells, and in rodent skeletal muscle, RSV exposure induces the activation of SIRT1, which translates into positive effects on mitochondrial biogenesis through deacetylation of SIRT1 target proteins (Lagouge et al. 2006; Baur et al. 2006; Park et al. 2012; Price et al. 2012; Menzies et al. 2013; Hubbard et al. 2013; Rodgers et al. 2005). Based on these results, several models have been proposed regarding RSV’s mechanism of action in skeletal muscle. Specifically, both indirect activation of SIRT1 by RSV, through cAMP dependent activation of AMPK and increased availability of SIRT1 substrate (Canto et al. 2009; Park et al. 2012), and direct activation of SIRT1 by RSV, consequently activating AMPK via LKB1 (Price et al. 2012; Hubbard et al. 2013), have been proposed. While the position of AMPK, either upstream (Park et al. 2012) or downstream (Price et al. 2012) of SIRT1 is unclear, its activation
remains an essential component of the any proposed RSV-SIRT1 pathway. In the current study, RSV had no effect on the acetylation status of p53 (Figure 7), a known SIRT1 target (Langley et al. 2002), suggesting that SIRT1 activity was not altered. In addition, as discussed above, neither the phosphorylation of AMPK, nor its activity (unchanged phosphorylation of AMPK target ACC) were elevated following in the RSV condition. Combined, the lack of change in both p53 and AMPK are inconsistent with SIRT1 being activated. Thus, in contrast to evidence from cellular and animal models, the current findings suggest a single 0.3 g dose of RSV does not activate SIRT1 in human skeletal muscle.

While our results do conflict with the majority of the data from cells and animals, they may help explain the relatively small and/or non-existent changes in mitochondrial content following chronic RSV supplementation in human trials. Poulsen et al. (2013) demonstrated no effect of 4 weeks of RSV on the phosphorylation of AMPK, or a number of markers of SIRT1 activity. Further Yoshino et al. (2012) observed no effect of 12 weeks of RSV on skeletal muscle SIRT1 protein content or evidence of SIRT1 activation, no effect on the phosphorylation of AMPK, and no activation of a number of biological pathways involved in mitochondrial biogenesis. While Timmers et al. (2011) did report an increase in AMPK phosphorylation, increased protein content of both SIRT1 and its downstream targets in skeletal muscle, and increased citrate synthase activity; there was no change in the expression of oxidative phosphorylation proteins, or PCr recovery, and only modest improvements in skeletal muscle fatty acid oxidation after 30 days of RSV supplementation.

The acute response presented here highlights the difficulty associated with beginning chronic interventions prior to confirming the acute mechanisms characterized in animal models. Our results suggest that RSV’s mechanism of action in humans differs from that characterized in
animals, and may help explain the controversial results derived from chronic RSV interventions. Future research should work to further elucidate RSV’s mechanism of action in human tissue, to better predict long term outcomes and maximize potential benefits to be gained from appropriate RSV applications.

4.5.4 Cellular signaling in Adipose Tissue

The results from the current study demonstrate no effect of RSV on the phosphorylation of AMPK, ACC, or the acetylation of p53 in adipose tissue (Figure 8). These results suggest that signaling associated with mitochondrial biogenesis is not activated with an acute dose of RSV in adipose tissue. Previous reports have shown no change in SIRT1 protein content, or marker of SIRT1 activity in adipose tissue following 12 weeks of RSV (Yoshino et al. 2012). Human trials involving RSV and adipose tissue are very limited, thus the bulk of our hypothesis stemmed from animal and cellular models, which contradict the current findings. Um et al. (2010) observed an increased phosphorylation of AMPK in white adipose tissue in mice following 3 months of RSV and postulated that this increased AMPK phosphorylation was required for RSV induced mitochondrial biogenesis. Additionally, Park et al. (2012) observed an increased concentration of cAMP with RSV, similar to that observed in skeletal muscle, indicative of RSV induced activation of pathways responsible for mitochondrial biogenesis in adipose tissue. While our conclusions are tempered due to the limited literature, we hypothesized that RSV’s mechanism of action in adipose tissue would mimic that observed in skeletal muscle (Park et al. 2012; Price et al. 2012; Hubbard et al. 2013; Um et al. 2010). Therefore, owing to similar logic presented in the skeletal muscle section, the current results contradict our hypothesis and suggest that a single 0.3g
dose of RSV does not activate SIRT1 and thus pathways responsible for mitochondrial biogenesis in human adipose tissue.

4.6 Limitations

Some limitations of the present work are apparent and worthy of discussion. First, the decision to provide an absolute dose of RSV, as opposed to a relative per kilogram dose, was largely dictated by the need to capsule RSV for ingestion. Due to the nature of the capsules, small individual alterations in RSV concentrations were not possible. A dose of 0.3g was chosen due to the positive reports from Timmers et al. (2011) using a dose of 0.15g. We hoped to significantly increase the dose above what the previous study had used in the hopes of eliminating the possibility of RSV bioavailability being a limiting factor. While there may have been slight differences in the relative concentrations given to our participants, the use of an absolute dose is common in human RSV trials (Crandall et al. 2011; Brasnyo et al. 2012; Poulsen et al. 2013; Timmers et al. 2011; Yoshino et al. 2012), although future studies could benefit from providing a relative dose to limit the possibility of these individual RSV bioavailability differences. Second, our results suggest RSV does not activate SIRT1 in skeletal muscle and adipose tissue due to the absence of activation of known SIRT1 targets and modulators, yet we have not directly measured SIRT1 intrinsic activity. The availability of a SIRT1 activity assay suggests this could be resolved, but the accuracy and validity of commercially available SIRT1 activity assay kits has been questioned in the past (Gurd et al. 2012), therefore its use may not be any more reliable than our current measures. Third, insulin sensitivity could have been better assessed using more traditionally established techniques such as a 2 h oral glucose tolerance test, or the gold standard, a hyperinsulinemic euglycemic clamp. The use of the latter, while resource intensive, would have
also allowed for the infusion of primed tracer substrates, enabling us to precisely measure whole body glucose and fatty acid oxidation, as opposed to estimating rates of substrate oxidation using formulae based upon gas exchange measurements. Fourth, owing to the discomfort of the significant number of biopsies being asked of our participants, we opted to perform only a post-absorptive adipose tissue biopsy, consequently we are unable to compare fasted to post-absorptive results. Further, due to the limited amount of tissue obtained from adipose biopsies, western blot analysis of proteins was restricted. Fifth and last, while our results suggest a single dose of RSV does not activate SIRT1 and its associated pathways responsible for mitochondrial biogenesis, due to the acute nature of the trial, we are unable to comment on the possibility of an additive effect of RSV doses, although the lack of consistent reports of mitochondrial biogenesis in human trials suggests this is not a significant concern.

4.7 Summary

The current study examined several metabolic outcomes and intracellular signaling pathways hypothesized to be affected by RSV in humans. Our results suggest that RSV can acutely improve late phase insulin sensitivity, yet has no effect on whole body fatty acid oxidation. The activation of p38 MAPK in skeletal muscle may provide an explanation for the acute insulin sensitizing effects of RSV, owing to p38 MAPK’s reported effect on GLUT4 catalytic activity. RSV had no effect on the activation of AMPK or ACC in skeletal muscle or adipose tissue, which may explain the unchanged contribution of fatty acid to total substrate oxidation, as AMPK and ACC play pivotal roles in the flux of fatty acid into the mitochondria for oxidation. Finally, RSV had no effect on activation of p53 in skeletal muscle or adipose tissue, which, combined with the unchanged activation of AMPK, suggests an acute dose of RSV did not
activate SIRT1 or its associated down stream pathways responsible for mitochondrial biogenesis in skeletal muscle and adipose tissue. Limitations associated with measuring SIRT1 activity and analysis limitations due to sampling techniques require future research be done to further elucidate the mechanism of RSV action on insulin sensitivity and SIRT1 activation in human skeletal muscle and adipose tissue.

4.8 References


Chapter 5

General Discussion

5.1 Summary of Key Findings

Our results suggest that a single dose of RSV can acutely improve late phase insulin sensitivity in humans, and has no effect on whole body fatty acid oxidation. The acute insulin sensitizing effect of RSV may be attributed to the observed increased activation of p38 MAPK in skeletal muscle (Furtado et al. 2002) and/or the activation of protein(s) upstream of AKT in the insulin-signaling cascade. RSV had no effect on AMPK activation in skeletal muscle or adipose tissue, which may explain RSV’s inability to acutely influence substrate selection, owing to the role of AMPK activation in the control of fatty acid oxidation (Hue and Taegtmeyer, 2009).

Lastly, while cellular and animal models have consistently demonstrated RSV mediated SIRT1 activation, the current results are not consistent with SIRT1 activation in human skeletal muscle and adipose tissue. RSV treatment had no effect on the activation of p53, a known SIRT1 target, or AMPK, a critical component of proposed RSV-SIRT1 pathway in cells and animals (Park et al. 2012; Price et al. 2012). This suggests the proposed pathways of RSV mediated SIRT1 activation are not conserved across species, and an acute dose of RSV does not activate SIRT1 and it’s associated downstream effectors in human skeletal muscle and adipose tissue.

5.2 Study Strengths

To our knowledge, this is the first study to investigate the acute effects of RSV on insulin sensitivity and fatty acid oxidation in humans. The impressive results from animal studies appear
to have inspired the desire to begin chronic dosage clinical trials, overlooking the importance of confirming the acute mechanism responsible for RSV effects in human tissue. Our study has taken a step back and is the first to evaluate whether the pathways of RSV action characterized in cellular and animal models are conserved in acute \textit{in vivo} human trials. Our results suggest these pathways are not conserved across species and provide justification for a new avenue of research aimed at uncovering RSV’s true mechanism in humans. Lastly, the use of a crossover design with a wash out period strengthens our observations, as we were able to compare individuals to their own control, therefore better compensating for individual variability.

5.3 Study Implications

The current study has demonstrated a single dose of RSV can acutely enhance late phase insulin sensitivity in overweight and obese males. Importantly, the participants in the current study were metabolically healthy at the time of the intervention; however, due to their classification as overweight and obese, they are at an elevated risk for the development of metabolic disease and dysfunction (Kahn et al. 2006). As outlined in Chapter 3, reports of RSV’s chronic effect on insulin sensitivity in humans are inconsistent, suggesting RSV may not induce adaptations responsible for improved insulin sensitivity, such as mitochondrial biogenesis. Moreover, the current study suggests this may not be RSV most relevant application. As an acute insulin sensitizer, RSV could potentially be used as a supplement to provide a proactive boost to endogenous insulin action, acting in a preventative manner before the manifestation of insulin insensitivity, as opposed to a treatment once an individual has become insulin insensitive. This becomes especially relevant in young overweight and obese populations, as these individuals may not yet be insulin insensitive, but they have a significantly elevated risk for the development of
insulin sensitivity dysfunction (Kopelman et al. 2006; Weiss et al. 2004). RSV may provide a protective effect and prevent or slow the development of metabolic dysfunction and insulin insensitivity by helping regulate glucose metabolism (Kahn et al. 2006). Therefore, a RSV supplement aimed at acutely increasing insulin sensitivity, such as following a meal, could hold clinical value and is worthy of future investigation.

5.4 Study Limitations

As mentioned previously, some limitations to the current work are apparent and worthy of discussion. Section 4.5 Limitations in Chapter 4 outlines the specific methodological limitations of the current study. This paragraph will focus on broader limitations of the work. First, while the use of human subjects is critical for understanding in vivo mechanisms, a number of limitations are imposed due to ethical and technical considerations. The timing of the biopsies was based on previous reports of RSV’s bioavailability and half-life in humans. Peak plasma RSV concentrations occur approximately 120 minutes following ingestion (Smoliga et al. 2011; Timmers et al. 2011; Walle et al. 2004), thus we took the post-absorptive biopsy at 120 minutes based on the hypothesis that at this time the concentration of RSV and its activation of intracellular pathways would be highest. It is possible that our hypothesis was incorrect; in which case we may have missed RSV’s mediated signaling events. Although not possible in the current study due to the discomfort and risk to the participant, taking continuous skeletal muscle and adipose tissue biopsies (every 10 minutes) over those 120 minutes would have allowed us to present a time course of intracellular signaling, potentially revealing novel RSV action in human tissue. Second, while techniques exist to quantify RSV’s concentration in plasma and urine (Boocock et al. 2007), we did not have the expertise or access to HPLC to perform this analysis.
Additionally, the presence of RSV in the plasma does not necessarily reflect tissue specific bioavailability, and to our knowledge the established techniques have not been used to quantify RSV uptake into skeletal muscle and adipose tissue. Third, while we were limited to an oral RSV supplement due to the established safety and tolerability guidelines in humans, a significantly higher dose of RSV is responsible for the significant improvements to metabolic health observed in animals. Previous clinical trials suggest that a dose above 1g/day of RSV increases the incidence of adverse events in humans quite drastically. The 0.3g dose of RSV given in the current study is equivalent to approximately 2.7mg/kg of body weight (based on mean participant weight). In animal studies, the best results were observed with 400mg/kg/day, or nearly 150x the concentration of our dose. This could explain the lack of intracellular signaling in humans; we simply are not giving enough RSV to activate the proposed pathways.

5.5 Future Research

Based on the study limitations discussed above, several areas would benefit from future research. First, the current study suggests RSV does not activate SIRT1 in human skeletal muscle and adipose tissue, although no insight into possible alternative mechanisms can be provided. Acute RSV trials should aim to clarify the actual signaling pathways involved in both insulin sensitivity and mitochondrial biogenesis, perhaps by exploring a wider number of proteins in the hopes of identifying novel RSV targets that may be implicated in its acute action in humans. Second, while there are some ethical and technical limitations to be addressed, providing a dose of RSV akin to that used in animals would be a very interesting study. Due to gastrointestinal considerations, the direct administration of a RSV solution into the arterial system may enable a significantly more concentrated dose without the associated adverse events. Obviously, the risk of
complications would have to be assessed (liver toxicity, kidney function etc.), but if possible, providing a dose of 400mg/kg/day may offer the same metabolic improvements seen in animal studies. Third, the use of specific SIRT1 activators, such as SRT1720, would be an interesting addition to an acute RSV study. While differing in molecular structure, SRT1720 is proposed to activate similar pathways to RSV, but with much greater potency and specificity (Feige et al. 2008). If SRT1720 was deemed suitable for humans, it may circumnavigate the difficulty of providing a concentrated dose that exists with RSV and could be a viable alternative as a pharmacological adjunct to improving metabolic health. Fourth, SIRT1 mediated mitochondrial biogenesis is reliant on its activation of PGC-1α through deacetylation, but no acetylated-PGC-1α antibody is available. While not currently possible in vivo in humans, the expression of flag-tagged PGC-1α in skeletal muscle and adipose tissue would allow for the immunoprecipitation of PGC-1α and quantification of its acetylation status, providing stronger conclusions about RSV action on the critical steps of the SIRT1-PGC-1α axis. Fifth and last, the use of chronic studies should continue, as the additive effect of RSV dosages are unknown. Furthermore, these chronic studies provide valuable data on the long-term effects of RSV and give insight into the potential role of RSV as a therapeutic aid, possibly identifying novel clinically relevant applications for this compound.

5.6 M.Sc. Research Experience

Over my two years of graduate school I have learned a number of valuable skills, both practical and abstract. From a practical perspective, my work in the muscle biochemistry lab has provided me the opportunity to develop a wide skill set and confidence to perform any number of laboratory techniques. Having worked on a number of different projects over the past 2 years I
have learned to effectively schedule and interact with participants, use and trouble shoot metabolic carts, grow and sustain viable cell cultures, assist in muscle and adipose tissue biopsies, and perform the analysis that comes along with these techniques. From a more abstract perspective, I have matured both as a person and student, and have learned how to ask the right questions, search for the answers and, most importantly, think critically. Finally, the opportunity to work and collaborate with a diverse group of people has been invaluable. Daily interactions with my lab mates, participants, Dr. Gurd, faculty members, SKHS staff, and other collaborators have fostered my ability to work as part of a team, negotiate and resolve conflict effectively, provide and receive constructive criticism, and has afforded me a new perspective on the value of collaboration within scientific enquiry.

5.7 Conclusions

An acute dose of RSV can improve insulin sensitivity, but has no effect on fatty acid oxidation in humans. The lack of effect observed on intracellular signaling suggests RSV does not acutely activate SIRT1 and that the pathways of RSV action on mitochondrial biogenesis proposed from cellular and animal models are not conserved in humans.

5.8 References


Appendix A

Research Ethics Boards - Letter of Informed Consent

Consent to Volunteer for Participation in a Study

TITLE: Comparing the effects of exercise training and a dietary resveratrol supplement on skeletal muscle and mitochondrial function.

PRINCIPAL INVESTIGATOR: Brendon J. Gurd, PhD
Queen’s University
School of Kinesiology and Health Studies
Kingston, Ontario, K7L 3N6
613-533-6000 ext. 79023

STUDENT INVESTIGATOR: Cameron Williams
Queen’s University
School of Kinesiology and Health Studies
Kingston, Ontario, K7L 3N6
613-540-0679
You are being invited to participate in a study examining the influence of different types of intervention, either exercise or dietary, on overall fitness (your ability to complete exercise tasks) and mitochondrial function (the ability of your muscle to produce energy). You have been invited to participate in this study because you are a healthy adult (18-45 years). The following brief is intended to provide you with the details you should be aware of prior to your consent as a participant in this study. Please read the following information carefully and feel free to ask any questions that you may have.

**BACKGROUND INFORMATION**

Exercise capacity (fitness) is an important predictor of long term health. In addition, the proper function of organs within your body also contributes to maintaining health. Exercise capacity, the ability of your muscle’s mitochondria to produce energy (mitochondrial function) and the ability of adipose cells (fat tissue) to release fuel are all impaired with obesity and are predictors of both weight gain, the development of type II diabetes and cardiovascular disease. While exercise is well established as an effective stimulus for increasing fitness and muscle/adipose tissue function, there is considerable interest in several dietary supplements that may mimic the beneficial effects of exercise. One such supplement, Resveratrol, is a naturally occurring compound found most commonly in grapes, wine, and peanuts. Recently, evidence suggests resveratrol supplementation can mimic positive changes in muscle mitochondrial function and overall health that are usually observed following regular exercise. This study will examine, and compare, the ability of both exercise and resveratrol supplementation to increase overall fitness, skeletal muscle mitochondrial function, and adipose tissue function.
You will not be able to participate in the study if you have been diagnosed previously with any respiratory, cardiovascular (e.g. High blood pressure, heart conditions), metabolic (e.g. Diabetes), neurological or musculoskeletal disease; or you are currently on medication; or you are a smoker; or you respond to the exercise protocol in an irregular manner (i.e. chest pains, dizziness, shortness of breath, excessive awareness of breathing).

EXPLANATION OF PROCEDURES

Participation:

Participation in the study is voluntary. You may refuse to participate or withdraw from the study at any time with no effect on your academic or employment status. Should you choose to participate you will take part in experimental procedures outlined below. These include 3 visits to the lab for exercise tests (approximately 1 hour each visit), 2 visits for muscle biopsies (approximately 2 hours), 14 visits for your dietary supplement (approximately 15 minutes, first thing each morning), and 8 visits for exercise training (approximately 1 hour per visit). All of these visits will occur within approximately 1 month of your enrolment in this study. The investigator will explain to you, in detail how each of these procedures will be conducted in the study in which you have agreed to participate.

Exercise tests:

During each of the exercise tests you will be required to wear a nose-clip (to prevent you from breathing through your nose) and a rubber mouthpiece (similar to breathing through a snorkel or diving mask). This will enable us to measure the volume of air that you breathe in and out, and measure the
gas concentration in that air. You may experience some initial discomfort from wearing the nose-clip and mouthpiece. You will also be required to wear a heart rate monitor around your chest during all tests. You will be asked to perform each of these tests on one occasion before, and one occasion following exercise training.

**Incremental exercise test:** This test is performed on a treadmill and is designed to measure your fitness level. During this test the intensity of exercise (speed and incline of the treadmill) will begin at a slow walk and will then increase gradually until you are physically unable to continue, or you choose to stop.

**Time trail test:** This test is also performed on a treadmill and is designed to measure your work capacity. This test will involve your completing 5 km as fast as you can. You will be able to select the speed you would like to walk/jog at and will be able to take as many breaks as you need.

**Experimental Interventions:**

Once you have agreed to participate in this study you will be randomly assigned to 1 of 3 experimental groups. All groups will complete the fitness testing and tissue sampling (detailed below) both before, and after the intervention. All interventions will last for 2 weeks.

While there are no reported complications associated with resveratrol treatment, it is recommended that you abstain from sexual activity that might result in pregnancy during the course of your participation in this study.

*Dietary Supplement Interventions:*
You may be randomly assigned into 1 of 2 dietary supplement groups, a resveratrol group, and a placebo control group. If you are put into either of these groups you will be asked to come to the lab each morning during the intervention period where you be provided with breakfast and a pill (containing either resveratrol or placebo). This component of the study will be double blinded, meaning that neither you, nor the researcher will know if you are taking resveratrol or placebo.

**Resveratrol supplement:** You will be given a capsule containing a given amount of pure (99.9%) trans-resveratrol. The dosage is relative to your body weight, but will be far below the recommended daily limit. You will be asked to take the capsule in the presence of the investigator following a standardized breakfast each morning for 14 days.

**Placebo supplement:** You will be given a matched capsule containing an inert placebo, meaning the capsule will contain cellulose fibre with no nutritional value and will have no physiological effect on your body. You will be asked to take the capsule in the presence of the investigator following a standardized breakfast each morning for 14 days.

There are some risks and/or adverse effects associated with the ingestion of the resveratrol supplement. The most common side effects are (but are not limited too) gastrointestinal discomforts, diarrhea, nausea, abdominal pain, and headache. These adverse effects are often mild and transient, and disappear within a day or 2 of discontinuing resveratrol supplements. Should any of these side effects occur you should let the investigator in charge of the study know immediately. Recent clinical trials have determined that resveratrol is generally well tolerated, especially at the relatively low doses being used in this intervention. There have been no
reported serious adverse effects in the published literature. The two possible interventions are outlined below. The investigator will explain to you exactly what is involved in the specific protocol you are being assigned to.

*Exercise Training Protocols:*

If you are randomly assigned into the exercise intervention group you will be asked to complete an exercise training program lasting 2 weeks. Any exercise carries a slight risk of heart attack or may be uncomfortable if you are unfit or not used to exercise. The risk of a cardiac event (heart attack, dysrhythmias etc.) in a mixed subject population (healthy low risk and unhealthy high risk patients together) is approximately 6:10 000, however this risk decreases in a previously healthy (i.e. moderately active) population. There may be some minor discomfort during exercise; you may experience increased awareness of breathing, muscle pain and/or fatigue, increased sweating, or a general feeling of fatigue or nausea, all of which are not unexpected consequences of exercise.

**Exercise Intervention:** The exercise intervention will include walking/jogging on a treadmill at ~75% of your maximum heart rate for 30 minutes, 4 days per week. This represents a moderate-to-vigorous intensity of exercise, similar to a light jog, or a fast bike ride.

The investigator will explain to you exactly what is involved in the exercise intervention if you are assigned to this group.

**Physiological tests:**

Prior to starting the intervention you have been assigned, and in the week following this intervention, you will have a blood sample taken, aswell as
having a muscle and a fat biopsy. The details of these procedures, and the risks associated with them, are detailed below.

**Blood sample:** Before and after the intervention you will be asked to have a sample of blood taken by an individual trained, and certified to take blood samples. You may experience some minor discomfort when this small blood sample is drawn from a vein in your arm. The blood sampling may be painful and minor bruising is possible following venous blood sampling but generally fades within a few days. Blood samples will be examined for the concentration of resveratrol circulating in your blood stream.

**Muscle Biopsy:** Before and after the intervention you will be asked to have small amounts of muscle removed from your thigh muscle (quadriceps muscle) by means of a needle biopsy. The muscle biopsies will be taken either by a medical doctor or a professor trained in this technique under the supervision of a medical doctor. While you are resting on a bed, an anesthetic will be applied locally to anesthetize the skin over your thigh muscle at the site where the biopsies will be taken. A small incision (approximately 1 cm each) will be made through your skin and into your muscle approximately midway between your hip and knee. A small sample of muscle will then be taken from the incision site. This procedure is referred to as a biopsy.

There may be some discomfort associated with the biopsy procedure (like someone pressing hard into your muscle) but you should experience no pain. Following the exercise there may be light bruising of the leg muscle but this will generally fade within a couple of days. There is also a slight risk of infection following a biopsy but proper sterilization of equipment and cleaning of the sampled area minimizes this risk. If the site of the muscle biopsy becomes more
tender and redness and/or swelling develops in that area over the next five to seven days you should let the investigator in charge of your study know immediately and seek medical attention as needed.

Muscle samples will be utilized to examine changes in protein that contribute to mitochondrial content. This examination will be done in our lab using standard biochemical techniques.

**Fat Biopsy:** Before and after the intervention you will be asked to have a small piece of fat tissue removed from your thigh. This procedure is referred to as a biopsy. The fat biopsies will be taken by a medical doctor or by an individual trained in the technique under the supervision of a medical doctor. This procedure is exactly the same as the muscle biopsy procedure described above, except only fat will be removed. Briefly, an area of skin on thigh will be frozen with local anaesthetic and a small incision will be made in your skin (less than 1 cm). As we are taking both the muscle and fat sample from the same site, only one incision will be required for both of these procedures. A needle will then be inserted into your fat layer and a small piece of tissue will be removed. During fat biopsies you may feel slight pressure and/or discomfort in your fat layer but this discomfort will pass very quickly.

Following your biopsies there may be light bruising in the area where the biopsies were taken but this will generally fade within a couple of days. There is also a slight risk of infection following a biopsy but proper sterilization of equipment and cleaning of the sampled area minimizes this risk. If the site of the fat biopsy becomes more tender and redness and/or swelling develops in the area over the next five to seven days, or if you have any concerns whatsoever you should contact the research person supervising your study immediately and seek medical attention as needed.
Please refer to the Biopsy Information Sheet for more information regarding both the muscle and fat biopsy procedures.

RISK OF INJURY

All exercise also carries a small risk of personal injury. Should any such injury occur during your participation in this study you will be initially cared for by the study administrators, all of whom are certified in first aid. Should further assistance be required you will be taken to the university health centre/hospital or emergency as required.

POTENTIAL BENEFITS OF PARTICIPATION

You will gain no direct benefit through participation in this study.

CONFIDENTIALITY

During the course of your participation in this study you will not be required to provide any personal information beyond your name and phone number (for study purposes only). All information obtained during the course of this study, including your name and fitness results, is strictly confidential and your anonymity will be protected at all times. All samples obtained, both blood and muscle, will be stored in a freezer, no personal information will be attached to your sample (all samples will be labeled with subject ID only). Samples will be destroyed following completion of analysis. Your information will be kept in locked files and will be available only to Dr. Brendon Gurd and those working within his laboratory. Your identity will not be revealed in any description or publication.

By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.
VOLUNTARY CONSENT

I have been given an opportunity to ask any questions concerning the procedures. All of my questions regarding the research project have been satisfactorily answered. I understand that my test results are considered confidential and will never be released in a form that is traceable to me. I do understand that I am free to deny consent if I so desire, and may withdraw from the study at any time without any effect on my academic or employment status.

Should I have any questions about the study, I know that I can contact Dr. Brendon Gurd (613 533-9023), Dr. Jean Coté, Head, School of Kinesiology and Health Studies (613 533-6601), or if I have any questions about my rights as a research participant, I can contact Dr. Albert Clark, Chair, Queen’s Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (613 533-6081). A copy of this consent form will be provided to me for my records. My signature below means that I freely agreed to participate in this study.

____________________________  _____________________
Volunteer’s Signature  Date

STATEMENT OF INVESTIGATOR

I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

____________________________  _____________________
Principal Investigator’s Signature  Date
Muscle Biopsy Information Sheet

You have volunteered to take part in a research study that requires you to undergo a muscle biopsy. This is a commonly performed procedure in research studies and for the medical diagnosis of muscle disease. The procedure will be performed by a medical doctor trained to perform muscle biopsies or a specially trained researcher directly supervised by a medical doctor.

The muscle biopsy involves the removal of a small piece of muscle tissue from one of the muscles in your leg using a sterile hollow needle. The area over the outside of your lower thigh muscle (vastus lateralis muscle) will be carefully cleaned. A small amount of local freezing (anesthetic) will be injected into and under the skin. You will likely experience a burning sensation while the freezing is injected. Then a small, 4 – 5 mm incision will be made in your skin in order to create an opening for the biopsy needle. There is often a small amount of bleeding from the incision, but this is usually minimal.

The biopsy needle will then be inserted through the incision into the thigh muscle and a small piece of muscle (100 – 200 mg), about the size of a pencil eraser, will be quickly removed and the needle taken out. During the time that the sample is being taken (about 5 seconds), you may feel the sensation of deep pressure in your thigh and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise and daily activities. There may be some minimal bleeding
when the needle is removed which may require application of pressure for a few minutes.

Following the biopsy, the incision will be closed with sterile tape (steri-strips), and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the freezing wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". Pain killers such as Acetaminophen (e.g. Tylenol) or Ibuprofen (e.g. Advil) are acceptable if you experience pain associated with the biopsy. It is also beneficial to periodically apply an ice pack to the biopsy site the following day, as this will help to reduce any swelling and any residual soreness. The following day your leg may feel uncomfortable when going down stairs. The tightness in the muscle usually disappears within 2 days and subjects routinely begin exercising at normal capacity within 2 days. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 4 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided for at least 4 days following the biopsy procedure.

Potential Risks

• The local freezing will likely result in a burning feeling in the thigh at the time of the injection. This will last only 5 – 10 seconds. There is an extremely low risk of allergic reaction to the local injection (1 in 1 million).
• The chance of a local skin infection in less than 1 in 1000. Carefully cleaning the skin and keeping the area clean and dry until the skin heals will minimize this.

• Most subjects experience local soreness and stiffness in the leg for two or three days after the biopsy similar to a deep bruise or Charlie Horse. There is a very low risk of internal bleeding at the biopsy site which can result in more prolonged pain and stiffness in the leg.

• On occasion, a small lump of scar tissue may form under the site of the incision, but this normally disappears within 2-3 months, or within a few weeks if massaged. A small visible scar often remains from the biopsy incision.

• There is the possibility of a small area of numbness (about the size of a one dollar coin) around the biopsy site. This usually resolves over 5 – 6 months. There is a very low risk (estimated at less than 1/5000) of damage to a small nerve branch to the muscle. This would result in partial weakness of the vastus lateralis muscle (one of four muscles that straightens the knee) and would likely have no impact on day-to-day activities. Nerve injuries like this usually resolve in 8 – 12 months, but there is a theoretical risk of mild leg weakness.

Concerns or Problems

Infection can be serious, if you experience excessive redness, swelling or infection around the biopsy site or pain or stiffness in your leg you must contact Dr. Simpson right away. Dr. Simpson will be available 24 hours a day to answer any of your concerns or questions about the biopsy.
Dr. Craig Simpson: Cell Phone (613) 532-3371

However, if for some reason, you are not able to contact Dr. Simpson then you should contact your family doctor or go to the Emergency Department.

Please keep this Information Sheet until such time as your biopsy site has fully healed.

MUSCLE BIOPSY SUBJECT SCREENING FORM
To help us ensure your safety and wellbeing please answer the following questions.

1. Have you ever had a negative or allergic reaction to local freezing (e.g. during dental procedures)?
   
   No    Yes

2. Do you have any tendency toward easy bleeding or bruising (e.g. with minor cuts or shaving)?
   
   No    Yes

3. Are you currently taking any medications that may increase the chance of bleeding or bruising (e.g. Aspirin, Coumadin, Anti-inflammatories, Plavix)?
   
   No    Yes
4. Have you ever fainted or do you have a tendency to faint when undergoing or watching medical procedures?

   No        Yes

5. Will you contact Dr. Craig Simpson directly if you have any concerns about the biopsy site including: excessive redness, swelling, infection, pain or stiffness of the leg?

   No        Yes

Subject Name (print): ________________________________

Subject Signature: ________________________________

Date: ___________________

Signature of Person
Conducting Assessment: ________________________________
Appendix C

Research Ethics Board Approval

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

October 03, 2012

Mr. Cameron Williams
Department of Unit Kinesiology & Health Studies
28 Division St. Kingston, ON

Dear Mr. Williams:

Study Title: PHE-126-12 Comparing the effects of exercise training and a dietary resveratrol supplement on skeletal muscle and mitochondrial function.
Co-Investigators: Dr. Brendon Gurd, Supervisor Full Board Meeting Date: September 10, 2012

The members of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board have examined the protocol for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of this letter. Please attend carefully to the following list of ethics requirements you must fulfill over the course of your study:

**Reporting of Amendments:** If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval. (see [http://www.queensu.ca/ors/researchethics/REB.html](http://www.queensu.ca/ors/researchethics/REB.html)).

**Reporting of Serious Adverse Events:** Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other
serious adverse events must be reported within 15 days after becoming aware of the information.

**Reporting of Complaints:** Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. **Note:** All documents supplied to participants must have the contact information for the Research Ethics Board.

**Annual Renewal:** Prior to the expiration of your approval (which is one year from the date of the Chair's signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

Chair, Research Ethics Board

**Study Code:** PHE-126-12 Romeo #6007357

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete.

The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards and operates in compliance with the Tri-Council Policy Statement; Part C Division 5 of the Food and Drug Regulations, OHRP, and U.S DHHS Code of Federal Regulations Title 45, Part 46 and carries out its functions in a manner consistent with Good Clinical Practices.

Federalwide Assurance Number: #FWA00004184, #IRB00001173

**Current 2012 membership of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board:**

Dr. A.F. Clark, Emeritus Professor, Department of Biochemistry, Faculty of Health Sciences, Queen's University (Chair)

Dr. H. Abdollah, Professor, Department of Medicine, Queen's University

Dr. R. Brison, Professor, Department of Emergency Medicine, Queen's University

Dr. M. Evans, Community Member

Dr. S. Horgan, Manager, Program Evaluation & Health Services Development, Geriatric Psychiatry Service, Providence Care, Mental Health Services, Assistant Professor, Department of Psychiatry
Ms. J. Hudacin, Community Member

Dr. B. Kisilevsky, Professor, School of Nursing, Departments of Psychology and Obstetrics and Gynaecology, Queen's University

Mr. D. McNaughton, Community Member

Ms. P. Newman, Pharmacist, Clinical Care Specialist and Clinical Lead, Quality and Safety, Pharmacy Services, Kingston General Hospital

Ms. S. Rohland, Privacy Officer, ICES-Queen's Health Services Research Facility, Research Associate, Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute

Dr. B. Simchison Assistant Professor, Department of Anesthesiology and Perioperative Medicine, Queen's University

Dr. A.N. Singh WHO Professor in Psychosomatic Medicine and Psychopharmacology, Professor of Psychiatry and Pharmacology, Chair and Head, Division of Psychopharmacology, Queen's University
Appendix D

Dove Medical Press Permission to Reproduce Publication

From: "sandra@dovepress.com" <sandra@dovepress.com>
Subject: The Application of Clinical Genetics
Date: 26 February, 2013 10:28:08 PM EST
To: Cameron Williams <c.williams@queensu.ca>
Cc: Brendon Gurd <gurdb@queensu.ca>

Dear Cameron

Thank you for your email which I have discussed with our Publisher. There would be no problem with you including the published review paper in your thesis manuscript.

Kind regards

Sandra

Sandra Walters

Administrator, Dove Medical Press Ltd
44 Corinthian Drive, Albany, Auckland, New Zealand PO Box 300-008, Albany, Auckland, 0752, New Zealand
p +649 443 3060/62 f +649 443 3061 e sandra@dovepress.com Live Chat http://www.dovepress.com/live_help.t
Peer-reviewed journals for professionals
www.dovepress.com - open access to scientific and medical research
Dove Medical Press is a member of the Open Access Scholarly Publishers Association

The information in this electronic message is proprietary and confidential and is exclusively addressed to the named recipient(s). Any use, copying or distribution of the above referred information by any unintended recipient may be illicit and result in damage, harm and loss to the sender and/or to the intended recipient(s). If you have received this message in error, please immediately notify us.

From: Cameron Williams [mailto:c.williams@queensu.ca]
Sent: Wednesday, 27 February 2013 8:03 a.m.
To: sandra@dovepress.com
Cc: Brendon Gurd
Subject: Williams and Gurd 2012 - The Application of Clinical Genetics

Hi Sandra,
I am the first author on the paper titled "Skeletal muscle SIRT1 and the genetics of metabolic health: therapeutic activation by pharmaceuticals and exercise" published in August 2012 in The Application of Clinical Genetics.

I am completing my MSc degree under the supervision of Dr. Brendon Gurd, and we plan on including the published review paper in my thesis manuscript. I'm unsure of the appropriate copy write arrangements that need to be made for this to be possible. I imagine there will be some release from the publisher (you) stating I am allowed to do this?

If you could provide some guidance as to your policy on reproduction of my article for educational purposes only I would appreciate it.

Thank you,

Cameron

Cameron Williams
MSc Candidate
Muscle Biochemistry Lab
School of Kinesiology and Health Studies Queen's University, Kingston ON
c.williams@queensu.ca