ABSTRACT

The Effects of Resveratrol Supplementation on Glucose/Insulin Kinetics and Transcription of the AMPK and Insulin Signaling Pathways at Rest and Following an Oral Glucose Tolerance Test and Graded Exercise Test in Overweight Women

Geoffrey M. Hudson, Ph.D.

Committee Co-Chairpersons:
Darryn S. Willoughby, Ph.D.
Matthew Cooke, Ph.D.

The AMPK pathway plays a critical role in glucose metabolism and can potentially improve insulin resistance. Resveratrol is a natural polyphenolic compound that activates this pathway. Therefore, the primary purpose of this study was to determine the effects of daily activation of the AMPK pathway by resveratrol supplementation on glucose/insulin kinetics and transcriptional changes in the AMPK and insulin signaling pathway at rest and following an oral glucose tolerance test (OGTT) and graded exercise test (GXT). Sixteen sedentary, overweight women were recruited for the study. In a randomized and double blind fashion, participants were divided into groups that consumed either 500 mg of resveratrol or a cellulose placebo twice daily for 7 days. On the sixth and seventh day, they returned for an OGTT and a GXT, respectively. Blood and muscle tissue was sampled prior to, and following both the OGTT and GXT. Multivariate analyses revealed no significant changes in resting serum glucose and insulin concentrations ($p = 0.255$), clinical chemistry safety markers ($p = 0.309$), or

lipid profile panels ($p = 0.051$) following 7 days of resveratrol supplementation. Following the OGTT, serum glucose concentration was significantly increased 30 min ($p < 0.001$) and at 1 hr compared to baseline ($p = 0.001$). However, only a strong trend ($p = 0.051$) for increased serum insulin concentration was observed following the OGTT. The GXT protocol produced significant changes in serum glucose and insulin concentration. Glucose concentrations were significantly decreased at 1 hr when compared to baseline ($p = 0.01$) and 30 min ($p = 0.032$) post-exercise. Insulin concentrations were decreased at 1 hr ($p = 0.012$) and 2 hrs ($p = 0.003$) post-exercise. Resveratrol supplementation was unable to enhance such responses. No significant changes were observed with any of the genes analyzed. As a result, it appears that one week of resveratrol supplementation (500 mg/day) is not sufficient to enhance glucose/insulin homeostasis or transcription of metabolically-relevant genes in sedentary, overweight women. Since diabetes is still an increasing global health concern, it is evident that more research is needed to find ways to prevent and/or treat insulin resistance.

The Effects of Resveratrol Supplementation on Glucose/Insulin Kinetics and Transcription of the AMPK and Insulin Signaling Pathways at Rest and Following an Oral Glucose Tolerance Test and Graded Exercise Test in Overweight Women

by

Geoffrey M. Hudson, B.S., M.A.

A Dissertation

Approved by the Department of Health, Human Performance, and Recreation

_____
Rafer S. Lutz, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Doctor of Philosophy

Approved by the Dissertation Committee

_____
Darryn S. Willoughby, Ph.D., Co-Chairperson

_____
Matthew Cooke, Ph.D., Co-Chairperson

_____
Rodney G. Bowden, Ph.D.

_____
Mike Greenwood, Ph.D.

_____
Robert R. Kane, Ph.D.

Accepted by the Graduate School
August 2010

_____
J. Larry Lyon, Ph.D., Dean

Page bearing signatures is kept on file in the Graduate School

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AICAR – 5-aminoimidazole-4-carboxamide-ribonucleoside

AKT – protein kinase B

AMP – adenosine monophosphate

AMPK – AMP-activated protein kinase

AS160 – Akt substrate of 160kD or TBC1D4

ATP – adenosine triphosphate

AUC – area under the curve

BSA – body surface area

C_T – threshold cycle

DNA – deoxyribonucleic Acid

DXA – dual energy x-ray absorptiometry

g – gram

GAP – GTPase-activating domain

GLUT4 – facilitative glucose transporter four

GXT – graded exercise test

HOMA-IR – homeostatic model assessment of insulin resistance

IGT – impaired glucose tolerance

IGTT – intravenous glucose tolerance test

IRS-1 – insulin receptor substrate one

IU – international units

JNK – c-Jun N-terminal kinase

kD – kilodalton

kg – kilogram

kJ – kilojoule

km - kilometer

m – meter

mg – milligram

mL – milliliter

mmol – millimolar

mRNA – messenger ribonucleic acid

ng – nanogram

OGTT – oral glucose tolerance test

PDK1 – 3-phosphoinositide-dependent kinase

PI3K – phosphotidylinositol three kinase

PIP_2 – phosphatidylinositol-4,5-bisphosphate

PIP_3 – phosphatidylinositol-3,4,5-trisphosphate

PPAR – peroxisome proliferator-activated receptor

PTB – phosphotyrosine-binding

RDA – recommended dietary allowance

RPE – rating of perceived exertion

Ser – serine

SH2 – Src homology 2

Tyr – tyrosine

TNF-α – tumor necrosis factor alpha

tRNA – transfer ribonucleic acid

U – units

VO_2 max – maximal oxygen uptake

µg – microgram

µL – microliter

ACKNOWLEDGMENTS

I would first like to thank God for giving me patience, humility, and wisdom throughout this process. I would also like to thank my dear wife, Laura, whose unconditional love, encouragement, and support have meant so much to me. I love you very much and am so very grateful for all of your many sacrifices over the past few years. I also want to thank my parents, David and Deborah Hudson, who have unwaveringly supported me throughout my educational career. Thank you so much for your loving guidance and support.

To my teachers, I want to thank you for providing me with the knowledge to make it to this point and to prepare me for the future. I would like to specifically thank Dr. Richard Kreider for the research opportunities and financial support that he provided in this graduate program. With regards to the specific members of my committee, thank you Dr. Willoughby for providing me with the opportunity to learn so much in the EBNL and for your time, insight, and patience throughout the dissertation process. A special thank you to Dr. Matthew Cooke for your friendship and support in the development and execution of this research project. You were literally always there for us. Know that your sacrifices did not go unnoticed and your friendship will not be forgotten. I would like to thank Dr. Mike Greenwood for his helpful academic advisement, committee service, and specifically for the financial support that he provided to acquire the supplements necessary for this research. To Dr. Rodney Bowden, it is always a pleasure and encouragement to talk with you and I thank you for your service on my committee.

Thank you, Dr. Robert Kane, for the perspective that you provided as my outside committee member.

Finally, I would like to thank my Baylor family with whom I shared many early mornings, late nights, and battles in between. Thank you to Beth Davis and the Ph.D. graduates that showed me the ropes and taught me so much about life in the lab. I would like to send a special thank you to Dr. Brian Shelmadine for tirelessly working with me on this project. It certainly could not have been done without you. Thank you for your help with the research conception and for enduring the many, many hurdles and difficulties alongside me. It has certainly been a pleasure to serve with you and an experience and friendship that will not be forgotten. Thank you Dr. Thomas Buford, Dr. Mike Iosia, and Dr. Paul La Bounty for the comic relief and many entertaining and enlightening lunches that we shared. Thank you as well to my other classmates and friends, Jen, Erika, Monica, Kristen, and Jean. You all have made this an entertaining and unforgettable educational experience.

CHAPTER ONE

Introduction

Type II diabetes mellitus is a critical health concern that has tripled in prevalence over the past 25 years, with 24.1 million Americans (8% of the population) suffering from this condition in 2007 (American Diabetes Association, 2008). If blood glucose levels are not properly managed, diabetes can result in serious consequences such as the development of hypertension, retinopathy, nephropathy, and peripheral neuropathy (Misra & Chakrabarti, 2007). Prediabetes (serum glucose of 100-125 mg/dl) is an insulin resistant state that significantly increases the risk of type II diabetes development. These prediabetic individuals are also at a much greater risk of developing numerous cardiovascular diseases (i.e. atherosclerosis, peripheral artery disease, etc.), which also increases their mortality risk (Balkau et al., 1998; Bjornholt et al., 1999; Coutinho, Gerstein, Wang, & Yusuf, 1999). In fact, diabetic individuals have a 110% greater mortality risk, while those classified as only prediabetic still have a 40% greater risk (Saydah, Loria, Eberhardt, & Brancati, 2001). Therefore, not only is treatment of insulin resistance important to prevent the development of type II diabetes, but to also provide direct health benefits as well. The initial insulin resistant state that precedes the development of type II diabetes is where the insulin-responsive cells in the body do not appropriately respond to circulating insulin, thereby resulting in increased blood glucose and insulin levels and disturbances in fatty acid metabolism. Within adipocytes and myocytes, insulin serves to lower blood glucose levels by an insulin-signaling pathway that results in glucose uptake into the cell due to the translocation of the glucose

transporter 4 (GLUT4) to the cell membrane. The first line of defense to prevent or impede the development of type II diabetes mellitus is lifestyle modification. Such lifestyle changes include weight-loss, increasing physical activity, and modifying diet to an anti-atherogenic type diet (American Diabetes Association, 2002a; American Diabetes Association, 2002b; Gillies et al., 2007; Klein et al., 2004; Sherwin et al., 2003; Tuomilehto et al., 2001). These lifestyle changes are not only recommended for weight reduction, but exercise and a proper diet also provide direct benefits for management of blood glucose levels.

At rest or following a meal, insulin binding is the primary stimulus for glucose uptake in myocytes and adipocytes. Insulin-mediated glucose uptake proceeds due to the phosphatidylinositol 3-kinase (PI3K) pathway and phosphorylation of protein kinase B (PKB; Akt) and Akt Substrate of 160kD (AS160). Phosphorylation of AS160 then serves to release GLUT4 from its intracellular storage site and promote its translocation to the cell membrane. Integration of GLUT4 into the cell membrane then facilitates transport of glucose into the cell. Conversely, during moderate to intense exercise, glucose can be taken into myocytes by insulin-independent signaling pathways. One such insulin-independent signaling pathway that is partially responsible for this increase in glucose uptake is the AMP-activated protein kinase (AMPK) pathway. AMPK is activated by increases in adenosine monophosphate (AMP) levels during exercise (particularly at higher intensities) and it also serves to promote GLUT4 translocation and glucose uptake by phosphorylation of AS160. Recent evidence points to this convergence between both insulin and contraction-mediated signaling pathways at the AS160 signaling intermediate. Therefore, since AS160 appears to be the ‘gatekeeper’ that releases GLUT4 and thus

stimulates glucose uptake, interventions that can either enhance insulin signaling or AMPK signaling should improve glucose tolerance. However, further research in human participants is required to validate these hypotheses (Rockl, Witczak, & Goodyear, 2008).

As mentioned, in addition to weight reduction and physical exercise as important lifestyle changes for overweight and/or diabetic populations, dietary adjustments are also essential. Included in these recommended dietary changes is increased intake of fruits and vegetables. Fruits and vegetables are important dietary components that can provide beneficial antioxidants and phytonutrients. Resveratrol is one such phytonutrient that is found in high concentrations in red wine and a variety of plant sources such as grape skin, berries, pomegranates, and peanuts (Remsberg et al., 2008; Su, Hung, & Chen, 2006). Resveratrol has been widely studied over the past decade due to its connection with the "French Paradox," which is the relatively low incidence of heart disease in the French population despite a diet high in saturated fat (Furimsky et al., 2008). Aside from the initial findings of the cardioprotective benefits obtained from resveratrol supplementation, current literature suggests that resveratrol can also improve insulin sensitivity, promote weight loss, cancer growth inhibition, and increase longevity (Athar et al., 2007; Frojdo, Cozzone, Vidal, & Pirola, 2007; Miyazaki et al., 2008; Park et al., 2007). Although interest in this phytonutrient has recently increased, most research has been conducted in cell culture (Miyazaki et al., 2008; Park et al., 2007) or animal models, (Deng et al., 2008; Su et al., 2006), while very limited research has examined their purported effects in clinically controlled human trials (Gresele et al., 2008). Of particular research importance is the possible mechanism by which resveratrol can improve insulin-sensitivity by enhancing GLUT4 translocation. Some studies have suggested that

improved insulin-sensitivity from resveratrol supplementation resembles that of metformin (a commonly used drug to treat diabetes), which enhances GLUT4 translocation via AMPK stimulation (Su et al., 2006). Initial mechanistic research has shown that resveratrol does not allosterically stimulate AMPK, since it does not affect cellular AMP levels. However, resveratrol's stimulation of AMPK currently appears to be dependent upon the upstream serine/threonine kinase, LKB1, which activates AMPK by phosphorylation at Thr^{172} (Chan et al., 2008). Another potent activator of AMPK is 5-aminoimidazole-4-carboxamide-ribonucleoside (AICAR), which has been shown to have a synergic effect with insulin on stimulating glucose uptake. Furthermore, daily AICAR administration has been shown to mimic the effects of endurance exercise training on improvements in glucose transport, insulin sensitivity, GLUT4 content, and mitochondrial enzyme activity (Holmes, Kurth-Kraczek, & Winder, 1999; Jessen, Pold, Buhl, Jensen, Schmitz, & Lund, 2003). Therefore, the purpose of the current study was to determine whether daily activation of the AMPK pathway by resveratrol supplementation would improve fasting glucose homeostasis and glucose tolerance following a bolus ingestion of glucose. It also assessed the possible effect that resveratrol may continue to stimulate the AMPK pathway following exercise and continue to additively work with insulin to promote glucose uptake following exercise. Finally, this study purposed to determine the effects of resveratrol supplementation on transcriptional rates of metabolically relevant genes.

Purposes of the Study

The primary purpose of this study was to determine the effects of a polyphenolic supplement, resveratrol, on glucose/insulin kinetics at rest and following an intense exercise bout. More specifically, this study assessed the effects of resveratrol supplementation on A) fasting serum glucose and insulin homeostatic levels, B) glucose/insulin kinetics as determined by an oral glucose tolerance test (OGTT), C) serum glucose and insulin responses to a graded-exercise test (GXT), D) skeletal muscle expression of metabolically responsive genes (AMPK, Akt2, AS160, IRS1, and GLUT4) at rest, following the OGTT, and the GXT, and E) serum analyses for general clinical safety markers.

Hypotheses

H_1: After 1 week of supplementation, there will be no statistically significant difference between groups in age, body mass, height, waist circumference, body composition, heart rate, or blood pressure.

H_2: During the course of the study, there will be no statistically significant difference in caloric intake or macronutrient intake in the experimental group as compared to the placebo group.

H_3: After 1 week of supplementation, there will be no statistically significant difference in serum levels of the clinical chemistry markers of kidney and liver function in the experimental group as compared to the placebo group.

H_4: After 1 week of supplementation, there will be a statistically significant reduction in serum levels of LDL, total cholesterol, and triglycerides and a significant increase in HDL levels in the experimental group as compared to the placebo group.

H_5: After 1 week of supplementation, there will be a statistically significant reduction in serum levels of glucose, insulin, and the homeostasis model assessment (HOMA-IR) index of insulin resistance in the experimental group as compared to the placebo group.

H_6: Following the OGTT, there will be a significantly lower increase in serum levels of glucose and insulin in the experimental group as compared to the placebo group.

Additionally, the calculated assessment of insulin sensitivity (Matsuda Index) during the OGTT will be significantly greater in the experimental group as compared to the placebo group.

H_7: Following the GXT, there will be a significantly greater reduction in serum levels of glucose and insulin in the experimental group as compared to the placebo group.

H_8: After 1 week of supplementation, there will be a statistically significant increase in skeletal muscle transcription of genes critical for regulation of substrate utilization (AMPK, Akt2, AS160, GLUT4, and IRS1) in the experimental group as compared to the placebo group.

H_9: Following the OGTT, there will be a statistically significant increase in skeletal muscle transcription of genes critical for regulation of substrate utilization (AMPK, Akt2, AS160, GLUT4, and IRS1) in the experimental group as compared to the placebo group.

H_{10}: Following the GXT, there will be a statistically significant increase in skeletal muscle transcription of genes critical for regulation of substrate utilization (AMPK, Akt2, AS160, GLUT4, and IRS1) in the experimental group as compared to the placebo group.

Delimitations

This study was completed using the following guidelines:

1) Sixteen, sedentary, overweight/obese women between the ages of 18 and 40 participated in this study.

2) Participants were recruited from the general population of Waco, TX and the student population at Baylor University by flyers posted throughout campus, through Baylor announcements, and through newspaper advertisements.

3) Participants were randomly assigned to one of two supplement groups: resveratrol or placebo.

4) Participants completed an OGTT during the second testing session and a Balke GXT during the third testing session.

5) All participants in the study did not participate in any other forms of vigorous exercise during the duration of the study and did not modify their nutritional intake in any manner.

6) Muscle biopsies from the lateral thigh were collected during the pre-supplementation testing session; collected prior to the OGTT and 1 hr post-OGTT

during the second testing session; and collected prior to the GXT and 1 hr post-GXT during the third testing session.

7) Venous blood was collected during the pre-supplementation testing session; prior to the OGTT, 30 min post-OGTT, 1 hr post-OGTT, and 2 hr post-OGTT during the second testing session; and collected prior to the GXT, 30 min post-GXT, 1 hr post-GXT, and 2 hr post-GXT during the third testing session.

8) All testing was performed in the Exercise and Biochemical Nutrition Lab and Exercise and Sport Nutrition Lab at Baylor University in the Marrs-McLean Gym according to all policies and procedures within each respective laboratory.

Limitations

1) The sample size was limited to those who came forward to participate in the study, which limited the scope of conclusions that could be inferred to a larger population.

2) The motivation and willingness of each participant to maximally exert themselves during the third testing session during the GXT.

3) The sensitivity of the technologies and protocols utilized to identify quantifiable changes in the criterion variables.

4) The ability of the HOMA-IR index of insulin resistance and Matsuda insulin sensitivity index to accurately estimate insulin function in this population.

5) The daily schedules of each participant and the inherent circadian rhythms that exist for all humans as a result of slightly different testing times, stresses, etc.

Assumptions

1) Participants fasted for 12 hr prior to reporting for each testing session.

2) Participants were prediabetic yet had no contraindications to any of the prescribed treatments involved with this protocol.

3) Participants were sedentary – had not regularly exercised for 30 min a day for at least 5 days a week in the past year.

4) All participants followed all instructions throughout the familiarization session, the OGTT testing session, the GXT, and maximally exerted themselves during the GXT.

5) All assay reagents and equipment used in the sample analysis were accurate and reliable in quantification of the criterion variables.

6) All methods were previously established and were accurate and reliable methods for determination of the criterion variables.

Definition of Key Terms

1) AMPK – AMP activated protein kinase is an energy sensitive kinase activated by low-energy states (i.e. fasting and exercise). This enzyme serves to activate energy-producing processes and inhibit energy-storage processes.

2) Akt – This protein is also known as protein kinase B (PKB) and is a downstream target of IRS-1 and PI3K signaling. Akt is a key protein in cell signaling and promoting GLUT4 translocation and glucose uptake.

3) AS160 – AS160 is a substrate of Akt whose phosphorylation allows for translocation of GLUT4. AS160 is also a downstream target of AMPK signaling.

4) Glucogenesis – The production of glucose via gluconeogenesis or from the breakdown of glycogen stores.

5) Hepatocyte – A liver cell.

6) Hyperglycemia – A state of abnormally high fasting levels of glucose in the blood ($\geq$126mg/dL).

7) Insulin – A peptide hormone that is released from the β-cells of the pancreas in response to high blood glucose levels. When insulin binds to its receptor, it stimulates the PI3K cell signaling pathway, GLUT4 translocation to the plasma membrane, and glucose uptake into the cell.

8) Insulin sensitivity – The responsiveness of the liver and peripheral tissue (muscle and adipose) to insulin that results in glucose production and/or glucose uptake.

9) IRS-1 – A cytoplasmic protein that is stimulated by insulin, insulin-like growth factor-1, and possibly growth hormone. IRS-1 is a tyrosine kinase that self-phosphorylates its intracellular domains upon extracellular ligand binding. It also has the ability to bind, recruit, and activate (phosphorylate) Akt.

10) mRNA – A gene's DNA is transcribed into messenger RNA. The mRNA is then transported to the ribosome and serves as a template for the synthesis of proteins via translation.

11) Myocyte – A muscle cell.

12) Phosphorylation – The process of adding a phosphate group to a compound or protein through a kinase enzyme. This addition of a phosphate group to a protein or enzyme can activate or deactivate that protein.

13) Prediabetic – An insulin-resistant state that leads to the development of type II diabetes. Clinically defined as a fasting blood glucose concentration between 100 and 125 mg/dL.

14) Resveratrol – This is a naturally occurring polyphenolic phytoestrogen found in grapes, berries, and wine that is utilized in Ayurvedic medicines.

15) Serine/threonine kinase – These kinases phosphorylate the hydroxyl group of the substrate's serine or threonine side chains.

16) Transcription – The process by which genetic information stored in a strand of DNA is copied to synthesize a strand of mRNA.

17) Translocation – The change of location from one cellular compartment to another.

CHAPTER TWO

Literature Review

Insulin Resistance and Type II Diabetes

Type II diabetes is a metabolic disorder characterized by a resistance to insulin in muscle, liver, and adipose tissue cells that results in abnormal glucose and lipid metabolism. Glucose is the primary source of fuel for skeletal muscles and many other tissues. Insulin is secreted from the β-cells of the pancreas in response to increases in blood glucose levels. The insulin resistance present in type II diabetics, however, results in disruption in signaling pathways that govern peripheral glucose uptake and hepatic glucose production (Kahn & Flier, 2000; Karlsson & Zierath, 2007). Hyperglycemia is the consequence of this signaling disruption as peripheral tissues (skeletal muscle and adipose tissue) do not efficiently remove the glucose from the blood and glucogenesis in the liver is not effectively regulated (Karlsson & Zierath, 2007; Shulman, 2000). Furthermore, pancreatic β-cells initially compensate for this decrease in insulin sensitivity and release more insulin to correct the increased blood glucose levels. Therefore, equations incorporating the product of blood glucose and insulin concentrations are typically used to assess whole-body sensitivity to insulin (Matsuda & DeFronzo, 1999). Due to limited insulin sensitivity, blood glucose and insulin concentrations are higher in type II diabetics. Insulin resistance is a metabolic condition that can lead to numerous health complications such as atherosclerosis, cardiovascular disease, nephropathy, neuropathy, and retinopathy (Balkau et al., 1998; Bjornholt et al., 1999; Coutinho, Gerstein, Wang, & Yusuf, 1999). As a result, it is imperative that these

individuals increase their responsiveness to insulin by exercise, appropriate dietary modifications, and possibly pharmaceutical interventions in order to maintain a state of euglycemia and normal pancreatic function.

GLUT4 Transporter and Glucose Uptake

Under insulin-stimulated conditions, skeletal muscle accounts for nearly 80% of glucose disposal in healthy individuals (Ryder, Chibalin, & Zierath, 2001). Since glucose is a hydrophilic molecule, it cannot traverse the lipid bilayer of the cell membrane, but must be facilitated by membrane transporters (Karlsson & Zierath, 2007). Glucose transport in a skeletal muscle cell proceeds by facilitated diffusion utilizing glucose transporter four (GLUT4). GLUT4 is sequestered within intracellular compartments under basal conditions, but exercise or insulin stimulation initiates GLUT4 vesicular translocation and promotes its exocytosis to the sarcolemma (Thong, Dugani, & Klip, 2005; Voet, Voet, & Pratt, 2008). GLUT4 is one member of a larger family of facilitative glucose transporters (GLUTs 1-4) present in various cell types throughout the body. These GLUT transporters function to shuttle glucose across the plasma membrane by integrating into the lipid membrane and forming an aqueous pore across the membrane through which the hydrophilic glucose molecule can be easily transported. Although GLUTs facilitate glucose transport into the cell, this transport across the sarcolemma is most likely the rate-limiting step in glucose utilization (Cline et al., 1999; Goodyear & Kahn, 1998; Ryder et al., 2001). There is a great deal of tissue specialization among the GLUT transporters as well. For example, those present in the liver and brain are typically constitutively active. In contrast, the GLUT4 transporters present in muscle and adipose tissue cells are inducible and highly regulated (Bryant,

Govers, & James, 2002). In fact, GLUT4 concentration at the myocellular surface can triple within the first 10 min of stimulation (Sweeney et al., 2004). These rapid changes can then result in a near immediate 10–40-fold increase in glucose transport within the cell (Shepherd & Kahn, 1999). This shuttling process of GLUT4 to and from the sarcolemma is intricately regulated by both the glucose concentration in the blood and the energy state of the cell. Insulin signaling and the PI3K pathway regulate the former, while the AMPK pathway is partially responsible for GLUT4's responsiveness to cellular energy states. Since total GLUT4 expression in skeletal muscle of type II diabetics is not reduced, their impaired glucose uptake must be a result of disruptions in the insulin-signaling pathway (Karlsson & Zierath, 2007; Shepherd & Kahn, 1999).

Insulin-Mediated Glucose Uptake: PI3K-Akt Signaling Pathway

Insulin is the hormone that is responsible for the majority of glucose removal from the bloodstream. When blood glucose concentration is high, insulin is secreted from the β-cells of the pancreas. Insulin then binds to the insulin receptor located on insulin-responsive cells (adipocytes, hepatocytes, and myocytes) to stimulate the uptake of glucose from the blood and to inhibit hepatic glucose production (Thong et al., 2005). Insulin signaling within the myocyte proceeds via the PI3K signaling pathway (Figure 1). The insulin receptor is a heterotetrameric member of the tyrosine kinase receptor family. Ligand (i.e. insulin) binding to the α-subunit of the receptor results in a conformational change in the β-subunit that spans the cell membrane (Karlsson & Zierath, 2007). This conformational change in the β-subunit then stimulates the tyrosine kinase activity that causes neighboring subunits to then dimerize and autophosphorylate their intracellular domains. These phosphorylated tyrosine residues then can recruit and phosphorylate

many other cytoplasmic substrates; most notable in stimulating glucose uptake is phosphorylation of the insulin response element-1 (IRS-1) (Ottensmeyer, Beniac, Luo, & Yip, 2000; Thong et al., 2005). The phosphotyrosine-binding (PTB) domain of IRS-1 interacts with the phosphorylated tyrosine residues of the insulin receptor, while the Src homology 2 (SH2) domain activates PI3K. PI3K then serves to phosphorylate its lipid-soluble and membrane-bound substrate, phosphatidylinositol-4,5-bisphosphate (PIP_2). PIP_2 phosphorylation then produces phosphatidylinositol 3,4,5-triphosphate (PIP_3), whose pleckstrin homology (PH) domain, in turn, attracts other signaling molecules such as 3-phosphoinositide-dependent kinase (PDK1) (Thong et al., 2005). PDK1 then serves to phosphorylate the $Thr^{308/309}$ residue in the activation loop of Akt, followed by subsequent phosphorylation of the $Ser^{473/474}$ residue by an additional kinase (Karlsson & Zierath, 2007; Ueki et al., 1998). Once activated, Akt can then serve to phosphorylate Akt substrate of 160 kDa (AS160), which promotes vesicular budding of GLUT4 vesicles from their intracellular compartments and their translocation to the plasma membrane. Phosphorylation of AS160 is then essentially the key that unlocks the gate and releases GLUT4 to be translocated to the plasma membrane to facilitate glucose uptake (Thong et al., 2005). AS160 has six sites that can be phosphorylated by Akt (Sano et al., 2003). AS160 phosphorylation at these sites then results in activation of a GTPase-activating protein (GAP) domain that then targets a Rab protein (Kane et al., 2002). Rab proteins are small molecular G proteins that are important for numerous intracellular trafficking processes. AS160 partially binds to the GLUT4 vesicle and it is believed that phosphorylation and activation of this Rab GAP domain of AS160 then results in GLUT4

vesicular budding and exocytosis to the plasma membrane (Larance et al., 2005; Ramm, Larance, Guilhaus, & James, 2006).

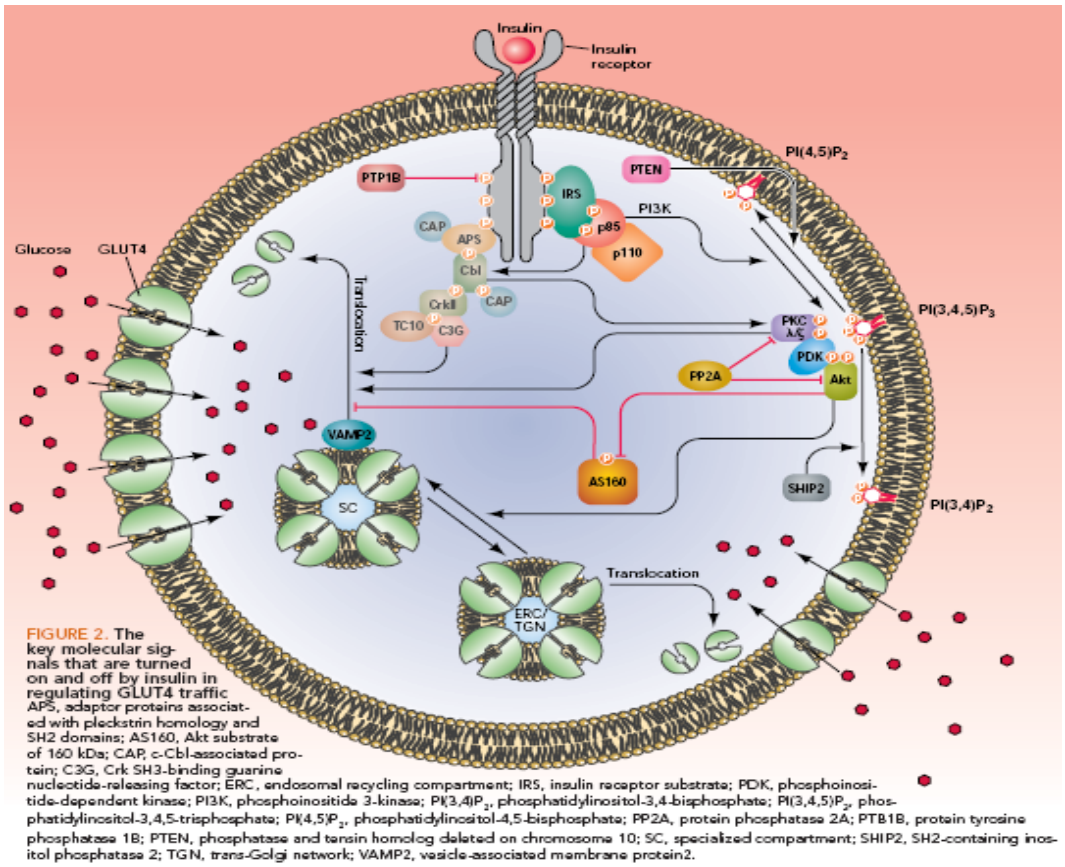


Figure 1. Schematic diagram depicting insulin-stimulated glucose uptake via the insulin-signaling pathway (Taken from Thong et al., 2005)

Although all intermediates in the PI3K/Akt pathway are ultimately affected in insulin resistant individuals, the mechanism of insulin resistance most likely originates with the intermediates at the beginning of the insulin-signaling cascade (Goodyear et al., 1995; Karlsson & Zierath, 2007; Krook et al., 2000). Discussion of this theory will begin with analysis of the terminal step in that sequence. Insulin-stimulated glucose uptake and GLUT4 translocation is inhibited in type II diabetics even though skeletal muscle GLUT4

levels are relatively normal (Karlsson & Zierath, 2007; Shepherd & Kahn, 1999). As a result, the diminished glucose uptake and GLUT4 trafficking most likely results from a disruption in upstream signaling (Garvey et al., 1998). Protein expression and activity of Akt (Akt1/2 and Akt3) has been shown to be normal in type II diabetics, despite diminished IRS1 and PI3K signaling (Y. B. Kim, Nikoulina, Ciaraldi, Henry, & Kahn, 1999). As a result, these authors concluded that Akt is most likely not the primary location of insulin-signaling disruption in type II diabetes. On the contrary, Brozinick et al. (Brozinick, Roberts, & Dohm, 2003) found that Akt2 and Akt3 activation was reduced in muscle collected from obese type II diabetics, while Akt1 isoform activation was unaffected in this group. These researchers also found that IRS1 expression was lower in obese muscle than muscle collected from lean participants. Furthermore, studies by Kim et al. (1999) showed a decrease in PI3-kinase activity and IRS1 activation upon insulin stimulation of muscle from type II diabetic patients. Other studies have also confirmed the findings that insulin resistant muscle typically has lower levels of IRS1 phosphorylation and decreased PI3K activity (Krook et al., 2000; Zierath, Krook, & Wallberg-Henriksson, 1998). Additionally, numerous other studies have shown defects in IRS1 function in type II diabetics, although these defects were not associated with altered IRS1 protein expression (Karlsson & Zierath, 2007; Krook et al., 2000). Analysis of the initial component of the insulin-signaling pathway, however, reveals that insulin binding to the insulin receptor and tyrosine phosphorylation of the insulin receptor has been reported to be normal or only slightly impaired in some insulin-resistance individuals (Goodyear et al., 1995; Karlsson & Zierath, 2007; Krook et al., 2000). Furthermore, it does not appear to be altered IRS1 phosphorylation by the insulin

receptor that causes the disruption, but phosphorylation at alternate IRS1 serine residues that results in its decreased activity (Karlsson & Zierath, 2007). At this point, it appears that these IRS1 phosphorylated serine residues that serve to limit IRS1 signaling and ultimately glucose uptake. As evidenced by the previously mentioned studies, isolation of the specific alterations in the insulin-signaling pathway in insulin-resistance is a quite complex process. However, identification of these specific signaling pathway alterations of insulin-resistant individuals would accelerate the development of more effective therapeutic means.

Exercise-Mediated Glucose Uptake: AMPK Pathway

Exercise is one of the primary recommendations for treating and/or preventing insulin resistance. It is typically thought that exercise is recommended to promote weight loss and, therefore, improve insulin resistance (Klein et al., 2004). The benefits of exercise with regards to insulin resistance reach much further than simply weight loss, however. Acute exercise produces transient enhancements in insulin sensitivity (Goodyear & Kahn, 1998; Hayashi, Wojtaszewski, & Goodyear, 1997; Santos, Ribeiro, Gaya, Appell, & Duarte, 2008), while exercise training can improve chronic insulin resistance (Gillies et al., 2007; O'Leary et al., 2006; Sherwin et al., 2003; Tuomilehto et al., 2001; Winnick et al., 2008). Not only does endurance exercise training improve glucose uptake by increases in GLUT4 and hexokinase activity (Hayashi et al., 1997; Host, Hansen, Nolte, Chen, & Holloszy, 1998), but 2 weeks of daily treadmill running in type II diabetic Zucker rats similarly resulted in an increase in GLUT4 content and reversal of insulin resistance of fast-twitch muscle (Etgen et al., 1997). Furthermore, Winnick et al. (2008) showed that whole-body improvements in insulin sensitivity after 7

days of aerobic exercise training were due to improvements in the insulin sensitivity of peripheral and not hepatic tissue. A study from Christ-Roberts and colleagues (2004) examined the effects of exercise training on the insulin-signaling pathway in humans. They compared the effects of 8 weeks of exercise training of insulin resistant nondiabetic participants to those of type II diabetic patients. After training, they found that insulin-stimulated glucose disposal and total glycogen synthase activity were increased in both groups. Protein expression of GLUT4 and Akt was also significantly increased in both groups, while training did not seem to affect IRS1 or PI3-kinase activity. The authors concluded that 8 weeks of exercise training improved glucose disposal by increasing GLUT4 protein levels without enhancing PI3K signaling and the increased glycogen synthase activity also contributed by shuttling excess glucose towards glycogen synthesis (Christ-Roberts et al., 2004). Although exercise training improves insulin resistance more than weight loss alone, its effects are also short-lived when training ceases. For example, Host et al. (1998) showed that the insulin sensitivity and GLUT4 content improvements in rats that trained (swimming 6 hr/day) for 5 weeks were significantly diminished in as little as 40 hr after the cessation of training.

As previously mentioned, insulin stimulated glucose uptake is inhibited in type II diabetic individuals due to alterations in the insulin-signaling pathway that results in suppressed GLUT4 transporter translocation to the sarcolemma. Although acute exercise increases insulin sensitivity, the benefits are transient and insulin resistance continues to limit glucose uptake in type II diabetic individuals. Aside from increasing post-exercise insulin sensitivity, glucose uptake is enhanced during exercise in both non-diabetic and insulin resistant individuals via insulin-independent pathways (Braun, Sharoff, Chipkin,

& Beaudoin, 2004; Goodyear & Kahn, 1998; Kennedy et al., 1999; Musi et al., 2001; Ryder et al., 2001; Santos et al., 2008). Kennedy and colleagues (1999) revealed that exercise-stimulated glucose uptake is a result of increased GLUT4 translocation even in type II diabetic patients. Type II diabetics have typically normal relative levels of GLUT4 protein (Musi et al., 2001), but impaired insulin-signaling inhibits the GLUT4 translocation to the sarcolemma. Consequently, the mechanism(s) responsible for exercise-stimulated glucose uptake somehow bypasses the suppressed insulin-signaling pathway and stimulates GLUT4 the same as a non-diabetic exercising individual. The study from Kennedy et al. (1999) involved 5 type II diabetic and 5 normal control participants that cycled for 45-60 min at 60-70% VO_2 max. Although plasma membrane GLUT4 content was 32% lower in this study in the diabetic patients at rest, the exercise bout produced a mean increase in GLUT4 content in both the diabetic (74%) and normal (71%) participants (Kennedy et al., 1999). In addition, exercise-stimulated uptake of glucose into the muscle was not affected by inhibition of the insulin signaling pathway. Even in the presence of wortmannin, a known PI3K inhibitor, GLUT4 translocation and glucose uptake is still stimulated by muscular contractions (Lee, Hansen, & Holloszy, 1995; Lund, Holman, Schmitz, & Pedersen, 1995).

The AMPK pathway is one of the proposed mechanisms responsible for this exercise-stimulated glucose uptake. AMPK serves as a 'fuel gauge' for the cell that is activated by cellular stresses that deplete ATP levels. Acute exercise stresses the cell and results in a hypoxic environment with increased levels of ADP and unphosphorylated creatine, all of which serve to activate AMPK (Fisher, Gao, Han, Holloszy, & Nolte, 2002; Holloszy, 2005; Musi & Goodyear, 2002). Figure 2 illustrates the various stimuli

and effects of AMPK activation. While both insulin and exercise stimulate glucose uptake and GLUT4 translocation, the connection between the two pathways eluded scientists for years. The two pathways seemed to work independently of each other since complete inhibition of the PI3K signaling pathway does not affect AMPK-stimulated glucose uptake (Lee et al., 1995; Lund et al., 1995). More recently it has been shown that AMPK stimulates glucose uptake in the same manner as Akt (a downstream target of the insulin signaling pathway) by phosphorylation of AS160 and resulting translocation of GLUT4 to the sarcolemma. This convergence of the PI3K and AMPK signaling pathways at AS160 is currently an emerging research area (Arias, Kim, Funai, & Cartee, 2007; Cartee & Funai, 2009; Deshmukh, Hawley, & Zierath, 2008; Santos et al., 2008). Arias and colleagues (2007) showed that immediate postexercise increase in glucose uptake and GLUT4 translocation in rat muscle is a result of increased AS160 phosphorylation via activation of the AMPK pathway and not the PI3K pathway. They also found that signaling of insulin-independent mechanisms is short lived as the improvements in glucose uptake 4 hr after exercise are a result of enhanced insulin-signaling and not activation of the AMPK pathway (Arias et al., 2007). In a more recent study, Treebak et al. (2009) showed that improvements in insulin sensitivity after acute exercise are partially a result of improved AS160 phosphorylation. Using a euglycaemic-hyperinsulinaemic clamp technique 4 hr after a one-legged exercise routine, these researchers showed that insulin stimulation increased glucose uptake in both legs with 80% higher increase in glucose uptake in the previously exercised leg. Similarly, AS160 phosphorylation was also increased after insulin stimulation to a greater degree in the previously exercised leg. However, AS160 phosphorylation at 4 specific serine sites

(Ser-318, Ser-341, Ser-588, and Ser-751) was higher in the previously exercised leg even in the absence of insulin. The researchers, therefore, speculated that AMPK signaling could perhaps be responsible for the insulin-independent increase in AS160 phosphorylation after the exercise bout (Treebak et al., 2009).

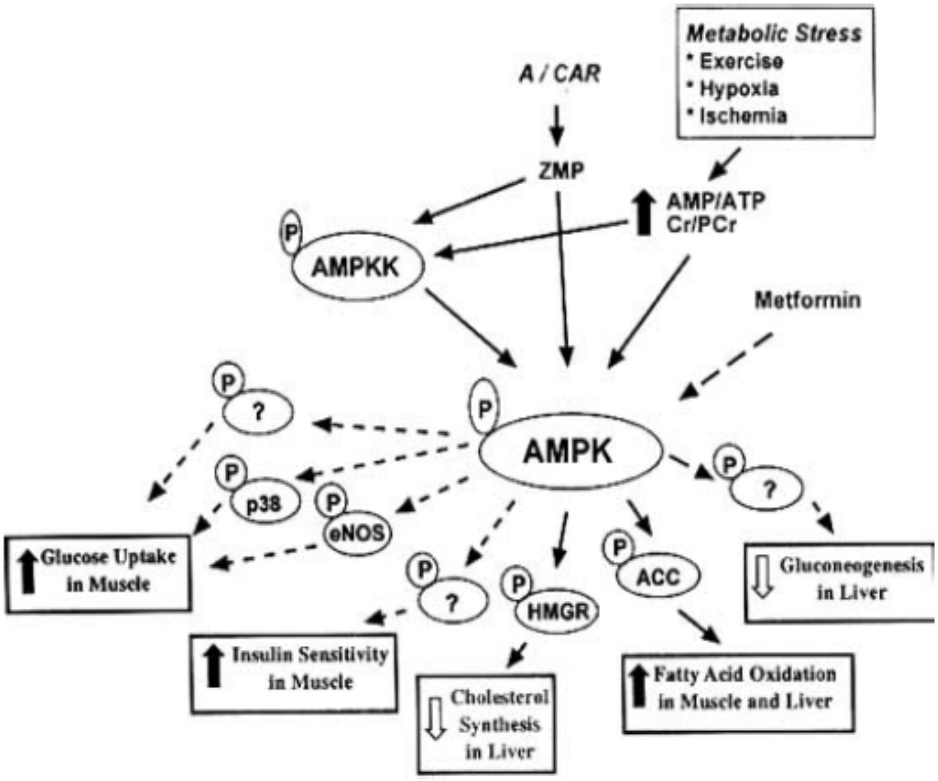


Figure 2. AMPK signaling pathway (Taken from Musi & Goodyear, 2002)

AMPK is considered to be the master energy-sensing switch in muscle cells that regulates both glucose and lipid metabolism. The AMPK pathway can be stimulated by a variety of stressors including muscle contraction, electrical stimulation of muscle tissue, hypoxia, various hormones and hormone analogs, and pharmaceutical agents. Many of the chemical and stress-related stimuli of AMPK and the metabolic target of this kinase can be seen above in Figure 2. In addition to allosteric regulation by AMP, upstream

kinases also regulate AMPK by phosphorylation (Misra & Chakrabarti, 2007). AMPK is a heterotrimeric protein whose activation by an upstream kinase is dependent upon the phosphorylation at the Thr^{172} residue of the catalytic domain in its α-subunit (Stein, Woods, Jones, Davison, & Carling, 2000). AMPK is activated in low energy states where ATP stores are being depleted and AMP levels are increasing. AMP serves to both allosterically activate AMPK and AMPK kinase (AMPKK). AMPKK then serves to phosphorylate and further increase AMPK activity. Furthermore, muscle contraction lowers levels of creatine phosphate, which is an allosteric inhibitor of AMPK (Hardie, Carling, & Carlson, 1998). Consequently, active AMPK then inhibits ATP consumption processes and stimulates glucose and lipid metabolism to restore cellular ATP levels and rectify the energy deficit that is present in the cell. Even though the present study will typically discuss AMPK in relation to muscle tissue, AMPK can also play an important role in the liver as well where its activation enhances fatty acid oxidation and inhibits production of glucose, cholesterol, and triglycerides (Hardie et al., 1998).

Although exercise stimulates GLUT4 translocation independent of insulin and the AMPK pathway is one possible mechanism for this action, it is imperative to also ask if insulin resistance affects AMPK activation. In 2001, Musi et al. were the first to investigate this relationship. In their study, 7 participants with type II diabetes and 8 matched controls exercised on a cycle ergometer for 45 min at 70% of their maximum workload. They found that blood glucose concentrations of the diabetic group were lowered from 7.6 mmol/L to 4.77 mmol/L after 45 min of exercise, while there was no change in blood glucose levels in the control group. Furthermore, exercise significantly increased AMPKα2 activity 2.7-fold in both groups after 20 min of exercise. AMPKα2

remained significantly doubled at least 30 min post-exercise (Musi et al., 2001). These results indicate that activation of the AMPK pathway through exercise or pharmaceutical intervention could provide therapeutic means to manage blood glucose levels in insulin resistant individuals. More recently, however, Sriwijitkamol et al. (2007) found that exercise-stimulated AMPK activity and AS160 phosphorylation was suppressed in obese and type II diabetic patients compared to lean control participants. The authors then concluded that obese type II diabetic patients might need to exercise at higher intensities to stimulate the AMPK signaling pathway to the same extent as the lean individuals.

While AMPK and glucose uptake are stimulated by exercise, the mode, intensity, and duration of the exercise are critical to determining the extent of exercise-stimulated AMPK activation and glucose uptake (Dreyer et al., 2008; Treebak et al., 2007). Sriwijitkamol et al. (2007) investigated the effects of varying exercise intensities (50% VO_{2max} and 70% VO_{2max}) on AMPK activation. They found that there was a significant time- and intensity-dependent relationship between AMPK activity and AS160 phosphorylation. Recently, results from a study from Treebak and colleagues (2007) were consistent with the Sriwijitkamol study. In the Treebak study, participants performed one of four cycling exercises: 90 min at 67% VO_{2peak}, 20 min at 80% VO_{2peak}, 110% of peak work rate, or a 30 sec maximal sprint. These investigators found that AS160 and AMPK phosphorylation increased in a time-dependent manner and were positively correlated in the 90 min trial, but did not change in the three shorter-duration trials. In contrast to the aerobic exercise studies, Dreyer et al. assessed the phosphorylation of AS160 following a resistance exercise session (leg extensions, 10 sets x 10 reps). Although glucose uptake increased during exercise and post-exercise

recovery, Akt phosphorylation did not increase until 1 hr and AMPKα2 until 2 hr post-exercise. As expected based on these results, AS160 phosphorylation was unchanged immediately post-exercise, but was significantly increased at 1 hr and 2 hr post-exercise (Dreyer et al., 2008). The results of these studies reveal that higher intensity, longer duration bouts of exercise are required to create the energy demand sufficient to significantly activate the AMPK pathway, particularly in obese, insulin resistant individuals.

Pharmaceutical Interventions: Introduction to Resveratrol

Due to its ability to stimulate glucose uptake and essentially bypass insulin signaling, the AMPK pathway provides a viable target for many pharmaceutical interventions for insulin resistance (Misra & Chakrabarti, 2007; Musi et al., 2001; Musi & Goodyear, 2006; Yamauchi et al., 2002; Zhou et al., 2001). Many experimental procedures utilize 5-aminoimidazole-4-carboxamide-ribonucleoside (AICAR) to stimulate the AMPK pathway. AICAR is an adenosine analog that is phosphorylated upon transport into the muscle cell to form an analog of AMP, 5-aminoimidazole-4-carboxamide-ribonucleotide (ZMP), and thus activate AMPK. AICAR has been shown to specifically activate the AMPK pathway and, therefore, increase peripheral glucose uptake and decrease hepatic gluconeogenesis (, Kurth-Kraczek, & Winder, 1999a; Jessen, Pold, Buhl, Jensen, Schmitz, & Lund, 2003; Jing, Cheruvu, & Ismail-Beigi, 2008). AICAR was previously studied in a phase I safety and pharmacokinetic trial in humans for its potential uses during myocardial ischemia (Nawarskas, 1999). The results of the study, however, revealed that AICAR has a short half-life and poor bioavailability, while also significantly elevating serum uric acid levels. As a result, AICAR is not a potential

intervention drug treatment for type II diabetes. However, due to its high potency in stimulating the AMPK pathway, AICAR is frequently used in animal model research studies investigating the AMPK pathway.

Metformin, on the other hand, is a drug that is commonly prescribed to treat insulin resistance as it has been shown to decrease blood glucose concentrations and improve blood lipid profiles (Misra & Chakrabarti, 2007; Santos et al., 2008; Zhou et al., 2001). Metformin's proposed mechanism of action to treat insulin resistance is also through activation of the AMPK pathway (Witczak, Sharoff, & Goodyear, 2008). Activation of AMPK by metformin in the liver results in inhibition of gluconeogenesis and acetyl-CoA carboxylase (ACC) and activation of carnitine palmitoyl transferase (CPT-1) and fatty acid oxidation. Metformin also activates AMPK in skeletal muscle where it serves to increase glucose uptake by the muscle. Although metformin is an effective treatment for diabetes, it has a low potency and is therefore taken in high doses. These larger doses can then produce moderate GI distress (i.e. diarrhea, nausea, vomiting) in some individuals (Zhou et al., 2001). Furthermore, the Diabetes Prevention Program compared the effects of metformin treatment to aggressive lifestyle intervention (supervised diet and exercise modification) in prediabetic persons and the lifestyle intervention reduced diabetes progression by 58%, while metformin (with traditional, unsupervised diet and exercise recommendations) only reduced it by 31% (compared to control group) (Knowler et al., 2002). The difference in effectiveness of the two treatments could be because of metformin's low efficacy or because the lifestyle modifications made by the lifestyle intervention group were much more dramatic than the volitional changes encouraged in the metformin group. Nonetheless, a potent AMPK

activator with high bioavailability and minimal adverse side effects is desirable for the treatment of insulin resistance.

Resveratrol could be a natural alternative therapeutic agent that could provide beneficial treatment to help type II diabetic or prediabetic individuals improve their glucose tolerance. Resveratrol (*3,5,4'-trihydroxystilbene;* Figure 3) is a polyphenolic extract of red wine that is also found in high concentrations in grape skins and the tree bark and root of certain tree species (Remsberg et al., 2008; Su et al., 2006). In addition to the many beneficial antioxidant, anti-inflammatory, cancer preventive, and cardiovascular effects of resveratrol (Athar et al., 2007; Frojdo et al., 2007; Miyazaki et al., 2008; Park et al., 2007), this compound has also been shown to improve insulin-sensitivity by enhancing GLUT4 translocation (Park et al., 2007; Su et al., 2006). Resveratrol derives the majority of its antioxidant properties from the two hydroxyl groups on the first benzene ring (Fang & Zhou, 2008). Additionally, Wright et al. have reviewed numerous studies that investigated the effects of antioxidant supplementation on insulin resistance (Wright & Sutherland, 2008). Based on the evidence from these studies, the antioxidant properties of resveratrol could also improve insulin sensitivity by preventing the oxidative stress-induced insulin resistance that results from decreased IRS-1 activity. Furthermore, resveratrol is also a known allosteric activator of the sirtuin, SIRT1 (Jiang, 2008). SIRT1 activation has also been shown to mediate insulin signaling by regulating IRS1 and IRS2 activity (Sun et al., 2007; Zhang, 2007). However, resveratrol has also been shown to stimulate glucose uptake independent of insulin-signaling pathways (Su et al., 2006). As a result, the primary effects of resveratrol supplementation on glucose homeostasis could likely be dependent upon its activation of

the AMPK pathway. Initial mechanistic research has shown that resveratrol does not allosterically stimulate AMPK, but it appears to activate the upstream serine/threonine kinase, LKB1, which then activates AMPK by phosphorylation at Thr^{172} (Chan et al., 2008). Although resveratrol has been shown to considerably activate the AMPK pathway, the precise mechanism by which resveratrol stimulates LKB1 and/or AMPK has yet to be elucidated.

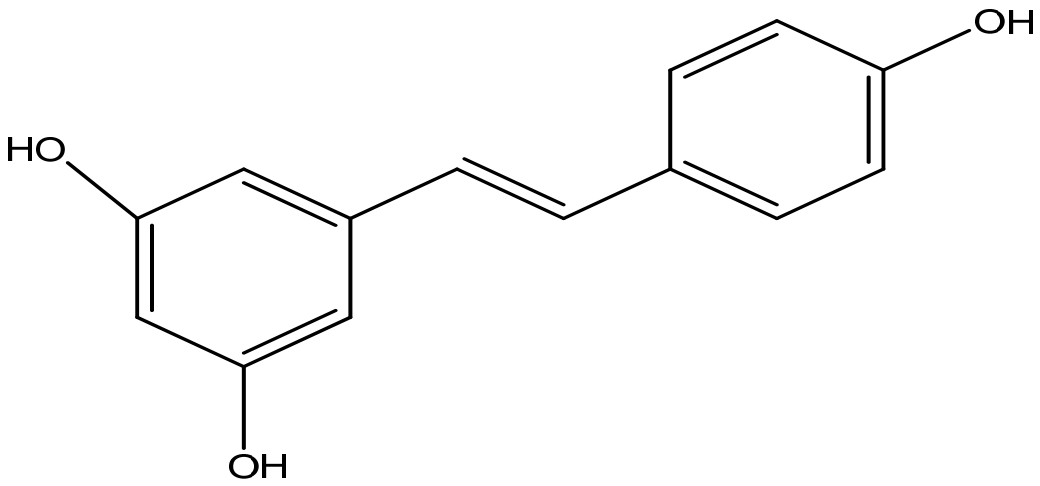


Figure 3. Chemical structure of *trans*-resveratrol

Resveratrol and the AMPK Pathway

One of the first studies to display the antidiabetic effects of resveratrol was published by Su et al. (2006). These researchers used male Sprague-Dawley rats treated with streptozotocin and nicotinamide, which provide a moderate insulin-deficient diabetic rodent model with stable hyperglycemia and reduced pancreatic insulin stores. The researchers found that acute resveratrol ingestion (0.5 mg/kg body weight) by these rats resulted in significant reductions in blood glucose concentration after 90 and 120 min.

Moreover, when orally fed resveratrol for 14 days, the rat's plasma glucose concentration was reduced by 20.3% and the triglyceride concentration by 33.3%. The plasma insulin levels of these rats was also significantly reduced when compared to the vehicle treated controls. Additional testing of the rat muscle under optimal insulin concentrations showed no attenuation of the resveratrol-induced effects upon glucose disposal. This finding ultimately led the authors to conclude that these anti-hyperglycemic properties of resveratrol act through a distinct mechanism that is different from the insulin-signaling / PI3K pathway (Su et al., 2006). Another study by Park et al. (2007) revealed that this distinct mechanism is in fact the AMPK signaling pathway. In order to study the effects of AMPK activation and glucose transport, these scientists incubated C2C12 myotubes with resveratrol and/or insulin and observed the effects upon AMPK. Resveratrol served to increase glucose uptake by activating AMPK, but not PI3K. Additionally, resveratrol amplified the effects of insulin on glucose uptake via AMPK activation, which led to further activation of the PI3K pathway. The authors also noted that leptin and adiponectin also improved insulin sensitivity by AMPK activation. Park et al. emphasized that AMPK seems to be a critical target for improving insulin resistance and that resveratrol has the ability to activate such an important signaling pathway. However, the exact mechanisms involved in the interaction of resveratrol, AMPK, and the PI3K/Akt pathways are not yet fully elucidated (Park et al., 2007).

Although the following studies investigate the effects of AMPK activation by AICAR and not resveratrol specifically, it is of great interest to see if resveratrol supplementation can evoke similar responses in humans. These studies revealed that short-term AICAR administration mimics exercise training effects on skeletal muscle in

terms of increasing insulin sensitivity, mitochondrial enzymes, GLUT4 content, and exercise-induced AMPK activation (Buhl et al., 2001; Holmes, Kurth-Kraczek, & Winder, 1999; Jessen, Pold, Buhl, Jensen, Schmitz, & Lund, 2003). Holmes, Kurth-Kraczek, and Winder (1999) showed that 5 day administration of AICAR (subcutaneous injection each morning) results in chronic activation of the AMPK pathway and a two-fold increase in GLUT4 content, hexokinase (HK) activity, and glycogen content of rat skeletal muscle. Although glucose uptake was not directly measured in this study, the authors speculated that AICAR treatment did increase glucose uptake and not just limit hepatic glucose production. Since acute administration of AICAR not only resulted in decreased blood glucose levels, but also increased muscle glycogen content and blood lactate levels, these data indicate an increase in glucose transport into the muscle (Holmes, Kurth-Kraczek, & Winder, 1999). Since increases in GLUT4 content, HK activity, and super-compensation of glycogen are all physiological adaptations that result from endurance training, other investigations have attempted to compare the effects of AICAR administration to short-term exercise training. In one such study, Wister rats underwent either a 5-day exercise training program on a treadmill, received daily AICAR injections, or received no treatment (Jessen, Pold, Buhl, Jensen, Schmitz, & Lund, 2003). Both exercise training and chronic AICAR treatment resulted in enhanced insulin signaling, improved glucose transport, and increased GLUT4 expression. It is interesting that only AICAR treatment enhanced glucose transport via both insulin-mediated and insulin-independent mechanisms. The AICAR-treated rat muscles showed a significant increase in insulin-stimulated IRS1-associated PI3-kianse, Akt1 and Akt2 activity, and phosphorylated Akt. Exercise trained rats, however, revealed no changes in these

markers of the insulin-signaling pathway. The authors concluded that these differences could be a result of shorter daily activation of the AMPK pathway in the 1 hr daily treadmill training than the daily AICAR injection, but further research is needed to confirm their speculations (Jessen, Pold, Buhl, Jensen, Schmitz, & Lund, 2003). These training and AICAR-induced glucose transport adaptations in skeletal muscle are isoform specific, however. Whereas the AMPKα-2 isoform is necessary for AICAR-induced improvements in glucose metabolism, the metabolic adaptations to exercise training are still present in AMPKα-2 knockout mice (Jorgensen et al., 2007). Therefore, this finding shows that AICAR's effects upon GLUT4 translocation are strictly dependent upon AMPK stimulation, while there are additional mechanisms involved in exercise-stimulated glucose uptake that are not solely dependent upon AMPK (i.e. calcium/calmodulin-dependent protein kinase, atypical protein kinase C). More recently, McConell and colleagues investigated the effects of exercise training or AICAR treatment on the attenuation of AMPK activation during acute exercise (McConell et al., 2008). These researchers found that 10 days of treadmill training (60 min/day) and 10 days of AICAR treatment in male Sprague-Dawley rats both attenuate immediate post-exercise (45 min on a treadmill) increases in AMPK and ACC phosphorylation. These results indicate that the muscle increases its metabolic efficiency and therefore decreases its AMPK activation during exercise in response to both chronic activation of AMPK via AICAR or through frequent stimulation by exercise training. As a result, future studies should compare the effects of AMPK activation by daily resveratrol supplementation to those of daily exercise training and determine the effectiveness of each to manage glucose homeostasis.

Resveratrol's Interactions with Other Hormones

Resveratrol can also improve insulin sensitivity through its interactions with other hormones. Since resveratrol is a phytoestrogen, many of its cardioprotective and anti-carcinogenic effects are mediated through interactions with estrogen and the estrogen receptor (ER). Additionally, the primary form of estrogen in the body, 17β-estradiol, is known to be a regulator of insulin sensitivity and glucose homeostasis. Resveratrol is a 17β-estradiol agonist that works through interaction with the ERα to up-regulate GLUT4 expression and thus improve glucose uptake (Deng, Hsieh, Huang, Lu, & Hung, 2008). One study showing how critical ERα is to glucose homeostasis revealed that male and female mice with a knockout (KO) ERα gene had insulin resistance, impaired glucose tolerance, and adipocyte hyperplasia and hypertrophy (Heine, Taylor, Iwamoto, Lubahn, & Cooke, 2000). Not only could the ER play a role in resveratrol's effects upon glucose uptake, but circulating estrogen levels could also affect glucose homeostasis as well. As a result, studies involving female participants should control for oral contraceptives and testing should be standardized to a time in the menstrual cycle where estrogen levels plateau.

Furthermore, research from the lab of Tomasz Szkudelski has shown that resveratrol not only improves insulin sensitivity measures by reducing hepatic gluconeogenesis and stimulating glucose disposal in skeletal muscle, but also by inhibiting pancreatic secretion of insulin (Szkudelski, 2006; Szkudelski, 2008). Throughout various studies with pancreatic islet cells, these authors have found that resveratrol suppresses insulin secretion from these cells. Large concentrations of resveratrol even suppress insulin secretion stimulated by somatostatin, protein kinase C,

or acetylcholine. Although these studies were performed in culture medium, it can be speculated that resveratrol's ability to improve insulin sensitivity (product of glucose and insulin concentrations), is a result of both improving glucose disposal and directly reducing insulin secretion (Szkudelski, 2006; Szkudelski, 2008). This insulin suppressive effect could also provide some therapeutic benefit to type II diabetic patients where β-cell rest is necessary to prevent eventual failure of the β-cells to produce insulin (development of type I diabetes). However, more *in vivo* research is needed to determine if physiologically feasible levels of resveratrol can suppress insulin secretion prior to the decrease in blood glucose concentration (which would also result in decreased insulin secretion).

Pharmacokinetics and Bioavailability of Resveratrol

While these studies about resveratrol seem promising and supplement companies use them to make numerous claims about the beneficial properties of resveratrol, some researchers question the ability to generalize these findings to a clinical human population. A recent review from Cottart and colleagues (Cottart, Nivet-Antoine, Laguillier-Morizot, & Beaudeux, 2010) summarized the information from studies regarding the bioavailability of resveratrol in human participants. While resveratrol is efficiently absorbed in the body (>70%), it is also promptly metabolized by the intestine and liver which then severely limits the bioavailability of free resveratrol (Walle, Hsieh, DeLegge, Oatis, & Walle, 2004). This process is further indicated by pharmacokinetic studies that found minimal free resveratrol concentrations and vast amounts of resveratrol conjugates in the plasma of mice and humans following oral resveratrol administration (Almeida et al., 2009; Boocock et al., 2007; Burkon & Somoza, 2008; Cottart et al.,

2010; Goldberg, Yan, & Soleas, 2003; Nunes et al., 2009; Walle et al., 2004; Wenzel & Somoza, 2005). While both sulfate and glucuronic acid conjugation of resveratrol is commonly seen, sulfation appears to be the main factor limiting free resveratrol's bioavailability in humans. Boocock et al. (2007) was the first to publish a phase I dose escalation pharmacokinetic study of resveratrol in human participants. This study investigated dosages of administration of 0.5, 1, 2.5, or 5 g of resveratrol on plasma levels of resveratrol and its related metabolites. Their results indicated that the area under the plasma concentration curve (AUC) for those two metabolites (resveratrol-3-sulfate and resveratrol monoglucuronide) were up to 23 times greater than the AUC of the parent compound with 77% of resveratrol and its metabolites being excreted in the urine within the first 4 hours. Based on these results, these authors concluded that it was not plausible for even high-dose resveratrol consumption (5 g) to provide a sufficient concentration of *trans*-resveratrol in the plasma to meet the 5 μmol/l concentration determined by *in vitro* mechanistic studies to be required to elicit chemopreventive effects (Boocock et al., 2007).

A previous study investigated the effects of administration medium on the absorption and bioavailability of resveratrol. In this cross-over study, participants were provided 25 mg/70kg *trans*-resveratrol in white wine (11.5% ethanol), grape juice, and vegetable juice/homogenate (Goldberg et al., 2003). Despite their hypothesis that alcohol could promote absorption, the administration matrice did not seem to affect the absorption or bioavailability of resveratrol with 1.7-1.9% of peak serum concentration being free *trans*-resveratrol. More recently, Nunes et al. (2009) assessed the effect of repeated resveratrol administration on plasma levels of resveratrol. Although 3 days of

200 mg resveratrol supplementation 3 times a day resulted in higher plasma concentrations (than a single 200 mg dose), these differences were not statistically or clinically relevant. Around the same time, another research group studied the pharmacokinetics of multiple-dose resveratrol administration (Almeida et al., 2009). This group had participants ingest 25, 50, 100, or 150 mg of *trans*-resveratrol every 4 hours for a total of 13 doses. While all levels were well tolerated and the higher dosages resulted in larger plasma concentrations, these concentrations were still markedly below the 5 μmol/l level necessary to elicit pharcologic effects. The researchers also noticed significant diurnal variations in the *trans*-resveratrol pharmacokinetics. They found that the *trans*-resveratrol minimum concentrations between doses were highest in the morning and lowest at night (Almeida et al., 2009). In another study, this group also found that oral ingestion of 400 mg of *trans*-resveratrol following a standard high fat meal significantly delays the absorption (time to peak concentration: 120 min vs. 30 min) of resveratrol, but does not affect the overall serum levels (AUC) when compared to ingestion following an 8 hr fast (Vaz-da-Silva et al., 2008). Other studies have attempted to circumvent this polyphenol conjugation by intravenous resveratrol administration (Walle et al., 2004). Since oral ingestion of resveratrol is conjugated during absorption and then is further metabolized in the liver before it reaches the systemic circulation, it was hypothesized that intravenous administration might result in enhanced delivery of *trans*-resveratrol to target tissues. Although it is distributed throughout the body before it reaches the liver, this study still revealed significant systemic metabolism of resveratrol with the intravenous administration (Walle et al., 2004).

While this system of conjugation is typically part of the body's defense mechanisms for protection against toxicity, the conjugated form of some polyphenols yields similar effects as their parent compound (Boocock et al., 2007; Yoshizumi et al., 2002). Yoshizumi et al. (2002) found that a glucuronidated form of quercitin work similarly to free quercitin in inhibition of angiotensin II-induced vascular smooth muscle hypertrophy through inhibiting c-Jun N-terminal kinase (JNK) activation and the activator protein-1 pathway. At this point, the pharmacological properties of resveratrol conjugates are unknown and could potentially augment the pharmacological activity demonstrated by pure *trans*-resveratrol (Boocock et al., 2007). Although there is still the possibility that conjugated forms of resveratrol could possess similar pharmacological properties, this conjugation also makes the generally lipophilic polyphenol more polar and water-soluble, which limits its ability to enter the cell and ultimately facilitates its excretion (Goldberg et al., 2003).

As mentioned in the previous studies, *trans*-resveratrol has high absorption rates, but very low bioavailability. As a result, serum levels of *trans*-resveratrol are not at a high enough concentration for cancer prevention based on existing *in vitro* mechanistic studies. However, the specific resveratrol concentration necessary to induce *in vivo* pharmacological effects related to glucose homeostasis, such as AMPK stimulation, has yet to determined (Boocock et al., 2007; Walle et al., 2004). Additionally, the fate of the remaining 30% that is not initially excreted has yet to be elucidated. Due to its lipophilic nature, a large portion of resveratrol could be bound to cellular membranes. As a result, the small quantities of *trans*-resveratrol measured in the plasma fraction might not

account for the resveratrol contained within the cellular fraction (Cottart et al., 2010; Soleas, Yan, & Goldberg, 2001).

Resveratrol and Glucose Homeostasis

The previously mentioned studies by Su et al. and Park et al. (Park et al., 2007; Su et al., 2006) revealed that resveratrol stimulates glucose uptake in insulin resistant animal models. It has also been shown that insulin-signaling and AMPK stimulation (exercise or resveratrol) can have additive effects on maintaining blood glucose levels (Holmes, Kurth-Kraczek, & Winder, 1999; Jessen, Pold, Buhl, Jensen, Schmitz, & Lund, 2003; Merrill, Kurth, Hardie, & Winder, 1997). However, motivating the general public to make the lifestyle modifications necessary to improve their insulin resistant state can be difficult. As a result, investigators examined the effects of resveratrol supplementation and a high-calorie diet on overall glucose homeostasis. Deng et al. (2008) specifically studied the effects of resveratrol on insulin sensitivity in rats fed a high cholesterol and fructose diet (HCF) for 15 weeks. This protocol was implemented to mimic a typical Western diet, with diet-induced weight gain chosen over genetic modification, so that anti-obesity factors did not complicate the insulin resistance findings in obese rats. The rats that were administered the HCF only diet acquired insulin resistance syndrome which was characterized by an increase in blood pressure, hyperlipidemia, hyperinsulinemia, and impaired glucose tolerance with a relatively normal body weight. Resveratrol supplementation in conjunction with the HCF diet, however, resulted in metabolic characteristics that were similar to those of rats fed a standard diet. Resveratrol also worked to potentiate the insulin-stimulated effects on whole body glucose uptake and steady-state glucose uptake of the liver, epididymal fat tissue, and isolated soleus muscle

in rats fed the HCF diet (Deng et al., 2008). In a similar study, mice fed a high-calorie diet plus resveratrol had significantly lower blood glucose levels and higher insulin sensitivity compared to rats fed a standard or a high-calorie diet (Baur et al., 2006). These studies reveal that resveratrol supplementation can improve insulin sensitivity and blood glucose homeostasis even in the presence of high-calorie diets.

Summary

In summary, insulin resistance is a serious metabolic disturbance that threatens the health of millions of individuals worldwide. Although lifestyle modifications such as exercise and an anti-atherogenic, calorie restrictive diet have been shown to improve insulin resistance and potentially prevent the onset of type II diabetics, pharmacological interventions are necessary to improve these conditions in some individuals under certain circumstances. While there are numerous pharmaceutical treatments for insulin resistance, each has its own flaws and disadvantages. Resveratrol, however, is a natural alternative with numerous health benefits that could also provide potential therapeutic advantages to insulin resistance. The current literature reveals that resveratrol improves insulin sensitivity by activating the AMPK pathway within skeletal muscle and thus stimulating glucose uptake into the muscle. However, the majority of the research has been performed in animal models and more research needs to be done to investigate whether resveratrol can generate these beneficial results at physiologically safe doses in humans. As a result, the current study investigated the effects of resveratrol supplementation on glucose/insulin kinetics and expression of insulin and AMPK signaling intermediates in skeletal muscle of overweight or obese females.

CHAPTER THREE

Methods

Participants

Sixteen sedentary, overweight or obese females between 18 and 40 years of age completed the study. An a priori sample size calculation revealed that 6 participants per group was necessary to detect a significant difference in the glucose and insulin response to the OGTT given a Type I error rate of 0.05 and a power of 0.80 (Braun et al., 2004; Tuomilehto et al., 2001). Twenty participants initially qualified and began the study, but four participants dropped out at some point during the study. Of those that dropped out, two participants were in the placebo group and two were in the resveratrol group. None of these participants failed to complete the study as a result of the supplement, but were a result of personal reasons and time constraints. Participants were declared sedentary if they did not exercise for 30 min a day at least 5 days a week for a year or more. Participants were declared overweight if they had a body mass index (BMI) greater than 25 kg/m^2. The calculation for BMI used was body mass (kg) divided by the square of the height (m) (Whaley, Brubaker, Otto, & Armstrong, 2006). Participants must have also had a body fat percentage ≥ 30%, as determined by dual energy x-ray absorptiometry (DEXA), to participate in this study. Initial eligibility requirements specified that participants had to be prediabetic. For participants to qualify as prediabetic, their fasting blood glucose had to be between 100-125 mg/dL. These measurements were taken on two separate occasions using capillary blood from the finger and a handheld glucometer. Prior to participation, all eligible participants obtained written approval from their

primary care physician. Participants who qualified for the study were cleared for participation by successfully completing a series of health screening examinations (i.e., health screening questionnaire, blood pressure assessment, fasting glucose assessment) by qualified study personnel. Additionally, since oral contraceptives appear to improve glucose tolerance by reducing peripheral tissue insulin insensitivity in young women and, therefore, all participants were women who had not taken oral contraceptives for at least two months prior to beginning the study (C. Kim et al., 2002). Furthermore, since estrogen is a hormone that also influences insulin sensitivity, then testing of insulin sensitivity was conducted when the participant had consistent levels of estrogen (Deng et al., 2008). As a result, all testing sessions took place during the luteal phase (days 16-26) of the participant's 28-day menstrual cycle. Participants were not allowed to participate if they met any of the following criteria: 1) had any metabolic disorders including known electrolyte abnormalities; heart disease, arrhythmias, diabetes, thyroid disease or hypogonadism; a history of medically controlled hypertension, hepatorenal, musculoskeletal, autoimmune, or neurologic disease; if they were taking thyroid, hyperlipidemic, hypoglycemic, anti-hypertensive, or androgenic medications; 2) had ingested any thermogenic nutritional supplements for a 2 month time period prior to beginning the study; 3) took any additional nutritional supplement or contraindicated prescription medication during the protocol. All participants meeting entrance criteria signed informed consent statements in compliance with the Human Participants Guidelines of Baylor University and the American College of Sports Medicine. Participants expressing interest in participating in this study were interviewed on the phone to determine whether they appeared to qualify to participate in this study.

Participants believed to meet eligibility criteria were then invited to attend an entry/familiarization session.

Study Site

All testing sessions were conducted in the Exercise & Sport Nutrition Laboratory (ESNL) and the Exercise & Biochemical Nutrition Laboratory (EBNL) in the Department of Health, Human Performance, and Recreation at Baylor University.

Independent and Dependent Variables

The independent variables included the nutritional supplementation protocol, oral glucose tolerance test, and graded exercise testing protocol. Dependent variables included body mass, body composition, waist circumference, measurements of serum glucose and insulin, muscle gene expression measures, serum clinical safety markers, and resting heart rate and blood pressure.

General Overview of the Testing Protocol

This study protocol included 1 familiarization session and 3 testing sessions. The first session was a familiarization session where the participants received verbal and written explanations of the study protocol. This session also included an initial fasting blood glucose screening and assessment of body mass and height to ensure that the participants met the entrance criteria. The second testing session began with another blood glucose screening to confirm that the participant was indeed prediabetic. If the second glucose reading confirmed that the participant was prediabetic, they then underwent body mass, body composition, and hemodynamic safety marker assessments. A baseline (T1) blood and muscle tissue sample was then taken and the participant was

randomized into a supplementation group and began the supplementation regimen. The participant then ingested the respective supplement each morning for the next 5 days, while also recording dietary intake each day. After 5 days of supplementation, the participant then returned to the lab for the second testing session (T2). At the beginning of this session, the participant ingested the supplement and body mass, total body water, resting heart rate, and resting blood pressure was assessed. Baseline blood and muscle biopsy samples were then collected and the participant then in ingested a 75 g glucose solution for the oral glucose tolerance test (OGTT). Following the glucose ingestion, the participant rested for 2 hr and a muscle biopsy sample was taken at 1 hr post-ingestion, while venous blood samples were taken at 30 min, 1 hr, and 2 hr post-ingestion. The participant would then return the following day for the final testing session (T3). At the beginning of this session, the participant ingested the supplement and body mass, resting heart rate, and resting blood pressure were initially assessed. Baseline blood and muscle biopsy samples were then collected and the participant performed a GXT. Following the conclusion of the GXT, the participant rested for 2 hr and a muscle biopsy sample was taken at 1 hr post-ingestion, while venous blood samples were taken at 30 min, 1 hr, and 2 hr post-ingestion. Table 1 provides a schematic overview of the testing protocol, while the following paragraphs provide more specific details about the testing procedures.

Table 1. *General overview of the testing protocol*

FAM Session & Initial Glucose Screening	Day 1 2nd Glucose Screening & Baseline Testing (T1)	Days 2-5 Supplementation Period	Day 6 Oral Glucose Tolerance Testing (T2)	Day 7 Exercise Testing (T3)
Familiarization session & Informed Consent Health Status quest. Review medical history	Second blood glucose screening Body mass, BIA, DEXA, waist girth, HR, & BP assessments	Continue supplementation with placebo or resveratrol	Ingest supplement Body Mass, BIA, HR, & BP assessment Blood & muscle biopsy sample Glucose (75 g) administration	Ingest supplement Body Mass, HR, & BP assessment Blood & muscle biopsy sample Graded exercise test (GXT)
Blood glucose screening Body mass, HR, & BP assessments Body mass & height assessments	Pre-supplementation blood & biopsy sampling Randomized, double-blind, placebo-controlled assignment to groups Begin supplementation with placebo or resveratrol	Monitor dietary intake	30 min post-glucose blood sample 1 hr post-glucose blood & biopsy sample 2 hr post-glucose blood sample Continue supplement intake and dietary monitoring	30 min post-GXT blood sample 1 hr post-GXT blood & biopsy sample 2 hr post-GXT blood sample

Familiarization and Initial Glucose Screening

During the familiarization session, participants completed a medical history questionnaire and personal information form (Appendix A) to help determine whether they met the eligibility criteria. The participants were familiarized to the study protocol through both verbal and written explanation outlining the study design. Individuals that wanted to participate in the study then read and signed the university-approved documents granted by the Institutional Review Board for Human Subjects of Baylor University (Appendix B).

Participants were instructed to refrain from exercise for 24 hours and fast for 12 hours prior to pre-testing and for all scheduled assessments. All participants reported to the lab in the morning for all scheduled assessments. Body mass was initially measured

in order to ensure that participants met the entry criteria for BMI. Fasting blood glucose was then assessed using a ReliOn® Ultima glucometer and testing strips (ReliOn®; Bedford, MA) to ensure that they were prediabetic (glucose: 100-125 mg/dL). The manufacturer states that the typical range of variation for the results of the ReliOn® Ultima glucometer and testing strips is from 2.9% - 5.1%. Resting heart rate (HR), and blood pressure (BP) measurements were also taken to screen for potential cardiovascular contraindications to exercise. Participants were finally instructed to return to the lab in two days, so that a second fasting blood glucose measurement could be taken to confirm that they were prediabetic.

Baseline (Pre-Supplementation) Testing Session (Day 1 – T1)

Participants had their fasting blood glucose checked upon arrival to ensure that their fasting glucose was between 100-125 mg/dL. Total body mass (kg), waist circumference, total body water (total, intracellular, and extracellular), percent body fat, fat mass, and fat-free mass were then assessed. Following these assessments, participants had resting heart rate and blood pressure determined using standard procedures. Participants then donated approximately 10-15 ml of fasting blood using venipuncture techniques of an antecubital vein in the forearm according to standard procedures. A muscle biopsy sample (10-15 mg) was then collected from the vastus lateralis muscle using the fine needle aspiration technique. The participants were then randomized into groups and immediately began the supplementation protocol.

Supplementation Protocol (Days 1-6)

Following baseline measurements, participants were matched based on body fat percentage and then randomly assigned in a double-blind manner to ingest capsules containing a cellulose placebo (250 mg) or resveratrol supplement (500 mg) twice daily for the duration of the study. RevGenetics brand resveratrol supplements were used for this study (Miami, FL). Each 500 mg capsule contained 495 mg of *trans*-resveratrol from the polygonum cuspidatum plant root. RevGenetics' supplements were made in the USA and underwent independent laboratory testing in the United States for purity and safety. RevGenetics provided a quality assurance report to verify that their product is 99% *trans*-resveratrol and this report is included in Appendix C. Nutricology® cellulose (Nutricology Inc.; Alameda, CA) placebo supplements were prepared in Capsuline® vegetarian gel capsules using a Capsuline® 60 capsule filling machine (Capsuline Inc.; Pompano Beach, FL). Placebo capsules were then packaged in generic containers for double-blind administration by an individual not associated with the study. Supplementation compliance was monitored by having the participants fill out supplement calendars recording the amount of supplement ingested during each day of the supplement period. Participants were instructed to consume the supplement for 5 days prior to the second testing session (T2).

Oral Glucose-Tolerance Test (Day 6 – T2)

Participants fasted for 12 hours and reported to the lab in the morning of the sixth day of supplementation. Upon arriving, participants ingested their respective supplement and then had their total body mass, total body water (total, intracellular, and extracellular), heart rate, and blood pressure measured. Participants then donated

approximately 10-15 ml of fasting blood using venipuncture techniques of an antecubital vein in the forearm according to standard procedures. Next, a muscle biopsy sample was collected from the vastus lateralis muscle using the fine needle aspiration technique. An OGTT was then performed, where the participant consumed a 75 g glucose solution (Casco-Nerl TRUTOL Glucose Tolerance Beverages; East Rutherford, RI) within a 5 min period. Additional venous blood samples (5 ml) were obtained from the antecubital vein at 30 min, 1 hr, and 2 hr after glucose ingestion. A 1 hr post-glucose ingestion muscle biopsy was also obtained. Participants were instructed to remain in a rested state during the 2 hr following the glucose administration. The OGTT was then used to determine the amount of time it took a bolus of glucose to be cleared from the blood. For instance, it takes more time and a greater amount of insulin to clear the same amount of glucose from an insulin resistant individual's blood. As a result, whole-body insulin sensitivity is best calculated from an equation that utilizes the reciprocal of the product of insulin and glucose concentrations (Matsuda & DeFronzo, 1999). In normal, non-insulin resistant individuals fasting plasma glucose levels should be below 6.1 mmol/L. Individuals with impaired glucose tolerance have fasting glucose concentrations between 6.1 and 7.0 mmol/L, while those with type II diabetes mellitus have concentrations above 7.0 mmol/L. Following an OGTT, the plasma glucose concentration should be below 7.8 mmol/L for a normal individual. Glucose concentrations between 7.8 and 11.1 mmol/L during an OGTT are indicative of impaired glucose tolerance, while those above 11.1 mmol/L are typically diagnosed as having diabetes mellitus. Again, although these criteria are typically utilized in clinical settings for diagnosis of insulin resistant states, research utilizing the euglycemic clamp technique has shown that more specialized

equations that incorporate the OGTT values are necessary to more accurately assess whole-body insulin sensitivity. As a result, the equation derived by Matsuda and DeFronzo (1999) was utilized in the current study to assess whole-body insulin sensitivity. The composite insulin sensitivity index derived by these researchers is:

$$\frac{10000}{\sqrt{(FPG \times FPI) \times (\mu OGTT[glu\cos e] \times \mu OGTT[insulin])}}$$

where FPG is the fasting plasma glucose concentration, FPI is the fasting plasma insulin concentration, and μOGTT[glucose] and μOGTT[insulin] are the mean plasma glucose and insulin concentrations during the OGTT. This equation incorporates both hepatic and peripheral insulin sensitivity in the basal state and after ingestion of a glucose load and is well correlated ($r = 0.73$, $p < 0.0001$) with direct measures of insulin sensitivity obtained from the euglycemic clamp technique (Matsuda & DeFronzo, 1999).

Graded Exercise Testing Session (Day 7 – T3)

Participants fasted for 12 hours and reported to the lab in the morning of the seventh day of supplementation. Upon arriving, participants ingested their respective supplement and then had their total body mass, heart rate, and blood pressure measured. A fasting blood sample (10-15 ml) was drawn using standard venipuncture techniques. Similarly, a muscle biopsy sample (10-15 mg) using the fine needle aspiration technique was also taken. It should be noted that both blood and muscle sampling was taken on the contralateral arm and leg that was tested during T2. A 12-lead electrocardiogram (ECG) was used to ensure that there are no contraindications during exercise testing based on the ACSM guidelines. The participant then performed a standard symptom-limited Modified Balke treadmill maximal exercise test to volitional exhaustion. The participant's oxygen

uptake (VO_2) and respiratory exchange ratio (RER) were also measured throughout the duration of the test to assess their aerobic capacity and substrate utilization. The specific testing procedure used during this test is detailed in the following section. Following the treadmill test, approximately 5 ml of blood was sampled using standard venipuncture techniques at 30 min, 1 hr, and 2 hrs following the cardiopulmonary exercise test to assess glucoregulatory and insulin sensitivity markers post-exercise. Muscle biopsy samples were obtained prior to and 1 hr post-exercise for assessment of gene expression changes of glucoregulatory intermediates. Again, participants remained in the laboratory in a rested state for the 2 hr following the exercise bout.

Experimental Testing Procedures

Graded Exercise Testing

At the beginning of the third testing session, participants performed a standard symptom-limited Modified Balke treadmill maximal exercise test to volitional exhaustion. VO_2 and the RER were measured every 15 sec via an open-circuit sampling system (Parvo Medics 2400 TrueMax Metabolic Measurement System, Sandy, UT). The VO_2 measure was used to assess a person's aerobic capacity, while RER (VCO_2/VO_2) was used to determine which substrates were being utilized for energy production. To obtain these measurements, the participants wore both noseclips and a cranial harness with a sterile mouthpiece. Once the participant was ready to begin the test protocol, they would straddle the treadmill with both legs while the treadmill was turned on at a speed of 2.0 mph and at a 0% grade. The participant would then use one foot to repeatedly swipe the belt in order to gauge the speed of the motion. Once the participant was familiar with this speed, they would step onto the belt while still gripping the handrail

with both hands. Once the participant became comfortable walking on the treadmill, they would let go of the handrail and begin walking freely. The participant would then perform a standard symptom-limited Modified Balke treadmill maximal exercise test using the utilizing the protocol detailed in Table 2. Throughout the treadmill test, blood pressure was accessed via standard procedures, while heart rate and rhythm was assessed using an ECG. The test would stop when the participant felt that she can no longer continue or if any of the ACSM's criteria for termination of an exercise stress test were observed (Whaley et al., 2006).

Table 2. *Modified Balke Protocol*

Stage	Speed	Grade (%)	Duration (min.)
1	3.4	0	1
2	3.4	1	1
3	3.4	2	1
4	3.4	3	1
5	3.4	4	1
6	3.4	5	1
7	3.4	6	1
8	3.4	7	1
9	3.4	8	1
10	3.4	9	1
11	3.4	10	1
12	3.4	11	1
13	3.4	12	1

Anthropometric and Body Composition Testing Procedures

Participants had height, total body mass, waist circumference, total body water (total, intracellular, and extracellular), and body composition measured during this study. Height was measured using standard anthropometry and total body mass (kg) was measured on a calibrated electronic scale with a precision of ± 0.02 kg (Cardinal Detecto Scale; Webb City, MO). Waist circumference was measured using a tension-regulated

retractable cloth tape measure (GF Health Products Inc.; Atlanta, GA) at the narrowest portion of the torso. Total body water (total, intracellular, and extracellular) was estimated using a Xitron 4200 bioelectrical impedance analyzer (BIA; Xitron Technologies Inc.; San Diego, CA). The BIA assessed these fluid volumes of the body by passing a small, alternating electrical current through the body and measuring the electrical impedance of the body tissues. More specifically, positive and negative surface electrodes were placed on the right hand and foot of the participant and a low energy, high frequency current (500 μ-amps at a frequency of 50 kHz) was transmitted through the body. The participant would lie in the supine position while the positive (red) electrode was placed on the dorsal surface of the medial portion of the right hand and the negative (black) electrode was placed on the dorsal surface at the distal end of the second metacarpals (just below the knuckles). The other positive electrode was then placed on the anterior surface of the right foot just distal to the talus, while the negative electrode was placed at the distal end of the first metatarsal. The BIA then measured the resistance of the body to the electrical current and calculated the intracellular fluid content, extracellular fluid content, and total body water (National Health and Nutrition Examination Survey (NHANES), 2000). Research has shown that the BIA is acceptable for assessing short-term changes in TBW within individuals over time with a precision of ±0.5% TBW (Wells et al., 1999). Fat mass, fat-free mass, bone mineral density, and percent body fat were determined with a Hologic® Discovery™ DEXA - QDR Series (Bedford, MA) by qualified personnel with limited radiation safety training (in compliance with state regulations). Quality control calibration procedures were performed on a spine phantom (Discovery W-CALIBER Model DPA/QDR-1

anthropometric spine phantom) prior to each testing session. This DEXA is a fan beam x-ray densitometer which uses an energy tube to produce two different energy levels that are then utilized to determine the density of the different body compartments. This test required the participant to lie in the supine position without any metal present on her clothing or body. A low dose of radiation was then used to scan her entire body for approximately 6 min. The radiation exposure from the DEXA for the whole body scan is ~1.5 mR per scan (National Health and Nutrition Examination Survey (NHANES), 2000). This radiation level is similar to the amount that a person would receive in one month while living in central Texas. After the scanning was complete, the DEXA segmented the scan of the body into 6 regions (head, right arm, left arm, trunk, right leg, and left leg) and into 3 tissue compartments (bone, muscle, and fat) based on the density of each tissue type. Test-retest reliability studies performed in the ESNL on male athletes have shown that this DEXA instrument has a precision of ±0.31% when measuring bone mineral content and total fat-free mass. Furthermore, previous research using DEXA and D_2O dilution has also shown a negligible mean error when measuring fat-free mass (Wells et al., 1999).

Assessment of Hemodynamic Safety Markers

Resting hemodynamic safety markers (heart rate and blood pressure) were assessed at the beginning of each testing session. Heart rate was determined by palpation of the radial artery using standard procedures. Blood pressure was assessed in the seated position using standard procedures with an aneroid sphygmomanometer (Whaley et al., 2006).

Venous Blood Sampling

Participants donated approximately 10-15 ml of fasting venous blood during each resting blood draw, approximately 5 ml during the post-OGTT blood draws, and approximately 5 ml during the post-GXT blood draws. All blood samples were obtained from the antecubital vein using standard phlebotomy procedures by study personnel trained in phlebotomy in compliance with guidelines established by the Texas Department of Health and Human Services. The phlebotomists and lab technicians wore personal protective clothing (gloves, lab coats, etc.) when handling blood samples. For each blood draw, participants were seated in a phlebotomy chair and their arm was cleaned with a sterile alcohol wipe and sterile gauze. A standard rubber tourniquet was then placed on the brachium (upper arm) and tightened enough to visibly indent the skin, but not cause the participant discomfort. For venipunctures, an antecubital vein was palpated and then a 22 gauge sterile needle attached to a plastic Vacutainer™ holder was inserted into the vein using standard procedures. One plasma tube, one serum separation tube, and one whole blood tube was inserted into the Vacutainer™ holder for blood collection in succession using multiple sample phlebotomy techniques. Once samples were obtained, the Vacutainer™ holder and needle were removed. The needle was then discarded as hazardous waste in a plastic sharps container. Once sampling was complete, the site of the blood draw was then cleaned with a sterile alcohol wipe and gauze and a sterile Band-Aid was placed on the site. The blood collection tubes were then be labeled and placed in a test tube rack. Laboratory technicians (who have received blood borne pathogen training and wore personal protective clothing) would centrifuge the serum

and/or plasma samples at 2,400 rpm for 15 min, transfer serum and/or plasma into labeled storage containers, and store at -80°C for later analysis.

Muscle Biopsy Sampling

Using a fine needle aspiration technique, percutaneous muscle biopsies (10-15 mg), were obtained from the middle portion of the vastus lateralis muscle of the leg at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. For subsequent biopsies, muscle tissue was extracted from the same location by using the previous location and depth markings on the needle. Pre-biopsy preparations included removing hair from the procedure area and wiping the area with 70% isopropyl alcohol prep pads. The biopsy site was numbed with 1 ml of 1% Lidocaine. After local anesthesia, the biopsy site was sterilized with 10% providone-iodine solution and a pilot hole was made with an 18-gauge needle. The biopsy procedure would then begin and take 15-20 sec to perform. Once the muscle sample had been obtained, pressure would be immediately applied to the biopsy site with sterile gauze. The site of the biopsy was then cleaned with a sterile alcohol wipe. The alcohol wipe and gauze then were discarded in an appropriately labeled biohazard waste receptacle. An adhesive bandage would then be immediately applied to the biopsy site. Bleeding was minimal due to the small puncture-type opening; therefore, only a standard band-aid type bandage was needed to cover the puncture. The biopsy needles were discarded as hazardous waste in an appropriately-labeled plastic sharps container. The tissue sample was placed in a cryogenic storage tube and immediately 'snap frozen' in liquid nitrogen. The frozen sample was then stored at -80°C for future analyses. Written instructions for post-biopsy care were given to the participants. The participant was then

instructed to leave the band-aid in place for 12 hr (unless unexpected bleeding or pain occurs) and asked to contact the lab immediately if they felt there was a problem. The time course of the study would then allow for follow-up to occur in order to ensure all biopsy locations are healing correctly and free of infection. Aside from the testing sessions, the participant was further advised to refrain from vigorous physical activity during the first 24 hours post-biopsy. These suggestions would minimize pain and possible bleeding of the area. If needed, the participant may have taken non-prescription analgesic medication such as Acetaminophen to relieve pain. However, medications such as aspirin, Nuprin, Bufferin, or Advil were discouraged as these medications may lead to ecchymosis at the biopsy site. Participants were informed that soreness of the area may occur for about 24 hours post-biopsy.

Serum Analyses

All samples were collected and immediately centrifuged for 15 min. Serum and plasma were then pippeted from the vials into microcentrifuge tubes and frozen at -80°C. Serum samples were sent to Quest Diagnostics (Irving, TX) for analysis. Glucose and lipid panels, consisting of LDL, HDL, total cholesterol, triglycerides, HDL/cholesterol ratio was analyzed from serum samples collected in serum-separator Vacutainer™ tubes.

Serum insulin levels (intra-assay CV 5.3%; inter-assay CV 5.6%) were analyzed using enzyme-linked immunoabsorbent assays (ELISA) kit (Alpha Diagnostic International; San Antonio, TX). The principle behind this ELISA is an enzymatically amplified sandwich type assay. Serum samples were diluted 1:10 with diluent buffer and dispensed into a 96-well microtiter plate whose wells were coated with an anti-insulin antibody that was used to 'capture' the insulin molecules in each sample. Next, 100 μL

of antibody-enzyme conjugate was then added to each well. This plate was then incubated for 30 min on an orbital shaker at room temperature. After this incubation, the remaining serum was washed from the plate five times with 300 μL of wash buffer and 200 μL of HRP-substrate mix was added to each well and allowed to incubate for 15 min. Finally, 50 μL of 0.2 M sulfuric acid stopping solution was added to each well and the absorbance of the solution was immediately read at a wavelength of 450 nm using a Wallac Victor^{2} ^{TM}1420 multilabel microplate reader (Perkin-Elmer Life Sciences; Boston, MA). A Jitterbug orbital microplate shaker (Boekel Scientific; Philadelphia, PA) was employed for all incubation periods and a MultiWash Advantage automated microplate washer (Tri Continent Scientific; Grass Valley, CA) during the plate washing steps for all insulin assays. All enzyme-linked immunoabsorbent assays was quantified using the Wallac Victor^{2} ^{TM}1420 multilabel microplate reader (Perkin-Elmer Life Sciences; Boston, MA). The plate reader measured the optical density of every standard, control, and unknown sample. All standards, controls, and unknowns were assayed in duplicate and were read at a wavelength of 450 nm, against a known standard curve. After the absorbances were measured, Mikrowin 2000 (Mikrotek Laborsysteme; Germany) data-reduction software was utilized to quantify the concentration of the specific analyte. Using the results from the fasting serum glucose and insulin analyses, insulin resistance was quantified using the homeostasis model assessment of insulin resistance (HOMA-IR). The following formula was used for HOMA-IR: (fasting serum glucose x fasting serum insulin)/405 (Matthews et al., 1985; Wallace, Levy, & Matthews, 2004).

RNA Isolation and Reverse Transcription

Approximately 10 mg of each muscle sample was removed, weighed, and subsequently placed in an autoclaved microcentrifuge tube. Using previous guidelines (Willoughby & Wilborn, 2006), the skeletal muscle samples were then homogenized in 500 µl of a monophasic solution of phenol and guanidine isothiocyanate contained within the TRI-reagent (Sigma Chemical Co., St. Louis, MO) and a plastic Kontes pestle. The muscle homogenate was then allowed to stand for 5 min at room temperature to allow for the complete dissociation of nucleoprotein complexes. At this point, 100 µl of chloroform was added and the solution was mixed and allowed to sit at room temperature for 10 min and then centrifuged at 12,000 x g for 15 min. The supernatant was then removed and transferred to a new microfuge tube, and then 250 µl of isoproponal was added. The solution was mixed and allowed to sit at room temperature for 10 min and again centrifuged at 12,000 x g for 10 min. The supernatant was then discarded and the RNA pellet was washed with 500 µl 75% ethanol and centrifuged for 5 min at 7,500 x g. This ethanol wash was then repeated a second time at which point the supernatant was removed and the RNA pellet allowed to air dry for 10 min. Finally, the RNA pellet was dissolved in 50 µl of nuclease-free water and the RNA samples were stored at -80°C until later analysis.

Total RNA extracted from each muscle sample was reverse transcribed and used to generate cDNA using an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) for the subsequent real-time quantitative polymerase chain reaction (RT-PCR) measurements. A reverse transcription reaction mixture [2 µg of cellular RNA, 5x reverse transcription buffer (20 mM Tris-HCL, pH 8.3; 50 mM KCl; 2.5 mM MgCl2; 100 µg of bovine serum

albumin/ml), a dNTP mixture containing 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.8 μM MgCl2, 0.5 μg/μl of oligo(dT) 15 primer, and 25 u/μg of MMLV RNAase H+ reverse transcriptase enzyme (Bio-Rad, Hercules, CA, USA)] was added to 2 μg of skeletal muscle RNA and incubated at 25°C for 5 min, 42°C for 30 min, and then heated to 85°C for 10 min. The resultant cDNA concentration was determined spectrophotometerically using (Helio γ, Thermo Electron, Milford, MA) by optical density (OD) at 260 nm using an OD_{260} equivalent to 50 μg/μl (Willoughby, Stout, & Wilborn, 2007) and the starting cDNA template concentration was standardized by adjusting all samples to 200 ng prior to amplification. Finally, the cDNA was then stored at -80°C until later analysis.

Assessment of Metabolic Gene Expression: Real-Time PCR Amplification

Expression of genes involved with glucose transport and substrate metabolism in skeletal muscle (GLUT4, IRS1, Akt, AMPK, and AS160) were assessed using real–time PCR. Oligonucleotide primers were designed according to known human mRNA sequences available online through the National Center for Biotechnology Information (NCBI) genome database. A list of the oligonucelotide primers utilized during RT-PCR is presented in Table 3. The sense and anti-sense primers were synthesized commercially (Integrated DNA Technologies, Coralville, IA). β-actin was used as an external control standard for each reaction due to its consideration as a constitutively expressed "housekeeping gene," and the fact that it has been shown to be an appropriate external reference standard in real-time PCR in human skeletal muscle following acute exercise (Willoughby et al., 2007).

Table 3. *Oligonucleotide Primers used for RT-PCR*

Primer Name	NCBI Accession Number	Sense Sequence (5' → 3')	Anti-Sense Sequence (5' → 3')	Amplicon Size (bp)
β-Actin	NM_001101	ATC GTG GAC ATT AAG	GTC ATC ACC ATT GGC AAT	102
AMPK alpha2	EF056019	TGA TGA TGA AGT GGA GCA GAG	GCC AGT GAG AGA GCC AGA AAG	139
GLUT4	M20747	CCA TTG TTA TCG GCA TTC	GGA TGA TGT AGA GGT AGC	165
AS160	AB449885	AGC AAT GAG TCC CTA AGT G	CTC GTT CCT GTC CAA TCC	144
AKT2	M95936	GTC GCC AAC AGC CTC AAG	ACC GCC ACT TCC ATC TCC	104
IRS-1	NM_005544	GAG AGC AGC GGT GGT AAG	GGC AAT GAG TAG TAG GAG AGG	149

Based on previous guidelines (Willoughby & Wilborn, 2006), 200 ng of cDNA was added to each of the 25 μl PCR reactions using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). Each reaction contained the following mixtures: [10x PCR buffer, 0.2 μM dNTP mixture, 2.0 μM of a cocktail containing both the sense and antisense RNA oligonucleotide primers, 2 mM $MgCL_2$, 1.0 u/μl of hot-start iTaq DNA polymerase, SYBR Green I dye, and nuclease-free dH_2O], and was amplified using RT-PCR (iCycler IQ Real-Time PCR Detection System, Bio-Rad, Hercules, CA, USA). SYBR green dye was added to each sample so that it could incorporate into the amplicon during the RT-PCR and allow for fluorescence to be measured after each RT-PCR cycle. The amplification profile was run for 40-cycles employing a denaturation step at 95°C for 30 sec, primer annealing at 58°C for 30 sec, and extension at 72°C for 30 sec. To help control for differences in amplification efficiency during thermocycling, all PCR reactions for each gene were prepared from the same SYBR green stock solution. The quantity of target gene mRNA was determined relative to the expression of β-actin, and

change in threshold cycle (ΔC_T) values were used to compare gene expression for the muscle samples collected during the testing sessions. The specificity of the PCR was demonstrated with an absolute negative control reaction containing no cDNA template or primers, and single gene products confirmed using DNA melt curve analysis. Test-retest reliability of performing this procedure of mRNA expression on samples in this laboratory has demonstrated low mean coefficients of variation and high reliability (1.6%, intraclass $r = 0.95$).

To assess positive mRNA amplification, 20 μl aliquots of the PCR reaction mixtures were electrophoresed in 1.5% agarose gels in 1X Tris-Acetate-EDTA (TAE) buffer. Ethidium bromide was present in the TAE buffer at 1 μg/ml and was used to stain the gel. This was then illuminated with UV transillumination (Chemi-Doc XRS, Bio-Rad, Hercules, CA, USA). Gene markers for an additional study were also included in this run. As shown in Figure 4, the agarose gel was arranged in this order form left to right: the molecular weight marker, β-actin, AMPK, PPARα, PGC-1α, PPARγ, GLUT4, Akt2, and AS160.

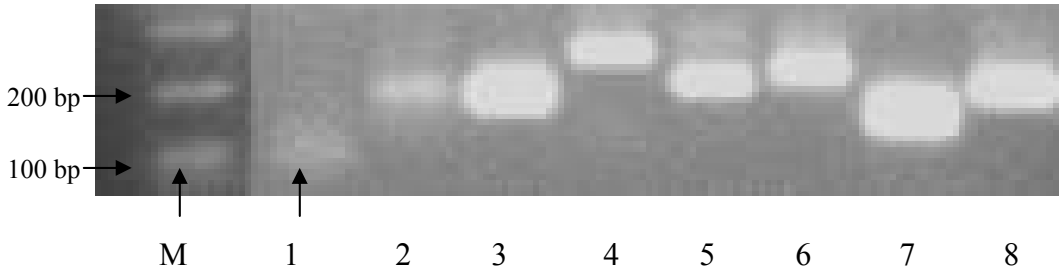


Figure 4. Agarose Gel Illustration of PCR amplicons
Illustration of PCR amplicons for the mRNA targets for one study participant run on a 1.5% agarose gel. M = base pair marker, 1 = β-Actin, 2 = AMPK, 3 = PPARα, 4 = PGC-1α, 5 = PPARγ, 6 = GLUT4, 7 = Akt2, 8 = AS160.

Dietary Records

The participants' diets were not standardized and participants were not asked to change their dietary habits during the course of the study. In order to assess the average daily macronutrient consumption of fat, carbohydrate, and protein, the participants were required to keep dietary records during the entire supplementation period. The dietary records were analyzed with the Food Processor Dietary Assessment Software program (ESHA Research Inc., Salem, OR).

Reported Side Effects from Supplements

To determine whether the participants suffered any negative side effects from the control or experimental supplements, participants reported by questionnaire whether they tolerated the supplement, supplementation protocol, as well as report any medical problems/symptoms they may have encountered throughout the supplementation period.

Statistical Analysis

Participants were matched according to body fat percentage and randomized into treatment groups in an attempt to alleviate possible baseline differences between groups. However, a one-way analysis of variance (ANOVA) was used to determine differences between groups for related demographic variables and blood and muscle samples taken at T1. A 2 x2 (group x time) factorial multivariate analysis of variance (MANOVA) with repeated measures was used to determine differences in related blood and muscle samples between groups from T1 to T3. Differences between groups over time in blood variables during T2 and T3 were determined via repeated measures, 2 x 4 factor MANOVA analyses. Similarly, differences between groups over time in gene expression variables

during T2 and T3 were determined via repeated measures, 2 x 2 factor MANOVA analyses. MANOVA with repeated measures was employed for these analyses because it reduces that likelihood of a Type I error that would result with the use of repeated univariate procedures. When the repeated measures analysis yielded a significant main effect or interaction, significant differences between means were determined using Bonferonni's post hoc test to further control for alpha inflation of the subsequent univariate ANOVA. Additionally, post-hoc tests of significant interaction effects were analyzed using independent sample t-tests. Finally, for the Matsuda Index calculations obtained the OGTT, an independent sample t-test was also utilized to analyze differences between groups. All statistical procedures were performed using SPSS version 17.0 software (Chicago, IL) and a probability level of ≤ 0.05 was used to determine statistical significance.

CHAPTER FOUR

Results

Participant Demographics

Sixteen sedentary, overweight or obese females volunteered to participate in this study and their demographic information is presented in Table 4. Each participant was randomized into either the placebo or RV group based on her body fat percentage. At baseline, independent-sample t-tests revealed that the RV group was significantly younger ($p = 0.035$) and taller ($p = 0.030$) than the placebo group. There were no significant differences in body mass ($p = 0.895$) or BMI ($p = 0.329$). Additional demographic variables are also presented in Table 5. Results revealed no significant differences between groups at baseline ($p > 0.05$) for resting energy expenditure or respiratory quotient (RQ). Results for body composition variables [TBW, extracellular fluid (ECF), intracellular fluid (ICF), fat mass, lean mass, and percent body fat] revealed no significant differences between groups at baseline ($p > 0.05$). Hypothesis one states that there would be no difference in height between the two groups; therefore, hypothesis one failed to be accepted.

Table 4. *Study Participant Demographics*

Variable	Placebo (n=8)	Resveratrol (n=8)	*p-value*
Age (yrs)*	31±5.3	25±5.2	0.035
Ht (cm)*	161.29±7.071	170.05±7.478	0.030
Body Mass (kg)	105.19±33.314	103.36±19.154	0.895
BMI (kg/m^2)	40.12±10.54	35.75±6.23	0.329

Note: Data are presented as means ± standard deviations. *denotes a significant difference between groups ($p < 0.05$)

Table 5. *Body Composition and Metabolism*

Variable	Placebo	Resveratrol	*p-value*
TBW (L)	36.4±7.45	38.42±4.79	0.529
ECF (L)	16.45±3.20	17.20±2.39	0.605
ICF (L)	19.94±4.38	21.22±2.81	0.499
Fat Mass (kg)	47.38±20.34	44.11±12.18	0.702
Lean Mass (kg)	49.57±10.81	50.39±7.81	0.880
% Body Fat	46.73±5.26	45.00±5.62	0.536
REE (kcal/d)	1614.9±415.3	1773.0±199.2	0.348
RQ	0.90±0.08	0.91±0.07	0.859

Note: Data are presented as means ± standard deviations

Dietary Intake and Supplement Compliance

All participants were required to complete a four-day dietary record during the supplementation period and submit this record prior to T3. Participants were also instructed to consume their usual diet, but refrain from red wine. The results of the dietary analysis are presented in Table 6. One-way ANOVA analysis revealed no significant differences between groups for total caloric or macronutrient intake ($p > 0.05$). Hypothesis two was thus accepted, since it stated that there would be no difference in caloric intake or macronutrient intake between groups during the study. In addition to the previous instructions, participants were required to use their dietary record to record the time at which they ingested both pills for each day of the supplementation protocol. Upon arrival for T2, 4 pills were still remaining, and hence, participants were instructed to ingest 2 pills prior to any testing on the day of T2 and T3. All participants that completed the study were compliant with the supplementation protocol.

Table 6. *Dietary Analysis*

Variable	Placebo	Resveratrol	*p-value*
Total Calories (kcal)	2033.95±647.68	2197.54±549.95	0.595
Protein (g)	79.90±35.37	74.40±17.00	0.698
Carbohydrate (g)	259.00±89.25	291.04±88.55	0.483
Fat (g)	73.86±21.85	75.63±25.32	0.883

Note: Data are presented as means ± standard deviations

Reported Side Effects

No significant side effects were reported following the resveratrol or cellulose placebo supplementation. One participant reported a single occurrence of mild gastrointestinal distress while taking the resveratrol supplement. Ninety-four percent of the participants reported that they did not experience any side effects during the supplementation protocol.

Serum Clinical Chemistry Markers

A two-way [group (2) x time point (2)] repeated measures MANOVA was used to evaluate the effects of supplementation on serum lipid levels and clinical chemistry markers of liver and kidney function. This data is presented in Tables 7-9. The within-subjects factor was time with two levels (baseline = T1 and immediately before a GXT = T3). The between-subjects factor was group with two levels (RV and PL).

Serum Lipid Levels

Serum lipid levels [total cholesterol, high-density lipoproteins (HDL), triglycerides, low-density lipoproteins (LDL)] were within normal physiological ranges for both group at each time point (Kratz, Ferraro, Sluss, & Lewandrowski, 2004). Multivariate analysis showed no main effect for group [Wilks' Lambda = 0.755, F = 0.893, p = 0.500, effect size (η^2) = 0.245]. There was a significant main effect for time [Wilks' Lambda = 0.314, F = 6.008, p = 0.008, effect size (η^2) = 0.686] and a strong trend for a group x time interaction [Wilks' Lambda = 0.453, F = 3.323, p = 0.051, effect size (η^2) = 0.547]. However, subsequent univariate ANOVA tests did not reveal any significant time effect for any of the lipid variables. Hypothesis four states that there

would be no difference in lipid panel variables between the two groups, and thus was accepted.

Table 7. *Serum Lipid Levels*

Variable	Placebo		Resveratrol	
	T1	T3Pre	T1	T3Pre
Total Cholesterol (mg/dL)	168.63 ±21.58	163.00 ±18.45	161.43 ±30.21	166.00 ±17.57
HDL (mg/dL)	44.88 ±15.04	42.63 ±11.70	44.14 ±17.18	41.13 ±8.87
Triglycerides (mg/dL)	132.88 ±140.17	125.75 ±98.18	140.57 ±101.09	125.38 ±59.42
LDL (mg/dL)	95.75 ±16.32	95.25 ±19.39	89.29 ±22.30	99.88 ±16.45

Note: Data are presented as means ± standard deviations

Electrolytes and Markers of Kidney Function

All of the electrolytes and kidney function variables [sodium, potassium, chloride, carbon dioxide, calcium, blood urea nitrogen (BUN), uric acid, creatinine, total protein, and albumin] were in normal physiological ranges for both groups at each time point (Kratz et al., 2004). Multivariate analysis revealed no main effect for group [Wilks' Lambda = 0.359, F = 0.894, p = 0.590, effect size (η^2) = 0.641], time [Wilks' Lambda = 0.119, F = 3.711, p = 0.080, effect size (η^2) = 0.881], or group x time interaction [Wilks' Lambda = 0.235, F = 1.625, p = 0.309, effect size (η^2) = 0.765].

Table 8. *Serum Clinical Chemistry Markers: Electrolytes & Kidney Function*

Variable	Placebo		Resveratrol	
	T1	T3Pre	T1	T3Pre
Sodium (mmol/L)	135.63 ±4.53	137.63 ±3.96	132.43 ±10.36	136.88 ±5.14
Potassium (mmol /L)	4.30 ±0.53	4.21 ±0.46	3.99 ±0.41	4.30 ±0.59
Chloride (mmol /L)	102.63 ±4.73	105.63 ±2.50	101.14 ±7.90	104.37 ±3.34
CO_2 (mmol /L)	21.75 ±4.98	19.50 ±2.73	23.71 ±4.40	22.75 ±3.37
Calcium (mg/dL)	8.86 ±0.51	8.90 ±0.48	8.64 ±0.71	8.91 ±0.67
BUN (mg/dL)	11.00 ±3.46	12.88 ±4.36	12.14 ±2.90	11.63 ±2.33
Uric Acid (mg/dL)	5.23 ±1.55	5.05 ±1.69	5.26 ±1.27	4.78 ±0.76
Creatinine (mg/dL)	0.73 ±0.11	0.74 ±0.14	0.76 ±0.14	0.79 ±0.09
Total Protein (g/dL)	7.08 ±0.65	6.95 ±0.56	6.66 ±0.54	6.76 ±0.62
Albumin (g/dL)	4.09 ±0.30	3.99 ±0.39	3.80 ±0.39	3.96 ±0.37

Note: Data are presented as means ± standard deviations

Markers of Liver Function

All of the liver function variables [total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] were in normal physiological ranges for both groups at each time point (Kratz et al., 2004). Multivariate analysis showed no main effect for group [Wilks' Lambda = 0.852, F = 0.479, p = 0.751, effect size (η_2) = 0.148], time [Wilks' Lambda = 0.895, F = 0.324, p = 0.856, effect size

(η^2) = 0.105], or group x time interaction [Wilks' Lambda = 0.930, F = 0.207, p = 0.929, effect size (η^2) = 0.070]. Hypothesis three states that there would be no difference in clinical chemistry markers of kidney and liver function between the two groups and therefore, was accepted.

Table 9. *Serum Clinical Chemistry Markers: Liver Function*

Variable	Placebo		Resveratrol	
	T1	T3Pre	T1	T3Pre
Total Bilirubin (mg/dL)	0.35 ±0.12	0.34 ±0.19	0.36 ±0.13	0.38 ±0.17
ALP (U/L)	65.63 ±21.50	67.13 ±23.09	60.00 ±23.77	60.50 ±21.02
AST (U/L)	15.13 ±5.11	16.25 ±3.69	17.00 ±4.34	17.37 ±4.98
ALT (U/L)	17.88 ±12.04	17.50 ±10.11	17.57 ±7.91	17.50 ±8.91

Note: Data are presented as means ± standard deviations

Serum Glucose and Insulin Dynamics

Serum glucose and insulin levels and HOMA-IR assessment of insulin resistance were assessed prior to supplementation intervention (T1), OGTT (T2 Pre) and GXT (T3 Pre) and are presented in Table 10. Despite the fact that all participants were screened as prediabetic using capillary glucometer measurements, the average fasting serum glucose concentration was below 100 mg/dL. Multivariate analysis showed no main effect for group [Wilks' Lambda = 0.661, F = 2.055, p = 0.160, effect size (η^2) = 0.339], time [Wilks' Lambda = 0.432, F = 1.97, p = 0.173, effect size (η^2) = 0.568], or group x time interaction [Wilks' Lambda = 0.485, F = 1.591, p = 0.255, effect size (η^2) = 0.515]. Hypothesis five states that there would be a statistically significant reduction in serum

levels of glucose, insulin, and the HOMA-IR in the RV group compared to placebo, and therefore hypothesis five failed to be accepted.

Table 10. *Analysis of Resting Glucose & Insulin Levels*

Variable	Placebo			Resveratrol		
	T1	T2Pre	T3Pre	T1	T2Pre	T3Pre
Serum Glucose (mg/dL)	95.63 ±14.11	99.75 ±18.67	98.88 ±18.24	86.57 ±9.83	87.00 ±6.85	90.13 ±8.29
Insulin (μIU/mL)	6.87 ±4.76	9.50 ±7.23	7.14 ±4.86	6.49 ±3.35	5.88 ±2.10	6.92 ±5.32
HOMA-IR	1.71 ±1.30	2.05 ±1.43	1.83 ±1.36	1.43 ±0.85	1.26 ±0.47	1.50 ±1.04

Note: Data are presented as means ± standard deviations

Glucose & Insulin Dynamics - OGTT (T2)

A 2 x 4 (group x time) factorial multivariate was used to analyze the effects of RV supplementation on glucose and insulin levels prior to and following an OGTT. Multivariate analysis revealed a significant main effect for time [Wilks' Lambda = 0.076, $F = 18.145$, $p < 0.001$, effect size (η_2) = 0.924]. Mauchly's test of sphericity was significant ($p < 0.10$) for both glucose and insulin, so sphericity was not assumed and the Huynh-Feldt correction factor was assessed for each variable.

Univariate analysis showed a significant time effect for glucose ($p < 0.001$), with subsequent pairwise comparison revealing significantly higher glucose concentrations at 30 min ($p < 0.001$) and at 1 hr ($p = 0.007$) following the OGTT. A strong trend for insulin ($p = 0.051$) was indentified following the OGTT. No main effect for group [Wilks' Lambda = 0.820, $F = 1.423$, $p = 0.276$, effect size (η_2) = 0.180] or group x time

interaction [Wilks' Lambda = 0.505, F = 1.472, p = 0.289, effect size (η^2) = 0.495], was indentified. These results are displayed in Figures 5 and 6.

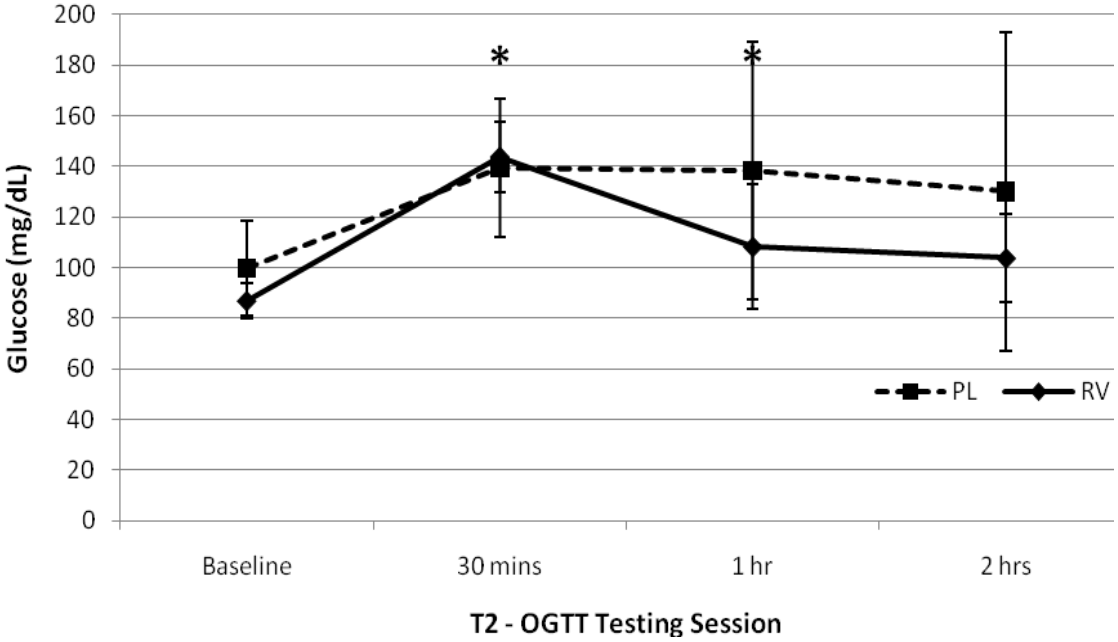


Figure 5. Glucose Dynamics During OGTT (T2)
Data are presented as means ± standard deviations and all glucose concentrations are expressed as mg/dL. * Significantly different from baseline ($p < 0.01$)

Additionally, the Matsuda Index was calculated in order to assess the participant's insulin sensitivity in response to the OGTT. A student's t-test was utilized to assess differences between groups and revealed that there was no significant difference (p = 0.166) in the Matsuda Index between the placebo (9.14 ± 4.83) and RV (12.65 ± 3.10) groups.

Hypothesis six states that there would be significantly lower increases in serum levels of glucose and insulin in the RV group compared to the placebo group following an OGTT. Additionally, the Matsuda Index was hypothesized to be significantly higher in the resveratrol group compared to the placebo group following an OGTT. Since no

significant differences between groups were observed, hypothesis six was failed to be accepted.

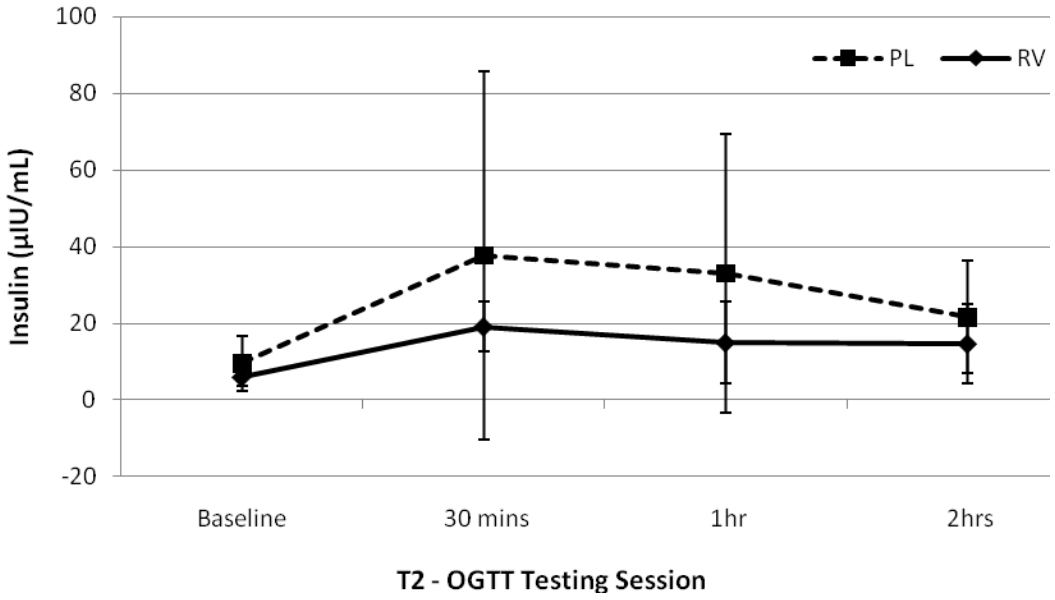


Figure 6. Insulin Dynamics During OGTT (T2)
Data are presented as means ± standard deviations and all insulin concentrations are expressed as µIU/mL.

Glucose & Insulin Dynamics - GXT (T3)

A 2 x 4 (group x time) factorial multivariate was used to analyze the effects of RV supplementation on glucose and insulin levels prior to and following a GXT. Multivariate analysis revealed a significant main effect for time [Wilks' Lambda = 0.127, $F = 10.324$, $p = 0.001$, effect size (η^2) = 0.873]. Mauchly's test of sphericity was significant ($p < 0.10$) for glucose, so sphericity was not assumed and the Huynh-Feldt correction factor was assessed for this variable.

Univariate analysis showed a significant time effect for glucose ($p = 0.002$), with subsequent pairwise comparison revealing significantly lower glucose concentrations at 1

hr compared to baseline (p = 0.01) and 30 min (p = 0.032) following the GXT. Univariate analysis showed a significant time effect for insulin ($p < 0.001$), with subsequent pairwise comparison revealing significantly lower insulin concentrations at 1 hr (p = 0.012) and 2 hr (p = 0.003) compared to baseline. No main effect for group [Wilks' Lambda = 0.840, F = 1.237, p = 0.322, effect size (η^2) = 0.160] or group x time interaction [Wilks' Lambda = 0.473, F = 1.668, p = 0.235, effect size (η^2) = 0.527] was identified. These results are displayed in Figures 7 & 8.

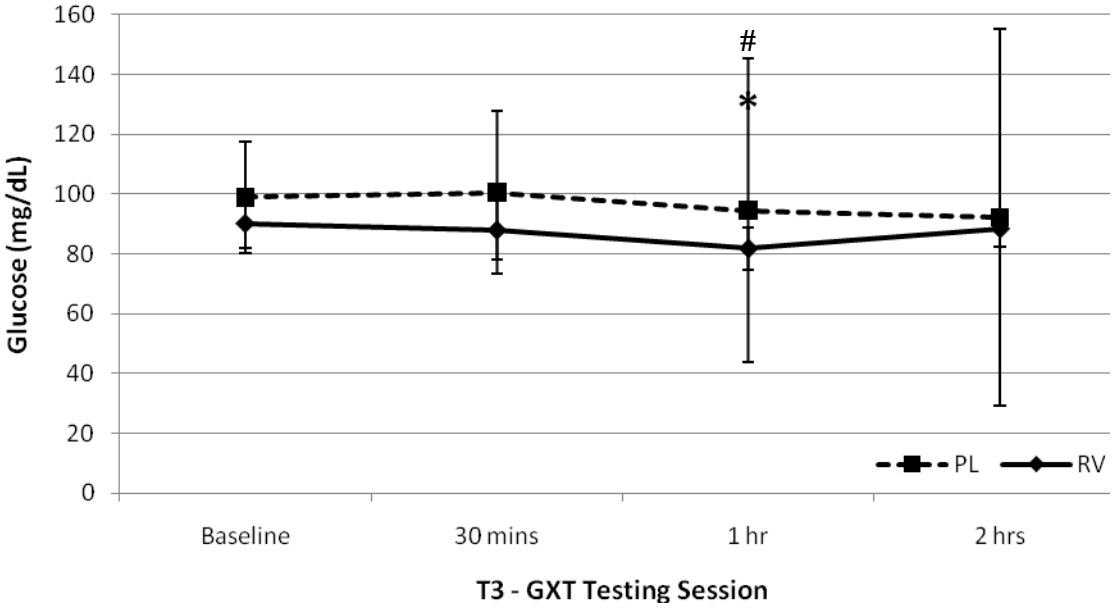


Figure 7. Glucose Dynamics - GXT (T3)
Data are presented as means ± standard deviations and all glucose concentrations are expressed as mg/dL. * indicates significantly different from baseline (p = 0.01). # indicates significantly different from 30 minutes (p = 0.032).

Hypothesis seven states that there would be a significantly lower reduction in serum levels of glucose and a greater reduction of insulin in the RV group compared to the placebo group following the GXT. Since no significant differences between groups were observed, hypothesis seven failed to be accepted.

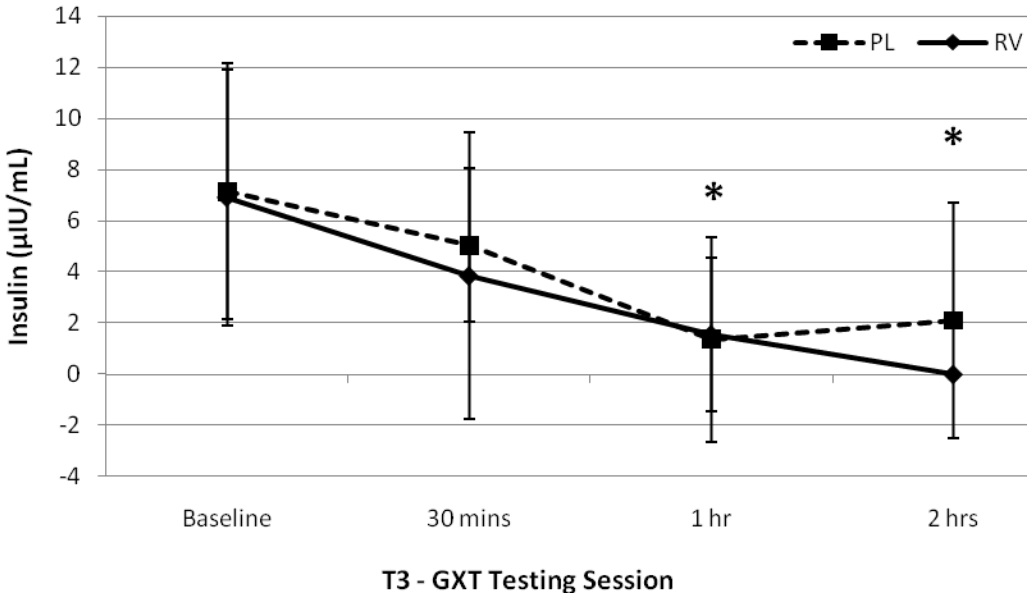


Figure 8. Insulin Dynamics - GXT (T3)
Data are presented as means ± standard deviations and all insulin concentrations are expressed as µIU/mL. * indicates significantly different from baseline ($p < 0.05$).

Gene Transcriptional Changes

The mRNA expression of five genes important to substrate utilization (Akt2, AMPK, AS160, GLUT4, and IRS1) were analyzed to determine the effects of RV supplementation on the baseline transcriptional rates of each gene and the transcriptional changes observed during an OGTT and following a GXT. These rates of expression are presented in Table 11. A 2 x 3 factorial MANOVA with repeated measures was utilized to analyze the effects of RV after 6 and 7 days of supplementation. This multivariate analysis showed no main effect for group [Wilks' Lambda = 0.935, F = 0.138, p = 0.979, effect size (η_2) = 0.065], time [Wilks' Lambda = 0.343, F = 0.957, p = 0.557, effect size (η_2) = 0. 657], or group x time interaction [Wilks' Lambda = 0.120, F = 3.65, p = 0.083, effect size (η_2) = 0.880]. A 2 x 2 factorial MANOVA with repeated measures was used to assess transcriptional changes following an OGTT (T2). Multivariate analysis

revealed no main effect for group [Wilks' Lambda = 0.836, F = 0.392, p = 0.843, effect size (η^2) = 0.164], time [Wilks' Lambda = 0.713, F = 0.804, p = 0.572, effect size (η^2) = 0. 287], or group x time interaction [Wilks' Lambda = 0.435, F = 2.601, p = 0.093, effect size (η^2) = 0.565]. A 2 x 2 factorial MANOVA with repeated measures was also utilized to assess transcriptional changes following an acute bout of intense exercise (T3). Again, multivariate analysis revealed no main effect for group [Wilks' Lambda = 0.525, F = 1.809, p = 0.199, effect size (η^2) = 0.475], time [Wilks' Lambda = 0.632, F = 1.166, p = 0.389, effect size (η^2) = 0. 368], and group x time interaction [Wilks' Lambda = 0.643, F = 1.110, p = 0.414, effect size (η^2) = 0.357].

Table 11. *Gene Expression Changes*

Variable	Placebo					Resveratrol				
	T1	T2 Pre	T2 1Hr	T3 Pre	T3 1Hr	T1	T2 Pre	T2 1Hr	T3 Pre	T3 1Hr
Akt2 / β-actin	0.94 ±0.08	0.95 ±0.06	1.02 ±0.12	0.96 ±0.98	1.00 0.96	0.98 ±0.05	0.92 ±0.07	0.98 ±0.13	0.98 ±0.11	0.96 ±0.12
AMPK / β-actin	1.11 ±0.11	1.12 ±0.11	1.17 ±0.09	1.12 ±0.06	1.17 ±0.10	1.14 ±0.06	1.12 ±0.11	1.12 ±0.11	1.10 ±0.06	1.09 ±0.10
AS160 / β-actin	0.91 ±0.07	0.93 ±0.05	0.97 ±0.07	0.97 ±0.09	0.98 ±0.05	0.95 ±0.07	0.92 ±0.07	0.98 ±0.10	0.92 ±0.05	0.91 ±0.05
GLUT4/ β-actin	1.11 ±0.13	1.18 ±0.05	1.21 ±0.03	1.21 ±0.03	1.19 ±0.04	1.19 ±0.09	1.19 ±0.07	1.15 ±0.07	1.16 ±0.05	1.16 ±0.05
IRS1 / β-actin	1.05 ±0.17	1.04 ±0.11	1.06 ±0.13	1.03 ±0.10	1.07 ±0.10	1.08 ±0.10	0.98 ±0.10	1.07 ±0.08	1.05 ±0.13	1.12 ±0.17

Note: Data are presented as means ± standard deviations. All data is expressed as the ratio of ΔC_T values between the variable of interest (Akt2, AMPK, AS160, GLUT4, or IRS1) and the internal standard (β-Actin).

Hypotheses eight, nine, and ten state that there would be a statistically significant increase in skeletal muscle transcription of genes critical for regulation of substrate utilization (AMPK, Akt2, AS160, GLUT4, and IRS1) in the experimental group as compared to the placebo group after 1 week of supplementation, following an OGTT, and following a GXT, respectively. Since no significant differences between groups were observed, hypotheses eight, nine, and ten failed to be accepted.

CHAPTER FIVE

Discussion

General Findings and Confounding Variables

The primary purpose of this study was to determine the acute effects of *trans*-resveratrol supplementation on glucose homeostasis and insulin sensitivity in sedentary, overweight females. More specifically, the aims of the current study were threefold. The first aim was to examine the acute effects of six days of resveratrol supplementation on insulin-mediated glucose disposal during the 2 hr following the bolus glucose ingestion of the OGTT. Another aim was to observe the impact of seven days of resveratrol supplementation on exercise-mediated glucose disposal up to 2 hr following an acute bout of intense exercise. Finally, the third aim was to investigate the effect of resveratrol supplementation on skeletal muscle mRNA expression of metabolically relevant proteins (AMPK, Akt2, AS160, GLUT4, and IRS1) at rest and following an OGTT and an acute bout of intense exercise. Since these proteins are involved in insulin-mediated and insulin-independent glucose uptake, increased transcription of these genes could provide a mechanism for the proposed improvements in insulin sensitivity and glucose homeostasis.

In cell culture and rodent models, resveratrol supplementation has been shown to increase glucose disposal in C2C12 myotubes and insulin resistant rats (Park et al., 2007; Su et al., 2006). Furthermore, Baur et al. (Baur et al., 2006) and Sun et al. (Sun et al., 2007) both found that resveratrol administration to rats on obesogenic diets significantly improved the insulin sensitivity of these animals. In the current study, serum glucose and

insulin levels were significantly elevated following ingestion of 75 g of glucose and subsequently returned back to baseline levels by 2 hr. In contrast, following an acute bout of intense exercise, serum glucose and insulin levels were significantly decreased, with only glucose levels returning to baseline by 2 hr. Despite this change and in contrast to previous findings, acute resveratrol supplementation was unable to enhance such normal physiological responses. Furthermore, resveratrol supplementation produced no significant effect on fasting glucose or insulin levels. Additionally, resveratrol supplementation was unable to significantly enhance the expression of the metabolically relevant genes at rest, and/or following an OGTT and acute bout of intense exercise.

Acute Effects of Resveratrol Supplementation on Clinical Safety Markers, Blood Lipid Profiles, and Insulin Resistance

Resveratrol has been shown to be safe for human consumption, with very limited adverse effects from acute supplementation within the range of 0.5 to 5 g (Almeida et al., 2009; Boocock et al., 2007; Cottart et al., 2010). In the current study, no significant changes in side effects or serum clinical safety markers for liver and kidney function were observed following an acute 7 day ingestion of 1000 mg of *trans*-resveratrol. Notwithstanding, conclusions regarding the safety of resveratrol supplementation for an extended period of time (i.e. years) have yet to be established (Boocock et al., 2007).

Numerous studies in rodent models have shown that chronic resveratrol supplementation improves insulin sensitivity and blood lipid levels (Baur et al., 2006; Su et al., 2006; Sun et al., 2007; Zang et al., 2006). A study from Baur et al. (2006) supplemented middle aged mice (1 yr old) on a high calorie diet with resveratrol (22.4 mg/kg/day) and found that resveratrol significantly improved insulin sensitivity and

AMPK activity compared to control mice on the high calorie diet only. Sun and colleagues (2007) performed a 16 wk study where mice ate a normal chow diet, a high fat diet, or a high fat diet with resveratrol (2.5 mg/kg/day) and found that the significant increases in plasma insulin, total cholesterol, and LDL levels in the high fat diet group were absent in the high fat diet / resveratrol group. Furthermore, this resveratrol group also had significantly better glucose tolerance and insulin response to an intravenous glucose tolerance test (IGTT) than the high fat diet group. These responses were actually similar to the IGTT responses of the normal chow group (Sun et al., 2007). Additionally, a study from Su et al. (2006) found that two weeks of oral resveratrol administration (0.5 mg/kg every 8 hr) to insulin resistant rats significantly decreased serum glucose, insulin, and triglyceride levels. In contrast to these findings, the current study found that one week of resveratrol supplementation was unable to lower serum glucose, insulin, or lipid levels in overweight, obese women. Serum glucose and insulin were measured and the HOMA-IR was calculated to determine the effects of resveratrol supplementation on fasting glucose homeostasis and insulin sensitivity, but none of these factors were significantly altered by one week of *trans*-resveratrol supplementation.

One reason for the lack of evidence for improvement in glucose homeostasis in the present study could be due to experimental design and blood glucose screening method utilized. This reason will be discussed later in the "Study Limitations" section. An additional explanation for failing to find any antidiabetic or lipid-lowering effects of resveratrol supplementation is the reported low bioavailability of *trans*-resveratrol in humans (Boocock et al., 2007; Cottart et al., 2010; Walle et al., 2004; Wenzel & Somoza, 2005). Although it was known that *trans*-resveratrol had a low bioavailability in humans,

there were no studies to indicate the effectiveness of resveratrol to improve glucose homeostasis in overweight or obese females. In addition, there was little evidence as to the appropriate supplementation administration and dosage necessary to produce therapeutic effects in humans. While the relative resveratrol dosage in the current study (~4.84 mg/kg/day) is comparable to the 1.5, 2.5, 22.4 mg/kg/day doses used in the previously mentioned rodent studies, Reagan-Shaw et al. (Reagan-Shaw, Nihal, & Ahmad, 2007) contend that animal doses cannot simply be extrapolated to human equivalent doses by conversions based on body weight. These authors advocate the use of the body surface area (BSA) normalization method when translating dosages from animal to human studies (Reagan-Shaw et al., 2007). In order to calculate the human equivalent dose with the BSA, the animal dose (mg/kg) is multiplied by the quotient of the animal model K_m factor (mouse: 3; rat: 6) divided by the human K_m factor (60 kg adult: 37). To find the K_m factor for the adults in the current study, one must divide the mean weight (102 kg) by the BSA. The BSA can be found using the Du Bois height-weight formula where BSA is equal to body weight $(kg)^{0.425}$ multiplied by height $(cm)^{0.725}$ multiplied by 0.007184 (Du Bois & Du Bois, 1916). Based on these BSA calculations, the human equivalent dosage for the Sun et al. study (2007) would be 0.20 mg/kg/day, 0.24 mg/kg/day for the study from Su et al. (2006), and 1.37 mg/kg/day for the Baur et al. study (2006). While the relative doses in the current study were higher than those presented in these rodent studies, the lack of significant treatment effects could be a result of the short duration of supplementation or because of different degrees of insulin resistance. These possibilities require further investigation, however.

While the length of the current study was shorter than the previously mentioned rodent studies, other research had indicated that short-term AICAR administration (AMPK stimulation) mimicked exercise training effects on skeletal muscle in terms of increasing insulin sensitivity, mitochondrial enzymes, GLUT4 content, and exercise-induced AMPK activation. Holmes and colleagues (Holmes, Kurth-Kraczek, & Winder, 1999) showed that 5 day administration of AICAR (subcutaneous injection each morning) resulted in chronic activation of the AMPK pathway and a two-fold increase in GLUT4 content, hexokinase (HK) activity, and glycogen content of rat skeletal muscle. In another study, Wister rats underwent either a 5-day exercise training program on a treadmill, received daily AICAR injections, or received no treatment (Jessen, Pold, Buhl, Jensen, Schmitz, & Lund, 2003). Both exercise training and chronic AICAR treatment resulted in enhanced insulin signaling, improved glucose transport, and increased GLUT4 expression. As a result, the current study hypothesized that chronic stimulation of the AMPK by *trans*-resveratrol supplementation would yield similar results in improving insulin sensitivity. It could be possible that more frequent ingestion of the supplement, higher overall dosage, and longer period of supplementation could yield greater improvements in glucose homeostasis. However, as the current study was the initial phase of a two month trial and there was no research on the effects of long term resveratrol supplementation in humans, the manufacturer recommended dosage was utilized.

This study also hypothesized that one week of resveratrol supplementation would upregulate transcription of the genes for AMPK, GLUT4, AS160, IRS1 and Akt2. Research indicates that the genes for these proteins can be induced by activation of the

AMPK and/or insulin-signaling pathways (Christ-Roberts et al., 2004; Ducluzeau et al., 2001; Lee-Young, Canny, Myers, & McConell, 2009; Long et al., 2005; Short et al., 2003). While some studies have shown that resveratrol is capable of altering gene transcription via AMPK activation (Chan et al., 2008; Ni et al., 2007), no study has investigated the effects of *trans*-resveratrol on the transcriptional rate of the genes examined in this study. The present study was unable to provide any evidence for *trans*-resveratrol supplementation to upregulate these genetic markers, however. Further in vivo research should investigate the dosage of *trans*-resveratrol required to sufficiently activate the AMPK to stimulate glucose uptake and gene transcription in human skeletal muscle.

Acute Effects of Resveratrol Supplementation on Insulin-mediated Glucose Disposal

Breen et al. (2008) demonstrated that resveratrol administration (25-100 μM) significantly increased AMPK phosphorylation and glucose uptake in L6 myotubes. Whereas Su et al. (2006) found that plasma glucose levels were significantly reduced (~30%) at 90 min after an acute dose (0.5 mg/kg) of resveratrol fed to insulin resistant rats. Based on the results of these studies and the expectation that an acute dose of resveratrol would enhance glucose disposal by stimulation of the AMPK pathway, it was hypothesized that the resveratrol group would have lower glucose and insulin responses in the OGTT trial. On the contrary, resveratrol supplementation did not induce any detectable difference in the glucose or insulin changes observed during the OGTT.

Based on the nature of the OGTT, the significant increases seen in serum glucose and insulin concentrations during the OGTT were expected; however, the magnitude of

these responses was below that which was hypothesized. The purpose of this test is to assess the body's ability to respond to a bolus ingestion of 75 g of glucose by adequately clearing the glucose from the bloodstream within the 2 hour time frame. As is the case in insulin resistant individuals, the inability of the body to adequately dispose of this excess glucose is a result of a failure of the hepatic and peripheral tissues to appropriately acknowledge the body's insulin response. The Matsuda Index is used to evaluate the glucose and insulin responses during the OGTT and assess whole-body insulin sensitivity (Matsuda & DeFronzo, 1999). When compared to the plasma glucose and insulin concentrations of the impaired glucose tolerance group (IGT) reported in the Matsuda et al. (1999) article, the mean serum glucose and insulin concentrations in the present study were lower during the OGTT. This discrepancy in glucose concentration is consistent with the fact that the participants in this study were not prediabetic according to their fasting glucose either. Similarly, Dagogo-Jack, Askari, and Tykodi (2009) studied the OGTT responses of individuals classified as low-normal fasting glucose (<90 mg/dL), high-normal fasting glucose or "pre-prediabetes" (90-99 mg/dL), and impaired fasting glucose or "prediabetes" (100-125 mg/dL). The mean serum glucose concentrations during the OGTT of the current study are most consistent with the glucose responses of the pre-prediabetes group in the Dagogo-Jack study. However, the only two participants that could be classified as having impaired glucose tolerance (2 hr post-OGTT [blood glucose] >140 mg/dL) were in the placebo group of the current study.

An interesting comparison can be observed when examining the glucose changes during the OGTT in the current study, however. Although there was not a significant group by time effect for this glucose response, Figure 5 shows that the peak glucose level

was reached at 30 min for the resveratrol group and the glucose concentration then returned to near baseline levels at the 1 hr time point. This response is consistent with the glucose response of the normoglycemic participants in the Matsuda study. Furthermore, the glucose levels of the placebo group during the OGTT appear to peak between 30 and 60 min and only minutely decrease at the 2 hr time point. This OGTT blood glucose response is similar to the response seen in the IGT of the Matsuda study. Again, although there was not a significant interaction effect in this glucose response, it could be speculated that the resveratrol group had more efficient glucose disposal ability than that of the placebo group. However, this does not necessarily mean that a possible improvement in glucose disposal is a direct result of the resveratrol supplementation; instead, this discrepancy may support the observation that the placebo group was more insulin resistant than the resveratrol group at the onset of the study. An altered study design where a pre-supplementation OGTT was performed would be the only way to determine if this response is actually a result of the supplementation or a result of the two groups not being initially homogenous with regards to their degree of insulin resistance. Clearly, these observations require further investigation.

While the mRNA expression of most genes involved in insulin and AMPK signaling increased following ingestion of a 75 g bolus of glucose, none of these increases were significant. Furthermore, there were no group or group by time interactions, and thus no effect of resveratrol supplementation on such gene expression rates in response to the OGTT. As the expression of the genes for both GLUT4 and AMPK have shown to be effected by diet (O'Brien & Granner, 1996; Palacios et al., 2009; Rodgers et al., 2005; Yeo et al., 2008), it was expected that the bolus glucose

ingestion and subsequent insulin response would stimulate the expression of some of these genes involved in both the insulin-mediated and AMPK signaling pathways. Some studies have shown that prolonged insulin exposure down-regulates the expression of the GLUT4 gene (Flores-Riveros, McLenithan, Ezaki, & Lane, 1993) in 3T3-L1 mouse adipocytes and L6 muscle cells (Koivisto et al., 1991). In a human study, however, Schalin-Janti et al. (1994) found that insulin administration (μ = 586 pmol/l) during a euglycemic-hyperinsulinemic clamp stimulated increased GLUT4 mRNA levels in control participants, but not type II diabetic patients or their first degree relatives. Furthermore, another study found that a 3 hr euglycemic-hyperinsulinemic clamp stimulated transcriptional changes in numerous metabolic genes in obese individuals, whereas these effects were blunted in type II diabetic patients (Ducluzeau et al., 2001). While none of the participants in the current study were type II diabetic, nearly half of them had first degree relatives that were type II diabetic. As a result, it is possible that the insulin-signaling mechanism needed to stimulate GLUT4 mRNA transcription was impaired for these participants. It is also possible that the insulin response induced by the OGTT was insufficient to stimulate transcription in these specific genes. To the author's knowledge, this is the first study to examine the effects of an OGTT on the transcriptional activity of these metabolically relevant genes. One study from Denmark did examine GLUT4 mRNA expression following an OGTT, but it only observed the difference of GLUT4 mRNA content between groups and did not examine within-subjects transcriptional changes (Holland-Fischer et al., 2007).

Acute Effects of Resveratrol Supplementation on Exercise-Mediated Glucose Disposal

It is well documented in the scientific literature that an acute exercise bout results in enhanced insulin sensitivity for 2-4 hr post-exercise (Cartee & Wojtaszewski, 2007; Cartee & Funai, 2009; Koshinaka, Kawasaki, Hokari, & Kawanaka, 2009). The significant reductions in glucose and insulin in the current study are consistent with these findings. The post-exercise reductions in serum insulin were quite considerable. However, it was hypothesized that insulin sensitivity would be further enhanced in the resveratrol group and the results of this study do not indicate such an impact. The previous explanations of low bioavailability, dosage, and a possibly more insulin resistant placebo could contribute to this finding. In addition, it is possible that a treatment effect might have been observed if a different exercise protocol was employed that utilized a longer exercise duration and created greater metabolic stress. This theory requires further investigation, however.

The final directional hypothesis in this study also failed to be accepted as there was not a significant time or group difference seen in the transcriptional rates of the genes for AMPK, GLUT4, AS160, IRS1 or Akt2. Aside from increasing post-exercise insulin sensitivity, exercise training also improves glucose homeostasis and resting insulin sensitivity over time (Cartee & Wojtaszewski, 2007; Cartee & Funai, 2009; Kennedy et al., 1999; Koshinaka et al., 2009; Short et al., 2003). Part of these improvements in insulin sensitivity is a result of increased GLUT4 expression (Etgen et al., 1997; Hayashi et al., 1997; Host et al., 1998; Kraniou, Cameron-Smith, & Hargreaves, 2006). While it was hypothesized in the current study that resveratrol would work synergistically with exercise to stimulate the AMPK pathway and upregulate the

genes of these metabolically relevant proteins to improve future insulin sensitivity, there was no group or time effect for these transcriptional rates. While exercise training has consistently shown improvements in GLUT4 protein expression, one bout of short duration (~8 min), intense exercise may not provide a significant metabolic stimulus to impact the expression of GLUT4 or any of these other gene markers. Using the vastus lateralis muscle samples of untrained men, Kraniou and colleagues (Kraniou et al., 2006) showed that 60 min of cycling at 39% VO_{2peak} or 27 min at 83% VO_{2peak} were both sufficient stimuli to significantly elevate GLUT4 mRNA expression immediately and 3 hr post-exercise. Although it was an intense bout of exercise, the short duration of the GXT limited the overall metabolic demand of the exercise session to an extent that there was no significant stimulation of GLUT4 mRNA expression. While another possibility could be that the biopsy time point of 1 hr was outside of the response window for these genes, Kraniou et al. (Kraniou et al., 2006) showed significantly elevated GLUT4 mRNA expression at 3 hr postexercise. As a result, insufficient metabolic stress from the GXT is the more likely explanation for the lack of transcriptional changes observed in the current study.

Study Limitations

One of the primary limitations of this study was the prediabetes screening method. The glucose screening for prediabetes was conducted using capillary blood from a finger and a ReliOn® Ultima glucometer. A glucose concentration between 100-125 mg/dL was a requirement for prediabetes and inclusion into the study. Each participant's blood was tested using this capillary glucometer on two separate occasions prior to entry into the study and every participant met the prediabetic entrance criterion at this point. A

few participants did have one value below the 100 mg/dL threshold, but they were still allowed to participate given the reported variation of precision testing of the glucometer (2.9% to 5.1%). Additionally, some participants had previously been a part of another study where their serum glucose levels indicated that they would qualify as prediabetic. Although the average blood glucose concentration during screening was 107 mg/dL, the baseline serum glucose concentration for the placebo group was actually 95.63±14.11 mg/dL and 86.57±9.83 mg/dL for the resveratrol group. Consequently, only three of the sixteen participants that were screened as prediabetic at the onset of the study were actually qualified as prediabetic based on their serum glucose concentrations. Although the difference in fasting serum glucose levels between groups is not statistically significant, the glucose level of the resveratrol group is considerably lower than that of the placebo group. Additionally, the only three participants with fasting serum glucose concentrations greater than 100 mg/dL were in the placebo group, while no participants in the resveratrol group could be considered as prediabetic. While these baseline differences in blood glucose levels may not have been statistically significant, it is possible that some of the hypothesized hypoglycemic responses from resveratrol supplementation were not seen in the experimental group because their blood glucose levels were already lower than that of the placebo group.

Furthermore, there was an additional lack of homogeneity between the groups at baseline as there were significant age and height differences. When randomized into treatment groups, participants were matched based on their body fat percentage and not age, height or blood glucose level. As a result, it is possible that the baseline differences

of age, height, and blood glucose concentration could have affected the group outcomes for the aims in the directional hypotheses of this study.

Conclusions & Future Research Directions

Ultimately this study concluded that seven days of resveratrol supplementation (250 mg twice daily) was insufficient to improve insulin sensitivity and lower fasting serum glucose levels in overweight or obese females. This supplementation protocol was also unsuccessful at improving serum insulin responsiveness and transcriptional rates of metabolically relevant genes during an OGTT. Finally, one week of resveratrol supplementation was also unable to improve post-exercise insulin sensitivity and transcriptional rates of metabolically relevant genes following an acute bout of intense exercise. It is important to note that a number of limitations exist in the present study. This study contained a relatively small sample size and a short supplementation period. Furthermore, despite being screened as prediabetic, the majority of participants in this study had serum fasting glucose measurements below those required to be considered prediabetic (100 – 125 mg/dL). All of these limitations may limit the scope of the findings in the current study. While an a priori power calculation (power of 0.80) revealed that a sample size of sixteen would be sufficient, this small sample size does increase the chance of committing a type II error and missing a difference that is actually present. Furthermore, as this study aimed to assess the impact of resveratrol supplementation on a population with an impaired fasting glucose, the majority of the participants were simply overweight or obese with relatively normal fasting glucose concentrations. Capillary blood glucometer analysis was chosen as the method of screening in this study because it is very time and cost efficient. Employing this method

allowed the researchers to immediately determine the eligibility of a participant, while it would have taken considerably more time to have serum samples shipped for analysis. While large scale studies have found that capillary glucometer measurements are efficient for screening of type II diabetes (despite an error range of 6-7%) the specific glucometer employed in this study does not appear to be an accurate screening tool for prediabetes (Blumenfeld, Hertelendy, & Ford, 1977; Colagiuri et al., 2003; Kruijshoop, Feskens, Blaak, & de Bruin, 2004). In addition to screening for insulin resistance, this study could have benefited from a pre-supplementation OGTT that would have provided additional information about the glucose tolerance of the participants prior to entry into the study.

While there are numerous studies that have evaluated the *trans*-resveratrol concentration necessary for the chemopreventive pharmacological effects, future research should also determine what serum levels of *trans*-resveratrol is required to improve insulin sensitivity. Since *trans*-resveratrol's low bioavailability is a limitation of resveratrol supplementation, it would be beneficial to find the minimal amount of *trans*-resveratrol in the blood that is necessary to stimulate glucose uptake and improve insulin sensitivity (Boocock et al., 2007; Walle et al., 2004). Additionally, a recent review of the literature (Frojdo, Durand, & Pirola, 2008) supports the contention that more research needs to be conducted on methods of improving *trans*-resveratrol bioavailability in human participants in order to maximize its therapeutic potential. In addition to insulin sensitivity, future studies should also examine phosphoprotein changes and determine the resveratrol dosage necessary to stimulate the AMPK pathway. Furthermore, future research should investigate the therapeutic properties of resveratrol metabolites such as the resveratrol sulfates and glucuronides. Although it is seen as a limitation to resveratrol

supplementation, these *trans*-resveratrol metabolites could potentially elicit similar therapeutic responses as their parent compound (Boocock et al., 2007; Yoshizumi et al., 2002). It would also be beneficial to assess the potential antidiabetic effects of similar polyphenols such as pterostilbene or quercitin that may have a greater bioavailability in humans (Bhathena & Velasquez, 2002; Hwang, Kwon, & Yoon, 2009). Additional studies should also be conducted assessing the effects of resveratrol supplementation on glucose regulation in type II diabetic patients. Resveratrol is believed to stimulate glucose uptake via activation of the AMPK pathway in a manner similar to that of metformin (Park et al., 2007). It would be of clinical significance if it was found that resveratrol supplementation could be used as an effective botanical treatment for hyperglycemia, but possess fewer side effects. It would also be prudent of future studies to examine the effects of resveratrol on the transcription or activity of other crucial proteins or enzymes involved in carbohydrate (i.e. hexokinase, glycogen synthase) and lipid (i.e. PPARs, carnitine palmitoyl transferase) metabolism. While the current study failed to yield any significant effects of one week of *trans*-resveratrol supplementation, the scientific literature does provide a foundation for future research investigations into the many possible therapeutic effects of resveratrol.

APPENDICES

APPENDIX A

IRB Proposal

Application to the Baylor IRB
For Review of Research/Activity Proposal

Part 1: Signature Page

1. Name Matthew Cooke, PhD

2. Email Address (optional) Matt_Cooke@baylor.edu

3. Complete Mailing Address P.O. Box 97313

4. Position Assistant Professor

5. Faculty Advisor (if researcher is Graduate Student) _____

6. Department/School HHPR/SOE

7. Telephone # x4025 FAX # _____

8. Are you using participants in research (***Y*** or N) or in teaching exercises (Y or ***N***)?

9. Title of the research project/teaching exercise:

Effects of Resveratrol and Pterostilbene Supplementation on Insulin and Exercise-Mediated Signaling Pathways for Glucose Uptake in Overweight Insulin-Resistant Females: A Double-Blind, Clinically-Controlled Study

10. Please return this signed form along with all the other parts of the application and other documentation to the University Committee for Protection of Human Subjects in Research; Dr. Matt Stanford, Chairman, Department of Psychology and Neuroscience, Baylor University, P.O. Box 97334, Waco, Texas 76798-7334. If you have questions, or if you would like to see a copy of the OHRP Report on protection of human subjects in research, contact Dr. Stanford at extension 2961.

_____ 11/26/08

Signature of Principal Investigator Date

_____

Signature of Faculty Advisor (required if researcher is a Graduate Student)

Departmental Review: _____

Department Chair or the Chair's Designate

Part 2: Introduction & Rationale

Type II diabetes mellitus is a critical health concern that has tripled in prevalence over the past 25 years, with 24.1 million Americans (8% of the population) suffering from this condition in 2007 (3). This condition is typically preceded by an initial insulin resistant state where the insulin-responsive cells in the body do not appropriately react to circulating insulin, which results in an increase in blood glucose levels and disturbances in fatty acid metabolism. Insulin serves to lower blood glucose levels via an insulin-signaling pathway within adipocytes and muscle cells that result in translocation of the GLUT4 glucose transporter to the cell membrane. The first line of defense to prevent the development or further progression of Type II diabetes mellitus is lifestyle change. Of particular importance is losing weight, increasing physical activity, and modifying diet to an anti-atherogenic type diet (2, 4, 13).

Insulin binding is the primary stimulus for glucose uptake in muscle cells and adipocytes at rest or after a meal. Glucose, however, is taken up into muscle cells during moderate to intense exercise via insulin-independent signaling pathways. Insulin-mediated glucose uptake proceeds via the phosphatidylinositol 3-kinase (PI3-K) pathway and phosphorylation of protein kinase B (PKB; Akt) and Akt Substrate of 160 kD (AS160). Glucose uptake into muscle cells during moderate to intense exercise, however, is mediated by insulin-independent signaling pathways, likely via activation of AMP-activated protein kinase (AMPK). AMPK, which responds to increases in adenosine monophosphate (AMP):adenosine triphosphate (ATP) ratio during exercise (particularly at higher intensities), serves to phosphorylate AS160 and relieve the GLUT4 sequestration to its intracellular storage site and facilitate its translocation to the plasma membrane. Additionally, AMPK has also been shown to increase insulin sensitivity by phosphorylating specific serine residues on the insulin receptor. Recent evidence points to a convergence between the insulin and contraction-mediated signaling pathways at AS160; however this concept requires further *in vivo* research. (11).

In addition to weight reduction and physical exercise, dietary adjustments are also essential to lifestyle modifications recommended for obese or diabetic individuals. Included in these recommended dietary changes is increased intake of fruits and vegetables. Fruits and vegetables are important dietary components that can provide beneficial antioxidants and phytonutrients. Resveratrol and pterostilbene are two such phytonutrients that are found in high concentrations in red wine and a variety of plant sources such as grape skin, berries, pomegranates, and peanuts (10, 12). Over the past 5 years, research into these 2 botanical compounds has expanded, with benefits from supplementation including: improved insulin sensitivity, weight loss, cardioprotection, cancer growth inhibition, and decreased mortality. Although interest in such compounds has increased, limited research has examined their purported effects in humans. In particular, the mechanism by which both supplements are said to improve insulin-sensitivity, by enhancing GLUT4 translocation, requires further investigation. A few studies have suggested that improved insulin-sensitivity from resveratrol and pterostilbene supplementation is similar to that of metformin (a commonly used drug to treat diabetes); enhancing GLUT4 translocation via AMPK stimulation (12). However, more research is needed to confirm such observations. Additionally, exercise also stimulates GLUT4 translocation, albeit via insulin-independent mechanisms. Administration of resveratrol and pterostilbene could provide a synergistic enhancement of glucose uptake during exercise, but more importantly, continue to stimulate glucose uptake after exercise and hopefully attenuate a post-exercise hyperglycemic response (a common observation in insulin-resistant individuals).

The primary purpose of this study is to determine the effects of two polyphenolic (resveratrol or pterostilbene) supplements on glucose/insulin kinetics and markers of inflammation at rest and

following an exercise bout. Further, the study will also determine the effects of prolonged polyphenolic supplementation on markers of the metabolic syndrome. In so doing, this study will involve three specific aims:

Specific Aim 1: To determine the acute (4 days) and chronic (8 weeks) effects of pterostilbene and resveratrol supplementation on A) glucose/insulin kinetics (serum glucose, insulin) as determined by an oral glucose tolerance test (OGTT), B) skeletal muscle expression of genes involved in GLUT4 translocation (GLUT4, AMPK, AS160, Akt), C) serum levels of other relevant hormones (estrogen, leptin, adiponectin, cortisol), and D) serum and whole blood analyses for general clinical safety markers will be assessed.

Specific Aim 2: To determine the acute (5 days) and chronic (8 weeks) effects of pterostilbene and resveratrol supplementation on A) glucose/insulin kinetics (serum glucose, insulin), B) plasma inflammatory markers (TNF-α, CNTF, IL-6, IL-16), C) lipid peroxidation, and D) skeletal muscle expression of genes involved in GLUT4 translocation (GLUT4, AMPK, AS160, Akt, aPKC, CAMK, PPARα,γ,δ) prior to and following an exercise bout.

Specific Aim 3: To determine the effects of 4 weeks and 8 weeks of polyphenolic supplementation in conjuction with an exercise training program on A) serum lipid profiles, B) muscle markers of lipid metabolism (ACC, CPT1) clinical safety markers (HR, BP, complete serum metabolic panel), C) gene expression of NF-kB, SIRT1, and PPARα,γ,δ D)body composition, E) waist circumference, F) resting energy expenditure, and G) cardiopulmonary fitness.

Part 3: Methodology

Participants

Approximately 45 sedentary, overweight, pre-diabetic [fasting plasma glucose: 100-125mg/dL (6)] premenopausal females between 18 and 35 years of age, will be recruited for this study. Participants will be declared sedentary if they have not exercised for 30 minutes a day at least 5 days a week for a year or more. Additionally, oral contraceptives appear to reduce glucose tolerance by reducing peripheral tissue insulin sensitivity. Since oral contraceptives may increase glucose intolerance, all participants will be women who have not taken oral contraceptives for at least two months. Prior to participation in the study, all potential participants must provide written physician approval. Participants who qualify for the study will be cleared for participation by successfully completing a series of health screening examinations (i.e., health screening questionnaire, blood pressure assessment, fasting glucose assessment) by qualified research staff members.

All participants will not be allowed to participate if they meet any of the following criteria: 1) have current or past history of anabolic steroid use; 2) have any metabolic disorders including known electrolyte abnormalities; heart disease, arrhythmias, diabetes, thyroid disease or hypogonadism; a history of medically controlled hypertension, hepatorenal, musculoskeletal, autoimmune, or neurologic disease; if they are taking thyroid, hyperlipidmeic, hypoglycemic, anti-hypertensive, or androgenic medications; 3) have ingested any ergogenic levels of creatine, HMB, thermogenics, ribose, pro-hormones (i.e., DHEA, androstendione, etc.) or other purported anabolic or ergogenic nutritional supplements for a 2-month time period prior to beginning the study; 4) do not take any additional nutritional supplement or contraindicated prescription medication during the protocol. All participants meeting entrance criteria will sign informed

consent statements in compliance with the Human Participants Guidelines of Baylor University and the American College of Sports Medicine.

Study Site
All exercise sessions will be supervised by qualified individuals and will take place at the Student Life Center or at Russell Gymnasium at Baylor University or at the WRS Athletic Club. The majority of the training sessions will take place at Russell Gymnasium and will be supervised by National Strength and Conditioning Association (NSCA) – Certified Strength and Conditioning Specialists (CSCS). Testing sessions will be conducted in the Exercise & Sport Nutrition Laboratory (ESNL) and the Exercise & Biochemical Nutrition Laboratory (EBNL) in the Department of Health, Human Performance, and Recreation at Baylor University.

Independent and Dependent Variables
Table 1 shows the general research design and time course for assessments. The independent variables will be physical activity, diet, nutritional supplementation, subject's age, and the number of testing/evaluation times during the study. Dependent variables will include body composition measurements (body weight, whole body DEXA scans and total body water), resting energy expenditure and respiratory quotient, maximal oxygen consumption, fasting whole blood and plasma analysis, inflammatory and anti-inflammatory cytokine measures, lipid metabolism status, muscle protein and gene expression measures, serum clinical safety markers, uric acid, triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein, resting blood pressure and heart rate, and estimated energy intake.

Entry
Participants expressing interest in participating in this study will be interviewed on the phone to determine whether they appear to qualify to participate in this study (Please refer to Selection Criteria listed below). Participants believed to meet eligibility criteria will then be invited to attend an entry/familiarization and baseline session.

Familiarization Session/Baseline Testing/Pre-Supplementation (Day 1- T1)
Participants will be instructed to refrain from exercise for 24 hours and fast for 12 hours prior to pre-testing and for all scheduled assessments. Upon reporting to the lab, participants will complete questionnaires regarding exercise habits and health status (Symptoms Inventory). All participants will report to the lab in the morning for all scheduled assessments. Fasting blood glucose will initially be assessed using a OneTouch® glucometer (Lifescan, Inc.) to ensure that they are prediabetic (glucose: 100-125mg/dL [6]). Participants meeting entry criteria will be familiarized to the study protocol by way of a verbal and written explanation outlining the study design. After meeting all inclusion criteria, familiarization of the study, and signing of the informed consent, participants will then begin baseline testing.

Resting energy expenditure (REE) will be determined by analysis of the gases expired by the participant while resting in a supine position (Parvo Medics, Provo, UT). Total body mass (kg), total body water (total, intracellular, and extracellular), percent body fat, fat mass, and fat-free mass, will be assessed. Following these assessments, participants will have resting heart rate and blood pressure determined using standard procedures. Participants will then donate approximately 10-15 ml of fasting blood using venipuncture techniques of an antecubital vein in the forearm according to standard procedures. Blood samples will be allowed to stand at room temperature for 10 min and then centrifuged. For each sample, the plasma and/or serum will be removed and frozen at -80°C for later analysis. Percutaneous muscle biopsies (10-15 mg), using a fine needle aspiration technique, will be obtained from the middle portion of the vastus lateralis muscle of the dominant leg at the midpoint between the patella and the greater trochanter of the femur at a

depth between 1 and 2 cm under local ansesthesia (2% Xylocaine with epinephrine). After removal, adipose tissue will be trimmed from the muscle specimens and immediately frozen in liquid nitrogen and then stored at -80°C for later analysis.

Supplementation Protocol

Following baseline measurements, subjects will be matched based on age, body composition, and fasting glucose to randomly and blindly ingest (meaning neither the investigators nor the participants will know which groups until the end of the study) capsules containing a cellulose placebo, 250mg of resveratrol, or 250mg of pterolstilbene twice daily for the duration of the study. Supplements will be prepared in gel capsule form and packaged in generic bottles for double blind administration by Sabinsa Corporation. Supplementation compliance will be monitored by having the participants fill out supplement logs recording the amount of supplement ingested during each day of the supplement period. Participants will be instructed to consume the supplement for 4 days prior to the second testing session (T2).

Testing Sessions T2

Upon arriving, participants will have their total body mass (kg), total body water (total, intracellular, and extracellular), heart rate and blood pressure assessed. An indwelling catheter will be inserted into the participant's antecubital vein of the forearm according to standard procedures, and approximately 10-15ml of blood will be sampled. Next a percutaneous muscle biopsy sample (10-15 mg), using a fine needle aspiration technique, will be obtained from the middle portion of the vastus lateralis muscle. An OGTT will then be performed, where the participant will ingest 1.75g of glucose per kilogram of body weight (max = 75 g glucose). Additional venous blood samples (5 ml) will be obtained by an indwelling venous catheter at 30 minutes, 1hr, and 2hrs after glucose ingestion. A 2hr post-glucose ingestion muscle biopsy will also be obtained. Participants will be instructed to remain in a rested state during the 2hrs following the glucose administration.

Testing Sessions T3

Participants will have total body mass (kg), total body water (total, intracellular, and extracellular), heart rate, and blood pressure measured. An indwelling catheter will again be inserted and approximately 10-15 ml of blood will be sampled. Similarly, a percutaneous muscle biopsy sample (10-15 mg), using a fine needle aspiration technique will also be taken. It should be noted that both blood and muscle sampling will be taken on the contralateral arm and leg that was tested during T2. A 12-lead electrocardiogram (ECG) will be used to ensure that there are no contraindications during exercise testing based on the ACSM guidelines. Oxygen uptake (VO_2) will be measured every 15 sec via an open-circuit sampling system. Throughout the treadmill test, blood pressure will be accessed via standard procedures, while heart rate and rhythm will be assessed using an ECG. Blood samples (10-15mL) via an indwelling venous catheter will be taken at 30min, 1hr, and 2hrs following the cardiopulmonary exercise test to assess glucoregulation, insulin sensitivity, inflammatory markers, and lipid peroxidation markers post-exercise. Muscle biopsy samples will be obtained prior to and 2hrs post-exercise for assessment of protein and gene expression of glucoregulatory intermediates and certain inflammatory markers. Again, subjects will remain in the laboratory in a rested state for the 2hrs following the exercise bout. Participants will be instructed to continue consuming the supplement and begin an 8 week structured, energy restricted, low glycemic index diet and supervised, structured, circuit-training exercise program that includes both aerobic and resistance-training elements. Participants will return to return to the laboratory in 4 weeks for blood donation, hemodynamic, body composition and cardiopulmonary assessment.

Testing Sessions T4

Upon arriving, participants will have their total body mass (kg), total body water (total, intracellular, and extracellular), heart rate and blood pressure assessed. Resting energy expenditure (REE) will be determined by analysis of the gases expired by the participant while resting in a supine position (Parvo Medics, Provo, UT). Participants will then donate approximately 10-15 ml of fasting blood using venipuncture techniques of an antecubital vein in the forearm according to standard procedures. Subjects will then perform a graded-exercise test on a treadmill following the same guidelines as mentioned above.

Testing Sessions T5 & T6

Following the 8 week training program, subjects will return to the laboratory for testing sessions 5 and 6. Testing sessions 5 and 6 will mirror testing sessions 2 and 3, respectively to determine the chronic effects of supplementation in conjunction with exercise training on insulin-mediated glucose uptake and proteins and genes involved in exercise-mediated glucose uptake. The only addition is that REE will also be assessed at the beginning of testing session 5. Further, clinical safety panels will be used to determine the safety of the supplement. Training volume, dietary intake and supplementation will be monitored during the 8 week study intervention by training and supplementation logs and 4-day dietary recall analysis sheets.

Nutritional Intervention

Each participant will follow a structured low glycemic index diet during the eight-week study. The diet will involve the following protocol in which each participant will be requested to: 1) consume two servings of low-fat dairy each day, 2) consume two pieces of fruit per day, or one cup of canned or frozen fruit, 3) at lunch and dinner, consume low-starch vegetables, eating at least two cups of different kinds at each meal, 4) consume four ounces of meat protein (beef, pork, veal, poultry, fish, shellfish) at lunch and dinner, and 5) limit starchy vegetables (potatoes, corn, rice), grain-based foods (breads, pasta, salty snacks), flour-based foods (pastries, cakes, cookies, donuts), candy, sugar-containing beverages including juices. A typical dietary meal plan is presented in Table 2.

Exercise Protocol

The exercise intervention will be 8 weeks in duration. All groups will participate in a resistance training circuit three times a week. Circuit training will consist of 12 stations with 8-12 repetitions, following intensity guidelines of the American College of Sports Medicine (ACSM). Aerobic activity will be three days a week and intensities will follow ACSM guidelines. An overview of the exercise training program is presented in Tables 3 and 4.

Assessment of Body Composition (T1 – T6)

Total body mass (kg) will be determined on a standard dual beam balance scale (Detecto). Total body water (total, intracellular, and extracellular) will be determined with bioelectrical impedance (BIA; Xitron) while percent body fat, fat mass, and fat-free mass, will be determined using a Dual Energy X-ray Absorptiometer (DXA; Hologic QDR-4500W).

Assessment of Hemodynamic Safety Markers (T2, T4, T5)

In order to assess any hemodynamic safety concerns regarding the effects of the diet, exercise program, and polyphenol supplement, after 1 week, 4 weeks, and 8 weeks participants will undergo the assessment of resting hemodynamic safety markers (heart rate and blood pressure). Heart rate will be determined by palpation of the radial artery using standard procedures. Blood pressure will be assessed in the supine position using a mercurial sphygmomanometer using standard procedures.

Assessment of Dietary Analysis (T2, T4, T5)
In an attempt to determine compliance with the low glycemic diet, and to also assess the average daily macronutrient consumption of fat, carbohydrate, and protein in the diet, each participant will be required to keep four-day dietary records during weeks 1, 4, and 8 of the study. The dietary records will be analyzed with the Food Processor dietary assessment software program (ESHA Research Inc., Salem, OR).

Assessment of Serum Lipids (T1, T3, T4, T6)
Using a Dade Dimension clinical chemistry analyzer (Dade-Behring, Inc., Newark, DE) blood samples will be assayed for serum lipid levels (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides). This assay will help evaluate the effects of the exercise and the supplementation regimen on serum lipids.

Reported Side Effects from Supplementation (T3, T4, T6)
To determine whether the participants suffered any side effects from the placebo or supplement, after weeks 1, 4, and 8 participants will report by questionnaire administered in a confidential manner whether they tolerated the supplement, supplementation protocol, as well as report any medical problems/symptoms they may have encountered throughout the study.

In Vivo Biochemical Analyses
A standard clinical chemistry panel will be run by Quest Diagnostics, Inc. (Madison, NJ), whereas all other analytical procedures will be conducted in line with established and published protocols in the Exercise and Biochemical Nutrition Laboratory at Baylor University. Clinical blood counts will be determined via Cell Dyne 3500. Serum insulin, cortisol, and estrogen will be determined by way of enzyme-linked immunoabsorbent assay (ELISA) at a wavelength of 450 nm. Insulin sensitivity will be determined by way of the Matsuda OGTT insulin sensitivity index using OGTT insulin values (9). In addition, insulin resistance will be determined Homeostatic model assessment (HOMA) of insulin resistance (8). Bioplex bead analysis will be used to determine plasma leptin, adiponectin, TNF-α, CNTF, IL-6, IL-10, and IL-16 levels. The skeletal muscle gene expression of GLUT4, AMPK, AS160, Akt, aPKC, CAMK, NF-kB, SIRT1, and PPARα,γ,δ will be performed using RT-PCR. Oligonucleotide primers will be designed using Primer Express from known human mRNA sequences available online through the NCBI database. The quantity of mRNA will be determined relative to the expression of β-actin, and ΔC_T values will be used to compare gene expression. The specificity of the PCR will be demonstrated with an absolute negative control reaction containing no cDNA template, and single gene products confirmed using DNA melt curve analysis. Protein levels of GLUT4, AMPK, AS160, Akt, PPARα,γ,δ, CAMK, and NF-kB will be measured with an ELISA kit.

Data Analysis, Presentation, & Publication
Data will be analyzed using with SPSS for Windows Version 16 software (SPSS Inc., Chicago, IL). Body composition, aerobic capacity, glucose/insulin kinetics, protein and mRNA expression data will be analyzed via separate (time x supplement) factorial multivariate, repeated measures analyses of variance (MANOVAs). Data will be considered significantly different when the probability of error is 0.05 or less. Tukey's honestly significant differences post-hoc procedures will be performed when a significant interaction is observed. Data will be presented at an appropriate scientific conference (e.g., American College of Sports Medicine, Experimental Biology, etc) and published in a peer reviewed scientific journal (e.g., Diabetes, Medicine & Science in Sport and Exercise, Nutrition, International Journal of Sport Nutrition and Exercise Metabolism, etc).

References

1) Boocock DJ, Faust GES, Patel KR, Schinas AM, Brown VA, et al. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiol Biomarkers Prev*. 16:1246-1252, 2007.
2) Diabetes mellitus and exercise. American Diabetes Association. *Diabetes Care*. 25:S64-S68, 2002.
3) Economic costs of diabetes in the U.S. in 2007. American Diabetes Association. *Diabetes Care.* 31:1–20, 2008.
4) Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. American Diabetes Association. *Diabetes Care*. 25:S50-60, 2002.
5) Hougee S, Faber J, Sanders A, de Jong RB, van den Berg WB, et al. Selective COX-2 inhibition by Pterocarpus marsupium extract characterized by pterostilbene, and its activity in healthy human volunteers. *Planta Medica*. 71:387-392, 2005.
6) How to tell if you have pre-diabetes. American Diabetes Association. Retrieved October 15, 2008. http://www.diabetes.org/pre-diabetes/pre-diabetes-symptoms.jsp
7) Kasdorf G, Kalkhoff RK. Prospective studies of insulin sensitivity in normal women receiving oral contraceptive agents. *J Clin Endocrinol Metab*, 1988; 66:846-52.
8) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 1985;28:412-419.
9) Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*, 1999;22:1462-1470.
10) Rembsberg CM, Yanez JA, Ohgami Y, Vega-Villa KR, Rimando AM, and Davies NM. Pharmacometrics of pterostilbene: Preclinical pharmacokinetics and metabolism, anticancer, antiinflammatory, antioxidant, and analgesic activity. *Phytotherapy Research*. 22:169-179, 2008.
11) Rockl KSC, Witczak CA, and Goodyear LJ. Signaling mechanisms in skeletal muscle: Acute responses and chronic adaptations to exercise. *Life*. 60:145-153, 2008.
12) Su H-C, Hung L-M, and Chen J-K. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. *American Journal of Endocrinology and Metabolism*. 290:E1339-E1346, 2006.
13) The prevention or delay of type 2 diabetes. American Diabetes Association. *Diabetes Care*. 26:S62-S69, 2003.

Research Team

Matt Cooke, PhD. Dr. Cooke is an Associate Professor in the Department of Health, Health, Human Performance, & Recreation at Baylor University. Dr. Cooke has previous research experience in the sport nutrition industry, specifically in the areas of protein supplementation and training adaptations. He will serve as one of the principal investigators of the study assisting with all aspects of data collection and analysis.

Geoffrey Hudson, MA, CSCS. Mr. Hudson is currently a Ph.D. student in Exercise, Nutrition and Preventative Health at Baylor University and a research assistant in the Exercise and Sport Nutrition Laboratory at Baylor University. Mr. Hudson has previous research experience in the

areas of sport nutrition supplementation, anaerobic performance enhancement, thermoregulation, and the metabolic syndrome. He will serve as one of the principal investigators of the study assisting with all aspects of data collection and analysis and manuscript preparation.

Brian Shelmadine, MA, CSCS. Mr. Shelmadine is currently a Ph.D. student in Exercise, Nutrition and Preventative Health at Baylor University and a research assistant in the Exercise and Sport Nutrition Laboratory at Baylor University. Mr. Shelmadine has previous research experience in the areas of sport nutrition supplementation, anaerobic performance enhancement, strength and conditioning, and the metabolic syndrome. He will serve as one of the principal investigators of the study assisting with all aspects of data collection and analysis and manuscript preparation.

Thomas Buford, MS, CSCS. Mr. Buford is currently a Ph.D. student in Exercise, Nutrition and Preventative Health at Baylor University and a research assistant in the Exercise and Sport Nutrition Laboratory at Baylor University. Mr. Buford has previous research experience in the areas of sport nutrition supplementation, anaerobic performance enhancement, strength and conditioning, and muscle physiology. He will assist with data collection.

Mike Greenwood, PhD, FNSCA, CSCS*D, FACSM, FISSN. Dr. Greenwood is Professor and Graduate/Research Coordinator in the Department of HHPR at Baylor University. Dr. Greenwood's research expertise is in exercise nutrition predominately creatine supplementation and strength related exercise programming. He will advise on experimental design and procedures.

Darryn S. Willoughby, PhD, FACSM, FISSN, CSCS, CISSN. Dr. Willoughby is an Associate Professor of Exercise and Muscle Physiology and Biochemistry in the Department of Health, Human Performance, & Recreation at Baylor University. He is also an Associate Professor of Baylor's Biomedical Science Institute. Dr. Willoughby is an internationally recognized exercise biochemist and molecular physiologist. He has conducted a vast amount of research focusing on the biochemical and molecular regulatory mechanisms regarding exercise performance and nutrition. Dr. Willoughby will be the principal supervisor of the project. He will oversee all aspects of the study and advise on the biochemical and clinical chemistry assays involved in the project.

Procedures

Medical Monitoring. Interested participants will be invited to familiarization sessions. During this time, participants will sign consent forms and complete medical history information. Participants will then undergo a general exam by trained laboratory personnel to determine whether the subject meets entry criteria to participate in the study. This exam will include evaluating the medical and training history questionnaires and performing a general physical examination according to ACSM exercise testing guidelines. Based on this examination, participants will be assessed for their risk of cardiovascular disease and contraindications to exercise and then a recommendation will be made on whether the participant meets entry criteria and may therefore participate in the study. Trained, non-physician exercise specialists certified in CPR will supervise participants undergoing testing and assessments. A telephone is in the laboratory in case of any emergencies, and there will be no less than two researchers working with each subject during testing sessions. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for

assistance. Participants will be informed to report any unexpected problems or adverse events they may encounter during the course of the study to Matthew Cooke, Ph.D. If clinically significant side effects are reported, the participants will be referred to discuss the problem with Ronald Wilson, MD for medical follow-up. Dr. Wilson is one of the Sports Medicine physicians for Baylor University and is associated with the Department of HHPR. He has agreed to provide medical support and consultation for this study and to the ESNL. Dr. Wilson will evaluate the complaint and make a recommendation whether any medical treatment is needed and/or whether the participant can continue in the study. If Dr. Wilson feels medical follow-up is necessary, the participant will be referred to obtain medical treatment from their personal physician. This is a similar referral/medical follow-up system that Baylor athletes are provided with the exception that participants in this study will not be provided medical care. New findings and/or medical referrals of unexpected problems and/or adverse events will be documented, placed in the participants research file, and reported to the Baylor IRB committee.

Screening for Cardiopulmonary Disease Risk and Exercise Contraindications. All participants will have their risk of cardiopulmonary disease and possible contraindications to exercise assessed by Exercise Physiologists in accordance to standard procedures described by the American College of Sports Medicine's (ACSM) (*ACSM's Guidelines for Exercise Testing and Prescription, 6th ed. Williams & Wilkins Publishers, 2000)*. These guidelines are outlined and presented below:

ACSM Risk Stratification Criteria for Cardiovascular Disease

Low Risk

Younger individuals (men < 45 years of age; women < 55 years of age) who are asymptomatic for
cardiovascular disease and possess no more than one positive cardiovascular disease risk factor.

Moderate Risk

Older individuals and/or those who are asymptomatic for cardiovascular disease and possess two or more cardiovascular disease risk factors.

High Risk

Individuals with one or more signs/symptoms suggestive of cardiovascular disease.

ACSM Criteria for Signs and Symptoms Suggestive of Cardiovascular Disease

1. Pain, discomfort (or other anginal equivalents) in the chest, neck, jaw, arms, or other areas that may be
   due to myocardial ischemia.
2. Shortness of breath at rest or with mild exertion.
3. Dizziness or syncope.
4. Orthopnea or paroxysmal nocturnal dyspnea.
5. Ankle edema.
6. Palpitations or tachycardia.
7. Intermittent claudication.

8. Known heart murmur.
9. Unusual fatigue or shortness of breath with usual activities.

ACSM Absolute and Relative Contraindications to Exercise

Absolute Contraindications
1. Unstable angina.
2. Uncontrolled dysrhythmias.
3. Recent EKG changes and cardiac events.
4. Acute myocarditis or pericarditis.
5. Acute pulmonary embolism or acute myocardial infarction.
6. Severe aortic stenosis.
7. Dissecting aneurysm.
8. Acute infections.

Relative Contraindications
1. Left main coronary stenosis.
2. Severe hypertension (> 200/110).
3. Tachycardia or bradycardia.
4. Uncontrolled metabolic disease.
5. High-degree AV block.
6. Chronic infectious disease.
7. Cardiomyopahty and outflow obstructions.
8. Stenotic valve disease.
9. Ventricular aneurysm.

Assessment of Hemodynamic Safety Markers (Heart Rate, Heart Rhythm, & Blood Pressure). Heart rate will be determined by an ECG. For the ECG, the subject's skin will be prepared for placement of 10 ECG electrodes. Electrode sites will be cleansed with sterile alcohol gauze using a circular motion. The site will be allowed to air dry or will be dried with a gauze pad. Electrodes will then be placed on the right subclavicular fossa (RA), left subclavicular fossa (LA), right abdomen (RL), left abdomen (LL), 4th intercostals space at the right sternal border (V1), 4th intercostals space at the left sternal border (V2), equidistant between V2 and V4 (V3), 5th intercostal space at the midclavicular line (V4), 5th intercostal space at the anterior axillary line (V5), and 5th intercostals space at the axillarly line (V6) of the chest. The subject will then be attached to a Quinton 710 ECG. Blood pressure will be assessed in the supine position after resting for 5-min using a mercurial sphygmomanometer using standard procedures.

Reported Side Effects from Supplements. Participants will report, by questionnaire administered in a blinded manner, whether they tolerated the supplement, supplementation protocol, as well as report any medical problems/symptoms they may have encountered throughout the protocol of the study.

Estimated Energy Intake/Dietary Inventories. The participants will be required to keep four-day dietary records at three time points (T2, T4, T5) that will be evaluated with the Food Processor dietary assessment software program (ESHA Research Inc., Salem, OR) to determine the average daily macronutrient consumption of fat, carbohydrate, and protein in the diet.

Resting Energy Expenditure Test. Participants will remove shoes and lie supine on a plinth. A sterile hood will be placed over the participant's head. Once the participant is ready to begin the

test protocol, the subject will begin to have their expired gases monitored continuously throughout the exercise test. Resting expired gases will be collected using the Parvo Medics 2400 TrueMax Metabolic Measurement System (Sandy, UT). Participants will be asked to remain motionless, yet awake for twenty minutes.

Body Composition Assessments. Participants will undergo body composition tests in the ESNL. Prior to each assessment, height will be measured using standard anthropometry and total body weight will be measured using a calibrated electronic scale with a precision of +/- 0.02 kg. Body composition will then be determined using a calibrated Hologic 4500W dual-energy x-ray absorptiometry (DEXA) by licensed personnel with limited x-ray technology. The DEXA body composition test will involve having the participant lie down on their back in a standardized position in a pair of shorts/t-shirt or a gown. A low dose of radiation will then scan their entire body for approximately six minutes. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass. Radiation exposure from DEXA for the whole body scan is approximately 1.5 mR per scan. This is similar to the amount of natural background radiation a person would receive in one month while living in Waco, TX. The maximal permissible x-ray dose for non-occupational exposure is 500 mR per year. Total radiation dose will be less than 5 mR for the entire study.

Total body water will then be estimated using a Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA) which measures bio-resistance of water and body tissues based on a minute low energy, high frequency current (500 micro-amps at a frequency of 50 kHz) transmitted through the body. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water and the current to be used has been deemed safe. This is measured through four electrodes placed on the body: one electrode will be placed on the posterior surface of the right wrist, in between the radial and ulna styloid processes (wrist bones), another electrode will be placed on the posterior surface of the right hand at the distal base of the second metacarpal; the third electrode will be placed on the anterior surface of the right foot at the distal end of the first metatarsal. Participants will lie on a table in the supine position and electrodes will be connected to the analyzer. After the subject is connected, age, gender, weight, height, and activity level are entered into the unit by the technician. After the unit has measured the resistance, which takes approximately 30 seconds, the unit then calculates total body water and body water percent.

Aerobic Exercise Capacity Test. Participants will first be seated next to the treadmill and resting heart rate and blood pressure will be measured. A sterile mouthpiece attached to a head harness will be secured on the participant. The participant will then have a noseclip placed on their nose. Resting expired gases will be collected using the Parvo Medics 2400 TrueMax Metabolic Measurement System (Sandy, UT). Once the participant is ready to begin the test protocol, he/she will straddle the treadmill with both legs while the treadmill is turned on at a speed of 2.0 mph and at a 0% grade. The participant will then use one foot to repeatedly swipe the belt in order to gauge the speed of the motion. Once the participant is familiar with this speed, he/she will step onto the belt while still gripping the handrail with both hands. Once the participant becomes comfortable walking on the treadmill, he/she will let go of the handrail and begin walking freely. The participant will then perform a standard symptom-limited Modified Balke treadmill maximal exercise test using the following speeds and grades:

Stage	Speed	Grade (%)	Duration (min.)
1	3.4	0	1
2	3.4	1	1
3	3.4	2	1
4	3.4	3	1
5	3.4	4	1
6	3.4	5	1
7	3.4	6	1
8	3.4	7	1
9	3.4	8	1
10	3.4	9	1
11	3.4	10	1
12	3.4	11	1
13	3.4	12	1

Blood Samples. Participants will donate approximately 3-4 teaspoons of fasting venous blood (approx 10-15 milliliters) during each baseline blood draw, ~5mL during the post-OGTT blood draws, and approx 10-15mL during the post-GXT blood draws. Blood samples obtained during testing sessions 2, 3, 5 and 6 will be via standard indwelling venous catheter, while blood samples obtained during testing session 1 and 4 will be via standard sterile venipuncture technique. All blood samples will be obtained from the antecubital vein using standard phlebotomy procedures by Matthew Cooke, Ph.D. or laboratory technician's trained in phlebotomy in compliance with guidelines established by the Texas Department of Health and Human Services. The phlebotomists and lab technicians will wear personal protective clothing (gloves, lab coats, etc.) when handling blood samples. Subjects will be seated in a phlebotomy chair. Their arm will be cleaned with a sterile alcohol wipe and sterile gauze. A standard rubber tourniquet will then be placed on the brachium (upper arm) and will be tight enough to visibly indent the skin, but not cause the patient discomfort. For venipunctures, an antecubital vein will be palpated and then a 22 gauge sterile needle attached to a plastic vacutainer holder will be inserted into the vein using standard procedures. One plasma vacutainer tube (Green top), one serum separation vacutainer tube (Red tops), and one whole blood vacutainer (Purple top) will be inserted into the vacutainer holder for blood collection in succession using multiple sample phlebotomy techniques. Once samples are obtained, the vacutainer holder and needle will be removed. The needle will then be discarded as hazardous waste in a plastic sharps container. For the indwelling catheter sampling, the entry site will be thoroughly cleaned with an alcohol prep pad and allowed to dry and also sterilized with 10% povidone-iodine solution and allowed to dry. The participant will be instructed to lower their arm and make a fist several times in order to maximize venous engorgement. The appropriate vein will be selected. If a suitable vein is difficult to identify, the arm may be covered with a warm, moist compress to help with peripheral vasodilatation. If after a meticulous search no suitable veins are found, then the tourniquet will be released from above the elbow and placed around the forearm to search in the distal forearm, wrist and hand. To puncture the vein, the 20 gauge catheter will be held in the dominant hand. With the bevel up, enter the skin at about a 30-degree angle and in the direction of the vein. After entering the skin, the angle of the catheter will be reduced until it is nearly parallel to the skin. If the vein appears to "roll" (move around freely under the skin), the venipuncture will begin by applying counter tension against the skin just below the entry site using the thumb of the non-dominant hand. The skin will be pulled distally toward the wrist in the opposite direction the needle will be advancing,

being careful not to press too hard which will compress blood flow in the vein and cause the vein to collapse. The catheter will be advanced into the vein until blood is seen in the "flash chamber" of the catheter. After entering the vein, the plastic catheter (which is over the needle) will be advanced into the vein while leaving the needle stationary. The hub of the catheter will be all the way to the skin puncture site. The tourniquet will be released. Gentle pressure will be applied over the vein just proximal to the entry site to prevent blood flow. The needle will be removed from within the plastic catheter and disposed in an appropriate sharps container. The catheter will be taped in place using strips of tape and a sterile dressing. Once sampling is complete, the site of the blood draw will then be cleaned with a sterile alcohol wipe and gauze and a sterile Band-Aid will be placed on the site. The blood collection tubes will be labeled and placed in a test tube rack. Laboratory technicians (who have received blood borne pathogen training and will be wearing personal protective clothing) will centrifuge the serum and/or plasma samples, transfer serum and/or plasma into labeled storage containers, and store at -80°C for later analysis.

Muscle Biopsies. Percutaneous muscle biopsies (10-15 mg), using a fine needle aspiration technique, will be obtained from the middle portion of the vastus lateralis muscle of the dominant leg at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. Muscle biopsies will be performed by Dr. Matt Cooke or an appropriately trained graduate student. Muscle biopsy training requires that graduate students successfully perform a minimum of 3 biopsy procedures on non-research participants under Dr. Willoughby or Dr. Cooke's direct supervision before they are allowed to perform them on research participants. Graduate students must then successfully perform 15 biopsies on research participants under direct supervision before they are allowed to perform muscle biopsies without supervision. The procedures used are previously-established procedures by Darryn Willoughby, Ph.D., who has extensive experience in performing muscle biopsies as a part of his research. For subsequent biopsies, muscle tissue will be extracted from the same location by using the previous location and depth markings on the needle. Pre-biopsy preparations will include removing hair from the procedure area and wiped with 70% isopropyl alcohol prep pads. The biopsy site will be numbed with 1cc of 2% Xylocaine with epinephrine. After local anesthesia, the biopsy site will be sterilized with 10% povidone-iodine solution and a pilot hole will be made with an 18-gauge needle. The biopsy procedure will then begin and take 15-20s to perform. Once the muscle sample has been obtained, pressure will be immediately applied to the biopsy site and an adhesive bandage immediately applied. Bleeding is minimal due to the small puncture-type opening; therefore, only a standard band-aid type bandage is needed to cover the puncture. The biopsy needles will be discarded as hazardous waste in an appropriately-labeled plastic sharps container. The site of the biopsy will be cleaned with a sterile alcohol wipe and gauze. The alcohol wipe and gauze then will be discarded in an appropriately labeled biohazard waste receptacle. The tissue sample will be stored at -80 degrees C for future analyses. Written instructions for post-biopsy care will be given to the subjects. The participant will be instructed to leave the band-aid in place for 12 hours (unless unexpected bleeding or pain occurs) and asked to contact the lab immediately if they feel there is a problem. The time course nature of the study will allow for daily follow-up to occur in order to ensure all biopsy locations are healing correctly and free of infection. Aside from the testing sessions, the participant will be further advised to refrain from vigorous physical activity during the first 24 hours post-biopsy. These suggestions will minimize pain and possible bleeding of the area. If needed, the subject may take non-prescription analgesic medication such as Acetaminophen to relieve pain if needed. However, medications such as aspirin, Nuprin, Bufferin, or Advil will be discouraged as these medications may lead to ecchymosis at the biopsy site. Soreness of the area may occur for about 24 hours post-biopsy.

Equipment

Digital Scale. Total body weight will be determined using a digital scale accurate to ±0.02 kg. The scale is calibrated by placing certified 25-kg weights and balancing the scale. Other than general instructions, special skills are not required to measure body weight.

Mercurial Sphygmomanometer. Blood pressure will be assessed by auscultation of the brachial artery using a mercurial sphygmomanometer using standard clinical procedures.

Bioelectrical Impedance Analyzer (BIA). The Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA) which measures bio-resistance and body composition based on a minute low energy, high frequency current transmitted through the body from surface electrodes attached to standardized anatomical locations on the dorsal surface of the right hand and foot while the subject lies motionless in a supine position. The analyzer is calibrated internally to a standard electrical current by pressing the calibration key located on the unit. A trained research assistant will perform this procedure.

Dual-Energy X-Ray Absorptiometer (DEXA). Body composition measurements will be determined by qualified personnel (in compliance with State Regulations) using a Hologic 4500W dual energy x-ray absorptiometer (Waltman, MA). This system segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments (i.e., bone mass, fat mass, and fat-free/soft tissue mass). Quality control (QC) calibration procedures will be performed on a spine phantom (Hologic X-CALIBER Model DPA/QDR-1 anthropometric spine phantom) prior to each testing session. In addition, weekly calibration procedures will be performed on a density step calibration phantom.

Resting Energy Expenditure. Resting energy expenditure measurements will be obtained using Parvo Medics 2400 TrueMax metabolic measurement system (Sandy, UT).

Aerobic Exercise Capacity Testing. Maximal cardiopulmonary measurements will be obtained using Parvo Medics 2400 TrueMax metabolic measurement system (Sandy, UT). Participants will be attached to the Quinton 710 ECG (Bothell, WA) and walk on a Quinton QStressTm65 treadmill (Bothell, WA).

Muscle Biopsy Needle. The muscle biopsy technique (as mentioned above) will be performed with a TRU-CORE® 1 Automatic Reusable Biopsy Instrument (Angiotech, Medical Device Technologies, INC., Gainsville, Florida, USA).

Clinical Chemistry Analysis. Serum samples will be shipped to Quest Diagnostics, Inc. (Madison, NJ) and a standard clinical chemistry panel will be run. Cell-Dyne 3500 (Abbott Diagnostics, Dallas, TX) hematology analyzer will be used to measure whole-blood markers [leukocytes, lymphocyte differentials (neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cells, hemoglobin, hematocrit, platelets].

Serum & Plasma Analysis. Blood samples will also be used to assess hormone profiles insulin, estrogen, and cortisol, and the levels of the inflammation-related, interleukin-1β (IL-1β), interleukin-6 (IL-6), ciliary neurotrophic factor (CNTF), and tumor necrosis factor-α (TNF-α) spectrophotometrically using either enzyme-linked immunoabsorbent assays (ELISA) or enzyme immunoassys (EIA) with a Wallac Victor-1420 micoplate reader (Perkin-Elmer Life Sciences, Boston, MA) or the Bio-Plex bead-based multiplex assays (Luminex xMAP technology) by Bio-

Rad Laboratories, Inc. (Hercules, CA; #7000005KYMR). The assays will be performed at either 405 or 450 nm wavlength against a known standard curve.

Muscle Protein Gene Expression. Muscle protein samples will be used to isolate total RNA, reverse transcribed, and then used to perform real-time polymerase chain reaction using specific oligonucleotide primers to amplify target genes using an iQ real-time PCR system (Bio-Rad, Hercules, CA).

Participants

Recruitment

Approximately 45 sedentary, overweight, pre-diabetic [fasting plasma glucose: 100-125mg/dL (4)] premenopausal females between 18 and 35 years of age, will be recruited for this study. Participants will be declared sedentary if they have not exercised for 30 minutes a day at least 5 days a week for a year or more. Additionally, oral contraceptives appear to reduce glucose tolerance by reducing peripheral tissue insulin sensitivity. Since oral contraceptives may increase glucose intolerance, all participants will be women who have not taken oral contraceptives for at least two months. Prior to participation in the study, all potential participants must provide written physician approval. Participants who qualify for the study will be cleared for participation by successfully completing a series of health screening examinations (i.e., questionnaire, blood pressure assessment) by qualified research staff members. A recruitment flyer that will be posted on campus, at area fitness centers, and on the Internet (http://www3.baylor.edu/HHPR/research/subjects/) and sent via campus mail is attached.

Selection Criteria

Participants will not be allowed to participate in the study if they:
have a body mass index less than 25.
have been involved in a habitual exercise training program (minimum of 2.5 hours/week for 1 year);
have any known metabolic disorder including heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism;
have a history of pulmonary disease, medically controlled hypertension, hepatorenal disease, musculoskeletal disorders, neuromuscular/neurological diseases, autoimmune disease, cancer, peptic ulcers, anemia, or bleeding disorders;
have liver, kidney disease, or heart disease;
are taking any heart, pulmonary, thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, endocrinologic (e.g, thyroid, insulin, etc), psychotropic, or neuromuscular/neurological medications;
have taken nutritional supplements or prescription medications that may affect body composition and/or muscle mass (e.g., creatine, HMB) within three months prior to the start of the study.
have any absolute or relative contraindication for exercise testing or prescription as outlined by the American College of Sports Medicine;
report any unusual adverse events associated with this study that in consultation with the supervising physician recommends removal from the study.

Compensation or Incentives

Participants completing all familiarization and testing sessions as well as turning in all required materials (i.e., dietary and training logs) in the study will be paid $150. Participants may receive

information regarding results of these tests if they desire. If participants are Baylor students, they will not receive any academic credit for participating in this study.

Potential Risks

The resveratrol and pterostilbene supplements to be investigated in this study have been studied for various medical uses in rats and in vitro, but limited research has been performed in humans. However, recent research has demonstrated that oral administration of these compounds is not associated with any significant medical side effects. Pterocarpus marsupium Roxb. extract (450 mg), one source of pterostilbene, was recently used to study Cox-2 inhibition in humans (5). No adverse side effects were reported in healthy human volunteers. Resveratrol is currently under study in Phase II clinical trials for use in prevention of colon cancer (www.cancer.org). Thus, resveratrol has passed Phase-I trials for safe dose ranges, any side effects, and how the body copes with the drug (1). Additionally, both compounds are found in ayurvedic medicines, which have been commonly used for hundreds of years, to treat hyperlipidemia and hyperglycemia. Moreover, these supplements are currently available in over the counter nutritional supplements sold in the United States. As with the vast majority of nutritional supplements, however, the FDA may not have evaluated the safety or marketing claims of resveratrol and pterostilbene.
Participants who meet eligibility criteria will be exposed to a low level of radiation during the DEXA body composition tests, which is similar to the amount of natural background radiation a person would receive in one month while living in Waco. In addition, a very low level of electrical current will be passed through each participant's body using a bioelectrical impedance analyzer. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of the BIA and DEXA analyzers has been shown to be safe methods of assessing body composition and total body water and is approved by the FDA.

Participants will donate samples of venous blood during the study using standard phlebotomy procedures. This procedure may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising. The subject may also experience some dizziness, nausea, and/or faint if they are unaccustomed to having blood drawn. Participants will likely experience short-term muscle soreness and moderate fatigue, and may experience muscle strains/pulls during their routine resistance-training program.

Subjects donating muscle biopsies may experience some anxiety before this procedure regarding a perception of pain or discomfort. The biopsy procedure may cause a small amount of bleeding and/or pain as the pilot hole is made and the sample is extracted from the muscle. However, once the anesthesia takes affect, there is usually only mild pressure and a small amount of bleeding as the needle is inserted and extracted. During the biopsy procedure, subjects may experience a slight localized cramping followed by brief and minor aching but these symptoms usually go away when the needle is withdrawn. Frequently, subjects feel little or no sensation at all. Although the muscle selected for biopsy (vastus lateralis) has no major blood vessels or nerves in the areas where the biopsy needle will be inserted, there is the rare occurrence of compressing or cutting small nerve branches, which can sometimes cause temporary tingling and numbness in the skin. These responses, when they have occurred, have dissipated in a few days or weeks. After the needle is withdrawn, pressure is applied to the site of the incision to prevent any unwarranted bleeding (there is usually very little bleeding). After the biopsy, the muscle is likely to be moderately sore for about 24 hours. This soreness is similar to muscle soreness following unusually vigorous exercise or a muscle injury especially if muscle is compressed against a bone (e.g., "charley horse"). Complications accompanying this procedure are rare and no complications have been observed in subjects who have donated biopsies in the EBNL in

previous studies. The primary risks, include bleeding, hematoma (bruising), infection, and slight scarring of the skin have occurred, however. Some individuals may develop keloid scarring at the site of incision. Also, some individuals may have an allergic reaction to the anesthetic, such as a local rash, or difficulty breathing. To minimize these risks, the amount of anesthetic used will be approximately 2-4% of the maximal dose for a normal sized individual. Every precaution will be made to keep these risks to a minimum. Additionally, these potential risks can be prevented and/or treated with rest, ice, compression, elevation, and adhering to post-biopsy care instructions. Taking a mild non-prescription pain medication such as Tylenol, providing the subject can tolerate these medications, is also recommended for pain. In all these procedures, care is taken to employ precautions to avoid infection, including the "universal precautions" for the handling of blood and infectious materials. Muscle biopsies do not cause any permanent damage with the exception of a small scar which should become undetectable over time, although some individuals may develop keloid scarring at the site of the incision.

Laboratory personnel represent trained, non-physician, certified exercise specialists (American Society of Exercise Physiologies Certified Exercise Physiologist, Certified Strength & Conditioning Specialists, and/or American College of Sports Medicine Health Fitness Instructor$_{SM}$, Exercise Technologist$_{SM}$, or Exercise Specialists$_{SM}$). All personnel involved in collecting data will be certified in CPR, which is also a condition to holding these professional certifications. A telephone and automated electronic defibrillator (AED) is located in the laboratory in case of any emergencies and there will be no less than two researchers working with each subject during testing. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for assistance.

Potential Benefits

The main benefit that participants may obtain from this study is that if these two thermogenic nutritional supplements are effective there is a possibility that they may experience decrements in percent body fat during training as well as improved health profiles. Participants may also gain insight about their health and fitness status from the assessments to be performed. However, even if no individual benefit is obtained, participating in this study will help to determine whether ingesting this nutritional supplement affects training adaptations and body composition. This information will be helpful to athletes and non-athletes alike who use thermogenic supplements during training to know whether they are effective or not.

Assessment of Risk

Because they are relatively new to the market and little research conducted in humans, the potential medical benefits of resveratrol and pterostilbene are not yet fully known; although, these compounds are available in a number of over the counter nutritional supplements. Initial results in humans and rats suggest that these supplements may provide benefit by reducing lipid levels, improving insulin sensitivity, weight loss, cardioprotection, cancer growth inhibition, and increased mortality (10-12). However, additional well-controlled research is necessary before conclusions can be drawn. This study will help determine whether ingesting either resveratrol or pterostilbene may prove to be effective at improving insulin sensitivity in overweight prediabetic females. The only known side effects associated with these polyphenols are found with extremely high and unrealistic doses and include: mild anaemia, an increased liver weight, and increased serum cholesterol.

The risk of supplementation of these compounds at the levels to be evaluated in this study is low. The greatest risk associated with participating in this study will be the participants initiating an

exercise-training program. Since the participants to be used in this study are prediabetic, are required to have physician clearance, and would be told to begin exercising and eating a healthier diet, these risks would be no different than if they participated in their own training program or exercised at a local gym. Additionally, each circuit training session will supervised by a Certified Strength and Conditioning Specialist. Therefore, the potential benefits of subjects participating in this study outweigh the potential risks.

Compensation for Illness or Injury

Each participant will agree to indemnify and hold harmless Baylor University, its officers, directors, faculty, employees, and students for any and all claims for any injury, damage or loss suffered as a result of participation in this study regardless of the cause of injury, damage, or loss.

Confidentiality

Information obtained from this research (including questionnaires, medical history, laboratory findings, or physical examination) will be kept confidential to the extent permitted by law. However, according to FDA regulations, records will be open to FDA representatives to review if necessary. This may include questionnaires, medical history, laboratory findings/reports, statistical data, and/or notes taken throughout this study. Records of the research may also be subpoenaed by court order or may be inspected by federal regulatory authorities. Data derived from this study may be used in reports, presentations and publications. However, participants will not be individually identified unless they give their written consent.

Data Presentation & Publication

Data will be presented at an appropriate scientific conference (e.g., American College of Sports Medicine, International Society of Sports Nutrition, Experimental Biology, etc.) and published in a peer reviewed scientific journal (e.g., Journal of Nutrition, International Journal of Sport Nutrition and Exercise Metabolism, etc.).

STATEMENT ON CONFLICT OF INTEREST

Funding for the study has been obtained from Sabinsa, Inc. (Piscataway, NJ) through research grants awarded to Baylor University. Researchers involved in collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

Table 1. Treatment Timeline

FAM Session & Pre-Supplementation Testing (T1)	Days 1-4 Pre-Testing Supplementation	Day 5 Oral Glucose Tolerance Testing (T2)	Day 6 Exercise Testing (T3)	Day 37 Training Assessment (T4)	Day 67 Oral Glucose Tolerance Testing (T5)	Day 68 Exercise Testing (T6)
Familiarization session & Informed Consent. Review medical history	Begin supplementation with placebo, resveratrol, or pterostilbene	Body Mass/ BIA/HR/BP	Body Mass/ BIA/HR/BP	Body Mass/REE/ BIA/HR/BP/ DXA	Body Mass/ BIA/HR/BP	Body Mass/REE/ BIA/HR/BP/ DXA
		Blood & muscle biopsy sample	Blood & muscle biopsy sample	Blood sample	Blood & muscle biopsy sample	Blood & muscle biopsy sample
Blood glucose screening		Glucose (75g) administration	Graded exercise test (GXT)	Graded exercise test (GXT)	Glucose (75g) administration	Graded exercise test (GXT)
Complete paperwork. Health Status		30min post-glucose blood sample	30min post-exercise blood sample		30min post-glucose blood sample	30min post-exercise blood sample
Body mass/REE/ BIA & DXA assessments	Monitor dietary intake	1hr post-glucose blood sample	1hr post-exercise blood sample		1hr post-glucose blood sample	1hr post-exercise blood sample
Pre-supplementation blood & biopsy sampling		2hr post-glucose blood & biopsy sample	2hr post-exercise blood & biopsy sample		2hr post-glucose blood & biopsy sample	2hr post-exercise blood & biopsy sample
Randomized, double-blind, placebo-controlled assignment		Continue supplement intake & dietary monitoring	Continue supplement intake		Continue supplement intake & dietary monitoring	

Table 2. Overview of a Typical Daily Meal Plan

MEAL	*EXAMPLE OF FOODS CONSUMED*
Breakfast	Meal replacement shake 1 cup low-fat milk 1 piece of fruit Coffee or tea
Mid-morning snack	½ snack bar Tea or coffee
Lunch	Large (> 2 cups) salad (a variety of raw vegetables) Low-fat salad dressing 4 ounces lean cold cuts 6 small crackers No-calorie carbonated beverage
Mid-afternoon snack	½ snack bar No-calorie beverage
Dinner	4 ounces meat ½ baked potato Small pat margarine 2 cups low-starch vegetables (two different kinds) Water
Evening snack	1 cup low-fat milk Cut up raw vegetables

Table 3. Overview of the Resistance Training Program

Weeks	Modalities (type of exercise)	Intensity (% 1-Rep Max)	Sets/Reps	Frequency (Days/Week)
1-2	upper-body exercises (chest, shoulders, back, arms, abs); lower-body exercises (gluteals, quadriceps, hamstrings)	50%-55%	2/15-20	3
3-4	upper-body exercises (chest, shoulders, back, arms, abs); lower-body exercises (gluteals, quadriceps, hamstrings)	50%-55%	2/15-20	3
5-6	upper-body exercises (chest, shoulders, back, arms, abs); lower-body exercises (gluteals, quadriceps, hamstrings)	55%-60%	3/12-15	3
7-8	upper-body exercises (chest, shoulders, back, arms, abs); lower-body exercises (gluteals, quadriceps, hamstrings)	55%-60%	3/12-15	3

Table 4. Overview of the Aerobic Training Program

Weeks	Modalities (type of exercise)	Intensity (% HRR)	Minutes	Frequency (Days/Week)
1-2	Walking, Jogging	45%-55%	35-40	3
3-4	Walking, Jogging	55%-60%	35-40	3
5-6	Walking, Jogging	55%-60%	40-45	3
7-8	Walking, Jogging	60%-65%	40-45	3

FEBRUARY 2009

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1	2 Supplement Workout Supplement	3 Supplement Walk Supplement	4 Supplement Workout Supplement	5 Supplement Walk Supplement	6 Supplement Workout Supplement	7 Supplement Walk Supplement
8 Supplement Supplement	9 Supplement Workout Supplement	10 Supplement Walk Supplement	11 Supplement Workout Supplement	12 Supplement Walk Supplement	13 Supplement Workout Supplement	14 Supplement Walk Supplement
15 Supplement Supplement	16 Supplement Workout Supplement	17 Supplement Walk Supplement	18 Supplement Workout Supplement	19 Supplement Walk Supplement	20 Supplement Workout Supplement	21 Supplement Walk Supplement
22 Supplement Supplement	23 Supplement Workout Supplement	24 Supplement Walk Supplement	25 Supplement Workout *Food Logs* Supplement	26 Supplement Walk *Food Logs* Supplement	27 Supplement Workout *Food Logs* Supplement	28 Supplement Walk *Food Logs* Supplement
1 Supplement Supplement	2 T4 Testing Session Supplement	3 Supplement Walk Supplement	4 Supplement Workout Supplement	5 Supplement Walk Supplement	6 Supplement Workout Supplement	7 Supplement Walk Supplement
8 Supplement Supplement	9 Supplement Workout Supplement				**Notes:** Take supplement at lunch & before bed Workout three times per week Walk three times per week 4 day food logs	

Figure 1. Sample Training Calendar given to the participant

Part 5: Informed Consent Form Checklist

When using humans as subjects in research you must obtain their informed consent. Check each of the following items as they appear on your Informed Consent Form and include this checklist with your protocol:

__ (a) A statement explaining the purpose of the research.

__(b) A statement of the expected duration of the subject's participation.

__(c) A description of the procedures to be followed.

__(d) A description of any reasonable foreseeable risks or discomforts to the subject, including invasion of privacy.

__(e) A description of any benefits resulting from the research, either to the subject or to others.

N/A (f) A statement that informs subject of his/her right not to be a subject in a research project that is also a teaching exercise.

__(g) A statement informing subject about how his/her anonymity will be guarded; i.e., that their confidentiality will be protected by assigned code numbers, by limitations of who has access to data, by data storage in locked cabinets, by locked computer files, etc.

__ (h) A statement that the subject's participation is voluntary, and that his/her refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

N/A (i) A disclaimer, if applicable, regarding the use of the Internet to collect data.

__(j) For research involving more than minimal risk, an explanation regarding the availability of any compensation or any medical treatments if injury occurs (if applicable, see OHRP Reports).

__(k) If written informed consent is required, a place for the subject to sign and date the form and a statement that a copy of the signed consent form will be given to the subject for his/her records.

N/A (l) If the subject is a minor, a statement of parental responsibility in consenting to the child's participation in the study with a place for the parent to sign and date the form in addition to the participant's signature.

__(m) Include a short summary of your expertise related to this research proposal.

__ (n) The name, address, and telephone number of the principal investigator of the research project, and his/her affiliation with Baylor University. If the principal investigator is a graduate student, the name and telephone number of the faculty advisor is also required.

__(o) A statement informing subject that inquiries regarding his/her rights as a subject, or any other aspect of the research as it relates to his/her participation as a subject, can be directed to Baylor's University Committee for Protection of Human Subjects in Research. The chairman is Dr. Ken Wilkins, Associate Dean Graduate Studies and Research, Graduate School, PO Box 97264, Waco, Texas 76798, phone number 254-710-3582.

Wound Care for the Muscle Biopsy Procedure

Complications resulting from the muscle biopsy procedure are rare. Furthermore, after the procedure, you can reduce your risk of chance of infection by adhering to the following course of action for wound care:

For approximately 24 hours post biopsy

- leave the bandage on for 24 hours (unless unexpected bleeding or pain occurs, which should be immediately reported to the lab)
- lightly clean around the bandage if necessary
- reapply a fresh adhesive bandage if necessary
- refrain from vigorous physical activity during the first 24 hours post biopsy

After approximately 24-48 hours post biopsy

- reapply a fresh adhesive bandage after 24-36 hours
- return to normal hygiene practices after 48 hours unless complications arise

Possible Pain Side Effects

Soreness of the area may occur for about 24 hours post biopsy. Following the procedures outlined above should significantly minimize pain and possible bleeding of the area. However, some subjects experience no significant pain post biopsy.

☺ If needed, the subject may take non-prescription analgesic medication such as Tylenol to relieve pain if needed.

☹ Medications such as aspirin, Advil, Bufferin, or Nuprin, are discouraged as they may lead to excess bruising at the biopsy site.

If any questions or complications arise please contact:

Matthew Cooke, Ph.D.
Room # 118 Marrs McLean Gym
(254) 710-4025
Matt_Cooke@baylor.edu

or

Geoffrey Hudson, MA.
Room # 117 Marrs McLean Gym
(254) 710-4012
Geoffrey_Hudson@baylor.edu

BAYLOR UNIVERSITY
EXERCISE & SPORT NUTRITION LABORATORY

Medical History Inventory

Directions. The purpose of this questionnaire is to enable the staff of the Exercise and Sport Sciences Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is CONFIDENTIAL as described in the Informed Consent Statement.

Name:_____ Age _____Date of Birth_____

Name and Address of Your Physician:_____

MEDICAL HISTORY

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in blank).

____ Heart murmur, clicks, or other cardiac findings?
____ Frequent extra, skipped, or rapid heartbeats?
____ Chest Pain of Angina (with or without exertion)?
____ High cholesterol?
____ Diagnosed high blood pressure?
____ Heart attack or any cardiac surgery?
____ Leg cramps (during exercise)?
____ Chronic swollen ankles?
____ Varicose veins?
____ Frequent dizziness/fainting?
____ Muscle or joint problems?
____ High blood sugar/diabetes?
____ Thyroid Disease?
____ Low testosterone/hypogonadism?
____ Gluacoma?
____ Asthma/breathing difficulty?
____ Bronchitis/Chest Cold?
____ Cancer, Melanoma, or Suspected Skin Lesions?
____ Stroke or Blood Clots?
____ Emphysema/lung disease?
____ Epilepsy/seizures?
____ Rheumatic fever?
____ Scarlet fever?
____ Ulcers?
____ Pneumonia?
____ Anemias?
____ Liver or kidney disease?
____ Autoimmune disease?
____ Nerve disease?
____ Psychological Disorders?

Do you have or have you been diagnosed with any other medical condition not listed?
_____
_____

Please provide any additional comments/explanations of your current or past medical history.
_____
_____

Please list any recent surgery (i.e., type, dates etc.).
_____
_____

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last 3 months.
_____

What was the date of your last complete medical exam?
_____

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study (including strength and maximal exercise tests) ____ If yes, please explain: _____
_____

Recommendation for Participation
____ No exclusion criteria presented. Subject is cleared to participate in the study.
____ Exclusion criteria is/are present. Subject is not cleared to participate in the study.

Signed: _____ Date: _____

Baylor University
Exercise & Sport Nutrition Laboratory

Personal Information

Name:

Address:

City: _____ State: _____ Zip Code: _____ SS# _____

Home Phone: ( ) _____ Work Phone: ( ) _____

Beeper: ( ) _____ Cellular ( ) _____

Fax:: ( ) _____ email address: _____

Birth date: _____ / _____ / _____ Age: _____ Height: _____ Weight: _____

Exercise History/Activity Questionnaire

1. Describe your typical occupational activities.

2. Describe your typical recreational activities.

3. Describe any exercise training that you routinely participate.

4. How many days per week do you exercise/participate in these activities?

5. How many hours per week do you train?

6. How long (years/months) have you been consistently training?

Baylor University
Exercise & Sport Nutrition Laboratory

NAME _____ **Date** _____

INSTRUCTIONS

1. Record everything you eat for 4 days (including one weekend day). If you eat pretzels, record how many. If you eat a bag of chips, record the number of ounces. For drinks, record the number of cups or ounces. Record everything you drink except water.
2. Record the Food, Amount, Brand Name, and Preparation Methods. For example: baked vs. fried chicken; 1 cup of rice; 2 teaspoons of margarine; 1 cup of 2% milk; McDonald's, Healthy Choice, or Frosted Flakes.
3. Record immediately after eating. Waiting until that night may make it difficult to remember all foods and quantities.

Food (include brand)	Method of Preparation	Quantity (cups, oz., no.)
BREAKFAST:		
LUNCH:		
DINNER:		
SNACKS:		

Baylor University
Exercise & Sport Nutrition Laboratory

NAME _____ **Date** _____

INSTRUCTIONS

1. Record all daily physical activity including type, duration and distance covered

Day	Type (i.e. cycling, running)	Duration	Distance covered

Reported Side Effects From Supplement Questionnaire
Follow-up Assessment

Subject #: _____ Date: _____

Testing Session	1	2	3
Rate the ***frequency*** of the following symptoms according to the scale where: 0 = none 1 = minimal (1-2 per/wk) 2 = slight (3-4 per/wk) 3 = occasional (5-6 per/wk) 4 = frequent (7-8 per/wk) 5 = severe (9 or more per/wk)			
Dizziness?			
Headache?			
Fast or racing heart rate?			
Heart skipping or palpitations?			
Shortness of breath?			
Nervousness?			
Blurred Vision?			
Any other unusual or adverse effects?			
Rate the ***severity*** of the following symptoms according to the scale where: 0 = none 1 = minimal 2 = slight 3 = moderate 4 = severe 5 = very severe			
Dizziness?			
Headache?			
Fast or racing heart rate?			
Heart skipping or palpitations?			
Shortness of breath?			
Nervousness?			
Blurred Vision?			
Any other unusual or adverse effects?			

PLEASE REMEMBER TO REPORT ANY SIDE EFFECTS IMMEDIATELY.

Directions: If necessary, please contact either Darryn Willoughby, Ph.D. at 254-710
3504 or Matt Cooke, Ph.D. at 254-710-4025. You may also e-mail either at Darryn_Willoughby@baylor.edu or Matt_Cooke@baylor.edu.

Thanks for your participation!

Exercise • Nutrition • Health • Performance
BU ESNL
BAYLOR UNIVERSITY
Exercise & Sport Nutrition Laboratory

BAYLOR
UNIVERSITY

Dear Medical Provider:

One of your patient's would like to participate in a study entitled "Effects of Resveratrol and Pterostilbene Supplementation on Insulin and Exercise-Mediated Signaling Pathways for Glucose Uptake in Overweight Insulin-Resistant Females: A Double-Blind, Clinically-Controlled Study" that is being conducted by Matthew Cooke, Ph.D. (254-710-4025; Matthew_Cooke@Baylor.edu) through the Exercise & Biochemical Nutrition Laboratory at Baylor University. In order to do so, she must meet the entrance criteria described below and have approval from her personal physician to participate in the study. The study will involve overweight, pre-diabetic, sedentary pre-menopausal females who have not taken oral contraceptives for the two months prior to the study. They also will be subjected to an oral glucose tolerance test and a maximal graded exercise test on a treadmill on two occasions. Assessments will include obtaining venous blood and muscle samples from the thigh, body composition measurements, and changes in maximal oxygen consumption. In addition, each participant will participate in a dietary intervention and circuit training exercise program for two months. Details about the study are described in the attached study outline and consent form. If you feel she meets the entrance criteria and/or any existing medical condition that she may have is under control and would not be a limitation for her to participate in the study, please sign the medical clearance below. If you feel the need to contact me, please feel free to do so.

Subjects

Approximately 45 overweight, sedentary, pre-diabetic, pre-menopausal females will participate in this study. I understand that in order to participate in this study, a trained individual will examine me to determine whether I qualify to participate. I understand that I will not be allowed to participate in this study if: 1) I do not have physician's approval; 2) I have been on hormone replacement therapy three months prior; 3) I do not have medically-managed hypertension, glucose-intolerance, type II diabetes, thyroid conditions, hyperlipidemia and/or other controlled medical conditions that my physician feels would benefit me from participation in this study; 4.) I have taken nutritional supplements that may affect my antioxidant status (e.g., green tea, fish oil, Vitamins C and E) for three months prior to the study 5.) I report any unusual adverse events associated with this study that in consultation with the supervising physician recommends removal from the study.

Medical Clearance

I medically clear _____ to participate in this study.

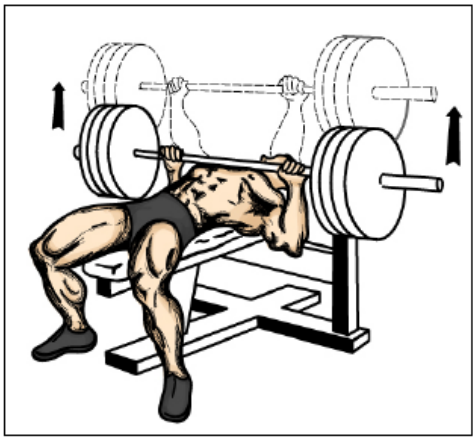
Physician's Name _____ Date _____

Physician's Signature _____

Exercise & Biochemical Nutrition Lab
Center for Exercise, Nutrition & Preventive Health Research
Department Of Health, Human Performance & Recreation
One Bear Place 97313 · Baylor University · Waco, TX 76798-7313 · (254) 710-3504 · Fax (254) 710-3527 ·
www3.baylor.edu/HHPR/EBNL

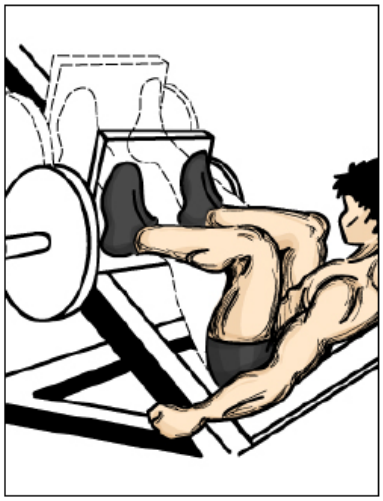
Resistance Training Examples

Bench Press



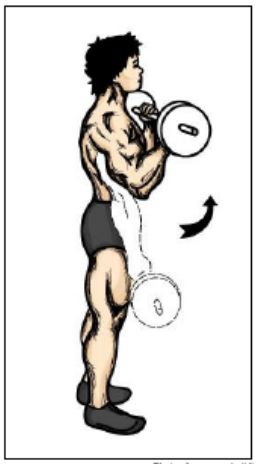
Photos from www.building-muscle101.com

Leg Press



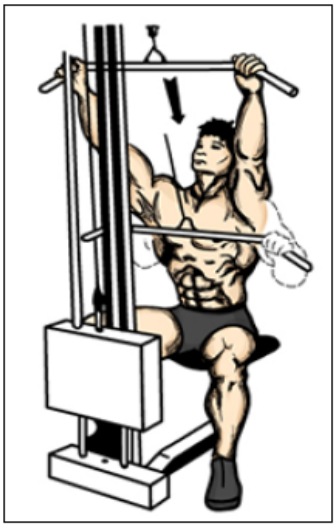
Photos from www.building-muscle101.com

Biceps Curl



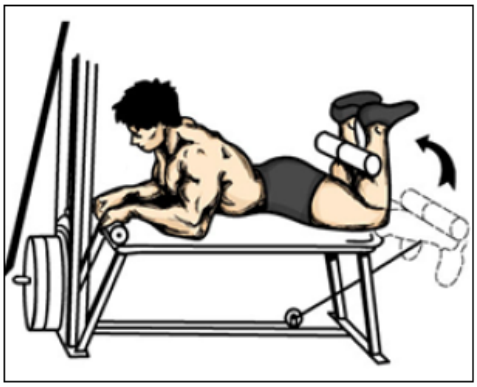
Photos from www.building-muscle101.com

Lat Pulls



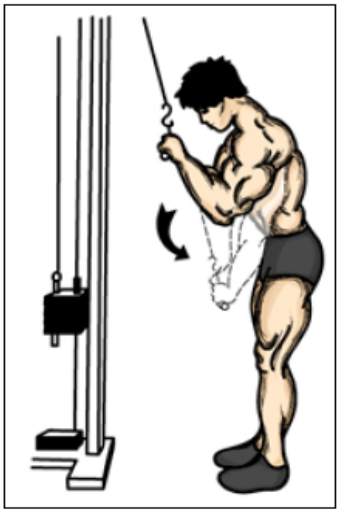
Photos from www.building-muscle101.com

Leg Curls



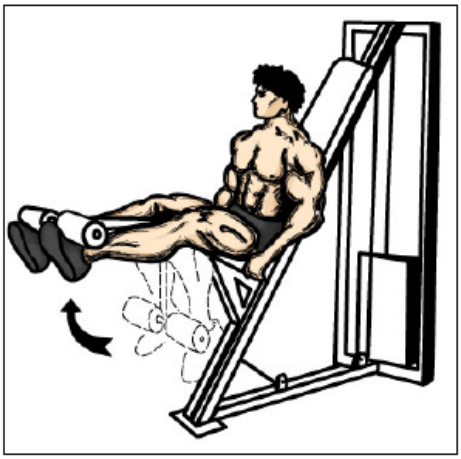
Photos from www.building-muscle101.com

Triceps Press



Photos from www.building-muscle101.com

Leg Extension



Photos from www.building-muscle101.com

Want to get paid to get in better shape & improve your health?

Inactive Women Needed for an Exercise & Nutritional Supplement Study

Researchers in the Center for Exercise, Nutrition, & Preventive Health Research at Baylor University are recruiting 36 inactive, overweight ***women*** *between the ages of* ***18-35*** *to participate in a study designed to evaluate the effects of botanical supplements and exercise on insulin sensitivity. Subjects will be required to ingest supplements, eat a healthy diet, and participate in an exercise program. The total duration of the study is 9 weeks. Participants will be asked to engage in a prescribed circuit training program for 3 days each week and walk 3 days each week. Participants will be required to undergo cardiopulmonary and body composition testing and to also submit to blood sampling and muscle biopsies. Eligible subjects will receive* ***$150*** *for completing the study in addition to body fat testing and nutritional counseling.*

For more information contact:

Center for Exercise, Nutrition, & Preventive Health Research
Department of HHPR at Baylor University

(254) 710-4012

Geoffrey_Hudson@baylor.edu or Brian_Shelmadine@baylor.edu

Exercise • Biochemistry • Nutrition • Health
BU EBNL
BAYLOR UNIVERSITY
Exercise & Biochemical Nutrition Laboratory

Did the holidays add too many pounds and too much debt?

Inactive Women Needed for an Exercise & Nutritional Supplement Study

Researchers in the Center for Exercise, Nutrition, & Preventive Health Research at Baylor University are recruiting 36 inactive, overweight ***women*** *between the ages of* ***18-35*** *to participate in a study designed to evaluate the effects of botanical supplements and exercise on insulin sensitivity. Subjects will be required to ingest supplements, eat a healthy diet, and participate in an exercise program. The total duration of the study is 9 weeks. Participants will be asked to engage in a prescribed circuit training program for 3 days each week and walk 3 days each week. Participants will be required to undergo cardiopulmonary and body composition testing and to also submit to blood sampling and muscle biopsies. Eligible subjects will receive* ***$150*** *for completing the study in addition to body fat testing and nutritional counseling.*

For more information contact:

Center for Exercise, Nutrition, & Preventive Health Research
Department of HHPR at Baylor University

(254) 710-4012

Geoffrey_Hudson@baylor.edu or Brian_Shelmadine@baylor.edu

Exercise • Biochemistry • Nutrition • Health
BU EBNL
BAYLOR UNIVERSITY
Exercise & Biochemical Nutrition Laboratory

New Years Resolutions

Earn $$$$$$!!

Get in Shape!!

Help Science!!!!

Inactive Women Needed for an Exercise & Nutritional Supplement Study

*Researchers in the Center for Exercise, Nutrition, & Preventive Health Research at Baylor University are recruiting 36 inactive, overweight **women** between the ages of **18-35** to participate in a study designed to evaluate the effects of botanical supplements and exercise on insulin sensitivity. Subjects will be required to ingest supplements, eat a healthy diet, and participate in an exercise program. The total duration of the study is 9 weeks. Participants will be asked to engage in a prescribed circuit training program for 3 days each week and walk 3 days each week. Participants will be required to undergo cardiopulmonary and body composition testing and to also submit to blood sampling and muscle biopsies. Eligible subjects will receive **$150** for completing the study in addition to body fat testing and nutritional counseling.*

APPENDIX B

Informed Consent Form

BAYLOR UNIVERSITY

Department of Health, Human Performance, & Recreation
Informed Consent Form

Title of Investigation:	Effects of Resveratrol and Pterostilbene Supplementation on Insulin and Exercise-Mediated Signaling Pathways for Glucose Uptake in Overweight Insulin-Resistant Females: A Double-Blind, Clinically-Controlled Study
Principal Investigator:	Mathew Cooke, PhD Assistant Professor, Department of HHPR, Baylor University
Co-investigators:	Geoffrey Hudson, MA, CSCS Exercise and Biochemical Nutrition Lab, Baylor University Brian Shelmadine, MA, CSCS Exercise and Biochemical Nutrition Lab, Baylor University Thomas Buford, MS, CSCS Exercise and Biochemical Nutrition Lab, Baylor University Mike Greenwood, PhD, FNSCA, CSCS*D, FACSM, FISSN Professor, Department of HHPR, Baylor University Darryn S. Willoughby, PhD, FACSM, FISSN, CSCS, CISSN Associate Professor, Department of HHPR, Baylor University Rodney Bowden, PhD Associate Dean, Graduate Studies and Research Associate Professor, Health Education Ronald Wilson, MD Exercise and Sport Nutrition Lab, Baylor University
Sponsors:	Sabinsa, Inc. (Piscataway, NJ)

Rationale

Diabetes mellitus is a critical health concern that has tripled in prevalence over the past 25 years. The CDC estimated 17.9 million Americans suffered from this condition in 2007 and that another 5.7 million Americans were estimated that to have diabetes but were undiagnosed. Of the two most common kinds of diabetes, Type 2 diabetes is the most prevalent, making up 90-95 percent all diagnosed cases in adults. Type 2 diabetes is preceded by an initial insulin resistant state

where the insulin-responsive cells in the body do not appropriately react to circulating insulin, which results in an increase in blood glucose levels and disturbances in fatty acid metabolism. Insulin binding is the primary stimulus for glucose uptake in muscle cells and fat cells at rest or after a meal. Insulin serves to lower blood glucose levels via an insulin-signaling pathway within fat cells and muscle cells that results in movement of the glucose transporter 4 (GLUT4) glucose transporter to the cell membrane. This initial insulin-resistant state leading to Type 2 diabetes is preceded by Pre-diabetes where people can have impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both. If a person has IFG, the fasting glucose level will be between 100-125 mg/dL. If a person has IGT, after given oral glucose, their blood glucose level is between 140-200 mg/dL after two hours.

It is not a given that those with pre-diabetes will progress to Type 2 diabetes. Progression can be prevented. The first line of defense to prevent the development or further progression of Type 2 diabetes mellitus is lifestyle change. Of particular importance is losing weight, increasing physical activity, and eating better. The goal is to improve insulin's ability to get glucose into muscle cells. One way to improve this ability is with exercise. However, during moderate-intense exercise glucose uptake is accomplished through insulin-independent mechanisms. During exercise, insulin levels in the blood decrease. Thus, glucose is taken into the cell via different means. Ultimately, either the insulin-mediated pathway or the non-insulin mediated pathway, induced by exercise, causes GLUT4 to move to the plasma membrane. During exercise the liver produces more glucose for the muscles to use as a fuel source. However, after exercise has stopped, muscles take in less glucose via the insulin-independent pathway, but the liver continues to produce glucose. At this time insulin levels in the blood begin to increase. However, insulin-resistant people are resistant to the effects of insulin and often suffer from post-exercise hyperglycemia.

Often drugs such as Metformin are prescribed to improve insulin sensitivity. Metformin, one of the most frequently prescribed diabetes medications, also enhances GLUT4 transport and insulin sensitivity in muscle and fat cells via insulin-independent mechanisms. Resveratrol and pterostilbene are two compounds found in high concentrations in red wine and a variety of plant sources such as grape skin, berries, pomegranates, and peanuts that are believed to improve insulin sensitivity in a manner similar to Metformin. These two compounds are found in a variety of nutritional supplements available at most health food stores. Over the past 5 years, research into these 2 botanical compounds has expanded, with benefits from supplementation including: improved insulin sensitivity, weight loss, cardioprotection, cancer growth inhibition, and increased mortality. Although interest in such compounds has increased, limited research has examined their purported effects in humans. Administration of resveratrol and pterostilbene could not only improve glucose uptake at rest, they could provide a synergistic enhancement of glucose uptake during exercise, but more importantly, continue to stimulate glucose uptake after exercise and hopefully attenuate a post-exercise hyperglycemic response.

Thus, the primary overall purpose of this study is to determine the effects of resveratrol or pterostilbene supplementation on glucose/insulin kinetics and markers of inflammation at rest and following an exercise bout. Further, this study will also determine the effects of prolonged supplementation and exercise training on additional cardiovascular risk factors often found with insulin resistance. These include elevated LDL, triglycerides, total cholesterol, decreased HDL levels, and an overall state of increased inflammation.

Description of the Study

I will be one of 45 eumenorrheic, physically inactive (not presently engaged in a regular, structured exercise program) females between the ages of 18-35 with a body mass index (BMI) ≥ 25 who will participate in this study. During an initial familiarization session, I will be informed of the requirements of the study and sign an informed consent statement in compliance with the Human Subjects Guidelines of Baylor University and the American College of Sports Medicine. A medically trained individual will examine me to determine if I am qualified to participate in this study. If I am cleared to participate in the study, I will be familiarized to the testing procedures. Familiarization will take approximately 30 minutes to complete. Once I complete the familiarization session, I will complete pre-supplementation testing.

I will not exercise for 48 hours nor eat for 12 hours prior to all testing sessions (including the initial familiarization session). On reporting to the lab, I will be weighed and seated in a comfortable chair where my resting blood pressure will be measured using a standard sphygmomanometer and heart rate determined by assessing pulse rate from my wrist. I understand that I will then have my resting energy expenditure measured. This will involve lying down on my back on an exam table for 20 minutes while a clear plastic bubble is placed over my head. I will then have my body composition determined by using a Hologic 4500W dual energy x-ray absorptiometer (DEXA). This will involve lying down on my back on the DEXA exam table in a pair of shorts or a gown for about 6 minutes. I understand that a low dose of radiation will scan my entire body to determine the amount of fat weight, muscle weight, and bone weight. I understand that I will be exposed to an X-ray dose that is similar to the amount of natural background radiation a person would receive in one month while living in Waco. I understand that I will then donate about 15 milliliters (3-4 teaspoons) of venous blood from a vein in my arm. Blood samples will be obtained using standard/sterile procedures using a needle inserted into a vein in my arm. I understand that personnel who will be taking my blood are experienced in phlebotomy (procedures to take blood samples) and are qualified to do so under guidelines established by the Texas Department of Health and Human Services. This will take about 5-minutes.

I understand that I will also donate a muscle biopsy sample prior to beginning supplementation. I understand that Matthew Cooke, PhD and trained research assistants will be responsible for collecting muscle biopsy samples. The muscle biopsy procedure basically involves sterilizing and anesthetizing or numbing (2% Xylocaine with epinephrine) the biopsy site on the outside middle of my thigh. Then, a pilot hole (about the diameter of hypodermic needle) is made in my skin and fascia in order to provide access to underlying muscle. Approximately 10 to 15 milligrams of muscle tissue is then extracted from the thigh muscle using a sterile muscle biopsy needle according to standard clinical procedures. Once the muscle sample has been obtained, pressure will be immediately applied to minimize bleeding. Since the site is small with minimal bleeding; I understand only a small bandage will be required to cover the biopsy site. I will then be given a list of post-biopsy instructions on how to clean and care for the incision in order to promote healing. I understand that I will have to return to the lab within 24 to 72 hours so that the biopsy sites can be checked for infection and improper healing. I understand that I will begin ingesting the appropriate supplement the following day.

I understand that after baseline testing, I will be matched based on age, body composition, and fasting glucose to orally ingest capsules containing a placebo, 250mg of resveratrol, or 250mg of pterolstilbene twice daily for the duration of the study. Resveratrol and pterostilbene are two polyphenolic botanical supplements that are found in high concentrations in red wine and a variety of plant sources such as grape skin, berries, pomegranates, and peanuts. Both resveratrol and pterostilbene are purported to improve insulin sensitivity, weight loss, cardioprotection,

cancer growth inhibition, and mortality. I understand that the supplements will be prepared in capsule form and presented to me packaged in a blinded format (where I don't know which one I am taking). I understand that my compliance in taking the supplements will be monitored by returning empty bottles to the EBNL when I report back for testing after 4 days, 4 weeks, and 8 weeks of supplementation. I understand that if I do not take my supplements I will be removed from the study.

I understand that during the supplementation period I will be required to participate in a structured exercise training program 3 times weekly, along with a daily energy-controlled diet. Study personnel will provide me with the guidelines for the exercise and diet program and I will be required to document my exercise training and dietary intake for review by one of the study's investigators. After baseline testing, I understand that following 4 days, 4 weeks, and 8 weeks of supplementation, I will be scheduled to return to the lab for subsequent testing.

I understand that after taking the supplement for four days I will return to lab for a second testing session. I understand that I will be required to have my heart rate, blood pressure, and total body water determined. I understand that an indwelling catheter will be inserted into my antecubital vein using standard techniques and a blood sample will be taken. I understand that I will also donate a muscle biopsy sample. I will then undergo an oral glucose tolerance test, The oral glucose tolerance test will require me to ingest 75g of glucose and remain in the laboratory for 2 hours. Additional blood samples will be drawn at 30 minutes and one hour post glucose ingestion. At 2 hours post glucose ingestion, a blood and muscle sample will be taken. In addition, I will turn in a 4-day dietary record and complete a report of side effects from supplementation questionnaire to determine if I have experienced any unexpected problems or adverse events from participating in this study.

I will return to the lab the following day for testing session number three. I understand that I will again have my heart rate, blood pressure, and body composition assessed. I understand that I will then donate another blood and muscle biopsy using the same techniques as described in testing session two. I understand that the blood and muscle biopsy sample will be taken on the contralateral arm and leg, respectively. I understand that I will then undergo a maximal cardiopulmonary treadmill test. For the maximal cardiopulmonary exercise treadmill test, I understand that I will then be positioned on the treadmill and a sterile mouthpiece will be placed in my mouth and a mouthpiece holder will be placed on my head. I understand that a nose clip will be placed on my nose and that the air I breathe will be measured for oxygen and carbon dioxide content. Once the equipment is attached, I will be given instructions to begin walking on the treadmill. I will then perform an exercise test that involves increasing the speed and grade I am walking on the treadmill until I reach my maximal effort. I understand that heart rate and my ratings of exertion will be monitored throughout the test. I understand that my heart rate and rhythm will be monitored by an electrocardiogram, where electrodes will be placed at standardized locations on my shoulders, hips, and chest. Once I reach my maximum, I understand that I will undergo a slow walking and seated recovery period. This test will take about 20 minutes to complete. Following the exercise test, additional blood samples will be drawn at 30 minutes and one hour post glucose ingestion. At 2 hours post glucose ingestion, a blood and muscle sample will be taken.

After testing session three I will then begin my exercise and nutrition program, and continue taking the supplement. I understand that after four weeks I will return to the laboratory for a fourth testing session. I understand I will be required to have my heart rate, blood pressure, and body composition determined, and undergo a maximal cardiopulmonary treadmill test. In addition, I will turn in a 4-day dietary record, four weeks of exercise training logs, and complete a

report of side effects from supplementation questionnaire to determine if I have experienced any unexpected problems or adverse events from participating in this study.

After testing session four, I will continue taking my supplement and resume my exercise and nutrition program for an additional four weeks. I understand that after a total of 8 weeks (four weeks following testing session four), I will return to the laboratory for a fifth and six testing session. I understand that I will be required to undergo the exact same battery of tests performed during the second and third testing sessions. In addition, I will turn in a 4-day dietary record, four weeks of exercise training logs, and complete a report of side effects from supplementation questionnaire to determine if I have experienced any unexpected problems or adverse events from participating in this study. I understand that if clinically significant side effects are reported, I will report those to Matthew Cooke, Ph.D, Geoffrey Hudson, MA, or Brian Shelmadine, MA. I may then be referred to discuss the problem with the ESNL physician, Dr. Ronald Wilson to determine whether any medical treatment is needed and/or whether I can continue in the study. I understand that if I fail to report my progress and health status to the research assistant I may be removed from the study.

I agree to do my best to: 1) follow the instructions outlined by the investigators; 2) show up to all scheduled testing times; 3) take the supplements as instructed, and 4) comply with the exercise training and diet protocol. I agree not to take any other nutritional supplements or performance enhancing aids during this study (i.e., vitamins/minerals, creatine, HMB, androstenedione, DHEA, etc). In addition, I agree not to take any non-medically prescribed medications and to report any medication that is prescribed for me to take during this study. I understand that if I take any other nutritional supplements or medications during the course of the study that may affect body composition, or blood hormone levels that I may be removed from the study.

Exclusionary Criteria

I understand that in order to participate in the study, a trained individual will examine me to determine whether I qualify to participate. I understand that I will not be allowed to participate in this study if: 1) my body mass index (BMI) is not at least 25; 2) my resting blood glucose is not between 100mg/dL and 120mg/dL; 3) I have taken oral contraceptives in the past 2 months; 4) I have menstrual irregularity (e.g., oligomenorrhea or amenorrhea); 5) I have any known metabolic disorder including heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism; 6) I have a history of pulmonary disease, medically controlled hypertension, liver or kidney disease, musculoskeletal disorders, neuromuscular or neurological diseases, autoimmune disease, cancer, peptic ulcers, or anemia; 7) I am taking any heart, pulmonary, thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, endocrinologic (e.g, thyroid, insulin, etc), psychotropic, neuromuscular/neurological; 8) I have a chronic infection (e.g., hepatitis, HIV, etc.); or 9) a known bleeding disorder.

I have reported all nutritional supplements, medically prescribed drugs, and non-medically prescribed drugs that I am presently taking. I have completed medical history questionnaires and am not aware of any additional medical problems that would prevent me from participating in this study. I agree to report all changes in medical status, nutritional and/or pharmacological agents (drugs) that I take during the course of the investigation to Matthew Cooke, Ph.D. (254-710-4025), Geoffrey Hudson, MA (254-710-4012), or Brian Shelmadine, MA (254-710-4012).

Risks and Benefits

I understand that the supplements to be investigated in this study have been studied for use as an anti-diabetic supplement in rats, have been used in few humans studies, but based on clinical trials it has been demonstrated that oral administration of these compounds is not associated with

any significant medical side effects. These nutrients are currently available in over the counter nutritional supplements sold in the United States. As with any food or nutritional supplement, possible side effects may include stomach upset, gastrointestinal distress, allergic reactions, changes in mood and vigor, and/or changes in training adaptations. However, as with the vast majority of nutritional supplements, I understand that the FDA may not have evaluated the safety or marketing claims of resveratrol or pterostilbene.

I understand that I will be exposed to a low level of radiation during the DEXA body composition tests, which is similar to the amount of natural background radiation a person would receive in one month while living in Waco, TX. This analyzer is commercially available and has been used in the health care industry as a means to assess body composition for over 10 years. The use of the DEXA analyzer has been shown to be a safe method of assessing body composition and is approved by the FDA.

I also understand that I will have about 3-4 teaspoons (~7-15milliliters) of blood drawn from a vein in my forearm using a sterile needle and blood tubes by an experienced phlebotomist 18 times over the nine weeks of this study. This procedure may cause a small amount of pain when the needle is inserted into my vein as well as some bleeding and bruising. I may also experience some dizziness, nausea, and/or faint if I am unaccustomed to having blood drawn.

I understand that I will have nine muscle biopsies performed by trained muscle physiologists over the nine weeks of this study. I understand that there may be some pain, minimal bleeding, and some residual bruising and soreness involved in this procedure. Specifically, I understand that there is a potential risk of contracting a community MRSA infection by participating in a muscle biopsy. I understand that if I feel it necessary I may take a non-prescription analgesic medication such as Tylenol to relieve pain if needed and that some soreness of the area may occur for about 24 hours after the biopsy. I will also be advised to avoid such medications such as aspirin, Advil, Bufferin, or Nuprin, as they may lead to bruising at the biopsy site.

I understand that my exercise program may cause symptoms of fatigue, shortness of breath, and/or muscular fatigue/discomfort and soreness. I understand that I may also experience muscle strains/pulls during my exercise program. However, these risks will be similar to the risk of participating in any exercise program. I also understand that trained, non-physician exercise specialists certified in CPR will supervise exercise assessments. I understand that a telephone and an automated electronic defibrillator are in the laboratory in case of any emergencies and that there will be no less than two researchers working with me during each testing session. I understand that emergency procedures are posted in the lab in the unlikely event that any emergency may arise.

I understand that the main benefit that may be obtained from this study is to determine whether providing 250mg of resveratrol or 250mg of pterostilbene, coupled with an exercise training and diet program, is effective at decreasing fat mass, and improving body composition, metabolism, cardiopulmonary performance, serum lipids, inflammatory markers, and hormones.

Alternative Treatments
This is not a medical treatment. Therefore, if medical treatment is needed, I must continue to obtain treatment for any medical problem I might have from my personal physician.

Costs and Payments
If I am a Baylor University student, I will not receive any academic credit for participating in this study. I understand that if I am an intercollegiate scholarship athlete I may not be eligible to

receive payment to participate in this study. If eligible I will be paid $150 for completing the familiarization and all experimental testing sessions over the course of the nine weeks. I also understand that I will be given free blood assessments during the course of the study as described above and may receive information regarding results of these tests if I desire.

New Information
Any new information obtained during the course of this research that may affect my willingness to continue participation in this study will be provided to me. In addition, I will be informed of any unusual/abnormal clinical findings in which medical referral to my personal physician may be warranted. If I desire, I may request that this information be provided to my physician.

Confidentiality
I understand that any information obtained about me in this research, including questionnaires, medical history, laboratory findings, or physical examination will be kept confidential to the extent permitted by law. However, I understand in order to ensure that FDA regulations are being followed, it may be necessary for a representative of the FDA to review my records from this study which may include questionnaires, medical history, laboratory findings/reports, statistical data, and/or notes taken about my participation in this study. In addition, I understand that my records of this research may be subpoenaed by court order or may be inspected by federal regulatory authorities. I understand that data derived may be used in reports, presentations, and publications. However, I will not be individually identified unless my consent is granted in writing. Additionally, that confidentiality will be maintained by assigning code numbers to my files, limiting access to data to research assistants, locking cabinets that store data, and providing passwords to limit access to computer files to authorized personnel only. I understand that once blood samples are analyzed that they will be discarded.

Right to Withdrawal
I understand that I am not required to participate in this study and I am free to refuse to participate or to withdraw from the study at any time. Further, that my decision to withdraw from the study will not affect my care at this institution or cause a loss of benefits to which I might be otherwise entitled. If there is concern about my medical safety, I may be referred to seek medical attention.

Compensation for Illness or Injury
I understand that if I am injured as a direct result of taking part in this study, I should consult my personal physician to obtain treatment. I understand that the cost associated with the care and treatment of such injury will be the responsibility of me or my insurance carrier. In some cases, insurers may not reimburse claims submitted for a research-related injury resulting from medical procedures or treatments performed as part of a research study. I understand that Baylor University, the investigator's institutions, and the grant sponsor have not budgeted funds to compensate me for injury or illness that may result from my participation in this study and thus will not be accountable for illness or injury acquired during the course of this study. However, I may be referred to my personal physician if any clinically significant medical/psychological findings are observed during the course of this study.

I agree to indemnify and hold harmless Baylor University, its officers, directors, faculty, employees, and students for any and all claims for any injury, damage or loss I suffer as a result of my participation in this study regardless of the cause of my injury, damage or loss.

Statement on conflict of interest

Funding for the study has been obtained from Sabinsa, Inc. (Piscataway, NJ) through research grants awarded to Baylor University. Researchers involved in collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

Voluntary Consent

I certify that I have read this consent form or it has been read to me and that I understand the contents and that any questions that I have pertaining to the research have been, or will be answered by Matthew Cooke, PhD (Assistant Professor, Department of Health, Human Performance & Recreation, 118 Marrs McLean Gymnasium, Baylor University, phone: 254-710-4025), Geoffrey Hudson, MA (Doctoral Research Assistant Department of Health, Human Performance & Recreation, 117 Marrs McLean Gymnasium, Baylor University, phone: 254-710-4012), or Brian Shelmadine, MA (Doctoral Research Assistant Department of Health, Human Performance & Recreation, 117 Marrs McLean Gymnasium, Baylor University, phone: 254-710-4012). My signature below means that I am at least 18 years of age and that I freely agree to participate in this investigation. I understand that I will be given a copy of this consent form for my records. If I have any questions regarding my rights as a research subject in this study, I may contact Baylor's University Committee for Protection of Human Subjects in Research. The chairman is Dr. Matt Stanford, Professor, Department of Psychology and Neuroscience, P.O. Box 97334, Waco, TX 76798-7334, phone number 254-710-2961.

Date _____ Participant's Signature _____

I certify that I have explained to the above individual the nature and purpose of the potential benefits and possible risks associated with participation in this study. I have answered any questions that have been raised and have witnessed the above signature. I have explained the above to the volunteer on the date stated on this consent form.

Date _____ Investigator's Signature _____

APPENDIX C

RevGenetic's Quality Assurance Report

American Analytical Chemistry Laboratories Corp.
JMT Technology Center - 711 Parkland Court - Champaign, IL 61821 - Phone: 217-352-6060 - Fax: 217-352-6052 - www.aaclabs.com

Report of Analysis

RevGenetics

Anthony Loera
650 nw 133 ct
Miami, FL 33182
305-938-0889
866-430-3953

AACL Control Number: 030609-7606
Product Name: Ttans-Resveratrol

Batch Number: 0902103-07
Lot Number:
Purchase Order:
Recieved On: 3/6/2009
Serving Size: 1 g
Report Date: 3/11/2009

Analysis Performed / Method		Result with Units	
Resveratrol Profile AACL-28AAAF (HPL(	Emodin	0.0944	%
	trans-Resveratrol	99.0	%
Coliforms BAM			cfu/g
E. Coli BAM			cfu/g
Pseudomonas BAM			cfu/g
Salmonella BAM			
Staphylococcus BAM			cfu/g
Total Plate Count BAM			cfu/g
Yeast and Molds BAM			cfu/g
Arsenic (As) AACL-22A (ICP-MS)		< 0.01	ppm
Cadmium (Cd) AACL-22A (ICP-MS)		< 0.01	ppm
Lead (Pb) AACL-22A (ICP-MS)		<0.05	ppm

Authorized by

Page 1 of 2

American Analytical Chemistry Laboratories Corp.
JMT Technology Center - 711 Parkland Court - Champaign, IL 61821 - Phone: 217-352-6060 - Fax: 217-352-6052 - www.aaclabs.com

Report of Analysis

RevGenetics

Anthony Loera
650 nw 133 ct
Miami, FL 33182
305-938-0889
866-430-3953

AACL Control Number: 030609-7606
Product Name: Ttans-Resveratrol

Batch Number: 0902103-07
Lot Number:
Purchase Order:
Recieved On: 3/6/2009
Serving Size: 1 g
Report Date: 3/11/2009

Analysis Performed / Method	Result with Units
Mercury (Hg) AACL-22G	< 0.025 ppm

Comments:

Authorized by

BIBLIOGRAPHY

Almeida, L., Vaz-da-Silva, M., Falcao, A., Soares, E., Costa, R., Loureiro, A. I., Fernandes-Lopes, C., Rocha, J. F., Nunes, T., Wright, L., & Soares-da-Silva, P. (2009). Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. *Molecular Nutrition & Food Research, 53 (*Suppl 1), S7-15.

American Diabetes Association. (2002a). Diabetes mellitus and exercise. *Diabetes Care, 25*(Supplement 1), S64-S68.

American Diabetes Association. (2002b). Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *Diabetes Care, 25*(1), 202-212.

American Diabetes Association. (2008). Economic costs of diabetes in the U.S. in 2007. *Diabetes Care, 31*(3), 596-615.

Arias, E. B., Kim, J., Funai, K., & Cartee, G. D. (2007). Prior exercise increases phosphorylation of akt substrate of 160 kDa (AS160) in rat skeletal muscle. *American Journal of Physiology: Endocrinology and Metabolism, 292*(4), E1191-200.

Athar, M., Back, J. H., Tang, X., Kim, K. H., Kopelovich, L., Bickers, D. R., & Kim, A. L. (2007). Resveratrol: A review of preclinical studies for human cancer prevention. *Toxicology and Applied Pharmacology, 224*(3), 274-283.

Balkau, B., Shipley, M., Jarrett, R. J., Pyorala, K., Pyorala, M., Forhan, A., & Eschwege, E. (1998). High blood glucose concentration is a risk factor for mortality in middle-aged nondiabetic men. 20-year follow-up in the Whitehall Study, the Paris Prospective Study, and the Helsinki Policemen Study. *Diabetes Care, 21*(3), 360-367.

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., Lakatta, E. G., Le Couteur, D., Shaw, R. J., Navas, P., Puigserver, P., Ingram, D. K., de Cabo, R., & Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature, 444*(7117), 337-342.

Bhathena, S. J., & Velasquez, M. T. (2002). Beneficial role of dietary phytoestrogens in obesity and diabetes. *The American Journal of Clinical Nutrition, 76*(6), 1191-1201.

Bjornholt, J. V., Erikssen, G., Aaser, E., Sandvik, L., Nitter-Hauge, S., Jervell, J., Erikssen, J., & Thaulow, E. (1999). Fasting blood glucose: An underestimated risk factor for cardiovascular death. Results from a 22-year follow-up of healthy nondiabetic men. *Diabetes Care, 22*(1), 45-49.

Blumenfeld, T. A., Hertelendy, W. G., & Ford, S. H. (1977). Simultaneously obtained skin-puncture serum, skin-puncture plasma, and venous serum compared, and effects of warming the skin before puncture. *Clinical Chemistry, 23*(9), 1705-1710.

Boocock, D. J., Faust, G. E., Patel, K. R., Schinas, A. M., Brown, V. A., Ducharme, M. P., Booth, T. D., Crowell, J. A., Perloff, M., Gescher, A. J., Steward, W. P., & Brenner, D. E. (2007). Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology, 16*(6), 1246-1252.

Braun, B., Sharoff, C., Chipkin, S. R., & Beaudoin, F. (2004). Effects of insulin resistance on substrate utilization during exercise in overweight women. *Journal of Applied Physiology, 97*(3), 991-997.

Breen, D. M., Sanli, T., Giacca, A., & Tsiani, E. (2008). Stimulation of muscle cell glucose uptake by resveratrol through sirtuins and AMPK. *Biochemical and Biophysical Research Communications, 374*(1), 117-122.

Brozinick, J. T., Jr, Roberts, B. R., & Dohm, G. L. (2003). Defective signaling through akt-2 and -3 but not akt-1 in insulin-resistant human skeletal muscle: Potential role in insulin resistance. *Diabetes, 52*(4), 935-941.

Bryant, N. J., Govers, R., & James, D. E. (2002). Regulated transport of the glucose transporter GLUT4. *Nature Reviews. Molecular Cell Biology, 3*(4), 267-277.

Buhl, E. S., Jessen, N., Schmitz, O., Pedersen, S. B., Pedersen, O., Holman, G. D., & Lund, S. (2001). Chronic treatment with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside increases insulin-stimulated glucose uptake and GLUT4 translocation in rat skeletal muscles in a fiber type-specific manner. *Diabetes, 50*(1), 12-17.

Burkon, A., & Somoza, V. (2008). Quantification of free and protein-bound trans-resveratrol metabolites and identification of trans-resveratrol-C/O-conjugated diglucuronides - two novel resveratrol metabolites in human plasma. *Molecular Nutrition & Food Research, 52*(5), 549-557.

Cartee, G. D., & Funai, K. (2009). Exercise and insulin: Convergence or divergence at AS160 and TBC1D1? *Exercise and Sport Sciences Reviews, 37*(4), 188-195.

Cartee, G. D., & Wojtaszewski, J. F. (2007). Role of akt substrate of 160 kDa in insulin-stimulated and contraction-stimulated glucose transport. *Applied Physiology, Nutrition, and Metabolism, 32*(3), 557-566.

Chan, A. Y., Dolinsky, V. W., Soltys, C. L., Viollet, B., Baksh, S., Light, P. E., & Dyck, J. R. (2008). Resveratrol inhibits cardiac hypertrophy via AMP-activated protein kinase and akt. *The Journal of Biological Chemistry, 283*(35), 24194-24201.

Christ-Roberts, C. Y., Pratipanawatr, T., Pratipanawatr, W., Berria, R., Belfort, R., Kashyap, S., & Mandarino, L. J. (2004). Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects. *Metabolism: Clinical and Experimental, 53*(9), 1233-1242.

Cline, G. W., Petersen, K. F., Krssak, M., Shen, J., Hundal, R. S., Trajanoski, Z., Inzucchi, S., Dresner, A., Rothman, D. L., & Shulman, G. I. (1999). Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *The New England Journal of Medicine, 341*(4), 240-246.

Colagiuri, S., Sandbaek, A., Carstensen, B., Christensen, J., Glumer, C., Lauritzen, T., & Borch-Johnsen, K. (2003). Comparability of venous and capillary glucose measurements in blood. *Diabetic Medicine: A Journal of the British Diabetic Association, 20*(11), 953-956.

Cottart, C. H., Nivet-Antoine, V., Laguillier-Morizot, C., & Beaudeux, J. L. (2010). Resveratrol bioavailability and toxicity in humans. *Molecular Nutrition & Food Research, 54*(1), 7-16.

Coutinho, M., Gerstein, H. C., Wang, Y., & Yusuf, S. (1999). The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care, 22*(2), 233-240.

Dagogo-Jack, S., Askari, H., & Tykodi, G. (2009). Glucoregulatory physiology in subjects with low-normal, high-normal, or impaired fasting glucose. *The Journal of Clinical Endocrinology and Metabolism, 94*(6), 2031-2036.

Deng, J. Y., Hsieh, P. S., Huang, J. P., Lu, L. S., & Hung, L. M. (2008). Activation of estrogen receptor is crucial for resveratrol-stimulating muscular glucose uptake via both insulin-dependent and -independent pathways. *Diabetes, 57*(7), 1814-1823.

Deshmukh, A. S., Hawley, J. A., & Zierath, J. R. (2008). Exercise-induced phospho-proteins in skeletal muscle. *International Journal of Obesity, 32 Suppl 4*, S18-23.

Dreyer, H. C., Drummond, M. J., Glynn, E. L., Fujita, S., Chinkes, D. L., Volpi, E., & Rasmussen, B. B. (2008). Resistance exercise increases human skeletal muscle AS160/TBC1D4 phosphorylation in association with enhanced leg glucose uptake during post-exercise recovery. *Journal of Applied Physiology, 105,* 1967-1974.

Du Bois, D., & Du Bois, E. F. (1916). A formula to estimate the approximate surface area if height and weight be known. *Archives of Internal Medicine, 17*, 863-871.

Ducluzeau, P. H., Perretti, N., Laville, M., Andreelli, F., Vega, N., Riou, J. P., & Vidal, H. (2001). Regulation by insulin of gene expression in human skeletal muscle and adipose tissue. Evidence for specific defects in type 2 diabetes. *Diabetes, 50*(5), 1134-1142.

Etgen, G. J., Jr, Jensen, J., Wilson, C. M., Hunt, D. G., Cushman, S. W., & Ivy, J. L. (1997). Exercise training reverses insulin resistance in muscle by enhanced recruitment of GLUT-4 to the cell surface. *The American Journal of Physiology, 272*(5 Pt 1), E864-9.

Fang, J. G., & Zhou, B. (2008). Structure-activity relationship and mechanism of the tocopherol-regenerating activity of resveratrol and its analogues. *Journal of Agricultural and Food Chemistry, 56*(23), 11458-11463.

Fisher, J. S., Gao, J., Han, D. H., Holloszy, J. O., & Nolte, L. A. (2002). Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *American Journal of Physiology.Endocrinology and Metabolism, 282*(1), E18-23.

Flores-Riveros, J. R., McLenithan, J. C., Ezaki, O., & Lane, M. D. (1993). Insulin down-regulates expression of the insulin-responsive glucose transporter (GLUT4) gene: Effects on transcription and mRNA turnover. *Proceedings of the National Academy of Sciences of the United States of America, 90*(2), 512-516.

Frojdo, S., Cozzone, D., Vidal, H., & Pirola, L. (2007). Resveratrol is a class IA phosphoinositide 3-kinase inhibitor. *The Biochemical Journal, 406*(3), 511-518.

Frojdo, S., Durand, C., & Pirola, L. (2008). Metabolic effects of resveratrol in mammals--a link between improved insulin action and aging. *Current Aging Science, 1*(3), 145-151.

Furimsky, A. M., Green, C. E., Sharp, L. E., Catz, P., Adjei, A. A., Parman, T., Kapetanovic, I. M., Weinshilboum, R. M., & Iyer, L. V. (2008). Effect of resveratrol on 17beta-estradiol sulfation by human hepatic and jejunal S9 and recombinant sulfotransferase 1E1. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 36*(1), 129-136.

Garvey, W. T., Maianu, L., Zhu, J. H., Brechtel-Hook, G., Wallace, P., & Baron, A. D. (1998). Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. *The Journal of Clinical Investigation, 101*(11), 2377-2386.

Gillies, C. L., Abrams, K. R., Lambert, P. C., Cooper, N. J., Sutton, A. J., Hsu, R. T., & Khunti, K. (2007). Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: Systematic review and meta-analysis. *British Medical Journal, 334*(7588), 299.

Goldberg, D. M., Yan, J., & Soleas, G. J. (2003). Absorption of three wine-related polyphenols in three different matrices by healthy subjects. *Clinical Biochemistry, 36*(1), 79-87.

Goodyear, L. J., Giorgino, F., Sherman, L. A., Carey, J., Smith, R. J., & Dohm, G. L. (1995). Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *The Journal of Clinical Investigation, 95*(5), 2195-2204.

Goodyear, L. J., & Kahn, B. B. (1998). Exercise, glucose transport, and insulin sensitivity. *Annual Review of Medicine, 49*, 235-261.

Gresele, P., Pignatelli, P., Guglielmini, G., Carnevale, R., Mezzasoma, A. M., Ghiselli, A., Momi, S., & Violi, F. (2008). Resveratrol, at concentrations attainable with moderate wine consumption, stimulates human platelet nitric oxide production. *The Journal of Nutrition, 138*(9), 1602-1608.

Hardie, D. G., Carling, D., & Carlson, M. (1998). The AMP-activated/SNF1 protein kinase subfamily: Metabolic sensors of the eukaryotic cell? *Annual Review of Biochemistry, 67*, 821-855.

Hayashi, T., Wojtaszewski, J. F., & Goodyear, L. J. (1997). Exercise regulation of glucose transport in skeletal muscle. *The American Journal of Physiology, 273*(6 Pt 1), E1039-51.

Heine, P. A., Taylor, J. A., Iwamoto, G. A., Lubahn, D. B., & Cooke, P. S. (2000). Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proceedings of the National Academy of Sciences of the United States of America, 97*(23), 12729-12734.

Holland-Fischer, P., Andersen, P. H., Lund, S., Pedersen, S. B., Vinter-Jensen, L., Nielsen, M. F., Kaal, A., Dall, R., Schmitz, O., & Vilstrup, H. (2007). Muscle GLUT4 in cirrhosis. *Journal of Hepatology, 47*(2), 212-219.

Holloszy, J. O. (2005). Exercise-induced increase in muscle insulin sensitivity. *Journal of Applied Physiology, 99*(1), 338-343.

Holmes, B. F., Kurth-Kraczek, E. J., & Winder, W. W. (1999). Chronic activation of 5'-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. *Journal of Applied Physiology, 87*(5), 1990-1995.

Host, H. H., Hansen, P. A., Nolte, L. A., Chen, M. M., & Holloszy, J. O. (1998). Rapid reversal of adaptive increases in muscle GLUT-4 and glucose transport capacity after training cessation. *Journal of Applied Physiology, 84*(3), 798-802.

Hwang, J. T., Kwon, D. Y., & Yoon, S. H. (2009). AMP-activated protein kinase: A potential target for the diseases prevention by natural occurring polyphenols. *New Biotechnology, 26*(1-2), 17-22.

Jessen, N., Pold, R., Buhl, E. S., Jensen, L. S., Schmitz, O., & Lund, S. (2003). Effects of AICAR and exercise on insulin-stimulated glucose uptake, signaling, and GLUT-4 content in rat muscles. *Journal of Applied Physiology, 94*(4), 1373-1379.

Jiang, W. J. (2008). Sirtuins: Novel targets for metabolic disease in drug development. *Biochemical and Biophysical Research Communications, 373*(3), 341-344.

Jing, M., Cheruvu, V. K., & Ismail-Beigi, F. (2008). Stimulation of glucose transport in response to activation of distinct AMPK signaling pathways. *American Journal of Physiology: Cell Physiology, 295*(5), C1071-82.

Jorgensen, S. B., Treebak, J. T., Viollet, B., Schjerling, P., Vaulont, S., Wojtaszewski, J. F., & Richter, E. A. (2007). Role of AMPKalpha2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. *American Journal of Physiology: Endocrinology and Metabolism, 292*(1), E331-9.

Kahn, B. B., & Flier, J. S. (2000). Obesity and insulin resistance. *The Journal of Clinical Investigation, 106*(4), 473-481.

Kane, S., Sano, H., Liu, S. C., Asara, J. M., Lane, W. S., Garner, C. C., & Lienhard, G. E. (2002). A method to identify serine kinase substrates. akt phosphorylates a novel adipocyte protein with a rab GTPase-activating protein (GAP) domain. *The Journal of Biological Chemistry, 277*(25), 22115-22118.

Karlsson, H. K., & Zierath, J. R. (2007). Insulin signaling and glucose transport in insulin resistant human skeletal muscle. *Cell Biochemistry and Biophysics, 48*(2-3), 103-113.

Kennedy, J. W., Hirshman, M. F., Gervino, E. V., Ocel, J. V., Forse, R. A., Hoenig, S. J., Aronson, D., Goodyear, L. J., & Horton, E. S. (1999). Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. *Diabetes, 48*(5), 1192-1197.

Kim, C., Siscovick, D. S., Sidney, S., Lewis, C. E., Kiefe, C. I., Koepsell, T. D., & CARDIA Study. (2002). Oral contraceptive use and association with glucose, insulin, and diabetes in young adult women: The CARDIA study. coronary artery risk development in young adults. *Diabetes Care, 25*(6), 1027-1032.

Kim, Y. B., Nikoulina, S. E., Ciaraldi, T. P., Henry, R. R., & Kahn, B. B. (1999). Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. *The Journal of Clinical Investigation, 104*(6), 733-741.

Klein, S., Sheard, N. F., Pi-Sunyer, X., Daly, A., Wylie-Rosett, J., Kulkarni, K., Clark, N. G., American Diabetes Association, North American Association for the Study of Obesity, & American Society for Clinical Nutrition. (2004). Weight management through lifestyle modification for the prevention and management of type 2 diabetes: Rationale and strategies: A statement of the American diabetes association, the north American association for the study of obesity, and the American society for clinical nutrition. *Diabetes Care, 27*(8), 2067-2073.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., Nathan, D. M., & Diabetes Prevention Program Research Group. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England Journal of Medicine, 346*(6), 393-403.

Koivisto, U. M., Martinez-Valdez, H., Bilan, P. J., Burdett, E., Ramlal, T., & Klip, A. (1991). Differential regulation of the GLUT-1 and GLUT-4 glucose transport systems by glucose and insulin in L6 muscle cells in culture. *The Journal of Biological Chemistry, 266*(4), 2615-2621.

Koshinaka, K., Kawasaki, E., Hokari, F., & Kawanaka, K. (2009). Effect of acute high-intensity intermittent swimming on post-exercise insulin responsiveness in epitrochlearis muscle of fed rats. *Metabolism: Clinical and Experimental, 58*(2), 246-253.

Kraniou, G. N., Cameron-Smith, D., & Hargreaves, M. (2006). Acute exercise and GLUT4 expression in human skeletal muscle: Influence of exercise intensity. *Journal of Applied Physiology, 101*(3), 934-937.

Kratz, A., Ferraro, M., Sluss, P. M., & Lewandrowski, K. B. (2004). Case records of the Massachusetts general hospital. weekly clinicopathological exercises. laboratory reference values. *The New England Journal of Medicine, 351*(15), 1548-1563.

Krook, A., Bjornholm, M., Galuska, D., Jiang, X. J., Fahlman, R., Myers, M. G., Jr, Wallberg-Henriksson, H., & Zierath, J. R. (2000). Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabetes, 49*(2), 284-292.

Kruijshoop, M., Feskens, E. J., Blaak, E. E., & de Bruin, T. W. (2004). Validation of capillary glucose measurements to detect glucose intolerance or type 2 diabetes mellitus in the general population. *Clinica Chimica Acta; International Journal of Clinical Chemistry, 341*(1-2), 33-40.

Larance, M., Ramm, G., Stockli, J., van Dam, E. M., Winata, S., Wasinger, V., Simpson, F., Graham, M., Junutula, J. R., Guilhaus, M., & James, D. E. (2005). Characterization of the role of the rab GTPase-activating protein AS160 in insulin-regulated GLUT4 trafficking. *The Journal of Biological Chemistry, 280*(45), 37803-37813.

Lee, A. D., Hansen, P. A., & Holloszy, J. O. (1995). Wortmannin inhibits insulin-stimulated but not contraction-stimulated glucose transport activity in skeletal muscle. *FEBS Letters, 361*(1), 51-54.

Lee-Young, R. S., Canny, B. J., Myers, D. E., & McConell, G. K. (2009). AMPK activation is fiber type specific in human skeletal muscle: Effects of exercise and short-term exercise training. *Journal of Applied Physiology, 107*(1), 283-289.

Long, Y. C., Barnes, B. R., Mahlapuu, M., Steiler, T. L., Martinsson, S., Leng, Y., Wallberg-Henriksson, H., Andersson, L., & Zierath, J. R. (2005). Role of AMP-activated protein kinase in the coordinated expression of genes controlling glucose and lipid metabolism in mouse white skeletal muscle. *Diabetologia, 48*(11), 2354-2364.

Lund, S., Holman, G. D., Schmitz, O., & Pedersen, O. (1995). Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proceedings of the National Academy of Sciences of the United States of America, 92*(13), 5817-5821.

Matsuda, M., & DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. *Diabetes Care, 22*(9), 1462-1470.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412-419.

McConell, G. K., Manimmanakorn, A., Lee-Young, R. S., Kemp, B. E., Linden, K. C., & Wadley, G. D. (2008). Differential attenuation of AMPK activation during acute exercise following exercise training or AICAR treatment. *Journal of Applied Physiology, 105*(5), 1422-1427.

Merrill, G. F., Kurth, E. J., Hardie, D. G., & Winder, W. W. (1997). AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *The American Journal of Physiology, 273*(6 Pt 1), E1107-12.

Misra, P., & Chakrabarti, R. (2007). The role of AMP kinase in diabetes. *The Indian Journal of Medical Research, 125*(3), 389-398.

Miyazaki, R., Ichiki, T., Hashimoto, T., Inanaga, K., Imayama, I., Sadoshima, J., & Sunagawa, K. (2008). SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. *Arteriosclerosis, Thrombosis, and Vascular Biology, 28*(7), 1263-1269.

Musi, N., Fujii, N., Hirshman, M. F., Ekberg, I., Froberg, S., Ljungqvist, O., Thorell, A., & Goodyear, L. J. (2001). AMP-activated protein kinase (AMPK) is activated in muscle of subjects with type 2 diabetes during exercise. *Diabetes, 50*(5), 921-927.

Musi, N., & Goodyear, L. J. (2002). Targeting the AMP-activated protein kinase for the treatment of type 2 diabetes. *Current Drug Targets: Immune, Endocrine and Metabolic Disorders, 2*(2), 119-127.

Musi, N., & Goodyear, L. J. (2006). Insulin resistance and improvements in signal transduction. *Endocrine, 29*(1), 73-80.

National Health and Nutrition Examination Survey (NHANES). (2000). *Body compositions procedures manual.* Retrieved February 22, 2008, from http://www.cdc.gov/nchs/data/nhanes/bc.pdf

Nawarskas, J. J. (1999). Acadesine: A unique cardioprotective agent for myocardial ischemia. *Heart Disease (Hagerstown, Md.),* 1(4), 255-260.

Ni, Y. G., Wang, N., Cao, D. J., Sachan, N., Morris, D. J., Gerard, R. D., Kuro-O, M., Rothermel, B. A., & Hill, J. A. (2007). FoxO transcription factors activate akt and attenuate insulin signaling in heart by inhibiting protein phosphatases. *Proceedings of the National Academy of Sciences of the United States of America, 104*(51), 20517-20522.

Nunes, T., Almeida, L., Rocha, J. F., Falcao, A., Fernandes-Lopes, C., Loureiro, A. I., Wright, L., Vaz-da-Silva, M., & Soares-da-Silva, P. (2009). Pharmacokinetics of trans-resveratrol following repeated administration in healthy elderly and young subjects. *Journal of Clinical Pharmacology, 49*(12), 1477-1482.

O'Brien, R. M., & Granner, D. K. (1996). Regulation of gene expression by insulin. *Physiological Reviews, 7*6(4), 1109-1161.

O'Leary, V. B., Marchetti, C. M., Krishnan, R. K., Stetzer, B. P., Gonzalez, F., & Kirwan, J. P. (2006). Exercise-induced reversal of insulin resistance in obese elderly is associated with reduced visceral fat. *Journal of Applied Physiology, 10*0(5), 1584-1589.

Ottensmeyer, F. P., Beniac, D. R., Luo, R. Z., & Yip, C. C. (2000). Mechanism of transmembrane signaling: Insulin binding and the insulin receptor. *Biochemistry, 3*9(40), 12103-12112.

Palacios, O. M., Carmona, J. J., Michan, S., Chen, K. Y., Manabe, Y., Ward, J. L.,3rd, Goodyear, L. J., & Tong, Q. (2009). Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. *Aging,* 1(9), 771-783.

Park, C. E., Kim, M. J., Lee, J. H., Min, B. I., Bae, H., Choe, W., Kim, S. S., & Ha, J. (2007). Resveratrol stimulates glucose transport in C2C12 myotubes by activating AMP-activated protein kinase. *Experimental & Molecular Medicine, 3*9(2), 222-229.

Ramm, G., Larance, M., Guilhaus, M., & James, D. E. (2006). A role for 14-3-3 in insulin-stimulated GLUT4 translocation through its interaction with the RabGAP AS160. *The Journal of Biological Chemistry, 28*1(39), 29174-29180.

Reagan-Shaw, S., Nihal, M., & Ahmad, N. (2007). Dose translation from animal to human studies revisited. *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 2*2(3), 659-661.

Remsberg, C. M., Yanez, J. A., Ohgami, Y., Vega-Villa, K. R., Rimando, A. M., & Davies, N. M. (2008). Pharmacometrics of pterostilbene: Preclinical pharmacokinetics and metabolism, anticancer, antiinflammatory, antioxidant and analgesic activity. *Phytotherapy Research: PTR, 2*2(2), 169-179.

Rockl, K. S., Witczak, C. A., & Goodyear, L. J. (2008). Signaling mechanisms in skeletal muscle: Acute responses and chronic adaptations to exercise. *IUBMB Life, 6*0(3), 145-153.

Rodgers, J. T., Lerin, C., Haas, W., Gygi, S. P., Spiegelman, B. M., & Puigserver, P. (2005). Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature, 43*4(7029), 113-118.

Ryder, J. W., Chibalin, A. V., & Zierath, J. R. (2001). Intracellular mechanisms underlying increases in glucose uptake in response to insulin or exercise in skeletal muscle. *Acta Physiologica Scandinavica, 17*1(3), 249-257.

Sano, H., Kane, S., Sano, E., Miinea, C. P., Asara, J. M., Lane, W. S., Garner, C. W., & Lienhard, G. E. (2003). Insulin-stimulated phosphorylation of a rab GTPase-activating protein regulates GLUT4 translocation. *The Journal of Biological Chemistry, 27*8(17), 14599-14602.

Santos, J. M., Ribeiro, S. B., Gaya, A. R., Appell, H. J., & Duarte, J. A. (2008). Skeletal muscle pathways of contraction-enhanced glucose uptake. *International Journal of Sports Medicine, 29,* 785-794.

Saydah, S. H., Loria, C. M., Eberhardt, M. S., & Brancati, F. L. (2001). Subclinical states of glucose intolerance and risk of death in the U.S. *Diabetes Care, 2*4(3), 447-453.

Schalin-Jantti, C., Yki-Jarvinen, H., Koranyi, L., Bourey, R., Lindstrom, J., Nikula-Ijas, P., Franssila-Kallunki, A., & Groop, L. C. (1994). Effect of insulin on GLUT-4 mRNA and protein concentrations in skeletal muscle of patients with NIDDM and their first-degree relatives. *Diabetologia, 3*7(4), 401-407.

Shepherd, P. R., & Kahn, B. B. (1999). Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. *The New England Journal of Medicine, 34*1(4), 248-257.

Sherwin, R. S., Anderson, R. M., Buse, J. B., Chin, M. H., Eddy, D., Fradkin, J., Ganiats, T. G., Ginsberg, H., Kahn, R., Nwankwo, R., Rewers, M., Schlessinger, L., Stern, M., Vinicor, F., Zinman, B., & American Diabetes Association. (2003). The prevention or delay of type 2 diabetes. *Diabetes Care, 26 (Suppl* 1), S62-9.

Short, K. R., Vittone, J. L., Bigelow, M. L., Proctor, D. N., Rizza, R. A., Coenen-Schimke, J. M., & Nair, K. S. (2003). Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. *Diabetes, 5*2(8), 1888-1896.

Shulman, G. I. (2000). Cellular mechanisms of insulin resistance. *The Journal of Clinical Investigation, 10*6(2), 171-176.

Soleas, G. J., Yan, J., & Goldberg, D. M. (2001). Measurement of trans-resveratrol, (+)-catechin, and quercetin in rat and human blood and urine by gas chromatography with mass selective detection. *Methods in Enzymology, 33*5, 130-145.

Sriwijitkamol, A., Coletta, D. K., Wajcberg, E., Balbontin, G. B., Reyna, S. M., Barrientes, J., Eagan, P. A., Jenkinson, C. P., Cersosimo, E., DeFronzo, R. A., Sakamoto, K., & Musi, N. (2007). Effect of acute exercise on AMPK signaling in skeletal muscle of subjects with type 2 diabetes: A time-course and dose-response study. *Diabetes, 5*6(3), 836-848.

Stein, S. C., Woods, A., Jones, N. A., Davison, M. D., & Carling, D. (2000). The regulation of AMP-activated protein kinase by phosphorylation. *The Biochemical Journal, 345 Pt* 3, 437-443.

Su, H. C., Hung, L. M., & Chen, J. K. (2006). Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. *American Journal of Physiology: Endocrinology and Metabolism, 29*0(6), E1339-46.

Sun, C., Zhang, F., Ge, X., Yan, T., Chen, X., Shi, X., & Zhai, Q. (2007). SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. *Cell Metabolism,* 6(4), 307-319.

Sweeney, G., Garg, R. R., Ceddia, R. B., Li, D., Ishiki, M., Somwar, R., Foster, L. J., Neilsen, P. O., Prestwich, G. D., Rudich, A., & Klip, A. (2004). Intracellular delivery of phosphatidylinositol (3,4,5)-trisphosphate causes incorporation of glucose transporter 4 into the plasma membrane of muscle and fat cells without increasing glucose uptake. *The Journal of Biological Chemistry, 279*(31), 32233-32242.

Szkudelski, T. (2006). Resveratrol inhibits insulin secretion from rat pancreatic islets. *European Journal of Pharmacology, 552*(1-3), 176-181.

Szkudelski, T. (2008). The insulin-suppressive effect of resveratrol - an in vitro and in vivo phenomenon. *Life Sciences, 82*(7-8), 430-435.

Thong, F. S., Dugani, C. B., & Klip, A. (2005). Turning signals on and off: GLUT4 traffic in the insulin-signaling highway. *Physiology, 20*, 271-284.

Treebak, J. T., Birk, J. B., Rose, A. J., Kiens, B., Richter, E. A., & Wojtaszewski, J. F. (2007). AS160 phosphorylation is associated with activation of alpha2beta2gamma1- but not alpha2beta2gamma3-AMPK trimeric complex in skeletal muscle during exercise in humans. *American Journal of Physiology: Endocrinology and Metabolism, 292*(3), E715-22.

Treebak, J. T., Frosig, C., Pehmoller, C., Chen, S., Maarbjerg, S. J., Brandt, N., MacKintosh, C., Zierath, J. R., Hardie, D. G., Kiens, B., Richter, E. A., Pilegaard, H., & Wojtaszewski, J. F. (2009). Potential role of TBC1D4 in enhanced post-exercise insulin action in human skeletal muscle. *Diabetologia, 52*(5), 891-900.

Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P., Keinanen-Kiukaanniemi, S., Laakso, M., Louheranta, A., Rastas, M., Salminen, V., Uusitupa, M., & Finnish Diabetes Prevention Study Group. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *The New England Journal of Medicine, 344*(18), 1343-1350.

Ueki, K., Yamamoto-Honda, R., Kaburagi, Y., Yamauchi, T., Tobe, K., Burgering, B. M., Coffer, P. J., Komuro, I., Akanuma, Y., Yazaki, Y., & Kadowaki, T. (1998). Potential role of protein kinase B in insulin-induced glucose transport, glycogen synthesis, and protein synthesis. *The Journal of Biological Chemistry, 273*(9), 5315-5322.

Vaz-da-Silva, M., Loureiro, A. I., Falcao, A., Nunes, T., Rocha, J. F., Fernandes-Lopes, C., Soares, E., Wright, L., Almeida, L., & Soares-da-Silva, P. (2008). Effect of food on the pharmacokinetic profile of trans-resveratrol. *International Journal of Clinical Pharmacology and Therapeutics, 46*(11), 564-570.

Voet, D. J., Voet, J. G., & Pratt, C. W. (2008). *Fundamentals of biochemistry: Life at the molecular level* (3rd ed.). New York, NY: John Wiley & Sons, Inc.

Wallace, T. M., Levy, J. C., & Matthews, D. R. (2004). Use and abuse of HOMA modeling. *Diabetes Care, 27*(6), 1487-1495.

Walle, T., Hsieh, F., DeLegge, M. H., Oatis, J. E., Jr, & Walle, U. K. (2004). High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 32*(12), 1377-1382.

Wells, J. C., Fuller, N. J., Dewit, O., Fewtrell, M. S., Elia, M., & Cole, T. J. (1999). Four-component model of body composition in children: Density and hydration of fat-free mass and comparison with simpler models. *The American Journal of Clinical Nutrition, 69*(5), 904-912.

Wenzel, E., & Somoza, V. (2005). Metabolism and bioavailability of trans-resveratrol. *Molecular Nutrition & Food Research, 49*(5), 472-481.

Whaley, M. H., Brubaker, P. H., Otto, R. M., & Armstrong, L. E. (2006). *ACSM's guidelines for exercise testing and prescription* (7th ed.). Philadelphia, Pa.: Lippincott Williams & Wilkins.

Willoughby, D. S., Stout, J. R., & Wilborn, C. D. (2007). Effects of resistance training and protein plus amino acid supplementation on muscle anabolism, mass, and strength. *Amino Acids, 3*2(4), 467-477.

Willoughby, D. S., & Wilborn, C. D. (2006). Estradiol in females may negated skeletal muscle myostatin mRNA expression and serum myostatin propeptide levels after eccentric muscle contractions. *Journal of Sport Science and Medicine,* 5(4), 672-681.

Winnick, J. J., Sherman, W. M., Habash, D. L., Stout, M. B., Failla, M. L., Belury, M. A., & Schuster, D. P. (2008). Short-term aerobic exercise training in obese humans with type 2 diabetes mellitus improves whole-body insulin sensitivity through gains in peripheral, not hepatic insulin sensitivity. *The Journal of Clinical Endocrinology and Metabolism, 9*3(3), 771-778.

Witczak, C. A., Sharoff, C. G., & Goodyear, L. J. (2008). AMP-activated protein kinase in skeletal muscle: From structure and localization to its role as a master regulator of cellular metabolism. *Cellular and Molecular Life Sciences: CMLS, 65*(23), 3737-3755.

Wright, D., & Sutherland, L. (2008). Antioxidant supplementation in the treatment of skeletal muscle insulin resistance: Potential mechanisms and clinical relevance. *Applied Physiology, Nutrition, and Metabolism, 33*(1), 21-31.

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S., Ueki, K., Eto, K., Akanuma, Y., Froguel, P., Foufelle, F., Ferre, P., Carling, D., Kimura, S., Nagai, R., Kahn, B. B., & Kadowaki, T. (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine,* 8(11), 1288-1295.

Yeo, W. K., Lessard, S. J., Chen, Z. P., Garnham, A. P., Burke, L. M., Rivas, D. A., Kemp, B. E., & Hawley, J. A. (2008). Fat adaptation followed by carbohydrate restoration increases AMPK activity in skeletal muscle from trained humans. *Journal of Applied Physiology, 105*(5), 1519-1526.

Yoshizumi, M., Tsuchiya, K., Suzaki, Y., Kirima, K., Kyaw, M., Moon, J. H., Terao, J., & Tamaki, T. (2002). Quercetin glucuronide prevents VSMC hypertrophy by angiotensin II via the inhibition of JNK and AP-1 signaling pathway. *Biochemical and Biophysical Research Communications, 293*(5), 1458-1465.

Zang, M., Xu, S., Maitland-Toolan, K. A., Zuccollo, A., Hou, X., Jiang, B., Wierzbicki, M., Verbeuren, T. J., & Cohen, R. A. (2006). Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes, 55*(8), 2180-2191.

Zhang, J. (2007). The direct involvement of SirT1 in insulin-induced insulin receptor substrate-2 tyrosine phosphorylation. *The Journal of Biological Chemistry, 282*(47), 34356-34364.

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., Musi, N., Hirshman, M. F., Goodyear, L. J., & Moller, D. E. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *The Journal of Clinical Investigation, 108*(8), 1167-1174.

Zierath, J. R., Krook, A., & Wallberg-Henriksson, H. (1998). Insulin action in skeletal muscle from patients with NIDDM. *Molecular and Cellular Biochemistry, 182*(1-2), 153-160.