Mechanisms Mediating Antidiabetic Effects of Serviceberry extracts, Curcumin, and Stilbenes

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 7, 2012

Keywords: curcumin, serviceberry, stilbene, obesity, diabetes

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Abstract

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. Hippocrates (460-377 B.C), said “Let food be thy medicine and medicine be thy food”. The goal of this study was to characterize potential antidiabetic mechanisms of serviceberry extracts, and two polyphenolic bioactive components, curcumin and stilbenes.

Serviceberry [*Amelanchier alnifolia* (Nutt.) Nutt. ex. M. Roem (Rosaceae)] has been traditionally used by the American Indians of Montana, in the management of diabetes. In this study, serviceberry plant samples consisting of leaves, twigs, and leaves with berries were extracted and fractionated. We demonstrate that serviceberry extracts activate AMPK (AMP-activated protein kinase), increase glucose uptake, and suppress dexamethasone-induced gluconeogenic gene expression. Additionally, water fractions from serviceberry leaves and twigs demonstrated significant inhibition of mammalian alpha-glucosidase activity, similar to that of acarbose, *in vitro* and in a diet-induced obesity mouse model of insulin resistance and diabetes. These findings validate traditional knowledge of the antidiabetic effects of serviceberry and indicate that serviceberry extracts delay intestinal absorption of carbohydrate and suppress hepatic glucose production.

Curcumin and stilbenes are polyphenolic bioactive components that have been demonstrated to possess significant antioxidant and anti-inflammatory effects. Several studies demonstrate that curcuminoids and stilbenes, including resveratrol, can contribute to improved

health, insulin sensitivity, and lower blood glucose levels in animal models of diabetes. In this study we demonstrated that curcuminoids increased the transcriptional activation of the nuclear receptor PPARγ. Consistent with PPARγ activation, curcumin-treatment increased gene expression and secretion of adiponectin. Interestingly, curcumin treatment did not induce differentiation of human subcutaneous preadipocytes and inhibited rosiglitazone-induced adipocyte differentiation in human subcutaneous preadipocytes. Natural stilbenes and synthetic analogs were investigated for alpha-glucosidase inhibitory effects *in vitro* and *in vivo*. Our studies demonstrate that resveratrol, a polyphenolic stilbene, and its naturally occurring analog, piceatannol, are potent inhibitors of mammalian alpha-glucosidase activity. These findings extend the range of the multifaceted beneficial properties of stilbene compounds, in blood glucose control and management of type 2 diabetes.

Acknowledgments

First of all, I would like to thank Dr. Suresh T. Mathews for providing the guidance in my research and studies. He has given me adequate help and encouragement that I needed to complete my Doctoral dissertation. Also, I would love to thank Dr. Doug White, Dr. Kevin W. Huggins and Dr. Floyd Woods for serving as my committee members and Dr. Robert L. Judd as my outside reader. Again, I want to mention specially Dr. Agnes Rimando, Mr. Wilbert Fish, Dr. Srinivasa Rao Mentreddy and Dr. Teayoun Kim's contribution to these projects. Thanks Mr. Wilbert Fish and Dr. Srinivasa Rao Mentreddy for indentifying serviceberry (*Amelanchier alnifolia*) as a medical plant for potential management of type 2 diabetes. Thanks Dr. Agnes Rimando for extracting serviceberry samples. It would be impossible for me to draw any conclusion without their previous work. I gratefully acknowledge the grant support (to Dr. Suresh T. Mathews, my major advisor) from the National Institute of Food and Agriculture (2009-38424-05766), the Alabama Agricultural Experiment Station (ALA095-001), and the Diabetes Action Research and Education Foundation, for carrying out these studies. In addition, I would love to express my gratitude to fellow graduate research assistants and faculty members in the Department of Nutrition, Dietetics, and Hospitality Management. Finally, special thanks are due to my family for their support, understanding, and love.

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PPAR	Peroxisome proliferator-activated receptor
THC	Tetrahydrocurcumin
AMPK	AMP-activated protein kinase
HGP	Hepatic glucose production
MAPK	Mitogen-activated protein kinase
GLUT4	Glucose Transporter 4
PI3K	Phosphatidylinositol 3-kinase
GLP	Glucagon-like peptide
ER	Endoplasmic Reticulum
DPP	Dipeptidyl peptidase

Chapter 1: Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2011). It affects over 25.8 million people in the United States and over 220 million people worldwide (CDC, 2011; WHO, 2011). The prevalence of insulin resistance, a condition characterized by impaired insulin action despite normal or elevated levels of circulating insulin, is even more widespread. Insulin resistance is a major causative factor in the early development of type 2 diabetes. It is also an independent risk factor for cardiovascular disease and the metabolic syndrome (DeFronzo, 1997; Reaven, 1988). Obesity, a major risk factor in the development of type 2 diabetes, further exacerbates this condition (Hill et al., 2003). Impaired insulin secretion from pancreatic β-cell, decreased glucose uptake in muscle and adipose tissue, increased hepatic glucose production (HGP) and decreased hepatic glucose uptake, prolonged elevation of free fatty acid and abnormal fat cell-secreted adipocytokines, decreased incretin effect, increased glucagon secretion from pancreatic α-cell, increased glucose reabsorption from kidney, and uncontrolled appetite by neurotransmitter dysfunction contribute to the metabolic dysfunction of type 2 diabetes (Defronzo, 2009).

Currently, in addition to recombinant insulin, there are eight classes of oral pharmacological agents: sulfonylureas, meglitinides, metformin, thiazolidinediones, α-glucosidase inhibitors, dipeptidyl peptidase IV (DPP-IV) inhibitors, bile acid sequestrants, and dopamine agonists and two classes of injectable medications: amylin analog and glucagon-like peptide

(GLP) agonist, available to treat type 2 diabetes as monotherapy and in combination (Bolen et al., 2007; DeFronzo, 1999; Inzucchi, 2002; Krentz and Bailey, 2005; Matthaei et al., 2000; Mudaliar and Henry, 2001; Scheen, 1998). These pharmacological treatments for diabetes usually aim at stimulating insulin secretion, decreasing glucagon secretion, delaying the absorption of carbohydrates, suppressing hepatic glucose production, improving glucose transport/uptake or increasing insulin sensitivity. Although, these contribute to the alleviation of diabetes, several complications and side effects persist.

Plant-based medicinal products or phytochemicals have been used by man since ancient times (Subbulakshmi and Naik, 2001). Functional foods, dietary supplements and nutraceuticals constitute a rapidly growing focus for research, product development, consumer interest, as well as regulatory efforts, in recent years. Bioactive components of functional foods, dietary supplements and nutraceuticals can reduce mounting health care costs for ailments such as diabetes, obesity, heart disease, cancer, etc. Functional food can be defined as a conventional food that is consumed as part of a usual diet, and which is demonstrated to have physiological benefit and/or reduce the risk of chronic disease, beyond basic nutritional function. The Dietary Supplement Health and Education Act (DSHEA) defines "dietary supplement" as a vitamin, a mineral, an herb or other plant-derived chemical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total dietary intake (including enzymes or tissues from organs or glands), or a concentrate, metabolite, constituent, or extract of these. Nutraceuticals can be defined as a product isolated or purified from food that is generally sold in supplement forms not usually associated with food and is demonstrated to have a physiological benefit or provide protection against chronic disease. Botanical substances have been used in the management of diabetes, with a considerable degree of success. Examples of herbs that may

regulate glucose homeostasis include *Aloe barbadensis*, *Eugenia jambolana*, *Gymnema sylvestre*, *Ocimum tenuiflorum*, *Trigonella foenum-graecum*, *Allum sativum*, *Galega officinalis* and *Curcuma longa L.* (Kim et al., 2009). Some of these are indigenous to the North American continent.

Serviceberry [*Amelanchier alnifolia* (Nutt.) Nutt. ex. M. Roem (Rosaceae)], also called Saskatoon Berry or Okinoki by the Native Indians, found in the North Glacier forests of the Rocky Mountains in Montana, has been and continues to be used by Native American Indians in the management of diabetes. The major functional components of Saskatoon berries are phenolic compounds, particularly the anthocyanins. In ripe saskatoon berries there are anthocyanins of cyanidin 3-galactoside, 3-glucoside, Cyanidin 3-xyloside, pelargonin 3-glucoside and malvidin derivatives (Mazza, 2008). Berries of *A. alnifolia* contain phenolic acids including 3-feruloylquinic, chlorogenic, and 5-feruloylquinic acids. Flavonoid compounds from the fruit include rutin, hyperoside, avicularin, and quercetin (Burns Kraft et al., 2008). Curcumin (diferuloylmethane), a polyphenolic compound and the bioactive component of turmeric, has been shown to possess potent antioxidant and anti-inflammatory properties. Despite its poor absorption, several studies demonstrate that curcuminoids (curcumin and its natural structural analogs) can lower blood glucose levels in animal models of diabetes. Stilbenes (1,2-diphenylethylene), naturally occurring compounds, are found in a wide range of plant sources and dietary supplements (Roupe et al., 2006). Resveratrol and piceatannol are two major kinds of stilbenes which are naturally occurring compounds and are found in a wide range of plant sources, especially grape, wine and kinds of berries. Many studies had shown their anti-cancer, anti-oxidant, anti-inflammatory actives. Recently, many studies focus on the anti-diabetic effect of resveratrol. Though several studies indicate blood glucose lowering properties for

serviceberries, curcumin, and stilbenes, the mechanisms that mediate such anti-diabetic effects are hitherto unknown. Therefore, the goal of this study was to investigate the potential anti-diabetic mechanisms of serviceberry extracts, curcumin, and stilbenes using *in vitro*, intact cells, and *in vivo* techniques.

Chapter 2: Review of Literature

2.1 Obesity

Obesity is a chronic disease that is causally related to serious medical complications, including hypertension, atherosclerosis, dyslipidemia, insulin resistance, and diabetes, which impairs the quality of life and lead to increased morbidity and premature death (Goralski and Sinal, 2007; Greenberg and McDaniel, 2002; NIH, 1998). Men and women with a BMI (Body Mass Index, weight in kilograms, divided by height in meters squared) of 25.0 to 29.9 kg/m^2 are considered overweight, and those with a BMI 30 kg/m^2 or greater are consider obese (NIH, 1998; WHO, 2000). However, the prevalence of obesity-related diseases, such as diabetes, begins to increase at BMI values below 25 kg/m^2 (Chan et al., 1994; Colditz et al., 1995). Ethnicity also influences BMI-associated health risk. The risk of diabetes is higher in Southeast Asian populations than in Whites when subjects are matched for BMI (McKeigue et al., 1991).

The interaction of genetic background and environmental factors affects body size, with an estimated 40% of the variance in body mass contributed by genetic background (Bouchard and Perusse, 1993). Behaviors that protect against obesity include controlling portion size, consumption of a diet low in fat and energy density, and increasing physical activity. The current prevalence of obesity has possibly resulted from alterations in environmental factors that increase energy intake and reduce physical activity (Fig. 1) because of sedentary lifestyle and work activities (Hill and Peters, 1998).

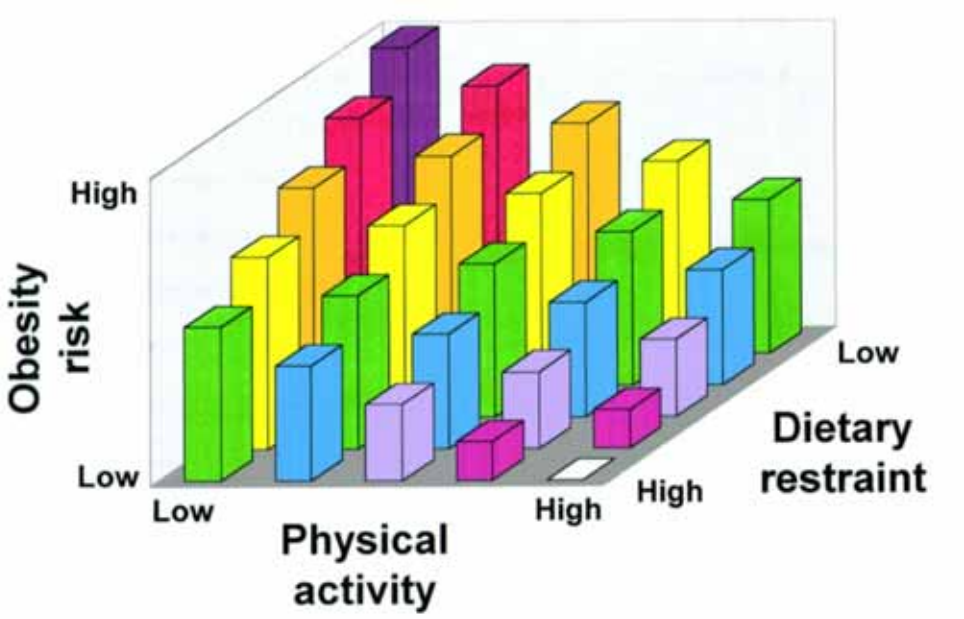


Figure 1. Hypothetical risk of obesity in individuals consuming a diet high in energy density and low physical activity (Hill and Peters, 1998).

2.1.1 Prevalence and trends in obesity

The prevalence of obesity in the United States is high and exceeds 30% in most age and sex groups based on the 1999-2008 National Health and Nutrition Examination Survey (NHANES) data (Flegal et al., 2010). Among men, age-adjusted obesity prevalence was 32.2% overall and prevalence of obesity within racial and ethnic groups was 31.9% among non-Hispanic White men, 37.3% among non-Hispanic Black men and 34.3% among all Hispanics men. For women, the age-adjusted prevalence was 35.5% and 33.0% among non-Hispanic White women, 49.6% among non-Hispanic Black women and 43.0% among all Hispanics women. Combining overweight and obesity, the age-adjusted prevalence was 68.0% overall, 72.3% among men, and 64.1% among women. Though the prevalence of obesity showed a striking increase in the 1980s and 1990s, the data from Flegal et al. (2010) suggest that obesity prevalence was relatively stable using 2007-2008 data (Flegal et al., 2010). One serious medical condition that affects children

and adolescents is childhood obesity that occurs when a child is well above the normal weight for his or her age and height. Childhood obesity puts children on the path to health problems that were once confined to adults, such as diabetes, high blood pressure and high cholesterol.

2.2 Diabetes

Diabetes mellitus is defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2011). It is a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin (Rao and Tiwari, 2002). Diabetes affects over 25.8 million people in the United States and over 220 million people worldwide. Over time diabetes leads to serious damage to many of the body's systems and cause serious complications, such as blindness, kidney damage, cardiovascular disease, stroke, atherosclerosis, lower-limb amputations and many others (CDC, 2011; NIH, 2008). Three types of diabetes mellitus exist. Type 1 diabetes, previously known as insulin-dependent diabetes mellitus (IDDM), juvenile or childhood-onset diabetes, is characterized by deficient or no insulin production and requires daily administration of insulin. It happens with birth or develops when the pancreatic β-cells are destroyed. Type 2 diabetes, which was formerly known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes, results from the ineffective use of insulin by the body. Ninety percent of people with diabetes around the world are diagnosed as type 2 diabetes. Insulin resistance, defined as the inability of peripheral tissues to respond to circulating insulin, and the attenuated ability of β-cells to respond to the body’s demand for insulin, preludes type 2 diabetes. The third type is called gestational diabetes which occurs during pregnancy, when hormones from the placenta block the action of insulin in the mother, leading to insulin resistance. Women with gestational

diabetes are at a greater risk for developing type 2 diabetes later in life (ADA, 2011). Impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG) are intermediate conditions between normality and diabetes. People with IGT or IFG are at high risk of progressing to type 2 diabetes, although this is not inevitable.

2.2.1 Prevalence and trends in diabetes

According to 2007 National Diabetes Fact Sheet, prediabetes is a condition in which individuals have blood glucose levels higher than normal, but not high enough to be classified as diabetes. People with prediabetes have an increased risk of developing type 2 diabetes, heart disease, and stroke. People with prediabetes have impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) and some people have both IFG and IGT. IFG is a condition in which the fasting blood sugar level is 100 to 125 milligrams per deciliter (mg/dL) after an overnight fast. IGT is a condition in which the blood sugar level is 140 to 199 mg/dL after a 2-hour oral glucose tolerance test. These two levels are higher than normal, but not high enough to be classified as diabetes. In 1988–1994, among U.S. adults aged 40–74 years, 33.8% had IFG, 15.4% had IGT, and 40.1% had prediabetes (IGT or IFG or both). In 2003–2006, 25.9% of U.S. adults aged 20 years or older had IFG (35.4% of adults aged 60 years or older). Applying this percentage to the entire U.S. population in 2007 suggests that an estimated 57 million American adults aged 20 years or older had IFG or prediabetes.

2.3 Insulin resistance

Insulin resistance is defined as the failure of the body to respond normally to insulin. Insulin resistance in liver leads to glucose overproduction in the basal state with fasting

hyperinsulinemia and impaired suppression of hepatic glucose production (HGP) (DeFronzo et al., 1989; Ferrannini et al., 1988; Groop et al., 1989). Insulin resistance in muscle is manifested by impaired glucose uptake after a meal leading to postprandial hyperglycemia (Ferrannini et al., 1988). Both type 2 diabetes and obesity are associated with insulin resistance, but hyperglycemia is not developed in most obese, insulin resistant individuals (Reaven, 1988). Under normal conditions, to maintain normal glucose tolerance, the pancreatic islet β-cells increase insulin release sufficiently to overcome the reduced efficiency of insulin action (Kahn et al., 1993; Perley and Kipnis, 1966; Polonsky et al., 1988). In obesity and insulin resistance associated with type 2 diabetes, the β-cell is unable to compensate fully for decreased insulin sensitivity. Dysfunction of β-cell exists in high risk of diabetes- developing individuals even when their glucose levels are still normal (Kahn, 2001).

The relationship between insulin levels and insulin sensitivity is reciprocal and nonlinear in nature. Two parameters, β-cell function and β-cell mass, make β-cell to adapt to changes in insulin sensitivity. Beta-cell volume is increased by about 50% in healthy human obese individuals as a result of hypertrophy of existing cells rather than proliferation (Butler et al., 2003; Kloppel et al., 1985). Beta-cell function decreases progressively as the fasting glucose level increases, even when the glucose level is still within the normal range (Utzschneider et al., 2006). In insulin resistant individuals of Pima Indians, in whom the prevalence of diabetes is higher than almost any other ethnic group in the world, the transition from normal to impaired glucose tolerance and then on to diabetes is characterized by a progressive loss of β-cell function (Weyer et al., 1999).

2.4 Mechanisms linking obesity to type 2 diabetes

2.4.1 Thrifty genotype

"Thrifty" genotype favors the economical use and storage of energy during periods of prolonged famine which plagued early human hunter-gatherers. During starvation, it was suggested that evolutionary pressure to preserve glucose for use by the brain led to a genetic propensity toward insulin resistance (Neel, 1962). In the modern-world setting of sedentary lifestyles and unrestricted access to high-caloric foods, the thrifty gene hypothesis may be causal to the *twin epidemics of obesity and diabetes* (Zimmet and Thomas, 2003). The "thrifty phenotype" hypothesis is an alternative to thrifty genes (Neel, 1962). It is posited that fetal malnutrition alters metabolic pathway and cause tissue adaptations favoring the thrifty use of nutrients in utero and in postnatal life, thereby leading to obesity and diabetes in the setting of subsequent adequate nutrition.

2.4.2 Abdominal obesity

Many prospective studies have shown that increased abdominal (visceral) fat accumulation is an independent risk factor for coronary artery disease, hypertension, stroke, and type 2 diabetes (Ducimetiere et al., 1986; Ferrannini et al., 1997; Fujimoto et al., 1999; Larsson et al., 1984). The mechanisms involved in these common clinical associations are not completely known, but include impaired free fatty acid metabolism, increased visceral production of glycerol, and abnormal production of adipose tissue-derived hormones and cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, leptin, adiponectin, and resistin. An impaired free fatty acid (FFA) or non-esterified fatty acid (NEFA) metabolism could affect insulin secretion and cause insulin resistance (Bergman et al., 2006; Lemieux, 2004; Mittelman et al., 2002). IL-6

and TNF-α, proinflammatory cytokines secreted by adipocyte, and C-reactive protein (CRP), are increased in patients with visceral obesity (Lemieux et al., 2001; Tsimikas et al., 2006; Weisberg et al., 2003). Adiponectin which can improve insulin signaling and potentially protect against atherosclerosis is reduced in obese individuals, especially in individuals with excess visceral adiposity (Berg and Scherer, 2005; Cote et al., 2005; Matsuzawa, 2006). Besides alteration in NEFA metabolism and endocrine function, excess caloric consumption and a sedentary lifestyle is other reason which contributes to the insulin-resistant state among individuals with visceral obesity.

2.4.3 Non-esterified fatty acids (NEFA)

In obesity, increased levels of adipocyte-derived free fatty acids contribute to insulin resistance in liver and muscle (Bergman and Ader, 2000; Boden and Shulman, 2002). Increased NEFA delivery or decreased intracellular metabolism of fatty acids activate a serine/threonine kinase cascade leading to serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2), and a reduced ability of these molecules to activate PI(3)K by increasing the intracellular content of fatty acid metabolites such as diacylglycerol (DAG), fatty acyl-coenzyme A (fatty acyl-CoA), and ceramides (Shulman, 2000). Elevated NEFA levels contribute to developing the insulin resistance and prevent the compensatory β-cell response in humans (Carpentier et al., 1999). Decreased insulin release could decrease suppression of hepatic glucose production and the efficiency of glucose uptake in insulin-sensitive tissues. Decreased insulin output could also increase lipolysis and elevate NEFA levels by impairing adipocyte metabolism. Both elevated NEFAs and glucose can occur simultaneously and are more deleterious to islet health and insulin action than either alone (Leung et al., 2004;

Prentki et al., 2002). Reduction in insulin release by β-cell dysfunction can decrease insulin action in brain region and cause weight gain and an aggravation of insulin resistance (Schwartz et al., 2000).

2.4.4 Adipocytokines and glucose homeostasis

More than an energy storage depot, adipocytes not only regulate fat mass and nutrient homeostasis but also is involved in the immune response, blood pressure control, hemostasis, bone mass, and thyroid and reproductive function, through the synthesis and release of peptide hormones (Trayhurn, 2005). Adipose tissue also communicates with the brain and peripheral tissues by secreting hormones regulating appetite and metabolism (Kershaw and Flier, 2004). Several adipocyte-derived factors contribute to insulin resistance, acting both the locally (autocrine/paracrine) and systemically (endocrine).

2.4.4.1 Tumor necrosis factor-α and other cytokines

Tumor necrosis factor-α (TNF-α) has pro-hyperglycemic effects on glucose homeostasis. Consistent with elevated TNF-α levels in obesity and in other insulin-resistant states (such as sepsis), addition of TNF-α to cells and administration to mice has been shown to reduce insulin action, and blockade of TNF-α action by biochemical or genetic means restores insulin sensitivity *in vivo* and *in vitro* (Hotamisligil, 1999). IL-6, produced by adipocytes, have both insulin-resistance-promoting and insulin-sensitizing effects, as conflicting evidence suggests (Carey et al., 2006; Rotter et al., 2003). Pro-inflammatory cytokines induce c-Jun N-terminal kinase 1 (JNK1)-mediated serine phosphorylation of insulin receptor substrate-1 (IRS-1) (Hirosumi et al., 2002), IκB kinase (IKK)-mediated nuclear factor-κB (NF-κB) activation

(Shoelson et al., 2003), suppressor of cytokine signaling 3 (SOCS3) (Howard and Flier, 2006) and production of ROS (Houstis et al., 2006), all of which can promote insulin resistance.

2.4.4.2 Leptin

Leptin, secreted primarily by adipocytes, improves insulin sensitivity in skeletal muscle and liver through lipid partitioning and decreases intra-myocellular lipid levels. These effects are mediated via a combination of direct activation of AMP-activated protein kinase (AMPK) and indirect actions mediated through central neural pathways (Kamohara et al., 1997; Minokoshi et al., 2002). An 'adipo-insular axis' has also been suggested with insulin promoting leptin secretion and leptin inhibiting insulin release (Kieffer and Habener, 2000).

2.4.4.3 Adiponectin

Adiponectin, a 30-kDa protein, with an amino-terminal collagen-like domain and a carboxy-terminal globular domain, can exist as a trimer, hexamer or a higher-order multimer with 12–18 subunits (Pajvani et al., 2003; Waki et al., 2003). Two receptor types have been indentified for adiponectin, one of which comprises two similar transmembrane proteins with homology to G-protein-coupled receptors, known as adipoR1 and adipoR2 (Yamauchi et al., 2003). The other, T-cadherin, without a transmembrane domain that has been proposed to act as a co-receptor for the high-molecular-weight forms of adiponectin on endothelial and smooth muscle cells (Hug et al., 2004). Adiponectin levels are inversely correlated with body mass (Hotta et al., 2001; Yatagai et al., 2003). Adiponectin stimulates AMPK activity in the liver and skeletal muscle, with profound effects on fatty acid oxidation and insulin sensitivity (Lago et al., 2007). Adiponectin enhances glucose-stimulated insulin secretion in islets from mice with diet-induced

obesity but has no effect on insulin secretion in islets from healthy mice or humans (Winzell et al., 2004).

2.4.4.4 Resistin

As one of a family of cysteine rich resistin-like molecules (RELMs) (Steppan et al., 2001b), resistin (also known as FIZZ3) was discovered as a secreted product of mouse adipocytes that was repressed by thiazolidinedione class of antidiabetic drugs (Steppan et al., 2001a). Resistin increases hepatic glucose output and reduces glucose uptake by muscle and fat (Banerjee et al., 2004; Steppan and Lazar, 2004). Although there are several multimeric forms of resistin circulating in plasma in mice, the lower molecular weight forms appear to be more active (Patel et al., 2004). Resistin, expressed exclusively in adipocytes in mice (Steppan et al., 2001a) but predominantly in macrophages in humans (Patel et al., 2003), was increased in insulin resistant states in mice and humans (Osawa et al., 2004; Rajala et al., 2004). Source of human resistin is debated and the data suggest that human resistin is the product of macrophages or other stromal cells with the fat pad (Kaser et al., 2003; Patel et al., 2003).

2.4.4.5 Retinol-binding protein 4

Retinol-binding protein 4 (RBP4), identified as a secreted member of the lipocalin superfamily, was coordinately regulated by changes in adipocyte glucose transporter 4 (GLUT4) levels in mice. Overexpression of RBP4 impairs hepatic and muscle insulin action in mice (Yang et al., 2005). High serum RBP4 levels are associated with insulin resistance in obese humans and in those with type 2 diabetes as well as in lean, nondiabetic people with a family history of diabetes (Graham et al., 2006).

2.4.5 Inflammation

Meta-flammation (metabolically triggered inflammation) principally triggered by nutrients and metabolic surplus, engages a set of molecules and signaling pathways involved in classical inflammation. Increasing adiposity and many of the more typical proinflammatory stimuli, including cytokines and TLRs, simultaneously activates both JNK (c-Jun N-terminal kinases) and IKKβ (IκB kinase-β). JNK promote insulin resistance through the phosphorylation of serine residues in IRS-1 (Aguirre et al., 2000; Aguirre et al., 2002; Cai et al., 2005; Hirosumi et al., 2002; Ozcan et al., 2004; Werner et al., 2004; Yuan et al., 2001). IKKβ phosphorylates the IκB protein inhibitors of NF-κB, targeting IκBα for proteasomal degradation, and liberates NF-κB for translocation into the nucleus, where it promotes the expression of numerous target genes whose products induce insulin resistance (Shoelson et al., 2006).

Cellular stress, including reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress, also activate JNK and NF-κB. Systemic markers of oxidative stress increases with adiposity. Lipid accumulation in the adipocyte activates NADPH oxidase which increases the ROS production. This can further increase the production of TNF-α, IL-6, and monocyte chemoattractant protein-1 (MCP-1), and decrease the production of adiponectin (Furukawa et al., 2004). Increased adiposity in obesity challenges ER function and capacity owing to architectural constraints that limit ER expansion as well as altered energy and nutrient availability and cause ER stress (inflammation and metabolic disorders). In both dietary and genetic obesity, ER stress is shown to be increased in adipose and liver tissue (Ozcan et al., 2004). ER stress activates JNK leading to serine phosporylation of insulin receptor substrate-1 (IRS-1) and also activates NF-κB (Ozcan et al., 2004). Cell stress and saturated fats may promote synthesis of ceramides, which

accumulate in tissue such as muscle and activate JNK and NF-κB, correlating with the degree of insulin resistance (Straczkowski et al., 2004).

2.5 Peripheral insulin resistance, progressive β-cell failure, and development of diabetes

2.5.1 Liver: Liver provides most of the glucose for utilization by the brain under basal or fasting conditions (Defronzo, 2009). A characteristic feature of type 2 diabetes is increased HGP. Insulin resistance in liver increases hepatic gluconeogenesis to increase HGP in type 2 diabetes (Consoli et al., 1990; DeFronzo and Ferrannini, 1987; Magnusson et al., 1992). Increased circulating glucagon levels and enhanced hepatic sensitivity to glucagon; increased expression and activity of phosphoenolpyruvate carboxykinase and pyruvate carboxylase (key enzyme of gluconeogenesis) because of elevated free fatty acids (lipotoxicity) (Gastaldelli et al., 2000); and increased expression and activity of glucose-6-phosphatase (key enzyme for glucose release from liver) by glucotoxicity (Clore et al., 2000) all contribute to accelerated HGP.

2.5.2 Skeletal muscle: Muscle insulin resistance accounts for 85-90% of impairment in total body glucose disposal in type 2 diabetic subjects (DeFronzo et al., 1985; Pendergrass et al., 2007). This impaired glucose transport and phosphorylation (Bonadonna et al., 1996; Cline et al., 1999; Mandarino et al., 1995; Pendergrass et al., 2007; Rothman et al., 1992), has been shown to reduce glycogen synthesis (Mandarino et al., 1987; Shulman et al., 1990; Shulman et al., 1985), and decrease glucose oxidation (Felber et al., 1987; Golay et al., 1988; Groop et al., 1989; Groop et al., 1991) in type 2 diabetic subjects.

2.5.3 Adipose tissue: In fat cells, resistance to insulin's antilipolytic effect causes day-long elevation in plasma FFA concentration (Bays et al., 2004; Bays et al., 2008; Bonadonna and De Fronzo, 1991; DeFronzo, 2004; Fraze et al., 1985; Groop et al., 1989; Groop et al., 1991). The elevation of plasma FFA levels (lipotoxicity) stimulate gluconeogenesis (Bevilacqua et al., 1987; Ferrannini et al., 1983; Williamson et al., 1966), induce hepatic/muscle insulin resistance (Felber and Vannotti, 1964; Golay et al., 1988; Roden et al., 1996; Thiebaud et al., 1982) and impair insulin secretion (Carpentier et al., 2000; Kashyap et al., 2003). Excessive amounts of insulin resistance-induced adipocytokines were secreted by dysfunctional fat cells (Bays et al., 2004; Bays et al., 2008). Enlarged fat cells are insulin resistant and lose the capacity to store fat which cause lipid "overflow" into muscle, liver, and β-cells to aggravate muscle/hepatic insulin resistance (Bays et al., 2004; Bays et al., 2008).

2.5.4 Gut: As a major endocrine organ, the gut also contributes to the pathogenesis of type 2 diabetes. The incretin effect in gastrointestinal tissues mainly depends on GLP-1 and GIP. Incretin can inhibit glucagon secretion (GLP-1) (Drucker, 2006; Drucker and Nauck, 2006; Meier and Nauck, 2006) and suppress HGP (Defronzo, 2009; Edgerton et al., 2009; Ionut et al., 2008). In type 2 diabetes, a deficiency of GLP-1 (Drucker, 2006; Drucker and Nauck, 2006; Meier and Nauck, 2006) and resistance to GIP were observed (Holst, 2006; Meier et al., 2001; Nauck et al., 1986; Nauck et al., 1993a).

2.5.5 Kidney: Kidney has also been implicated in the pathogenesis of type 2 diabetes because the ability of the diabetic kidney to reabsorb glucose appears to be augmented by an absolute

increase in the renal reabsorptive capacity for glucose (Dominguez et al., 1994; Kamran et al., 1997; Mogensen, 1971; Noonan et al., 2001).

2.5.6 Brain: brain tissue is also implicated in the pathogenesis of type 2 diabetes. In a study using functional magnetic resonance imaging (MRI) examining the cerebral response to an ingested glucose load, it was found that in two areas in hypothalamus (key centers for appetite regulation), the magnitude of the inhibitory response following glucose ingestion was found to be reduced in obese, insulin-resistant, normal glucose tolerance subjects even though the plasma insulin response was increased in the obese group (Matsuda et al., 1999). Cerebral insulin resistance leading to increased HGP and reduced muscle glucose uptake was observed in rodent studies (Obici et al., 2002; Obici et al., 2001).

2.5.7 Progressive β-cell failure: Insulin resistance is important in the progressive β-cells failure of type 2 diabetes because of the increased demand on β-cells to hypersecrete insulin. Elevated plasma free fatty acid (FFA) levels (lipotoxicity) impair insulin mRNA expression, decline of glucose-stimulated insulin release and reduction of islet insulin secretion (Higa et al., 1999). Chronically elevated plasma glucose levels (glucotoxicity) also impair β-cells function (Rossetti et al., 1990). Abnormalities in incretins (GLP-1 and glucose-dependent insulinotrophic polypeptide or gastric inhibitory polypeptide, GIP) play an important role in β-cells failure of type 2 diabetes, too. There is a deficiency of GLP-1 (Drucker, 2006; Drucker and Nauck, 2006; Meier and Nauck, 2006) and resistance to GIP in type 2 diabetes (Holst, 2006; Meier et al., 2001; Nauck et al., 1986; Nauck et al., 1993a).

Thus, as described above, insulin resistance in peripheral tissues, and the impaired ability of β-cells to compensate, leads to progressive β-cell failure, and the development of type 2 diabetes.

2.6 Antidiabetic drugs and their site of action

2.6.1 Alpha-glucosidase inhibitor

To achieve blood glucose level (especially postprandial hyperglycemia, PPHG) as close to normal as possible is the most challenging goal in the treatment of patients with type 2 diabetes. To control PPHG, much attention has recently been given to delay the absorption of glucose and transport across the intestinal brush border membrane after a meal (Rao and Tiwari, 2002). Alpha-glucosidase (EC 3.2.1.0) is the key enzyme of the metabolism of carbohydrate. Inhibition of intestinal α-glucosidases decreases postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption. Thus, α-glucosidase is a key therapeutic target in the management of type 2 diabetes mellitus (Shinde et al., 2008). Without causing hyperinsulinemia, body weight gain and hypoglycemic threat, α-glucosidases inhibitors are currently the most commonly used oral agent for ameliorating PPHG. *Acarbose, miglitol and voglibose* are three α-glucosidases inhibitors currently available to treat type 2 diabetes.

2.6.2 Dipeptidyl peptidase (DPP)-IV inhibitors

Incretin hormone has potent glucose-dependent insulinotropic properties, β cells' tropic effects, and intestinal motility inhibitory effects, all of which contribute to the reduction of plasma glucose (Deacon, 2004). Glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic peptide (GIP) account for the majority of incretin action (Drucker, 2003). As a gut hormone, GLP-1 plays a key role in glucose homeostasis via its incretin effect. Produced from the enteroendocrine L-cell of small intestine, GLP-1 is secreted in response to meal and

nutrients. Actions of GLP-1 include: (a) stimulate insulin release from the pancreatic islets and suppress post-prandial glucagon release in a glucose dependent manner, (b) delay gastric emptying, increase satiety and decrease appetite (Drucker, 2002; Kieffer and Habener, 1999; Nauck et al., 1993b; Nauck et al., 1997), and (c) stimulate beta-cell proliferation and differentiation to increase pancreatic beta cell mass and function (Abraham et al., 2002; Zhou et al., 1999). However, GLP-1 has a very short half-life and is rapidly degraded inside our body by the enzyme dipeptidyl peptidase (DPP)-IV. DPP-IV inhibitor that can block the DPP-IV enzyme can increase the endogeneous GLP-1 level and thus enhances the incretin action. Incretin mimetics such as *exanatide* mimics the action of GLP-1. While GLP-1 lasts only 2 minutes, *exanatide* lasts nearly 10 hours. Exanatide has been shown to improve diabetes control and to produce weight loss in most patients. DPP-IV inhibitors (for example, *sitagliptin* and *vildagliptin*) may be used alone or in combination with other antidiabetic drugs. These drugs are fairly well tolerated by most patients, have minimal side effects and are usually not associated with weight changes during therapy.

2.6.3 5'-AMP-activated protein kinase (AMPK) activators

Described as a “metabolic master switch” that regulates the cellular adaptation to nutritional and environmental variations, AMPK has a crucial function to mediate cellular and whole-body energy homeostasis. Response to pathological conditions such as severe metabolic shock (hypoxia or ischaemia) or other stresses (heat, starvation or prolonged exercise) activate AMPK to inhibit energy-consuming biosynthetic or anabolic pathways (such as fatty acid synthesis in liver and adipocytes, cholesterol synthesis in liver, protein synthesis in liver and muscle and insulin secretion from β-cell) and to promote ATP-producing catabolic pathways (such as fatty

acid uptake and oxidation in multiple tissue, glycolysis in heart and mitochondrial biogenesis in muscle) (Rutter et al., 2003; Viollet et al., 2009).

Mammalian AMPK is a trimeric enzyme composing of three different subunits, catalytic α (α1 or α2) and non-catalytic β (β1 or β2) and γ (γ1, γ2 or γ3), each encoded by a different gene. Expression and distribution of the three subunits are different. The α1 subunit is rich in the kidney, the lung and the adipose tissue, while α2 subunit is rich in heart and skeletal muscles. The regulatory β1 subunit is strongly expressed in the liver, whereas β1 subunit is predominantly found in skeletal muscle. The regulatory γ1 and γ2 subunits can be found in most tissue but γ3 is preferentially expressed in skeletal muscle (Viollet et al., 2009). There are three domains in the primary structure of α subunit: an N-terminal catalytic domain that contain a serine/threonine protein kinase (typical of the protein kinase superfamily) and a key site for activating AMPK by upstream kinases allowing phosphorylation at threonine residue Thr172 within the activation loop (T-loop), a central domain that seems to have an inhibitory function and a C-terminal regulatory binding domain which allow β and γ to bind (Hawley et al., 1996; Rutter et al., 2003; Stein et al., 2000). Interaction between α and γ subunit seems to be stabilized by the β subunit through its binding domain in the C-terminal part (Crute et al., 1998; Pang et al., 2007).

AMPK activity is regulated by both direct allosteric activation (by AMP) and reversible phosphorylation of AMPKα subunit on Thr172 by upstream kinases (AMPKK). AMPK activity is increased (> 1000 fold) by the combination of the allosteric and phosphorylation effects which allow small changes in cellular energy status to respond in a highly sensitive manner. After high cellular energy demand, intracellular ATP decreases while AMP increases which cause an increase of AMP/ATP ratio. Allosteric activation, phosphorylation of Thr172 by AMPKK and inhibition of dephosphorylation of Thr172 by protein phosphatases are promoted by the binding

of AMP to the AMPK γ subunit. One known AMPKK is Ca^{2+}/calmodulin-dependent kinase kinase β (CaMKKβ) which response to changes of cytoplasmic Ca^{2+} levels and indicate an alternative AMPK activation pathway in the absence of AMP increment (Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005).

Activation of AMPK has been shown to phosphorylate and inactivate ACC (Acetyl-CoA carboxylase), resulting in the inhibition of conversion of acetyl CoA to malonyl CoA for fatty acid synthesis, and de-inhibition of carnitine palmitoryltransferase I (CPT1), increasing fatty acid (long-chain acyl CoAs) β-oxidation by mitochondria (especially in muscle) (Park et al., 2002; Winder, 1998). The glucose homoeostasis role of AMPK is shown by the use of the cell-permeant adenosine analogue, AICAR (5-Aminoimidazole-4-carboxamide-1-β-D-ribonucleoside). It is rapidly taken up into cells and metabolized to form the AMP analog, ZMP. AICAR does not change the intracellular levels of AMP or ATP (Hardie et al., 1998). AICAR recruits GLUT4 to the plasma membrane in both heart and skeletal muscle cells, indicating a role for AMPK in promoting GLUT4 transduction through an insulin-independent pathway (Bergeron et al., 1999; Kurth-Kraczek et al., 1999). Glucose uptake can be increased efficiently in an insulin-independent manner (as in diabetes subjects) by stimulating AMPK in skeletal muscle (Hardie, 2004). Akt substrate of 160kDa (AS160/TBC1D4, Akt downstream) and it homolog, TBC1D1, both as Rab-GTPase activating protein, are recently identified downstream effectors of AMPK to regulate the translocation of GLUT4 from intracellular vesicles to the plasma membrane by interacting with small G-proteins, such as Rab, in a GDP-bound state (Sakamoto and Holman, 2008). Exercise or muscle contraction is a prototypical AMPK activator (Hayashi et al., 1998). Reversely, activation of AMPK also show exercise mimetic effects. Administration of AICAR increases running endurance in untrained mice (Narkar et al., 2008).

Hepatic glucose production (HGP), a major cause of fasting hyperglycemia in diabetic subjects, is found to be inhibited by activation of AMPK. AICAR-treated normal and insulin-resistance obese rats show inhibition of HGP (Bergeron et al., 2001). Metformin, a widely used antidiabetic drug, activates AMPK through activation of its upstream kinase LKB1, effectively lowering blood glucose (Shaw et al., 2005). Metformin shows HGP inhibitory effect in cultured rat hepatocytes (Zhou et al., 2001). Activation of AMPK by AICAR shows gluconeogenesis inhibitory effect by down regulating PEPCK (phophoenolpyruvate carboxykinase) and G6Pase (glucose-6-phosphatase) gene expression in hepatoma cells (H4IIE) which further inhibit HGP (Lochhead et al., 2000). Including ACC, AMPK inhibits 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, key enzyme for hepatic cholesterol synsthesis) in liver, and suppresses expression of lipogenesis-related genes such as fatty acid synthase (FAS) and L-type pyruvate kinase (Foretz et al., 2005; Foretz et al., 1998; Leclerc et al., 1998; Leclerc et al., 2001; Woods et al., 2000). Increased plasma triglyceride levels and enhanced hepatic lipogenesis showing in AMPKα2KO mice (deleted AMPKα2 gene specifically in the liver) emphasizes the critical role of AMPK in the control of hepatic lipid deposition through decreased lipogenesis and increased lipid oxidation to improve lipid profile in type 2 diabetes (Andreelli et al., 2006). AMPK activation has been shown to improve nonalcoholic fatty liver disease (NAFLD, a disorder of triacyglycerol accumulation in the liver) by decreasing fatty acid biosynthesis and increasing mitochondrial fatty acids oxidation. This is confirmed by an AICAR-treated decline in liver triglycerides in lean and obese rodents (Bergeron et al., 2001).

Insulin resistance, insulin secretion and different degrees of β-cell failure are associated with the pathogenesis of type 2 diabetes. Prolonged β-cell function failure leads to glucose intolerance and chronic high glucose dramatically influences β-cell metabolism and ultimately leads to

impaired insulin secretory response to glucose and β-cell apoptosis. Surprisingly, activation of AMPK suppresses glucose-stimulated insulin secretion in β-cell. AICAR or metformin treatment markedly reduces glucose-stimulated insulin secretion in β-cell lines and in rodent and human islets (Eto et al., 2002; Leclerc et al., 2004). This is thought to provide β-cell rest by reducing insulin release to protect against the negative effects of overstimulation (Tsuboi et al., 2003).

Several cytokines, such as leptin and adiponectin, have been shown to stimulate glucose transport in muscle in an AMPK dependent manner. Together, these offer a mechanistic basis to AICAR's effects in the prevention of the development of hyperglycemia in Zucker diabetic fatty (ZDF) rats, improvement of peripheral insulin sensitivity in skeletal muscle, and delay in β-cell dysfunction associated with type 2 diabetes (Pold et al., 2005).

Phytochemicals or polyphenols such as resveratrol (mainly from red wine and grape) and epigallocatechin-3-gallate (EGCG, mainly from green tea) were identified as potent activators of AMPK *in vitro* and *in vivo* (Baur et al., 2006; Collins et al., 2007; Zang et al., 2006). Activation of AMPK by resveratrol decreased lipid accumulation in the liver of diabetic mice (Baur et al., 2006).

2.6.4 Peroxisome Proliferator-Activated Receptor-gamma **(**PPAR-γ**)**

Peroxisome proliferator-activated receptors are members of the nuclear hormone receptor superfamily and ligand-active transcription factors that regulate several metabolic pathways (Braissant et al., 1996). In the presence of a ligand, PPARs form heterodimers with members of the retinoid X receptor (RXR, subfamily of nuclear hormone receptors) to cause a conformational change and recruit a co-activator to form a complex which binds to peroxisome proliferator response elements (PPRE) in target genes and stimulates their expression (Gampe et al., 2000; Nolte et al., 1998). In addition, PPARs can also function independently in the absence

of a hetero-partner or can regulate gene expression independently of PPRE, either by suppressing other transcription factor (TF) like GHP-1 (growth hormone protein-1, a transcription factor involved in pituitary specific gene expression), or by interfering with other signal transducer and activator like AP-1 (activator protein-1), signal transducer and activator of transcription (STAT)-1 and NF-κB (Feige et al., 2006; Feinstein et al., 2005; Ricote et al., 1998; Tan et al., 2005; Tolon et al., 1998).

Three isotypes of human PPAR (α, β/δ referred as δ, and γ) have been identified so far, each showing distinct tissue distributions, physiological roles and ligand specificity. Found in liver, kidney, heart, and muscle, tissues that exhibit high levels of fatty acid (FA) catabolism, PPARα is important for the uptake and oxidation of FA and lipoprotein metabolism and is the target for the lipid lowering fibrates which cause decrease triglycerides (TG) levels in plasma and an enhancement of high density lipoprotein cholesterol (HDL-C). Localized in fat, large intestine and macrophages, PPARγ plays an important role in adipocyte differentiation, lipid storage or redistribution and is the receptor for a well-known class of antidiabetic insulin sensitizer drugs, thiazolidinediones (TZD), such as ciglitazone, pioglitazone, rosiglitazone, and troglitazone (Berger and Moller, 2002; Desvergne et al., 2004; Feige et al., 2006; Gross and Staels, 2007a; Kersten et al., 2000; Michalik et al., 2004; Michalik and Wahli, 2006; Willson et al., 2000). Expressed in most cell types, PPAR*δ* agonists play important roles in the regulation of lipid and lipoprotein metabolism, treatment in dyslipidemia and cancer, and cell differentiation within the central nervous system.

Controlled by ligand binding, PPARγ has a lot of ligand-dependent transcriptional activity because it has a large ligand binding pocket which binds both natural and synthetic molecules (Gelman et al., 2007; Ulivieri and Baldari, 2007). Many natural ligands like fatty acids or fatty

acid derivatives are obtained through the diet or from intracellular signaling pathways (Garcia-Bates et al., 2008). The two natural ligands prostaglandin D2 (PGD2), and 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ2) are derived from arachidonic acid by the catalytic activities of the cyclooxygenase-2 (Cox-2) and prostaglandin D synthase (Feldon et al., 2006; Forman et al., 1995; Kliewer et al., 1995; Wigren et al., 2003). 15d-PGJ2 activates PPAR*γ* at low micromolar concentrations and is thought to be the most potent endogenous ligand for PPAR*γ* (Forman et al., 1995; Wang et al., 2006; Willson et al., 2000). TZD is a synthetic PPAR*γ* ligand with known side effects. These include hepatotoxicity (troglitazone), weight gain, edema, increased lipoprotein(a) concentrations, and probably enhanced risk of heart failure and cardiac hypertrophy (Gelman et al., 2007; Staels and Fruchart, 2005). Generally, the structure of natural and synthetic ligands comprise a polar head (carboxylate function or TZD group, for instance) and a hydrophobic tail (Zoete et al., 2007). Two isomers of PPAR*γ* (*γ*1 and *γ*2) are formed by alternative splicing and promoter usage which make PPAR*γ2* differ from PPAR*γ*1 by only 30 additional amino acids in the N-terminal (Fajas et al., 1997).

Activation of PPARγ by TZDs in type 2 diabetes patients decrease glycated hemoglobin (HbA1c), fasting and postpradial glucose levels, and lower circulating insulin levels by largely improving insulin sensitivity (Gross and Staels, 2007b). TZD treatment remodels adipocyte as a result of selective pre-adipocyte differentiation in subcutaneous depots and apoptosis of older and larger insulin-resistant visceral adipocytes. This makes the newer adipocytes smaller in size and more sensitive to insulin (Arner, 2003; Kahn et al., 2000). Lipolysis of circulating TGs and their storage in adipose tissue are enhanced by TZDs. TZD reduce FFA release by stimulating the use of glycerol for TG production. The hypoglycemic effect of TZDs is because of this reduction in FFAs which alleviates lipotoxicity in skeletal muscle, liver and pancreas, leading to

a reduction in hepatic glucose production and improved glucose utilization in skeletal muscles (Arner, 2003; Kahn et al., 2000). To allow the release of fatty acids from plasmatic transport proteins and to promote their cellular uptake (key roles of PPARs in lipid metabolism), both PPARα and PPARγ directly regulate the expression of lipoprotein lipase (LPL) to enhance fatty acid release from lipoproteins (Martin et al., 1997). Besides passing plasma membrane by passive diffusion, the FA (or lipophilic molecules) uptake in tissues with high capacities of consumption or storage is increased by the activity of specific transporters. The expression of the fatty acid translocase (FAT, also called CD36 in macrophages), a membrane receptor that binds oxidized low-density lipoproteins (oxLDL) and promotes fatty acid and cholesterol uptake, is also under the control of PPARα and PPARγ. Furthermore, PPARγ directly controls the transcription of the oxidized LDL receptor 1 (OLR1) in adipocytes. PPARα and PPARγ also activate the expression of fatty acid transport proteins (FATP) to promote fatty acid import (cross the plasma membrane) after which fatty acid is activated in acyl-CoA for further utilization. In this process, acyl-CoA synthetase (ACS) expression is regulated by PPARα and PPARγ. Interestingly, FATP-1 has been shown to be a very long chain acyl-CoA synthetase to facilitate mammalian fatty acid uptake via esterification coupled influx (Coe et al., 1999). The genes encoding fatty acid binding proteins, such as adipocyte-FABP (aP2) or liver-FABP, a family of intra-cellular lipid carriers expressed in a tissue-specific manner, are some of the best characterized targets of PPAR (Coe et al., 1999).

PPARγ activation increases glucose uptake in adipocytes and muscle cells and reduce plasma glucose levels partly by a direct action on insulin-stimulated glucose disposal which increases the expression and the translocation to the cell surface of the glucose transporters GLUT1 and GLUT4 (Kramer et al., 2001). PPARγ activation enhances the expression of

intracellular proteins such as c-Cbl-associated protein (CAP) to modulate the insulin signal pathway (Baumann et al., 2000). PPARγ agonists inhibit the expression of cytokines such as resistin, tumor necrosis factor α (TNFα), and interleukin-6 which promote insulin resistance. PPARγ agonists increase adiponectin concentrations in plasma. Adiponectin increases FA oxidation in liver and skeletal muscle and improves insulin sensitivity in skeletal muscle and liver, and decreases glucose production in liver, resulting in decreased circulating FFAs and TG and glucose levels (Yamauchi et al., 2001).

2.7 Functional foods, dietary supplements, and phytochemicals

Functional foods transmit specific beneficial properties above their basic nutritional contribution. The US dietary Supplement Health and Education Act (DSHEA, passed in 1994) defines the term "dietary supplement" as a vitamin or mineral, a herb or other plant-derived chemical, an amino acid, a dietary supplement by increasing the total dietary intake (including enzymes or tissues from organs or glands), or a concentrate, metabolite, constituent, or extract of these. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are biologically active plant-derived compounds which structurally and functionally mimic hormones such as insulin, estrogen, etc., (Cederroth and Nef, 2009). The beneficial actions of phytochemicals include antioxidant, hormonal action, enzyme activation, interference with DNA replication, anti-bacterial effect and physical action. Oral administration of American Ginseng Berry Juice, containing ginsenosides Re (0.88 mg/g), Rb_2 (0.77 mg/g), Rb_3 (2.90 mg/g), Rb (0.39 mg/g), reduces blood glucose and body weight in *ob/ob* mice (Xie and Foo, 2007). Black soy peptide (BSP) treatment significantly decreases bodyweight, and liver and epididymal adipose tissue weight gain in dietary obese rats. Moreover, BSP groups had a lower

total cholesterol concentration and low-density lipoprotein/high-density lipoprotein (LDL/HDL) ratio in serum and a lower level of hepatic triglycerides but higher excretion of feces than casein group suggesting BSP may be a potent bioactive component for anti-obesity and hypolipidemic benefits though modulation of lipid composition (Rho, 2007). Recent studies have shown that the hypoglycemic effect of the exopolysaccharides (EPS, from two different mushrooms, *Tremella fuciformis* and *Phellinus baumii*) in *ob/ob* mice was mediated through PPAR-γ activition which suggested that the EPS derived from the mushroom might be used as potential oral hypoglycemic material or functional foods for the management of type 2 diabetes (Cho et al., 2007). Significant decrease in body weight gain, concentration of plasma triglyceride (TG), plasma cholesterol (TC) was found in FIBL-treated (flavone from *Ipomoea Batatas***,** sweet potato leaf) type 2 diabetic rats. Moreover, FIBL treatment lowered fasting plasma insulin level, blood glucose (FBG) level, low density lipoprotein cholesterol (LDL-C), and malondialdehyde (MDA) levels, and increased insulin sensitive index and superoxide dismutase (SOD) level in diabetic rats with the dose of 50 mg/kg body weight FIBL being found to give the optimal effect (Zhao, 2007). Recent studies using Roquefort cheese demonstrated high water soluble phenolic content, DPPH radical inhibition activity, α-glucosidase and α-amylase inhibitory activity. All three herb, fruit, and fungal-enriched cheese samples in the study had significant ACE-I inhibitory activity, indicative of potential anti-diabetic and anti-hypertensive effects of Roquefort cheese (Apostolidis et al., 2007).

Plant derivatives with purported anti-diabetic properties have been used in folk medicine, traditional healing systems, and as complementary and alternative therapy. Several comprehensive reviews cite evidence of plants used in the treatment of diabetes (Marles and Farnsworth, 1995; Yeh et al., 2003). The discovery and synthesis of the hypoglycemic drug,

metformin (Glucophage), can be attributed to early findings (1918) of hypoglycemic activity of guanidine in French lilac (*Galega officinalis*) extracts (Watanabe, 1918). Some examples of botanicals that are thought to lower blood glucose include gymnema, fenugreek, bitter melon, ginseng, and nopal (Shane-McWhorter, 2001). A wide range of plant-derived principles belonging to compounds, mainly alkaloids, glycosides, galactomannan gum, polysaccharides, hypoglycans, peptidoglycans, guanidine, steroids, phenolics, glycopeptides and terpenoids, have demonstrated bioactivity against hyperglycaemia (Marles and Farnsworth, 1995).

2.8 Curcumin

Curcumin (diferuloylmethane) is an orange-yellow substance and the principal bioactive component of turmeric (*curcuma longa*). Turmeric is a traditional remedy and an ancient spice that has been used as condiment, flavoring agent, and medicine. Turmeric (*Curcuma longa*) plant is a member of the *curcuma* botanical group and ground turmeric is obtained from the crushed and powered root and rhizome (underground stem) of *Curcuma longa* plant. Curcumin constitutes approximately about 2-5% of turmeric powder. Curcuminoids in the dried rhizome of *C. longa* is a rich

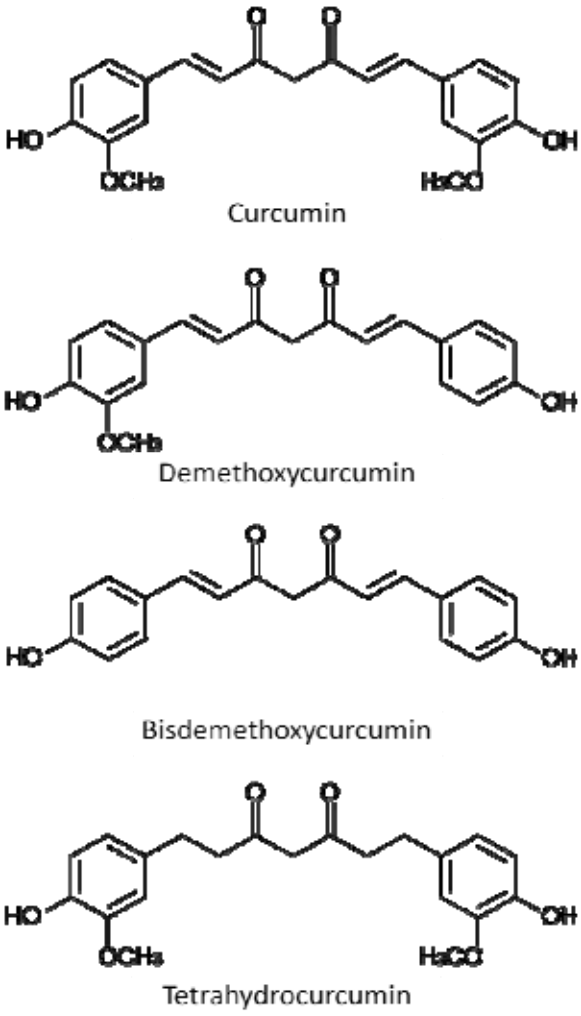


Figure 2: Chemical structure of curcuminoids

source of phenolic compounds, which include curcumin, demethoxycurcumin and bisdemethoxycurcumin (Kiuchi et al., 1993). Tetrahydrocurcumin (THC) is one of metabolites of curcumin (Fig. 2). Commercial curcumin contains approximately 77% curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin. Due to its ability to treat a wide variety of disorders without any side effect, curcumin has been of considerable interest to researchers. Curcumin has been shown to regulate numerous transcription factors, cytokines, protein kinase, adhesion molecules, redox status and enzymes linked to inflammation. Curcumin is soluble in ethanol, dimethylsulfoxide, and acetone but insoluble in water and ether. Its melting point is 183˚C and molecular formula is $C_{21}H_{20}O_6$.

Several reports have shown that curcumin inhibits NF-κB as shown in Table 1. It has been shown that curcumin can suppress blood glucose, increase the antioxidant status of pancreatic β-cells, and increase the activity of PPAR-γ (Nishiyama et al., 2005). Other reports showed that curcumin and its metabolite THC (tetrahydrocurcumin) can lower blood glucose, modulate hepatic enzymes and increase insulin levels in STZ-induced diabetic rats (Murugan and Pari, 2005) by decreasing peroxidation status and modulation of oxidative stress (Murugan and Pari, 2006). These authors also showed that the effect of curcumin was not better than that of THC (Murugan and Pari, 2007b).

Table 1: Inhibition of NF-κB Signaling Pathway

Effects of curcumin	Reference
Inhibits NF-κB	(Chuang et al., 2002)
Inhibits TNF-α production and release	(Han et al., 2002)
Regulation of proinflammatory cytokine expresstion	(Literat et al., 2001)
Downregulates chemokine expression and release	(Hidaka et al., 2002)
Inhibits IL-1-stimulated NF-κB and downregulates MMP gene expression	(Onodera et al., 2002)
Inhibits COX-2 transcription and expression	(Surh et al., 2001)
Inhibits NOS expression and nitrite production	(Pan et al., 2000)

One complication of diabetes is hyperlipidemia. The same authors examined the ability of curcumin to modulate the lipid profile in STZ-nicotinamide-induced diabetes. Curcumin significantly increased the plasma insulin levels and decreased blood glucose, cholesterol, triglycerides, free fatty acids, phospholipids, HMG-CoA reductase activity, VLDL, and LDL cholesterol levels in serum and liver of these rats (Murugan and Pari, 2007a).

The capability of curcumin to ameliorate diabetes and inflammation insulin resistance was examined in a mouse obesity model (Weisberg et al., 2008). It was found that curcumin treatment ameliorated diabetes in high-fat diet-induced obese and leptin-deficient ob/ob male C57BL/6J mice, as evidenced by glucose and insulin tolerance tests and hemoglobin A1c levels (Table 2). Curcumin treatment also significantly decreased macrophage infiltration of white adipose tissue, improved adipose tissue adiponectin production, and reduced hepatic nuclear NF-κB activity, hepatomegaly, and marker of hepatic inflammation.

Curcumin was reported to retard islet ROS generation and inhibit apoptosis which indicate that curcumin protects islets against STZ-induced oxidative stress by scavenging free radicals (Meghana et al., 2007). Pancreatic islet cells are protected from death in this manner, which may be beneficial in the treatment of diabetes mellitus. Another report indicated that curcumin treatment enhances islet recovery by inducing heat-shock protein 70, a response protein, and heme oxygenase-1 during cryopreservation (Kanitkar and Bhonde, 2008).

One of the most devastating microvascular complications of long-standing type 1 and type 2 diabetes is diabetic retinopathy which is caused by the oxidative stress and inflammation (Haidara et al., 2006; Kowluru and Chan, 2007). Curcumin was found to stop the development of STZ-induced diabetic cataracts in rats by inhibition of hyperglycemia-induced aggregation and insolubilization of lens proteins (Suryanarayana et al., 2005; Suryanarayana et al., 2007).

Curcumin also has the ability to inhibit VEGF expression in rats with STZ-induced diabetic retina. The hyperglycemia-mediated induction of VEGF can stimulate neovascualrization which has been implicated in the pathogenesis of diabetic retinopathy (Mrudula et al., 2007).

Table 2: Effect of curcumin treatment in animal models of diabetes

Animal	Dose	Effects of curcumin	Reference
Mice	3% diet	Anti-diabetic and anti-inflammatory in murine models of insulin-resistance and obesity	(Weisberg et al., 2008)
Rats	0.05% diet	Suppress retinal oxidative stress and inflammation	(Kowluru and Kanwar, 2007)
Rats	0.002, 0.01% diet	Inhibits hyperglycemia-induced VEGF expression in diabetic retina	(Mrudula et al., 2007)
Rats	0.002, 0.01% diet	Prevents diabetes-induced oxidative stress	(Suryanarayana et al., 2007)
Rats	80 mg/kg, p.o	Reduces serum and liver lipids level, HMG CoA reductase activity, and increased HDL. Decreases blood glucose, glycosylated haemoglobin and erythrocyte TBARS and increases plasma insulin, HG, erythrocyte antioxidants and the activities of membrane bound enzymes	(Pari and Murugan, 2007)
Rats	80 mg/kg, p.o	Improves specific insulin binding to the receptors, with receptor numbers and affinity binding reaching near-normal levels	(Murugan et al., 2008)
Rats	60 mg/kg, orally	Restores the decrease in gene expression of muscarinic M1, insulin receptor, superoxide dismutase (SOD), choline acetyl transferase and increase in gene expression of muscarinic M3,α7-nicotinic acetylcholine receptor, acetylcholine esterase and GLUT3 in cerebral cortex.	(Peeyush et al., 2009; Peeyush Kumar et al., 2010)
Rats	15, 30 mg/kg p.o	Ameliorates diabetic nephropathy in rats	(Sharma et al., 2006a; Sharma et al., 2006b)
Mice	0.2, 1.0 g/100g of diet	Induces hypoglycemia in genetically diabetic KK-Ay mice via binding to PPAR-γ	(Kuroda et al., 2005)
Rats	0.002, 0.01% diet	Delays diabetic cataract	(Suryanarayana et al., 2005)

Diabetic nephropathy is caused by chronic hyperglycaemia in diabetes. Curcumin was found to increase hepatic and renal function biomarkers and protein levels in experimental type 2 diabetic rats (Murugan and Pari, 2007b). Curcumin reversed the diabetes-induced total protein, albumin, globulin, and albumin/globulin ratio.

The above studies suggest that curcumin or dietary curcuminoids may have beneficial effects in diabetes, with the potential to lower or normalize blood glucose levels in animal models of diabetes. However, the mechanism(s) mediating curcumin effects on glucose metabolism are poorly understood.

2.9 Serviceberry

Serviceberry (*Amelanchier alnifolia* Nutt.), also called Saskatoon berry, Juneberry, is a deciduous shrub that is native to the western United States and Canada. It is distributed in the southern Yukon and Northwest Territories, the Canadian prairie province and northern prairies of the United States (Hu et al., 2005). It belongs to the Rosaceae family, and the Amelanchier genus. Other fruits belonging to Rosaceae family include apples, pears, prunes, plums, cherries, apricots, strawberries, raspberries and blackberries. The mature fruit is a purple berry-like pome, 1–1.5 cm in diameter, similar to a blueberry. A serving (a cup) of mature fruit weighs approximately 130–135 g, and a single berry commonly contains 1–5 very small seeds (Rogiers, 1997). *A. alnifolia* was extensively used by native cultures both medicinally (as a disinfectant and to prevent miscarriages) and as a food source, often in forms such as pemmican (a mixture of dried meat, dried berries, and fat) (Burns Kraft et al., 2008). The Native people of North US and Canada used sun- and smoke-dried Saskatoon berries to flavor foods. Also, Canada's aboriginal people used the fruit in soups, stews, meat dishes, pemmican and dried cakes. The juice from

Saskatoon berries was used to cure stomach ailments, and eye and eardrops were made from mature berries. It was also used as a food source by early explorers and pioneer settlers in these regions for preventing malnutrition-related diseases such as scurvy. Serviceberry has been cultivated and grown in orchards similar to blueberries (Hu et al., 2005; Mazza, 2008).

Chemical studies on Saskatoon berries have shown that they contain about 82–84% water (major constituent), 15–20% sugar, small amounts of protein and fat, a fair amount of fiber and relatively large amounts of potassium, iron, magnesium and phosphorous. Vitamin C, thiamin, riboflavin, pantothenic acid, vitamin B-6, folate, vitamin A and vitamin E are found in saskatoon berries. The major functional components of Saskatoon berries are phenolic compounds, particularly the anthocyanins. In ripe saskatoon berries there are at least four anthocyanins of which cyanidin 3-galactoside and 3-glucoside account for about 61% and 21% of the total anthocyanins, respectively (Mazza, 2008). Cyanidin 3-xyloside, pelargonin 3-glucoside, and malvidin derivatives are other anthocyanins present in the fruit. Berries of *A. alnifolia* contain phenolic acids including 3-feruloylquinic, chlorogenic, and 5-feruloylquinic acids. Flavonoid compounds from the fruit include rutin, hyperoside, avicularin, and quercetin (Burns Kraft et al., 2008). Anthocyanin content of Saskatoon berries ranges from 25 to 179 mg/100 g of berries and total phenolics range from 0.17% to 0.52% because content of total anthocyanin and total phenolics of fruit is affected by cultivar, maturity level and year of production. Benzaldehyde is the predominant aroma component of saskatoon berry and malic and citric are the predominant acids. Saskatoon berries are excellent sources of iron supplying 22.3% of the established Recommended Dietary Allowance (RDA) in a 100 g serving. The occurrence of carotene was reported earlier in Saskatoon berries; but, no reliable quantitative data on the carotenoid composition of Saskatoon berries have been reported. Prunasin, a cyanogenic glycoside, found in

trace amounts in prunes, plums, cherries, apricots, maize, beans and lettuce has been found in leaves and twigs of the Saskatoon shrubs during the bloom stage; however, there are no reports on the qualitative and quantitative composition of cyanogenic glycosides in Saskatoon berries.

More recently, it was found that a concentrated crude extract of *A. alnifolia* berries inhibited nitric oxide production in activated macrophages, indicating a potential protective role against cardiovascular disease and chronic inflammation (Wang and Mazza, 2002). It was also found the berries of *A. alnifolia* to be strong scavengers of free radicals without reducing cell viability (Hu et al., 2005). Fruit from the similar species, *A. canadensis* and *A. arborea*, inhibit cyclooxygenase-1 and -2 in vitro, indicating a role in moderating inflammation (Adhikari et al., 2006).

Serviceberry (*Amelanchier alnifolia*), found in the North Glacier forests of the Rocky Mountains in Montana, have been used by American Indians for treating diabetes (Johnston, 1987). This species continues to be used to-date, by the Blackfeet tribe of Native Americans, in the management of diabetes. However, there have been no scientific studies to validate this anecdotal evidence.

2.10 Stilbenes

Stilbenes (1,2-diphenylethylene) have two isomeric forms. (*E*)-stilbenes (trans-stilbenes), which are not sterically hindered, are more stable than (*Z*)-stilbenes (cis-stilbenes). Stilbenes, classified as phytoestrogen, are phenolics which include isoflavonoids and flavonoids (Roupe et al., 2006). Resveratrol (3,5,4′-trihydroxy stilbene, naturally occurring phytoalexin) and piceatannol (3,3′,4,5′- tetrahydroxy-trans-stilbene, naturally occurring polyphenol) have shown cardioprotective, anti-cancer, anti-inflammatory, antioxidant, and neuroprotective activity.

Recently, anti-diabetic activity of resveratrol and piceatannol has been also discussed by many studies. Resveratrol can enhance glucose uptake in skeletal muscle by activating PI3K-Akt-signaling and increasing GLUT4 expression (Chi et al., 2007). Resveratrol stimulates glucose uptake in muscle cell through mechanisms that involve sirtuin (silent mating type information regulation 2 homolog) and AMPK to stimulate GLUT4 transporter intrinsic activity (Breen et al., 2008). Resveratrol was found to improve insulin sensitivity *in vitro* in a SirT1-dependent manner by downregulating PTP1B (protein-tyrosine phosphatase 1B, a key insulin receptor phosphatase) at both the protein and the mRNA Level. Sirtuin 1 (SirT1), a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase, is one of seven of the mammalian homolog of yeast silent information regulator 2 (Sir2) (Sun et al., 2007). Activation of estrogen receptor is was a crucial factor for resveratrol-stimulating muscle glucose uptake via both insulin-dependent and insulin-independent pathways (Deng et al., 2008). Piceatannol and resveratrol isolated from the EtOH extract of the seeds of *Syagrus romanzoffiana* possess potent inhibitory activity against alpha-glucosidase type IV from *Bacillus stearothermophilus* with the IC_{50} value of 23.2 and 23.9 μM, respectively (Lam et al., 2008). These authors also showed that the anti-α-glucosidase activity was increased along with the increment of phenolic substitutions in the same skeleton Recently, piceatannol has been shown to inhibit adipogenesis in 3T3-L1 preadipocytes via modulation of mitotic clonal expansion and insulin signal transduction (Kwon et al., 2012). Piceatannol has strong anti-inflammatory effect by inhibiting TNF-induced NF-κB activation and NF-κB-mediated gene expression through suppression of IκBα kinase and p65 phosphorylation (Ashikawa et al., 2002). Pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystilbene), is another natural stilbene found in several plant species that show anti-cancer (Chiou et al., 2010; Paul et al., 2010), anti-inflammatory (Pan et al., 2008; Paul et al., 2009), and antioxidant (Chakraborty et

al., 2010; Mikstacka et al., 2010) activity. Pterostilbene can lower plasma lipoproteins and cholesterol in hypercholesterolemic hamsters by being an agonist for the peroxisome proliferator-activated receptor alpha-isoform (Rimando et al., 2005). A recent study showed that pterostilbene can decrease cell population growth and cause cell cycle arrest at the G2/M phase in 3T3-L1 preadipocytes (Hsu et al., 2012).

2.11 Study objectives:

Recent U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) withdrawals, recalls, and restricted access of several medications, including antidiabetic medications, have heightened safety issues related to the use of some conventional drugs. On the other hand, interest in and use of plant extracts, herbal preparations, and natural products, including bioactive compounds, as prophylactic and therapeutic agents for many diseases have grown considerably in the past few decades. In 2007, almost 4 out of 10 adults had used complementary and alternative medicine (CAM) therapies in the past 12 months, with the most commonly used CAM therapy being nonvitamin, nonmineral, natural products (17.7%) (Barnes et al., 2008).

A search of current literature indicates a limited understanding of potential mechanisms of how bioactive components present in medicinal plants, and purified natural compounds influence insulin action and glucose metabolism. Therefore, these studies were focused on characterizing the mechanisms of antidiabetic effects of serviceberry, curcumin (active ingredient from turmeric, *Curcuma longa*) and stilbenes (resveratrol and its analogs). Our studies offer insights in the evaluation and assessment of these plant extracts and natural compounds as complementary and alternative approaches, in the management of diabetes.

References

Abraham, E.J., Leech, C.A., Lin, J.C., Zulewski, H., and Habener, J.F. (2002). Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. Endocrinology *143*, 3152-3161.

ADA (2011). Diagnosis and classification of diabetes mellitus. Diabetes care *34 Suppl 1*, S62-69.

Adhikari, D.P., Schutzki, R.E., DeWitt, D.L., and Nair, M.G. (2006). Effects of Amelanchier fruit isolates on cyclooxygenase enzymes and lipid peroxidation. Food Chemistry *97*, 56-64.

Aguirre, V., Uchida, T., Yenush, L., Davis, R., and White, M.F. (2000). The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). J Biol Chem *275*, 9047-9054.

Aguirre, V., Werner, E.D., Giraud, J., Lee, Y.H., Shoelson, S.E., and White, M.F. (2002). Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. Journal of Biological Chemistry *277*, 1531-1537.

Andreelli, F., Foretz, M., Knauf, C., Cani, P.D., Perrin, C., Iglesias, M.A., Pillot, B., Bado, A., Tronche, F., Mithieux, G.*, et al.* (2006). Liver adenosine monophosphate-activated kinase-alpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. Endocrinology *147*, 2432-2441.

Apostolidis, E., Kwon, Y.I., and Shetty, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innovative Food Science & Emerging Technologies *8*, 46-54.

Arner, P. (2003). The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. Trends in Endocrinology and Metabolism *14*, 137-145.

Ashikawa, K., Majumdar, S., Banerjee, S., Bharti, A.C., Shishodia, S., and Aggarwal, B.B. (2002). Piceatannol inhibits TNF-induced NF-kappaB activation and NF-kappaB-mediated gene expression through suppression of IkappaBalpha kinase and p65 phosphorylation. J Immunol *169*, 6490-6497.

Banerjee, R.R., Rangwala, S.M., Shapiro, J.S., Rich, A.S., Rhoades, B., Qi, Y., Wang, J., Rajala, M.W., Pocai, A., Scherer, P.E.*, et al.* (2004). Regulation of fasted blood glucose by resistin. Science *303*, 1195-1198.

Barnes, P.M., Bloom, B., and Nahin, R.L. (2008). Complementary and alternative medicine use among adults and children: United States, 2007. National health statistics reports; no 12. Hyattsville, MD: National Center for Health Statistics. 2008.

Baumann, C.A., Chokshi, N., Saltiel, A.R., and Ribon, V. (2000). Cloning and characterization of a functional peroxisome proliferator activator receptor-gamma-responsive element in the promoter of the CAP gene. The Journal of Biological Chemistry *275*, 9131-9135.

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.*, et al.* (2006). Resveratrol improves health and survival of mice on a high-calorie diet. Nature *444*, 337-342.

Bays, H., Mandarino, L., and DeFronzo, R.A. (2004). Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. J Clin Endocrinol Metab *89*, 463-478.

Bays, H.E., Gonzalez-Campoy, J.M., Bray, G.A., Kitabchi, A.E., Bergman, D.A., Schorr, A.B., Rodbard, H.W., and Henry, R.R. (2008). Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. Expert Rev Cardiovasc Ther *6*, 343-368.

Berg, A.H., and Scherer, P.E. (2005). Adipose tissue, inflammation, and cardiovascular disease. Circ Res *96*, 939-949.

Berger, J., and Moller, D.E. (2002). The mechanisms of action of PPARs. Annual Review of Medicine *53*, 409-435.

Bergeron, R., Previs, S.F., Cline, G.W., Perret, P., Russell, R.R., 3rd, Young, L.H., and Shulman, G.I. (2001). Effect of 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside infusion on in vivo glucose and lipid metabolism in lean and obese Zucker rats. Diabetes *50*, 1076-1082.

Bergeron, R., Russell, R.R., 3rd, Young, L.H., Ren, J.M., Marcucci, M., Lee, A., and Shulman, G.I. (1999). Effect of AMPK activation on muscle glucose metabolism in conscious rats. The American Journal of Physiology *276*, E938-944.

Bergman, R.N., and Ader, M. (2000). Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends in Endocrinology and Metabolism *11*, 351-356.

Bergman, R.N., Kim, S.P., Catalano, K.J., Hsu, I.R., Chiu, J.D., Kabir, M., Hucking, K., and Ader, M. (2006). Why visceral fat is bad: mechanisms of the metabolic syndrome. Obesity (Silver Spring, Md) *14 Suppl 1*, 16S-19S.

Bevilacqua, S., Bonadonna, R., Buzzigoli, G., Boni, C., Ciociaro, D., Maccari, F., Giorico, M.A., and Ferrannini, E. (1987). Acute elevation of free fatty acid levels leads to hepatic insulin resistance in obese subjects. Metabolism *36*, 502-506.

Boden, G., and Shulman, G.I. (2002). Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. European Journal of Clinical Investigation *32 Suppl 3*, 14-23.

Bolen, S., Feldman, L., Vassy, J., Wilson, L., Yeh, H.C., Marinopoulos, S., Wiley, C., Selvin, E., Wilson, R., Bass, E.B.*, et al.* (2007). Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. Annals of Internal Medicine *147*, 386-399.

Bonadonna, R.C., and De Fronzo, R.A. (1991). Glucose metabolism in obesity and type 2 diabetes. Diabete Metab *17*, 112-135.

Bonadonna, R.C., Del Prato, S., Bonora, E., Saccomani, M.P., Gulli, G., Natali, A., Frascerra, S., Pecori, N., Ferrannini, E., Bier, D.*, et al.* (1996). Roles of glucose transport and glucose phosphorylation in muscle insulin resistance of NIDDM. Diabetes *45*, 915-925.

Bouchard, C., and Perusse, L. (1993). Genetics of obesity. Annual Review of Nutrition *13*, 337-354.

Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (1996). Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology *137*, 354-366.

Breen, D.M., Sanli, T., Giacca, A., and Tsiani, E. (2008). Stimulation of muscle cell glucose uptake by resveratrol through sirtuins and AMPK. Biochem Biophys Res Commun *374*, 117-122.

Burns Kraft, T.F., Dey, M., Rogers, R.B., Ribnicky, D.M., Gipp, D.M., Cefalu, W.T., Raskin, I., and Lila, M.A. (2008). Phytochemical composition and metabolic performance-enhancing activity of dietary berries traditionally used by Native North Americans. J Agric Food Chem *56*, 654-660.

Butler, A.E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R.A., and Butler, P.C. (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes *52*, 102-110.

Cai, D., Yuan, M., Frantz, D.F., Melendez, P.A., Hansen, L., Lee, J., and Shoelson, S.E. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nature Medicine *11*, 183-190.

Carey, A.L., Steinberg, G.R., Macaulay, S.L., Thomas, W.G., Holmes, A.G., Ramm, G., Prelovsek, O., Hohnen-Behrens, C., Watt, M.J., James, D.E.*, et al.* (2006). Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes *55*, 2688-2697.

Carpentier, A., Mittelman, S.D., Bergman, R.N., Giacca, A., and Lewis, G.F. (2000). Prolonged elevation of plasma free fatty acids impairs pancreatic beta-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. Diabetes *49*, 399-408.

Carpentier, A., Mittelman, S.D., Lamarche, B., Bergman, R.N., Giacca, A., and Lewis, G.F. (1999). Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. The American Journal of Physiology *276*, E1055-1066.

CDC (2011). National Diabetes Fact sheet.

Cederroth, C.R., and Nef, S. (2009). Soy, phytoestrogens and metabolism: A review. Molecular and Cellular Endocrinology *304*, 30-42.

Chakraborty, A., Gupta, N., Ghosh, K., and Roy, P. (2010). In vitro evaluation of the cytotoxic, anti-proliferative and anti-oxidant properties of pterostilbene isolated from Pterocarpus marsupium. Toxicol In Vitro *24*, 1215-1228.

Chan, J.M., Rimm, E.B., Colditz, G.A., Stampfer, M.J., and Willett, W.C. (1994). Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care *17*, 961-969.

Chi, T.C., Chen, W.P., Chi, T.L., Kuo, T.F., Lee, S.S., Cheng, J.T., and Su, M.J. (2007). Phosphatidylinositol-3-kinase is involved in the antihyperglycemic effect induced by resveratrol in streptozotocin-induced diabetic rats. Life sciences *80*, 1713-1720.

Chiou, Y.S., Tsai, M.L., Wang, Y.J., Cheng, A.C., Lai, W.M., Badmaev, V., Ho, C.T., and Pan, M.H. (2010). Pterostilbene inhibits colorectal aberrant crypt foci (ACF) and colon carcinogenesis via suppression of multiple signal transduction pathways in azoxymethane-treated mice. Journal of agricultural and food chemistry *58*, 8833-8841.

Cho, E.J., Hwang, H.J., Kim, S.W., Oh, J.Y., Baek, Y.M., Choi, J.W., Bae, S.H., and Yun, J.W. (2007). Hypoglycemic effects of exopolysaccharides produced by mycelial cultures of two different mushrooms Tremella fuciformis and Phellinus baumii in ob/ob mice. Applied Microbiology and Biotechnology *75*, 1257-1265.

Chuang, S.E., Yeh, P.Y., Lu, Y.S., Lai, G.M., Liao, C.M., Gao, M., and Cheng, A.L. (2002). Basal levels and patterns of anticancer drug-induced activation of nuclear factor-kappaB (NF-kappaB), and its attenuation by tamoxifen, dexamethasone, and curcumin in carcinoma cells. Biochemical Pharmacology *63*, 1709-1716.

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

Clore, J.N., Stillman, J., and Sugerman, H. (2000). Glucose-6-phosphatase flux in vitro is increased in type 2 diabetes. Diabetes *49*, 969-974.

Coe, N.R., Smith, A.J., Frohnert, B.I., Watkins, P.A., and Bernlohr, D.A. (1999). The fatty acid transport protein (FATP1) is a very long chain acyl-CoA synthetase. The Journal of Biological Chemistry *274*, 36300-36304.

Colditz, G.A., Willett, W.C., Rotnitzky, A., and Manson, J.E. (1995). Weight gain as a risk factor for clinical diabetes mellitus in women. Annals of Internal Medicine *122*, 481-486.

Collins, Q.F., Liu, H.Y., Pi, J., Liu, Z., Quon, M.J., and Cao, W. (2007). Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5'-AMP-activated protein kinase. The Journal of Biological Chemistry *282*, 30143-30149.

Consoli, A., Nurjhan, N., Reilly, J.J., Jr., Bier, D.M., and Gerich, J.E. (1990). Mechanism of increased gluconeogenesis in noninsulin-dependent diabetes mellitus. Role of alterations in systemic, hepatic, and muscle lactate and alanine metabolism. J Clin Invest *86*, 2038-2045.

Cote, M., Mauriege, P., Bergeron, J., Almeras, N., Tremblay, A., Lemieux, I., and Despres, J.P. (2005). Adiponectinemia in visceral obesity: impact on glucose tolerance and plasma lipoprotein and lipid levels in men. J Clin Endocrinol Metab *90*, 1434-1439.

Crute, B.E., Seefeld, K., Gamble, J., Kemp, B.E., and Witters, L.A. (1998). Functional domains of the alpha1 catalytic subunit of the AMP-activated protein kinase. The Journal of biological chemistry *273*, 35347-35354.

Deacon, C.F. (2004). Therapeutic strategies based on glucagon-like peptide 1. Diabetes *53*, 2181-2189.

DeFronzo, R.A. (1997). Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. Neth J Med *50*, 191-197.

DeFronzo, R.A. (1999). Pharmacologic therapy for type 2 diabetes mellitus. Annals of Internal Medicine *131*, 281-303.

DeFronzo, R.A. (2004). Dysfunctional fat cells, lipotoxicity and type 2 diabetes. Int J Clin Pract Suppl, 9-21.

Defronzo, R.A. (2009). Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes *58*, 773-795.

DeFronzo, R.A., and Ferrannini, E. (1987). Regulation of hepatic glucose metabolism in humans. Diabetes Metab Rev *3*, 415-459.

DeFronzo, R.A., Ferrannini, E., and Simonson, D.C. (1989). Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. Metabolism *38*, 387-395.

DeFronzo, R.A., Gunnarsson, R., Bjorkman, O., Olsson, M., and Wahren, J. (1985). Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest *76*, 149-155.

Deng, J.Y., Hsieh, P.S., Huang, J.P., Lu, L.S., and 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*, 1814-1823.

Desvergne, B., Michalik, L., and Wahli, W. (2004). Be fit or be sick: peroxisome proliferator-activated receptors are down the road. Mol Endocrinol *18*, 1321-1332.

Dominguez, J.H., Camp, K., Maianu, L., Feister, H., and Garvey, W.T. (1994). Molecular adaptations of GLUT1 and GLUT2 in renal proximal tubules of diabetic rats. Am J Physiol *266*, F283-290.

Drucker, D.J. (2002). Biological actions and therapeutic potential of the glucagon-like peptides. Gastroenterology *122*, 531-544.

Drucker, D.J. (2003). Enhancing incretin action for the treatment of type 2 diabetes. Diabetes care *26*, 2929-2940.

Drucker, D.J. (2006). The biology of incretin hormones. Cell Metab *3*, 153-165.

Drucker, D.J., and Nauck, M.A. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet *368*, 1696-1705.

Ducimetiere, P., Richard, J., and Cambien, F. (1986). The pattern of subcutaneous fat distribution in middle-aged men and the risk of coronary heart disease: the Paris Prospective Study. Int J Obes *10*, 229-240.

Edgerton, D.S., Johnson, K.M., Neal, D.W., Scott, M., Hobbs, C.H., Zhang, X., Duttaroy, A., and Cherrington, A.D. (2009). Inhibition of dipeptidyl peptidase-4 by vildagliptin during glucagon-like Peptide 1 infusion increases liver glucose uptake in the conscious dog. Diabetes *58*, 243-249.

Eto, K., Yamashita, T., Matsui, J., Terauchi, Y., Noda, M., and Kadowaki, T. (2002). Genetic manipulations of fatty acid metabolism in beta-cells are associated with dysregulated insulin secretion. Diabetes *51 Suppl 3*, S414-420.

Fajas, L., Auboeuf, D., Raspe, E., Schoonjans, K., Lefebvre, A.M., Saladin, R., Najib, J., Laville, M., Fruchart, J.C., Deeb, S.*, et al.* (1997). The organization, promoter analysis, and expression of the human PPARgamma gene. The Journal of biological chemistry *272*, 18779-18789.

Feige, J.N., Gelman, L., Michalik, L., Desvergne, B., and Wahli, W. (2006). From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. Prog Lipid Res *45*, 120-159.

Feinstein, D.L., Spagnolo, A., Akar, C., Weinberg, G., Murphy, P., Gavrilyuk, V., and Dello Russo, C. (2005). Receptor-independent actions of PPAR thiazolidinedione agonists: is mitochondrial function the key? Biochem Pharmacol *70*, 177-188.

Felber, J.P., Ferrannini, E., Golay, A., Meyer, H.U., Theibaud, D., Curchod, B., Maeder, E., Jequier, E., and DeFronzo, R.A. (1987). Role of lipid oxidation in pathogenesis of insulin resistance of obesity and type II diabetes. Diabetes *36*, 1341-1350.

Felber, J.P., and Vannotti, A. (1964). Effects of Fat Infusion on Glucose Tolerance and Insulin Plasma Levels. Med Exp Int J Exp Med *10*, 153-156.

Feldon, S.E., O'Loughlin C, W., Ray, D.M., Landskroner-Eiger, S., Seweryniak, K.E., and Phipps, R.P. (2006). Activated human T lymphocytes express cyclooxygenase-2 and produce proadipogenic prostaglandins that drive human orbital fibroblast differentiation to adipocytes. American Journal of Pathology *169*, 1183-1193.

Ferrannini, E., Barrett, E.J., Bevilacqua, S., and DeFronzo, R.A. (1983). Effect of fatty acids on glucose production and utilization in man. J Clin Invest *72*, 1737-1747.

Ferrannini, E., Natali, A., Bell, P., Cavallo-Perin, P., Lalic, N., and Mingrone, G. (1997). Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). J Clin Invest *100*, 1166-1173.

Ferrannini, E., Simonson, D.C., Katz, L.D., Reichard, G., Jr., Bevilacqua, S., Barrett, E.J., Olsson, M., and DeFronzo, R.A. (1988). The disposal of an oral glucose load in patients with non-insulin-dependent diabetes. Metabolism *37*, 79-85.

Flegal, K.M., Carroll, M.D., Ogden, C.L., and Curtin, L.R. (2010). Prevalence and trends in obesity among US adults, 1999-2008. JAMA *303*, 235-241.

Foretz, M., Ancellin, N., Andreelli, F., Saintillan, Y., Grondin, P., Kahn, A., Thorens, B., Vaulont, S., and Viollet, B. (2005). Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. Diabetes *54*, 1331-1339.

Foretz, M., Carling, D., Guichard, C., Ferre, P., and Foufelle, F. (1998). AMP-activated protein kinase inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. The Journal of biological chemistry *273*, 14767-14771.

Forman, B.M., Tontonoz, P., Chen, J., Brun, R.P., Spiegelman, B.M., and Evans, R.M. (1995). 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell *83*, 803-812.

Fraze, E., Donner, C.C., Swislocki, A.L., Chiou, Y.A., Chen, Y.D., and Reaven, G.M. (1985). Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance. J Clin Endocrinol Metab *61*, 807-811.

Fujimoto, W.Y., Bergstrom, R.W., Boyko, E.J., Chen, K.W., Leonetti, D.L., Newell-Morris, L., Shofer, J.B., and Wahl, P.W. (1999). Visceral adiposity and incident coronary heart disease in Japanese-American men. The 10-year follow-up results of the Seattle Japanese-American Community Diabetes Study. Diabetes Care *22*, 1808-1812.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., and Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest *114*, 1752-1761.

Gampe, R.T., Jr., Montana, V.G., Lambert, M.H., Miller, A.B., Bledsoe, R.K., Milburn, M.V., Kliewer, S.A., Willson, T.M., and Xu, H.E. (2000). Asymmetry in the PPARgamma/RXRalpha crystal structure reveals the molecular basis of heterodimerization among nuclear receptors. Mol Cell *5*, 545-555.

Garcia-Bates, T.M., Lehmann, G.M., Simpson-Haidaris, P.J., Bernstein, S.H., Sime, P.J., and Phipps, R.P. (2008). Role of peroxisome proliferator-activated receptor gamma and its ligands in the treatment of hematological malignancies. PPAR Res *2008*, 834612.

Gastaldelli, A., Baldi, S., Pettiti, M., Toschi, E., Camastra, S., Natali, A., Landau, B.R., and Ferrannini, E. (2000). Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study. Diabetes *49*, 1367-1373.

Gelman, L., Feige, J.N., and Desvergne, B. (2007). Molecular basis of selective PPARgamma modulation for the treatment of Type 2 diabetes. Biochimica et Biophysica Acta *1771*, 1094-1107.

Golay, A., Felber, J.P., Jequier, E., DeFronzo, R.A., and Ferrannini, E. (1988). Metabolic basis of obesity and noninsulin-dependent diabetes mellitus. Diabetes Metab Rev *4*, 727-747.

Goralski, K.B., and Sinal, C.J. (2007). Type 2 diabetes and cardiovascular disease: getting to the fat of the matter. Canadian Journal of Physiology and Pharmacology *85*, 113-132.

Graham, T.E., Yang, Q., Bluher, M., Hammarstedt, A., Ciaraldi, T.P., Henry, R.R., Wason, C.J., Oberbach, A., Jansson, P.A., Smith, U*., et al.* (2006). Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. The New England journal of medicine *354*, 2552-2563.

Greenberg, A.S., and McDaniel, M.L. (2002). Identifying the links between obesity, insulin resistance and beta-cell function: potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. European journal of clinical investigation *32 Suppl 3*, 24-34.

Groop, L.C., Bonadonna, R.C., DelPrato, S., Ratheiser, K., Zyck, K., Ferrannini, E., and DeFronzo, R.A. (1989). Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest *84*, 205-213.

Groop, L.C., Saloranta, C., Shank, M., Bonadonna, R.C., Ferrannini, E., and DeFronzo, R.A. (1991). The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab *72*, 96-107.

Gross, B., and Staels, B. (2007a). PPAR agonists: multimodal drugs for the treatment of type-2 diabetes. Best Practice & Research Clinical Endocrinology & Metabolism *21*, 687-710.

Gross, B., and Staels, B. (2007b). PPAR agonists: multimodal drugs for the treatment of type-2 diabetes. Best Pract Res Clin Endocrinol Metab *21*, 687-710.

Haidara, M.A., Yassin, H.Z., Rateb, M., Ammar, H., and Zorkani, M.A. (2006). Role of oxidative stress in development of cardiovascular complications in diabetes mellitus. Curr Vasc Pharmacol *4*, 215-227.

Han, S.S., Keum, Y.S., Seo, H.J., and Surh, Y.J. (2002). Curcumin suppresses activation of NF-kappaB and AP-1 induced by phorbol ester in cultured human promyelocytic leukemia cells. J Biochem Mol Biol *35*, 337-342.

Hardie, D.G. (2004). The AMP-activated protein kinase pathway--new players upstream and downstream. J Cell Sci *117*, 5479-5487.

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

Hawley, S.A., Davison, M., Woods, A., Davies, S.P., Beri, R.K., Carling, D., and Hardie, D.G. (1996). Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. The Journal of biological chemistry *271*, 27879-27887.

Hawley, S.A., Pan, D.A., Mustard, K.J., Ross, L., Bain, J., Edelman, A.M., Frenguelli, B.G., and Hardie, D.G. (2005). Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. Cell Metabolism *2*, 9-19.

Hayashi, T., Hirshman, M.F., Kurth, E.J., Winder, W.W., and Goodyear, L.J. (1998). Evidence for 5' AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. Diabetes *47*, 1369-1373.

Hidaka, H., Ishiko, T., Furuhashi, T., Kamohara, H., Suzuki, S., Miyazaki, M., Ikeda, O., Mita, S., Setoguchi, T., and Ogawa, M. (2002). Curcumin inhibits interleukin 8 production and enhances interleukin 8 receptor expression on the cell surface:impact on human pancreatic carcinoma cell growth by autocrine regulation. Cancer *95*, 1206-1214.

Higa, M., Zhou, Y.T., Ravazzola, M., Baetens, D., Orci, L., and Unger, R.H. (1999). Troglitazone prevents mitochondrial alterations, beta cell destruction, and diabetes in obese prediabetic rats. Proc Natl Acad Sci U S A *96*, 11513-11518.

Hill, J.O., and Peters, J.C. (1998). Environmental contributions to the obesity epidemic. Science *280*, 1371-1374.

Hill, J.O., Wyatt, H.R., Reed, G.W., and Peters, J.C. (2003). Obesity and the environment: where do we go from here? Science (New York, NY *299*, 853-855.

Hirosumi, J., Tuncman, G., Chang, L., Gorgun, C.Z., Uysal, K.T., Maeda, K., Karin, M., and Hotamisligil, G.S. (2002). A central role for JNK in obesity and insulin resistance. Nature *420*, 333-336.

Holst, J.J. (2006). Glucagon-like peptide-1: from extract to agent. The Claude Bernard Lecture, 2005. Diabetologia *49*, 253-260.

Hotamisligil, G.S. (1999). The role of TNFalpha and TNF receptors in obesity and insulin resistance. Journal of internal medicine *245*, 621-625.

Hotta, K., Funahashi, T., Bodkin, N.L., Ortmeyer, H.K., Arita, Y., Hansen, B.C., and Matsuzawa, Y. (2001). Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes *50*, 1126-1133.

Houstis, N., Rosen, E.D., and Lander, E.S. (2006). Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature *440*, 944-948.

Howard, J.K., and Flier, J.S. (2006). Attenuation of leptin and insulin signaling by SOCS proteins. Trends in endocrinology and metabolism: TEM *17*, 365-371.

Hsu, C.L., Lin, Y.J., Ho, C.T., and Yen, G.C. (2012). Inhibitory effects of garcinol and pterostilbene on cell proliferation and adipogenesis in 3T3-L1 cells. Food Funct *3*, 49-57.

Hu, C., Kwok, B.H.L., and Kitts, D.D. (2005). Saskatoon berries (Amelanchier alnifolia Nutt.) scavenge free radicals and inhibit intracellular oxidation. Food research international *38*, 1079-1085.

Hug, C., Wang, J., Ahmad, N.S., Bogan, J.S., Tsao, T.S., and Lodish, H.F. (2004). T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proceedings of the National Academy of Sciences of the United States of America *101*, 10308-10313.

Hurley, R.L., Anderson, K.A., Franzone, J.M., Kemp, B.E., Means, A.R., and Witters, L.A. (2005). The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. The Journal of biological chemistry *280*, 29060-29066.

Inzucchi, S.E. (2002). Oral antihyperglycemic therapy for type 2 diabetes: scientific review. Jama *287*, 360-372.

Ionut, V., Zheng, D., Stefanovski, D., and Bergman, R.N. (2008). Exenatide can reduce glucose independent of islet hormones or gastric emptying. Am J Physiol Endocrinol Metab *295*, E269-277.

Johnston, A. (1987). Plants and the Blackfoot. Lethbridge, Alberta: Lethbridge Historical Society, Occasional Paper No 15.

Kahn, C.R., Chen, L., and Cohen, S.E. (2000). Unraveling the mechanism of action of thiazolidinediones. J Clin Invest *106*, 1305-1307.

Kahn, S.E. (2001). Clinical review 135: The importance of beta-cell failure in the development and progression of type 2 diabetes. J Clin Endocrinol Metab *86*, 4047-4058.

Kahn, S.E., Prigeon, R.L., McCulloch, D.K., Boyko, E.J., Bergman, R.N., Schwartz, M.W., Neifing, J.L., Ward, W.K., Beard, J.C., Palmer, J.P.*, et al.* (1993). Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes *42*, 1663-1672.

Kamohara, S., Burcelin, R., Halaas, J.L., Friedman, J.M., and Charron, M.J. (1997). Acute stimulation of glucose metabolism in mice by leptin treatment. Nature *389*, 374-377.

Kamran, M., Peterson, R.G., and Dominguez, J.H. (1997). Overexpression of GLUT2 gene in renal proximal tubules of diabetic Zucker rats. J Am Soc Nephrol *8*, 943-948.

Kanitkar, M., and Bhonde, R.R. (2008). Curcumin treatment enhances islet recovery by induction of heat shock response proteins, Hsp70 and heme oxygenase-1, during cryopreservation. Life Sci *82*, 182-189.

Kaser, S., Kaser, A., Sandhofer, A., Ebenbichler, C.F., Tilg, H., and Patsch, J.R. (2003). Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. Biochemical and Biophysical Research Communications *309*, 286-290.

Kashyap, S., Belfort, R., Gastaldelli, A., Pratipanawatr, T., Berria, R., Pratipanawatr, W., Bajaj, M., Mandarino, L., DeFronzo, R., and Cusi, K. (2003). A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. Diabetes *52*, 2461-2474.

Kershaw, E.E., and Flier, J.S. (2004). Adipose tissue as an endocrine organ. The Journal of clinical endocrinology and metabolism *89*, 2548-2556.

Kersten, S., Desvergne, B., and Wahli, W. (2000). Roles of PPARs in health and disease. Nature *405*, 421-424.

Kieffer, T.J., and Habener, J.F. (1999). The glucagon-like peptides. Endocr Rev *20*, 876-913.

Kieffer, T.J., and Habener, J.F. (2000). The adipoinsular axis: effects of leptin on pancreatic beta-cells. American journal of physiology *278*, E1-E14.

Kim, T., Davis, J., Zhang, A.J., He, X., and Mathews, S.T. (2009). Curcumin activates AMPK and suppresses gluconeogenic gene expression in hepatoma cells. Biochemical and Biophysical Research Communications *388*, 377-382.

Kiuchi, F., Goto, Y., Sugimoto, N., Akao, N., Kondo, K., and Tsuda, Y. (1993). Nematocidal activity of turmeric: synergistic action of curcuminoids, Vol 41, 1993/09/01 edn.

Kliewer, S.A., Lenhard, J.M., Willson, T.M., Patel, I., Morris, D.C., and Lehmann, J.M. (1995). A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell *83*, 813-819.

Kloppel, G., Lohr, M., Habich, K., Oberholzer, M., and Heitz, P.U. (1985). Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Survey and synthesis of pathology research *4*, 110-125.

Kowluru, R.A., and Chan, P.S. (2007). Oxidative stress and diabetic retinopathy. Exp Diabetes Res *2007*, 43603.

Kowluru, R.A., and Kanwar, M. (2007). Effects of curcumin on retinal oxidative stress and inflammation in diabetes. Nutr Metab (Lond) *4*, 8.

Kramer, D., Shapiro, R., Adler, A., Bush, E., and Rondinone, C.M. (2001). Insulin-sensitizing effect of rosiglitazone (BRL-49653) by regulation of glucose transporters in muscle and fat of Zucker rats. Metabolism *50*, 1294-1300.

Krentz, A.J., and Bailey, C.J. (2005). Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs *65*, 385-411.

Kuroda, M., Mimaki, Y., Nishiyama, T., Mae, T., Kishida, H., Tsukagawa, M., Takahashi, K., Kawada, T., Nakagawa, K., and Kitahara, M. (2005). Hypoglycemic effects of turmeric (Curcuma longa L. rhizomes) on genetically diabetic KK-Ay mice. Biol Pharm Bull *28*, 937-939.

Kurth-Kraczek, E.J., Hirshman, M.F., Goodyear, L.J., and Winder, W.W. (1999). 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. Diabetes *48*, 1667-1671.

Kwon, J.Y., Seo, S.G., Heo, Y.S., Yue, S., Cheng, J.X., Lee, K.W., and Kim, K.H. (2012). Piceatannol, a natural polyphenolic stilbene, inhibits adipogenesis via modulation of mitotic clonal expansion and insulin receptor-dependent insulin signaling in the early phase of differentiation. J Biol Chem.

Lago, F., Dieguez, C., Gomez-Reino, J., and Gualillo, O. (2007). The emerging role of adipokines as mediators of inflammation and immune responses. Cytokine & growth factor reviews *18*, 313-325.

Lam, S.H., Chen, J.M., Kang, C.J., Chen, C.H., and Lee, S.S. (2008). alpha-Glucosidase inhibitors from the seeds of Syagrus romanzoffiana. Phytochemistry *69*, 1173-1178.

Larsson, B., Svardsudd, K., Welin, L., Wilhelmsen, L., Bjorntorp, P., and Tibblin, G. (1984). Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. Br Med J (Clin Res Ed) *288*, 1401-1404.

Leclerc, I., Kahn, A., and Doiron, B. (1998). The 5'-AMP-activated protein kinase inhibits the transcriptional stimulation by glucose in liver cells, acting through the glucose response complex. FEBS Lett *431*, 180-184.

Leclerc, I., Lenzner, C., Gourdon, L., Vaulont, S., Kahn, A., and Viollet, B. (2001). Hepatocyte nuclear factor-4alpha involved in type 1 maturity-onset diabetes of the young is a novel target of AMP-activated protein kinase. Diabetes *50*, 1515-1521.

Leclerc, I., Woltersdorf, W.W., da Silva Xavier, G., Rowe, R.L., Cross, S.E., Korbutt, G.S., Rajotte, R.V., Smith, R., and Rutter, G.A. (2004). Metformin, but not leptin, regulates AMP-activated protein kinase in pancreatic islets: impact on glucose-stimulated insulin secretion. American Journal of Physiology-Endocrinology and Metabolism *286*, E1023-1031.

Lemieux, I. (2004). Energy partitioning in gluteal-femoral fat: does the metabolic fate of triglycerides affect coronary heart disease risk? Arteriosclerosis, Thrombosis, and Vascular Biology *24*, 795-797.

Lemieux, I., Pascot, A., Prud'homme, D., Almeras, N., Bogaty, P., Nadeau, A., Bergeron, J., and Despres, J.P. (2001). Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. Arterioscler Thromb Vasc Biol *21*, 961-967.

Leung, N., Sakaue, T., Carpentier, A., Uffelman, K., Giacca, A., and Lewis, G.F. (2004). Prolonged increase of plasma non-esterified fatty acids fully abolishes the stimulatory effect of 24 hours of moderate hyperglycaemia on insulin sensitivity and pancreatic beta-cell function in obese men. Diabetologia *47*, 204-213.

Literat, A., Su, F., Norwicki, M., Durand, M., Ramanathan, R., Jones, C.A., Minoo, P., and Kwong, K.Y. (2001). Regulation of pro-inflammatory cytokine expression by curcumin in hyaline membrane disease (HMD). Life Sci *70*, 253-267.

Lochhead, P.A., Salt, I.P., Walker, K.S., Hardie, D.G., and Sutherland, C. (2000). 5-aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. Diabetes *49*, 896-903.

Magnusson, I., Rothman, D.L., Katz, L.D., Shulman, R.G., and Shulman, G.I. (1992). Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. J Clin Invest *90*, 1323-1327.

Mandarino, L.J., Printz, R.L., Cusi, K.A., Kinchington, P., O'Doherty, R.M., Osawa, H., Sewell, C., Consoli, A., Granner, D.K., and DeFronzo, R.A. (1995). Regulation of hexokinase II and glycogen synthase mRNA, protein, and activity in human muscle. Am J Physiol *269*, E701-708.

Mandarino, L.J., Wright, K.S., Verity, L.S., Nichols, J., Bell, J.M., Kolterman, O.G., and Beck-Nielsen, H. (1987). Effects of insulin infusion on human skeletal muscle pyruvate dehydrogenase, phosphofructokinase, and glycogen synthase. Evidence for their role in oxidative and nonoxidative glucose metabolism. J Clin Invest *80*, 655-663.

Marles, R.J., and Farnsworth, N.R. (1995). Antidiabetic plants and their active constituents. Phytomedicine *2*, 137-189.

Martin, G., Schoonjans, K., Lefebvre, A.M., Staels, B., and Auwerx, J. (1997). Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. The Journal of biological chemistry *272*, 28210-28217.

Matsuda, M., Liu, Y., Mahankali, S., Pu, Y., Mahankali, A., Wang, J., DeFronzo, R.A., Fox, P.T., and Gao, J.H. (1999). Altered hypothalamic function in response to glucose ingestion in obese humans. Diabetes *48*, 1801-1806.

Matsuzawa, Y. (2006). Therapy Insight: adipocytokines in metabolic syndrome and related cardiovascular disease. Nat Clin Pract Cardiovasc Med *3*, 35-42.

Matthaei, S., Stumvoll, M., Kellerer, M., and Haring, H.U. (2000). Pathophysiology and pharmacological treatment of insulin resistance. Endocr Rev *21*, 585-618.

Mazza, G.C.T. (2008). Carotenoids and cyanogenic glucosides in saskatoon berries (Amelanchier alnifolia Nutt.). Journal of food composition and analysis *21*, 249-254.

McKeigue, P.M., Shah, B., and Marmot, M.G. (1991). Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. Lancet *337*, 382-386.

Meghana, K., Sanjeev, G., and Ramesh, B. (2007). Curcumin prevents streptozotocin-induced islet damage by scavenging free radicals: a prophylactic and protective role. Eur J Pharmacol *577*, 183-191.

Meier, J.J., Hucking, K., Holst, J.J., Deacon, C.F., Schmiegel, W.H., and Nauck, M.A. (2001). Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. Diabetes *50*, 2497-2504.

Meier, J.J., and Nauck, M.A. (2006). Incretins and the development of type 2 diabetes. Curr Diab Rep *6*, 194-201.

Michalik, L., Desvergne, B., and Wahli, W. (2004). Peroxisome-proliferator-activated receptors and cancers: complex stories. Nat Rev Cancer *4*, 61-70.

Michalik, L., and Wahli, W. (2006). Involvement of PPAR nuclear receptors in tissue injury and wound repair. J Clin Invest *116*, 598-606.

Mikstacka, R., Rimando, A.M., and Ignatowicz, E. (2010). Antioxidant effect of trans-resveratrol, pterostilbene, quercetin and their combinations in human erythrocytes in vitro. Plant foods for human nutrition (Dordrecht, Netherlands) *65*, 57-63.

Minokoshi, Y., Kim, Y.B., Peroni, O.D., Fryer, L.G., Muller, C., Carling, D., and Kahn, B.B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature *415*, 339-343.

Mittelman, S.D., Van Citters, G.W., Kirkman, E.L., and Bergman, R.N. (2002). Extreme insulin resistance of the central adipose depot in vivo. Diabetes *51*, 755-761.

Mogensen, C.E. (1971). Maximum tubular reabsorption capacity for glucose and renal hemodynamcis during rapid hypertonic glucose infusion in normal and diabetic subjects. Scand J Clin Lab Invest *28*, 101-109.

Mrudula, T., Suryanarayana, P., Srinivas, P.N., and Reddy, G.B. (2007). Effect of curcumin on hyperglycemia-induced vascular endothelial growth factor expression in streptozotocin-induced diabetic rat retina. Biochem Biophys Res Commun *361*, 528-532.

Mudaliar, S., and Henry, R.R. (2001). New oral therapies for type 2 diabetes mellitus: The glitazones or insulin sensitizers. Annual Review of Medicine *52*, 239-257.

Murugan, P., and Pari, L. (2005). Effect of tetrahydrocurcumin on erythromycin estolate-induced lipid peroxidation in rats. J Basic Clin Physiol Pharmacol *16*, 1-15.

Murugan, P., and Pari, L. (2006). Effect of tetrahydrocurcumin on lipid peroxidation and lipids in streptozotocin-nicotinamide-induced diabetic rats. Basic Clin Pharmacol Toxicol *99*, 122-127.

Murugan, P., and Pari, L. (2007a). Influence of tetrahydrocurcumin on erythrocyte membrane bound enzymes and antioxidant status in experimental type 2 diabetic rats. J Ethnopharmacol *113*, 479-486.

Murugan, P., and Pari, L. (2007b). Influence of tetrahydrocurcumin on hepatic and renal functional markers and protein levels in experimental type 2 diabetic rats. Basic Clin Pharmacol Toxicol *101*, 241-245.

Murugan, P., Pari, L., and Rao, C.A. (2008). Effect of tetrahydrocurcumin on insulin receptor status in type 2 diabetic rats: studies on insulin binding to erythrocytes. J Biosci *33*, 63-72.

Narkar, V.A., Downes, M., Yu, R.T., Embler, E., Wang, Y.X., Banayo, E., Mihaylova, M.M., Nelson, M.C., Zou, Y., Juguilon, H.*, et al.* (2008). AMPK and PPARdelta agonists are exercise mimetics. Cell *134*, 405-415.

Nauck, M., Stockmann, F., Ebert, R., and Creutzfeldt, W. (1986). Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia *29*, 46-52.

Nauck, M.A., Heimesaat, M.M., Orskov, C., Holst, J.J., Ebert, R., and Creutzfeldt, W. (1993a). Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest *91*, 301-307.

Nauck, M.A., Kleine, N., Orskov, C., Holst, J.J., Willms, B., and Creutzfeldt, W. (1993b). Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia *36*, 741-744.

Nauck, M.A., Niedereichholz, U., Ettler, R., Holst, J.J., Orskov, C., Ritzel, R., and Schmiegel, W.H. (1997). Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. American Journal of Physiology-Endocrinology and Metabolism *273*, E981-988.

Neel, J.V. (1962). Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? American journal of human genetics *14*, 353-362.

NIH (1998). Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health. Obes Res *6 Suppl 2*, 51S-209S.

NIH (2008). National diabetes statistics, 2007.

Nishiyama, T., Mae, T., Kishida, H., Tsukagawa, M., Mimaki, Y., Kuroda, M., Sashida, Y., Takahashi, K., Kawada, T., Nakagawa, K.*, et al.* (2005). Curcuminoids and sesquiterpenoids

in turmeric (Curcuma longa L.) suppress an increase in blood glucose level in type 2 diabetic KK-Ay mice. J Agric Food Chem *53*, 959-963.

Nolte, R.T., Wisely, G.B., Westin, S., Cobb, J.E., Lambert, M.H., Kurokawa, R., Rosenfeld, M.G., Willson, T.M., Glass, C.K., and Milburn, M.V. (1998). Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. Nature *395*, 137-143.

Noonan, W.T., Shapiro, V.M., and Banks, R.O. (2001). Renal glucose reabsorption during hypertonic glucose infusion in female streptozotocin-induced diabetic rats. Life Sci *68*, 2967-2977.

Obici, S., Feng, Z., Karkanias, G., Baskin, D.G., and Rossetti, L. (2002). Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci *5*, 566-572.

Obici, S., Feng, Z., Tan, J., Liu, L., Karkanias, G., and Rossetti, L. (2001). Central melanocortin receptors regulate insulin action. J Clin Invest *108*, 1079-1085.

Onodera, S., Nishihira, J., Iwabuchi, K., Koyama, Y., Yoshida, K., Tanaka, S., and Minami, A. (2002). Macrophage migration inhibitory factor up-regulates matrix metalloproteinase-9 and -13 in rat osteoblasts. Relevance to intracellular signaling pathways. J Biol Chem *277*, 7865-7874.

Osawa, H., Yamada, K., Onuma, H., Murakami, A., Ochi, M., Kawata, H., Nishimiya, T., Niiya, T., Shimizu, I., Nishida, W*., et al.* (2004). The G/G genotype of a resistin single-nucleotide polymorphism at -420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. American journal of human genetics *75*, 678-686.

Ozcan, U., Cao, Q., Yilmaz, E., Lee, A.H., Iwakoshi, N.N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L.H., and Hotamisligil, G.S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science *306*, 457-461.

Pajvani, U.B., Du, X., Combs, T.P., Berg, A.H., Rajala, M.W., Schulthess, T., Engel, J., Brownlee, M., and Scherer, P.E. (2003). Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. The Journal of biological chemistry *278*, 9073-9085.

Pan, M.H., Chang, Y.H., Tsai, M.L., Lai, C.S., Ho, S.Y., Badmaev, V., and Ho, C.T. (2008). Pterostilbene suppressed lipopolysaccharide-induced up-expression of iNOS and COX-2 in murine macrophages. Journal of agricultural and food chemistry *56*, 7502-7509.

Pan, M.H., Lin-Shiau, S.Y., and Lin, J.K. (2000). Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of IkappaB kinase and NFkappaB activation in macrophages. Biochemical pharmacology *60*, 1665-1676.

Pang, T., Xiong, B., Li, J.Y., Qiu, B.Y., Jin, G.Z., Shen, J.K., and Li, J. (2007). Conserved alpha-helix acts as autoinhibitory sequence in AMP-activated protein kinase alpha subunits. The Journal of biological chemistry *282*, 495-506.

Pari, L., and Murugan, P. (2007). Tetrahydrocurcumin prevents brain lipid peroxidation in streptozotocin-induced diabetic rats. J Med Food *10*, 323-329.

Park, H., Kaushik, V.K., Constant, S., Prentki, M., Przybytkowski, E., Ruderman, N.B., and Saha, A.K. (2002). Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. The Journal of biological chemistry *277*, 32571-32577.

Patel, L., Buckels, A.C., Kinghorn, I.J., Murdock, P.R., Holbrook, J.D., Plumpton, C., Macphee, C.H., and Smith, S.A. (2003). Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun *300*, 472-476.

Patel, S.D., Rajala, M.W., Rossetti, L., Scherer, P.E., and Shapiro, L. (2004). Disulfide-dependent multimeric assembly of resistin family hormones. Science *304*, 1154-1158.

Paul, S., DeCastro, A.J., Lee, H.J., Smolarek, A.K., So, J.Y., Simi, B., Wang, C.X., Zhou, R., Rimando, A.M., and Suh, N. (2010). Dietary intake of pterostilbene, a constituent of blueberries, inhibits the beta-catenin/p65 downstream signaling pathway and colon carcinogenesis in rats. Carcinogenesis *31*, 1272-1278.

Paul, S., Rimando, A.M., Lee, H.J., Ji, Y., Reddy, B.S., and Suh, N. (2009). Anti-inflammatory action of pterostilbene is mediated through the p38 mitogen-activated protein kinase pathway in colon cancer cells. Cancer prevention research (Philadelphia, Pa *2*, 650-657.

Peeyush, K.T., Gireesh, G., Jobin, M., and Paulose, C.S. (2009). Neuroprotective role of curcumin in the cerebellum of streptozotocin-induced diabetic rats. Life Sci *85*, 704-710.

Peeyush Kumar, T., Antony, S., Soman, S., Kuruvilla, K.P., George, N., and Paulose, C.S. (2010). Role of curcumin in the prevention of cholinergic mediated cortical dysfunctions in streptozotocin-induced diabetic rats. Mol Cell Endocrinol *331*, 1-10.

Pendergrass, M., Bertoldo, A., Bonadonna, R., Nucci, G., Mandarino, L., Cobelli, C., and Defronzo, R.A. (2007). Muscle glucose transport and phosphorylation in type 2 diabetic, obese nondiabetic, and genetically predisposed individuals. Am J Physiol Endocrinol Metab *292*, E92-100.

Perley, M., and Kipnis, D.M. (1966). Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes *15*, 867-874.

Pold, R., Jensen, L.S., Jessen, N., Buhl, E.S., Schmitz, O., Flyvbjerg, A., Fujii, N., Goodyear, L.J., Gotfredsen, C.F., Brand, C.L.*, et al.* (2005). Long-term AICAR administration and exercise prevents diabetes in ZDF rats. Diabetes *54*, 928-934.

Polonsky, K.S., Given, B.D., and Van Cauter, E. (1988). Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. J Clin Invest *81*, 442-448.

Prentki, M., Joly, E., El-Assaad, W., and Roduit, R. (2002). Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. Diabetes *51 Suppl 3*, S405-413.

Rajala, M.W., Qi, Y., Patel, H.R., Takahashi, N., Banerjee, R., Pajvani, U.B., Sinha, M.K., Gingerich, R.L., Scherer, P.E., and Ahima, R.S. (2004). Regulation of resistin expression and circulating levels in obesity, diabetes, and fasting. Diabetes *53*, 1671-1679.

Rao, J.M., and Tiwari, A.K. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. Curr Sci *83*, 30-38.

Reaven, G.M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. Diabetes *37*, 1595-1607.

Rho, S.J.P.S.A.C.W.S.J.K.L.H.G. (2007). Dietetic and hypocholesterolaemic action of black soy peptide in dietary obese rats. Journal of the science of food and agriculture *15. 87*, 908-913.

Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J., and Glass, C.K. (1998). The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature *391*, 79-82.

Rimando, A.M., Nagmani, R., Feller, D.R., and Yokoyama, W. (2005). Pterostilbene, a new agonist for the peroxisome proliferator-activated receptor alpha-isoform, lowers plasma lipoproteins and cholesterol in hypercholesterolemic hamsters. J Agric Food Chem *53*, 3403-3407.

Roden, M., Price, T.B., Perseghin, G., Petersen, K.F., Rothman, D.L., Cline, G.W., and Shulman, G.I. (1996). Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest *97*, 2859-2865.

Rogiers, S.Y.K.N.R. (1997). Physical and chemical changes during growth, maturation, and ripening of saskatoon (Amelanchier alnifolia) fruit. Canadian Journal of Botany = Journal Canadien de Botanique *75*, 1215-1225.

Rossetti, L., Giaccari, A., and DeFronzo, R.A. (1990). Glucose toxicity. Diabetes Care *13*, 610-630.

Rothman, D.L., Shulman, R.G., and Shulman, G.I. (1992). 31P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. J Clin Invest *89*, 1069-1075.

Rotter, V., Nagaev, I., and Smith, U. (2003). Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in

human fat cells from insulin-resistant subjects. The Journal of biological chemistry *278*, 45777-45784.

Roupe, K.A., Remsberg, C.M., Yanez, J.A., and Davies, N.M. (2006). Pharmacometrics of stilbenes: seguing towards the clinic. Current clinical pharmacology *1*, 81-101.

Rutter, G.A., Da Silva Xavier, G., and Leclerc, I. (2003). Roles of 5'-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. Biochem J *375*, 1-16.

Sakamoto, K., and Holman, G.D. (2008). Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. American Journal of Physiology-Endocrinology and Metabolism *295*, E29-37.

Scheen, A.J. (1998). Clinical efficacy of acarbose in diabetes mellitus: a critical review of controlled trials. Diabetes Metab *24*, 311-320.

Schwartz, M.W., Woods, S.C., Porte, D., Jr., Seeley, R.J., and Baskin, D.G. (2000). Central nervous system control of food intake. Nature *404*, 661-671.

Shane-McWhorter, L. (2001). Biological Complementary Therapies: A Focus on Botanical Products in Diabetes. Diabetes Spectrum *14*, 199-208.

Sharma, S., Kulkarni, S.K., Agrewala, J.N., and Chopra, K. (2006a). Curcumin attenuates thermal hyperalgesia in a diabetic mouse model of neuropathic pain. Eur J Pharmacol *536*, 256-261.

Sharma, S., Kulkarni, S.K., and Chopra, K. (2006b). Curcumin, the active principle of turmeric (Curcuma longa), ameliorates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol *33*, 940-945.

Shaw, R.J., Lamia, K.A., Vasquez, D., Koo, S.H., Bardeesy, N., Depinho, R.A., Montminy, M., and Cantley, L.C. (2005). The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science (New York, NY *310*, 1642-1646.

Shinde, J., Taldone, T., Barletta, M., Kunaparaju, N., Hu, B., Kumar, S., Placido, J., and Zito, S.W. (2008). Alpha-glucosidase inhibitory activity of Syzygium cumini (Linn.) Skeels seed kernel in vitro and in Goto-Kakizaki (GK) rats. Carbohydrate research *343*, 1278-1281.

Shoelson, S.E., Lee, J., and Goldfine, A.B. (2006). Inflammation and insulin resistance. J Clin Invest *116*, 1793-1801.

Shoelson, S.E., Lee, J., and Yuan, M. (2003). Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. Int J Obes Relat Metab Disord *27 Suppl 3*, S49-52.

Shulman, G.I. (2000). Cellular mechanisms of insulin resistance. J Clin Invest *106*, 171-176.

Shulman, G.I., Rothman, D.L., Jue, T., Stein, P., DeFronzo, R.A., and Shulman, R.G. (1990). Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. N Engl J Med *322*, 223-228.

Shulman, G.I., Rothman, D.L., Smith, D., Johnson, C.M., Blair, J.B., Shulman, R.G., and DeFronzo, R.A. (1985). Mechanism of liver glycogen repletion in vivo by nuclear magnetic resonance spectroscopy. J Clin Invest *76*, 1229-1236.

Staels, B., and Fruchart, J.C. (2005). Therapeutic roles of peroxisome proliferator-activated receptor agonists. Diabetes *54*, 2460-2470.

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

Steppan, C.M., Bailey, S.T., Bhat, S., Brown, E.J., Banerjee, R.R., Wright, C.M., Patel, H.R., Ahima, R.S., and Lazar, M.A. (2001a). The hormone resistin links obesity to diabetes. Nature *409*, 307-312.

Steppan, C.M., Brown, E.J., Wright, C.M., Bhat, S., Banerjee, R.R., Dai, C.Y., Enders, G.H., Silberg, D.G., Wen, X., Wu, G.D.*, et al.* (2001b). A family of tissue-specific resistin-like molecules. Proceedings of the National Academy of Sciences of the United States of America *98*, 502-506.

Steppan, C.M., and Lazar, M.A. (2004). The current biology of resistin. Journal of internal medicine *255*, 439-447.

Straczkowski, M., Kowalska, I., Nikolajuk, A., Dzienis-Straczkowska, S., Kinalska, I., Baranowski, M., Zendzian-Piotrowska, M., Brzezinska, Z., and Gorski, J. (2004). Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. Diabetes *53*, 1215-1221.

Subbulakshmi, G., and Naik, M. (2001). Indigenous foods in the treatment of diabetes mellitus. Bombay Hospital Journal *43*, 548-561.

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

Surh, Y.J., Chun, K.S., Cha, H.H., Han, S.S., Keum, Y.S., Park, K.K., and Lee, S.S. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutation research *480-481*, 243-268.

Suryanarayana, P., Saraswat, M., Mrudula, T., Krishna, T.P., Krishnaswamy, K., and Reddy, G.B. (2005). Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest Ophthalmol Vis Sci *46*, 2092-2099.

Suryanarayana, P., Satyanarayana, A., Balakrishna, N., Kumar, P.U., and Reddy, G.B. (2007). Effect of turmeric and curcumin on oxidative stress and antioxidant enzymes in streptozotocin-induced diabetic rat. Med Sci Monit *13*, BR286-292.

Tan, N.S., Michalik, L., Desvergne, B., and Wahli, W. (2005). Multiple expression control mechanisms of peroxisome proliferator-activated receptors and their target genes. J Steroid Biochem Mol Biol *93*, 99-105.

Thiebaud, D., DeFronzo, R.A., Jacot, E., Golay, A., Acheson, K., Maeder, E., Jequier, E., and Felber, J.P. (1982). Effect of long chain triglyceride infusion on glucose metabolism in man. Metabolism *31*, 1128-1136.

Tolon, R.M., Castillo, A.I., and Aranda, A. (1998). Activation of the prolactin gene by peroxisome proliferator-activated receptor-alpha appears to be DNA binding-independent. The Journal of biological chemistry *273*, 26652-26661.

Trayhurn, P. (2005). Endocrine and signalling role of adipose tissue: new perspectives on fat. Acta Physiologica Scandinavica *184*, 285-293.

Tsimikas, S., Willerson, J.T., and Ridker, P.M. (2006). C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. J Am Coll Cardiol *47*, C19-31.

Tsuboi, T., da Silva Xavier, G., Leclerc, I., and Rutter, G.A. (2003). 5'-AMP-activated protein kinase controls insulin-containing secretory vesicle dynamics. The Journal of biological chemistry *278*, 52042-52051.

Ulivieri, C., and Baldari, C.T. (2007). The potential of peroxisome proliferator-activated receptor gamma (PPARgamma) ligands in the treatment of hematological malignancies. Mini Rev Med Chem *7*, 877-887.

Utzschneider, K.M., Prigeon, R.L., Carr, D.B., Hull, R.L., Tong, J., Shofer, J.B., Retzlaff, B.M., Knopp, R.H., and Kahn, S.E. (2006). Impact of differences in fasting glucose and glucose tolerance on the hyperbolic relationship between insulin sensitivity and insulin responses. Diabetes care *29*, 356-362.

Viollet, B., Lantier, L., Devin-Leclerc, J., Hebrard, S., Amouyal, C., Mounier, R., Foretz, M., and Andreelli, F. (2009). Targeting the AMPK pathway for the treatment of Type 2 diabetes. Front Biosci *14*, 3380-3400.

Waki, H., Yamauchi, T., Kamon, J., Ito, Y., Uchida, S., Kita, S., Hara, K., Hada, Y., Vasseur, F., Froguel, P.*, et al.* (2003). Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. The Journal of biological chemistry *278*, 40352-40363.

Wang, J., and Mazza, G. (2002). Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-gamma-activated RAW 264.7 macrophages. J Agric Food Chem *50*, 850-857.

Wang, T., Xu, J., Yu, X., Yang, R., and Han, Z.C. (2006). Peroxisome proliferator-activated receptor gamma in malignant diseases. Crit Rev Oncol Hematol *58*, 1-14.

Watanabe, C.K. (1918). Studies in the metabolic changes induced by the administration of guanidine bases. V. The change of phosphate and calcium content in serum in guanidine tetany and the relation between the calcium content and sugar in the blood. J Biol Chem *36*, 531-546.

Weisberg, S.P., Leibel, R., and Tortoriello, D.V. (2008). Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology *149*, 3549-3558.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., and Ferrante, A.W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest *112*, 1796-1808.

Werner, E.D., Lee, J., Hansen, L., Yuan, M., and Shoelson, S.E. (2004). Insulin resistance due to phosphorylation of insulin receptor substrate-1 at serine 302. The Journal of Biological Chemistry *279*, 35298-35305.

Weyer, C., Bogardus, C., Mott, D.M., and Pratley, R.E. (1999). The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest *104*, 787-794.

WHO (2000). Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser *894*, i-xii, 1-253.

WHO (2011). Fact Sheets,Diabetes

Wigren, J., Surapureddi, S., Olsson, A.G., Glass, C.K., Hammarstrom, S., and Soderstrom, M. (2003). Differential recruitment of the coactivator proteins CREB-binding protein and steroid receptor coactivator-1 to peroxisome proliferator-activated receptor gamma/9-cis-retinoic acid receptor heterodimers by ligands present in oxidized low-density lipoprotein. J Endocrinol *177*, 207-214.

Williamson, J.R., Kreisberg, R.A., and Felts, P.W. (1966). Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. Proc Natl Acad Sci U S A *56*, 247-254.

Willson, T.M., Brown, P.J., Sternbach, D.D., and Henke, B.R. (2000). The PPARs: from orphan receptors to drug discovery. J Med Chem *43*, 527-550.

Winder, W.W. (1998). Intramuscular mechanisms regulating fatty acid oxidation during exercise. Advances in Experimental Medicine and Biology *441*, 239-248.

Winzell, M.S., Nogueiras, R., Dieguez, C., and Ahren, B. (2004). Dual action of adiponectin on insulin secretion in insulin-resistant mice. Biochemical and Biophysical Research Communications *321*, 154-160.

Woods, A., Azzout-Marniche, D., Foretz, M., Stein, S.C., Lemarchand, P., Ferre, P., Foufelle, F., and Carling, D. (2000). Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. Mol Cell Biol *20*, 6704-6711.

Woods, A., Dickerson, K., Heath, R., Hong, S.P., Momcilovic, M., Johnstone, S.R., Carlson, M., and Carling, D. (2005). Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell Metabolism *2*, 21-33.

Xie, J.T.W.C.Z.N.M.W.J.A.M.S.R.A.H.H., and Foo, A.Y.C.S. (2007). American Ginseng Berry Juice Intake Reduces Blood Glucose and Body Weight in ob/ob Mice. an official publication of the Institute of Food Technologists *72*, S590-S594.

Yamauchi, T., Kamon, J., Ito, Y., Tsuchida, A., Yokomizo, T., Kita, S., Sugiyama, T., Miyagishi, M., Hara, K., Tsunoda, M.*, et al.* (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature *423*, 762-769.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N.*, et al.* (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nature medicine *7*, 941-946.

Yang, Q., Graham, T.E., Mody, N., Preitner, F., Peroni, O.D., Zabolotny, J.M., Kotani, K., Quadro, L., and Kahn, B.B. (2005). Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature *436*, 356-362.

Yatagai, T., Nagasaka, S., Taniguchi, A., Fukushima, M., Nakamura, T., Kuroe, A., Nakai, Y., and Ishibashi, S. (2003). Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. Metabolism *52*, 1274-1278.

Yeh, G.Y., Eisenberg, D.M., Kaptchuk, T.J., and Phillips, R.S. (2003). Systematic review of herbs and dietary supplements for glycemic control in diabetes. Diabetes Care *26*, 1277-1294.

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z.W., Karin, M., and Shoelson, S.E. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science *293*, 1673-1677.

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

Zhao, R.L.Q.L.L.L.J.Y.R.G.D. (2007). Antidiabetic activity of flavone from Ipomoea Batatas leaf in non-insulin dependent diabetic rats. International journal of food science & technology *42*, 80-85.

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N.*, et al.* (2001). Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest *108*, 1167-1174.

Zhou, J., Wang, X., Pineyro, M.A., and Egan, J.M. (1999). Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. Diabetes *48*, 2358-2366.

Zimmet, P., and Thomas, C.R. (2003). Genotype, obesity and cardiovascular disease--has technical and social advancement outstripped evolution? Journal of internal medicine *254*, 114-125.

Zoete, V., Grosdidier, A., and Michielin, O. (2007). Peroxisome proliferator-activated receptor structures: ligand specificity, molecular switch and interactions with regulators. Biochimica et Biophysica Acta *1771*, 915-925.

Chapter 3: Serviceberry [*Amelanchier alnifolia* (Nutt.) Nutt. ex. M. Roem (Rosaceae)] leaf extract inhibits mammalian α-glucosidase activity and suppresses postprandial glycemic response in a mouse model of diet-induced obesity and hyperglycemia

Abstract

Ethnopharmacological relevance: Serviceberry or Saskatoon berry [*Amelanchier alnifolia* (Nutt.) Nutt. ex. M. Roem (Rosaceae)], native to the North Glacier forests of the Rocky Mountains in Montana, has been used by the Blackfeet Indian tribe in the management of diabetes. Anecdotally, tea made from twigs and leaves have been used for optimum health and diabetes management. However, such traditional knowledge of the medicinal properties of *Amelanchier alnifolia* has not been validated by adequate scientific studies. The objective of this study was to validate and identify potential antidiabetic mechanisms of serviceberry using *in vitro* and *in vivo* studies.

Materials and Methods: Serviceberry plant samples consisting of leaves, twigs, and leaves with berries were extracted and fractionated. Ethyl acetate and water fractions were tested for inhibition of α-glucosidase activity *in vitro*. C57Bl6 mice fed a high-fat diet, were administered serviceberry leaf extract prior to sucrose-, starch-, or glucose-loading to test for α-glucosidase inhibition and decreased post-prandial glycemic response.

Results: *In vitro* studies demonstrated that leaf extracts of serviceberry are potent inhibitors of mammalian intestinal α-glucosidase activity (EC 3.2.1.20). Further, in an animal model of diet-induced obesity and hyperglycemia, serviceberry extracts demonstrated significant inhibition of intestinal α-glucosidase activity, and delayed the absorption of carbohydrates, resulting in significant lowering of post-prandial blood glucose concentrations, similar to the antidiabetic drug Acarbose™.

Conclusions: These findings indicating that serviceberry leaf extract may lower post-prandial glycemic response validate traditional knowledge of the Blackfeet Indians of Montana, and offer a complementary approach in the treatment and management of diabetes.

Introduction

Diabetes, a group of metabolic chronic disease affecting over 25.8 million people in the United States and over 346 million people worldwide, is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2011; WHO, 2011). Prolonged diabetes leads to serious damage to many of the body's physiological processes and causes serious complications, such as cardiovascular disease, stroke, atherosclerosis, blindness, kidney damage, lower-limb amputations among many others (CDC, 2011; WHO, 2011). Native American Indians have been shown to have a higher incidence of type 2 diabetes and related complications compared to other ethnic populations, including non-Hispanic whites, in the USA (CDC, 2011). Native American tribes, have experienced rapidly increasing rates of glucose intolerance and type 2 diabetes with prevalence rates as high as 50% in some tribes (Knowler et al., 1983). A sedentary lifestyle, combined with the consumption of high-energy foods, and a thrifty genotype may be attributed to this dramatic and rapid increase in type 2 diabetes (Neel, 1999). Native American Indians tend to shun conventional medicine and instead resort to alternative forms of therapy. In the US, American Indians continue to use plant sources for treating several types of ailments ranging from inflammation to diabetes and cancer (Swerdlow, 2000). Plant derivatives with purported anti-diabetic properties have been used in folk medicine, traditional healing systems, and as complementary and alternative medicine (CAM). Several botanical, herbal, and biological products have been claimed to lower blood glucose or decrease complications of diabetes (Yeh et al., 2003, and Mentreddy, 2007). *Aloe barbadensis*, *Eugenia jambolana*, *Gymnema sylvestre*, *Ocimum tenuiflorum*, *Trigonella foenum-graecum*, *Allum sativum*, *Galega officinalis* and *Curcuma longa L. are* examples of herbs that have been reported

to modulate blood glucose levels (Aggarwal et al., 2007; Grover et al., 2002; Mentreddy, 2007). Some of these are indigenous to the North American continent.

Serviceberry [*Amelanchier alnifolia* (Nutt.) Nutt. ex. M. Roem (Rosaceae)], also known as sarvis berry, Saskatoon berry, Juneberry, shadbush, or Okinoki by the Native Indians, are found in the North Glacier forests of the Rocky Mountains in Montana. Serviceberry is one of the traditional foods of the Blackfeet tribe. The berries are eaten raw, made into a stew, soup, or mixed into pemmican with dried meat (McClintock, 2004). Furthermore, a tea made from serviceberry twigs was used by the Blackfeet Indians for treating diabetes (Johnston, 1987). While there has been some interest in identifying bioactive compounds from leaves, stems, and berries of serviceberry (Burns Kraft et al., 2008; Lavola et al., 2012), there are limited reports on the scientific validation of traditional claims of its antidiabetic properties (Burns Kraft et al., 2008).

α-Glucosidase inhibitors are a class of drugs used to treat diabetes by inhibiting α-glucosidase, a membrane-bound intestinal enzyme that hydrolyzes polysaccharides to glucose and other monosaccharides. Liberated glucose is absorbed from the intestine and contributes to postprandial hyperglycemia. α-Glucosidase inhibitors prevent or delay the hydrolysis or absorption of carbohydrates and suppress postprandial hyperglycemia, making such inhibitors useful in the management of type 2 diabetes (Bell, 2004). Currently, acarbose and voglibose, are two such α-glucosidase inhibitors used clinically in combination with either diet or other anti-diabetic agents to control blood glucose levels of patients (Van de Laar et al., 2005).

In this study we have examined the effects of serviceberry leaf extracts on glucose metabolism. We demonstrate, using *in vitro* experiments, and in an animal model of obesity and

hyperglycemia that serviceberry leaf extracts significantly inhibit intestinal α-glucosidase activity, suppress carbohydrate absorption, and lower postprandial hyperglycemia.

Materials and Methods

Reagents. Acarbose was purchased from LKT Laboratories (St. Paul, MN); rat intestinal acetone powder, sucrose, maltose, starch were purchased from Sigma Chemical Co. (St. Louis, MO); glucose color reagent was from Raichem, (Columbia, MD); all other reagents were of analytical grade.

Preparation and treatment of herb extract: Fresh plants of serviceberry were collected from Browning, MT. The dates, location and conditions when plant samples were gathered were recorded *(*Table 1*)*. Plants were separated into different parts, as shown in Table 1, and air dried, powdered, before being extracted with aqueous methanol [50g powdered sample, 200mL H_2O: MeOH (20:80)], by sonication for 30 min. Samples were filtered and extraction was repeated twice. Filtrates were combined, and methanol was removed using a rotary evaporator. The aqueous extract was partitioned with ethyl acetate (25 mL, three times). The aqueous layer was lyophilized and the ethyl acetate layer was concentrated under vacuum. Dried ethyl acetate ('A' samples) and water ('B' samples) fractions were tested for inhibition of α-glucosidase activity (Fig. 1A and 1B). Sub-fractionations were performed using medium pressure chromatography (Biotage Isolera™, Biotage, LLC; Charlotte, NC) using a 40+M, C18 column, and eluted in a gradient manner with 5% MeOH in water to 100% MeOH. Eluates from medium pressure chromatographic separation were monitored online by their UV profiles. Eight subfractions were obtained for sample 6A (2901 through 2908) and 9 subfractions for sample 6B (3301 through 3309) (Fig. 1C).

α-Glucosidase inhibitory assay in vitro: Mammalian intestinal α-glucosidases is a complex consisting of three individual enzymes, namely sucrase, maltase, and isomaltase (Adachi et al., 2003). In this study, mammalian intestinal α-glucosidases from rat intestinal acetone powder were prepared. Rat intestinal acetone powder (200 mg) was hand-homogenized using 10 mL of ice-cold 50 mM phosphate buffer. After the contents were centrifuged at 8,000 rpm for 25 min, the supernatant was applied to a Sephadex G-100 column and eluted with ice-cold 50 mM phosphate buffer. The activity of rat α-glucosidase extract was verified using p-nitrophenyl-α-D-glucopyranoside as substrate by comparing with pure yeast α-glucosidase (Sigma). An aliquot of enzyme extract was incubated in the presence or absence of serviceberry extracts (5 mg/ml) using either sucrose (56 mM) or maltose (5 mM) as substrate (Shinde et al., 2008). The amount of glucose liberated from the substrate was assayed by glucose oxidase method using a commercially available Autokit (Raichem, Columbia, MD). Acarbose, a widely used anti-diabetic drug and synthetic inhibitor of mammalian α-glucosidase, was used as positive control.

α-Glucosidase inhibitory activity in animals: Animal experimental protocols were evaluated and approved by the Institutional Animal Care and Use Committee at Auburn University. Male C57Bl/6 mice, 4 weeks old, were purchased from the Charles River Laboratories, Inc. (Wilmington, MA). These mice were fed a high-fat diet (D-12451, 45% kcals from fat), Research Diets, Inc. (New Brunswick, NJ) for a period of 8 weeks. After an overnight fast (food deprivation for at least 12 h, but with free access to water), the mice were administrated serviceberry combined subfraction 3307 and 3308 orally, 60 min prior to an oral gavage of sucrose (4 g/kg), starch (3 g/kg), or glucose (2 g/kg). Blood samples were obtained at -60, 0, 15, 30, 60, and 120 min to assay glucose concentrations. Blood glucose levels were measured using

the Accu-Chek® glucometer (Roche Diagnostics, Indianapolis, IN). Control animals received vehicle (water) instead of serviceberry. Acarbose (5 mg/kg body weight) was used as positive control, and administered 60 minutes prior to sucrose-, starch-, or glucose-loading. Data were expressed as mean ± standard error of the mean (SEM). Statistical significance in $AUC_{Glucose}$ between treated and control animals were determined using Student's t-test.

Table 1: Location and conditions during plant sample collection

ID#	Plant part	Location/Sample collection conditions
1	Leaves	Birchcreek, Browning, dry rocky hillside; 1:30 PM, 62°F, patchy clouds
2	Leaves with few berry buds	East Glacier, hill side; 10:30 AM, 90°F, slightly cloudy.
3	Leaves and few buds	East Glacier Lodge (10 miles W of Browning) hillside; 10:30 AM, 62°F, slightly cloudy.
4	Leaves and berry buds	Cut Bank Creek (20 miles NW of Browning) at bridge off Highway 89 and Star School Rd.; 9:30 AM, 88°F, no clouds.
5	Twigs (cut)	Birchcreek area, 30 m of Browning. Dry rocky hillside, no wind. 1:30 PM, 62°F; Mission area 10m S of Browning; 3:00 PM, 67°F; humid.
6	Leaves	East Glacier, hillside; 10:30 AM 90°F, slightly cloudy Also collected from Cut Bank Creek (*see ID#4*)

Results

Serviceberry extract and subfractions inhibited mammalian α-glucosidase activity in vitro: Serviceberry leaf extract (5 mg/ml) samples 6A (ethyl acetate extract) and 6B (aqueous extract) (Table 1) showed considerable rat intestinal α-glucosidase inhibitory activity, using sucrose as substrate, at doses comparable to the positive control, acarbose (*Fig. 1A, B*). Samples 6A and 6B were further sub-fractionated (*Fig. 1C*) and the assay was repeated using sub-fractions 2901 through 2908, and 3301 through 3309. From these, samples 2905 (*Fig. 1D*), and 3307 and 3308 (*Fig. 1E*) demonstrated significant mammalian α-glucosidase inhibition, using sucrose as substrate. Following these results, the α-glucosidase inhibitory activity using maltose as substrate was tested. Samples 3307 and 3308 showed substantial α-glucosidase inhibitory activity (*Fig. 1F*), indicating that samples 3307 and 3308 were effective inhibitors of maltase and sucrose activities. Samples 3307 and 3308 were combined for further testing in an animal model of diet-induced obesity and diabetes.

Serviceberry subfractions lowered post-prandial blood glucose concentrations in high fat diet mice: C57Bl6 mice, fed a high-fat diet (45% kcals from fat) for 8 weeks, showed increased body weights (10.2 ± 1.4 g heavier than control chow-fed mice) and had significantly elevated fasting blood glucose levels (169.4 ± 14.8 mg/dl) compared to mice fed regular chow (132.8 ± 6.9 mg/dl). Serviceberry subfraction (50 mg/kg and 100 mg/kg body weight) was orally administered 60 minutes prior to an oral challenge of sucrose (4 g/kg body weight). Serviceberry subfraction-administered animals showed lower blood glucose levels compared to control animals. Area under the curve for glucose ($AUC_{Glucose}$) in animals administered serviceberry subfraction (50 mg/kg and 100 mg/kg) were significantly lower compared to $AUC_{Glucose}$ of

control group ($p = 0.0096$ and $p = 0.0408$, respectively), and was similar to that of the acarbose group (*Fig. 2A*). We next sought to examine the effect of serviceberry subfraction on starch loading in high-fat fed C57Bl/6 mice. Administration of serviceberry extract (50 mg/kg body weight) prior to starch loading (3 g/kg) resulted in lower blood glucose levels. $AUC_{Glucose}$ of serviceberry subfraction administered group was significantly lower ($p = 0.0017$) than control group, and was similar to that of the acarbose group (Fig.2B). To confirm that the lower $AUC_{Glucose}$ was because of serviceberry subfraction-mediated inhibition of intestinal α-glucosidase, high-fat fed mice were administered glucose and serviceberry extracts. As expected (since α-glucosidase has no effect on the monosaccharide glucose), $AUC_{GLUCOSE}$ of serviceberry subfraction- and glucose-loaded mice were not significantly different from vehicle and glucose-loaded mice ($p = 0.8466$) (*Fig. 2C*). These data indicate that serviceberry leaf subfraction delays or suppresses the absorption of glucose *in vivo* through a mechanism that inhibits sucrase, maltase, and isomaltase activities of the small intestinal α-glucosidases.

Discussion

Serviceberry or Saskatoon berry [*Amelanchier alnifolia* (Nutt.) Nutt. ex. M. Roem (Rosaceae)], native to the North Glacier forests of the Rocky Mountains in Montana, has been used by the Blackfeet Indian tribe in the management of diabetes. Blackfeet Indians pick ripe serviceberries in the fall season and consume them raw, or dry these for use later. Anecdotally, tea made from twigs and leaves have been indicated for optimum health. However, such traditional knowledge of the medicinal properties of *Amelanchier alnifolia* has not been validated by adequate scientific studies. Therefore, using traditional knowledge as a basis, we posited that bioactive components present in *Amelanchier alnifolia* extracts can lower blood glucose levels and potentially mediate anti-diabetic effects. To test this hypothesis, we initially screened serviceberry extracts for α-glucosidase inhibition using yeast α-glucosidase (data not shown), as this was readily available in pure form and widely used (Gowri et al., 2007; Kim et al., 2005). However, several authors have reported inconsistencies in yeast versus mammalian α-glucosidase inhibition, which may be attributed to structural differences (Babu et al., 2004; Lee and Lee, 2001; Shai et al., 2011). Further, mammalian intestinal α-glucosidases are more biologically relevant than yeast α-glucosidase. Therefore, we tested all extracts for inhibitory effects using purified α-glucosidases from rat intestinal acetone powder. We showed that ethyl acetate and aqueous extracts of serviceberry leaves (fractions 6A and 6B) had α-glucosidase inhibitory effects, similar to acarbose. Sub-fractionation of these extracts revealed 2 fractions (3307 and 3308) that showed significant inhibition of both sucrose and maltase activities. However, the α-glucosidase inhibitory activities for these subfractions (5 mg/ml) were not as potent as acarbose. This may be because the subfractions consist of a mixture of compounds and

the activity is masked by non-active compounds. Our findings are comparable to reported rat intestinal α-glucosidase inhibitory activity of *X. zambesiaca, P.africanum, M.mochisia, and C.abbreviata* (IC_{50} > 2.5 mg/ml compared to acarbose IC_{50} = 0.4 mg/ml) (Shai et al., 2011). Recent studies showed that Norton grape skin extract (IC_{50}: 0.384 mg/ml), green tea extract (IC_{50} = 1.34 mg/ml) and oolong tea extract (IC_{50} = 0.735 mg/ml) are potent inhibitors of intestinal α-glucosidases (Oki et al., 1999; Zhang et al., 2011).

Since serviceberry leaf extracts demonstrated potent α-glucosidase inhibitory activity *in vitro*, the extent to which serviceberry extracts can inhibit α-glucosidase *in vivo*, limit or delay starch digestion and absorption, and subsequently reduce post-prandial glycemic response were examined. Acute administration of serviceberry extracts (50 mg/kg body weight) significantly lowered post-prandial blood glucose levels after sucrose loading or a starch meal. Interestingly, $AUC_{Glucose}$ for animals administered the serviceberry subfraction were similar to that of acarbose-administered (5 mg/kg body weight) mice. However, serviceberry subfraction had no effect on glucose-loaded mice, strengthening the view that serviceberry inhibit α-glucosidase activity. While these studies have explored the α-glucosidase inhibitory activity of serviceberry leaf extracts and subfractions, it is currently not known if these also inhibit pancreatic α-amylase activity (Fig.2D). In fact, commercial inhibitors, including acarbose have significant adverse gastrointestinal side effects due to their non-specific inhibition of α-amylase, causing excessive accumulation of carbohydrates in the large intestine (Madar, 1989). Additional studies are needed to identify the bioactive component(s) that mediate the α-glucosidase inhibitory effect. Recently, Lavola et al. (2012) have reported bioactive polyphenols in leaves, stems, and berries of Saskatoon cultivars. *Amelanchier alnifolia* leaf components include quercetin- and

kaempferol-derived glycosides (42% of the phenols), hydroxycinnamic acids (36%), catechins, and neolignans (Lavola et al., 2012).

In conclusion, our findings indicate that serviceberry leaf extracts delay or suppress the absorption of glucose *in vivo* through a mechanism that inhibits sucrase, maltase, and isomaltase activities of the small intestinal α-glucosidases. Furthermore, these findings validate traditional knowledge and suggest that serviceberry leaf extracts may potentially offer a complementary approach in the treatment and management of diabetes.

Acknowledgement

This study was funded by grants from the National Institute of Food and Agriculture (2009-38424-05766) and (ALA095-001).

References

ADA, (2011). Diagnosis and classification of diabetes mellitus. Diabetes Care 34 Suppl 1, S62-69.

Adachi, T., Mori, C., Sakurai, K., Shihara, N., Tsuda, K., Yasuda, K., (2003). Morphological changes and increased sucrase and isomaltase activity in small intestines of insulin-deficient and type 2 diabetic rats. Endocrine Journal 50, 271-279.

Aggarwal, B.B., Sundaram, C., Malani, N., Ichikawa, H., (2007). Curcumin: the Indian solid gold. Advances in Experimental Medicine and Biology 595, 1-75.

Babu, K.S., Tiwari, A.K., Srinivas, P.V., Ali, A.Z., China Raju, B., and Rao, J.M. (2004). Yeast and mammalian alpha-glucosidase inhibitory constituents from Himalayan rhubarb *Rheum emodi* Wall.ex Meisson. Bioorg Med Chem Lett 14, 3841-3845.

Bell, D.S., (2004). Type 2 diabetes mellitus: what is the optimal treatment regimen? Am J Med 116 Suppl 5A, 23S-29S.

Burns Kraft, T.F., Dey, M., Rogers, R.B., Ribnicky, D.M., Gipp, D.M., Cefalu, W.T., Raskin, I., Lila, M.A., (2008). Phytochemical composition and metabolic performance-enhancing activity of dietary berries traditionally used by Native North Americans. Journal of Agricultural and Food Chemistry 56, 654-660.

CDC, (2011). National Diabetes Fact Sheet: national estimates and general information on diabetes and prediabetes in the United States. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention.

Gowri, P.M., Tiwari, A.K., Ali, A.Z., Rao, J.M., (2007). Inhibition of alpha-glucosidase and amylase by bartogenic acid isolated from *Barringtonia racemosa* Roxb. seeds. Phytotherapy Research : PTR 21, 796-799.

Grover, J.K., Yadav, S. and Vats, V., (2002). Medicinal plants of India with anti-diabetic potential. Journal of Ethnopharmacology 81, 81-100.

Johnston, A., (1987). Plants and the Blackfoot. Lethbridge Historical Society (Lethbridge, Alta.).

Kim, Y.M., Jeong, Y.K., Wang, M.H., Lee, W.Y., Rhee, H.I., (2005). Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. Nutrition 21, 756-761.

Knowler, W.C., Pettitt, D.J., Bennett, P.H., Williams, R.C., (1983). Diabetes mellitus in the Pima Indians: genetic and evolutionary considerations. American Journal of Physical Anthropology 62, 107-114.

Lavola, A., Karjalainen, R., Julkunen-Tiitto, R., (2012). Bioactive Polyphenols in Leaves, Stems, and Berries of Saskatoon (*Amelanchier alnifolia* Nutt.) Cultivars. Journal of Agricultural and Food Chemistry 60, 1020-1027.

Lee, D.S., Lee, S.H., (2001). Genistein, a soy isoflavone, is a potent alpha-glucosidase inhibitor. FEBS Letters 501, 84-86.

Madar, Z., (1989). The effect of acarbose and miglitol (BAY-M-1099) on postprandial glucose levels following ingestion of various sources of starch by nondiabetic and streptozotocin-induced diabetic rats. The Journal of Nutrition 119, 2023-2029.

McClintock, W., (2004). The Old North Trail: Or Life, Legends and Religions of the Blackfeet Indians. Kessinger Publishing.

Mentreddy, S.R., (2007). Medicinal plant species with potential antidiabetic properties. Journal of the Science of Food and Agriculture 87, 743-750.

Neel, J.V., (1999). The "thrifty genotype" in 1998. Nutrition Reviews 57, S2-9.

Oki, T., Matsui, T. and Osajima, Y., (1999). Inhibitory effect of alpha-glucosidase inhibitors varies according to its origin. Journal of Agricultural and Food Chemistry 47, 550-553.

Shai, L.J., Magano, S.R., Lebelo, S.L., Mogale, A.M., (2011). Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. Journal of Medicinal Plants Research 5, 2863-2867.

Shinde, J., Taldone, T., Barletta, M., Kunaparaju, N., Hu, B., Kumar, S., Placido, J., Zito, S.W., (2008). Alpha-glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel in vitro and in Goto-Kakizaki (GK) rats. Carbohydr Res 343, 1278-1281.

Swerdlow, J.L., (2000). Nature's medicine : plants that heal. National Geographic Society, Washington, D.C.

Van de Laar, F.A., Lucassen, P.L., Akkermans, R.P., Van de Lisdonk, E.H., Rutten, G.E., Van Weel, C., (2005). Alpha-glucosidase inhibitors for type 2 diabetes mellitus. Cochrane Database Syst Rev, CD003639.

WHO, 2011. Diabetes. Fact Sheet N°312.

Zhang, L., Hogan, S., Li, J., Sun, S., Canning, C., Zheng, S.J., Zhou, K., (2011). Grape skin extract inhibits mammalian intestinal alpha-glucosidase activity and suppresses postprandial glycemic response in streptozocin-treated mice. Food chemistry 126, 466-471.

Figure legends

Fig 1. ***Serviceberry extracts inhibit mammalian α-glucosidase:*** Ethyl acetate (A) or aqueous (B) extracts of serviceberry leaf samples were tested for inhibition of mammalian α-glucosidase activity by incubating with purified rat intestinal α-glucosidase and 56 mM sucrose for 15 min at room temperature. The liberated glucose was measured by glucose kit from RAICHEM at 500 nm. Acarbose (Acar) was used as a positive control. All samples, including acarbose, were used at a concentration of 5 mg/ml. Since fractions 6A and 6B demonstrated significant inhibitory activity, these were further fractionated (C). Ethyl acetate sub-fractions (2901 through 2908) and aqueous subfractions (3301 through 3309) were tested for their ability to inhibit mammalian α-glucosidase using either 56 mM sucrose (D, E) or 5 mM maltose (F) as substrate. Subfractions 3307 and 3308 showed pronounced inhibition of rat intestinal α-glucosidase activity. These subfractions (3307 and 3308) were combined and used for further animal studies.

Fig 2. ***Serviceberry extracts lower post-prandial blood glucose concentrations*****:** Male, C57Bl/6 mice (4 weeks old) were fed a high-fat diet (45% kcals from fat) for a period of 8 weeks. Mice were administered serviceberry combined subfraction 3307 and 3308 [50 mg/kg (Ser1) or 100 mg/kg (Ser2)] orally, 60 min prior to an oral gavage of sucrose (4 g/kg) (A), starch (3 g/kg) (B), or glucose (2 mg/kg) (C). Blood samples were obtained at -60, 0, 15, 30, 60, and 120 min to assay glucose concentrations. Control animals received vehicle (water) instead of serviceberry extracts. Acarbose (5 mg/kg) was used as positive control, and administered 60 minutes prior to sucrose loading. Specificity of α-glucosidase, α-amylase, and β-amylase (D): α-glucosidase catalyzes the hydrolysis of terminal 1,4-linked α-D-glucose residues successively from the non-reducing ends of maltooligosaccharides.

Fig.1

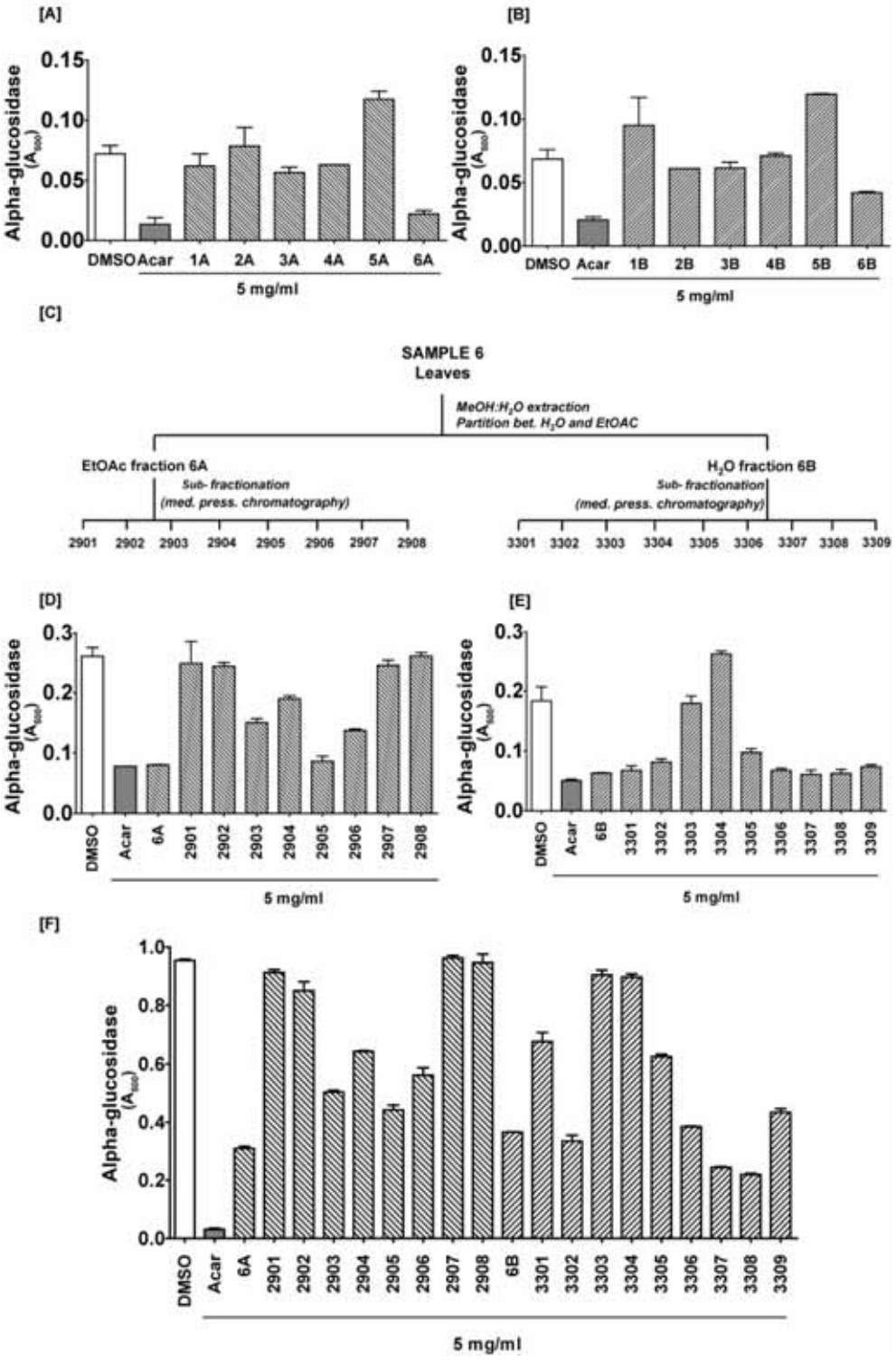
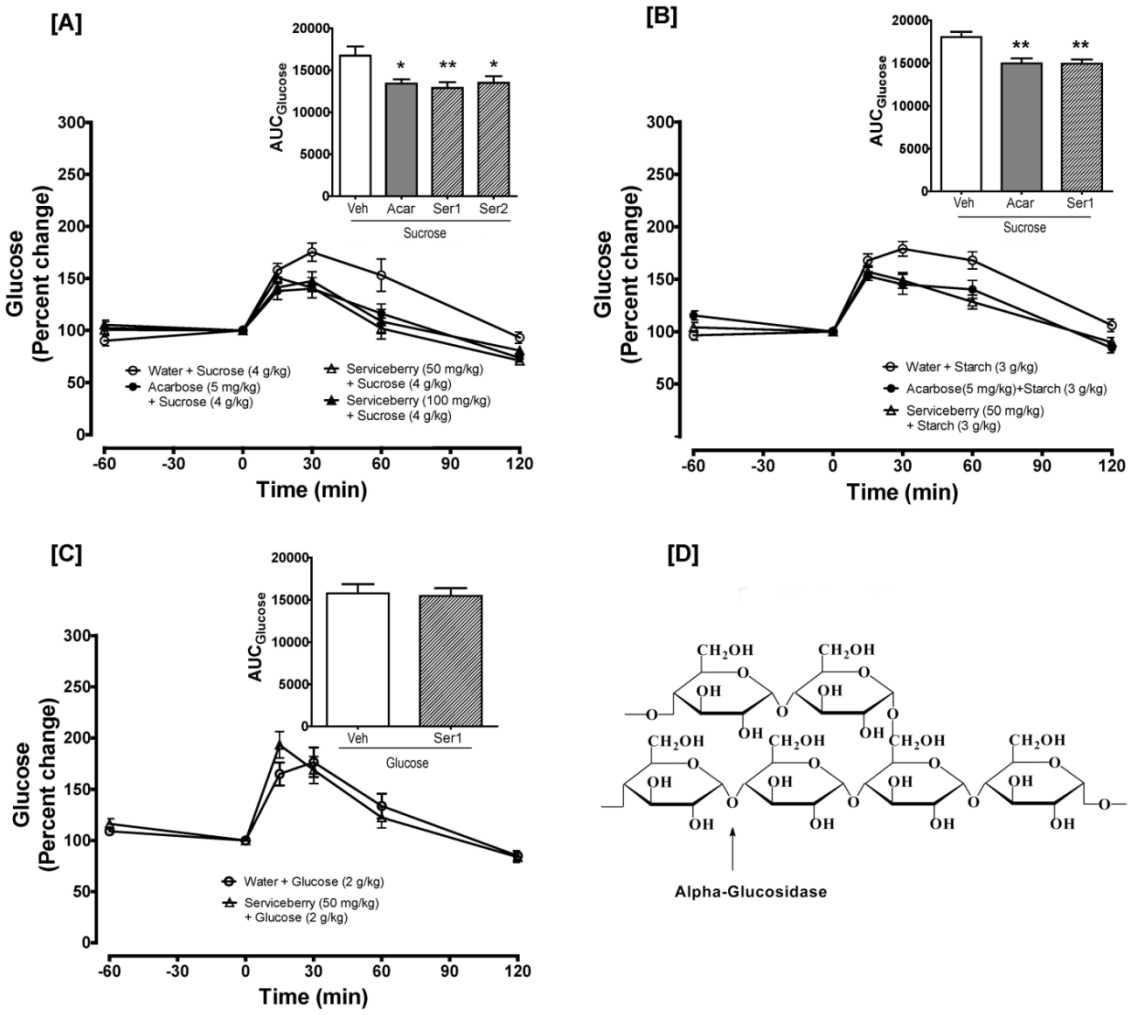

[A]
Alpha-glucosidase (A_{500})
0.15
0.10
0.05
0.00
DMSO Acar 1A 2A 3A 4A 5A 6A
5 mg/ml
[B]
Alpha-glucosidase (A_{500})
0.15
0.10
0.05
0.00
DMSO Acar 1B 2B 3B 4B 5B 6B
5 mg/ml
[C]
SAMPLE 6
Leaves
MeOH:H_2O extraction
Partition bet. H_2O and EtOAC
EtOAc fraction 6A
Sub- fractionation
(med. press. chromatography)
2901 2902 2903 2904 2905 2906 2907 2908
H_2O fraction 6B
Sub- fractionation
(med. press. chromatography)
3301 3302 3303 3304 3305 3306 3307 3308 3309
[D]
Alpha-glucosidase (A_{500})
0.3
0.2
0.1
0.0
DMSO Acar 6A 2901 2902 2903 2904 2905 2906 2907 2908
5 mg/ml
[E]
Alpha-glucosidase (A_{500})
0.3
0.2
0.1
0.0
DMSO Acar 6B 3301 3302 3303 3304 3305 3306 3307 3308 3309
5 mg/ml
[F]
Alpha-glucosidase (A_{500})
1.0
0.8
0.6
0.4
0.2
0.0
DMSO Acar 6A 2901 2902 2903 2904 2905 2906 2907 2908 6B 3301 3302 3303 3304 3305 3306 3307 3308 3309
5 mg/ml

Fig.2


[A]
Glucose (Percent change)
Time (min)
AUCGlucose
Veh
Acar
Ser1
Ser2
Sucrose
Water + Sucrose (4 g/kg)
Acarbose (5 mg/kg) + Sucrose (4 g/kg)
Serviceberry (50 mg/kg) + Sucrose (4 g/kg)
Serviceberry (100 mg/kg) + Sucrose (4 g/kg)
[B]
Water + Starch (3 g/kg)
Acarbose(5 mg/kg)+Starch (3 g/kg)
Serviceberry (50 mg/kg) + Starch (3 g/kg)
[C]
Water + Glucose (2 g/kg)
Serviceberry (50 mg/kg) + Glucose (2 g/kg)
Glucose
[D]
Alpha-Glucosidase

Chapter 4: Anti-diabetic activity of serviceberry [*Amelanchier alnifolia* (Nutt.) Nutt. ex. M. Roem (Rosaceae)], traditionally used by Native American Indian Blackfeet Tribe , associated with activation of AMPK pathway and suppression of hepatic gluconeogenic gene expression

Abstract

Several plant-based remedies offer cost-effective management of diabetes, but few plant species adapted to North America have been validated for their antidiabetic properties. One such species is serviceberry [*Amelanchier alnifolia* (Nutt.) Nutt. ex. M. Roem (Rosaceae)], found in the North Glacier forests of the Rocky Mountains in Montana, which has been traditionally used by the American Indians for the management of type 2 diabetes. However, there have been no scientific studies to validate this anecdotal evidence. The objective of this study was to validate and identify potential anti-diabetic mechanisms of serviceberry. Serviceberry plant samples consisting of leaves, twigs, and leaves with berries were extracted and fractionated. Aqueous extracts of serviceberry demonstrated an increase in glucose uptake in L6GLUT4myc skeletal muscle cells. Chromatographic fractions of serviceberry extracts showed that the ability to stimulate glucose uptake was associated with an increase in the activity of AMP-activated protein kinase (AMPK), and this was independent of insulin receptor activation or intracellular signaling through protein kinase B (PKB/Akt). In H4IIE rat hepatoma cells, serviceberry extracts demonstrated a similar increase in the phosphorylation of AMPK. Consistent with these findings, serviceberry extracts demonstrated a dose-dependent suppression of dexamethasone-induced gluconeogenic gene expression of PEPCK and G6Pase in H4IIE rat hepatoma cells. These findings, in addition to validating traditional knowledge of the antidiabetic properties of serviceberry, indicate that activation of AMPK and suppression of hepatic glucose production may be potential mechanisms that confer the antidiabetic effect of serviceberry extracts.

Introduction

While the prevalence of diabetes in the United States, and globally, is 8.3%, in 2011 (CDC, 2011; IDF, 2011), certain populations have experienced a disproportionately elevated prevalence of diabetes. Compared to the general US population, American Indians are 2.3 times more likely to be diagnosed with diabetes (Gittelsohn and Rowan, 2011). This is most likely caused by factors such as recent adoption of a sedentary lifestyle and a non-traditional diet (Berkes and Farkas, 1978; Hegele, 2001), as well as a genetic predisposition towards obesity (Knowler et al., 1983; Neel, 1999; Skyler, 2004). Currently, there is growing interest in herbal remedies due to side effects associated with therapeutic agents (oral hypoglycemic agents and insulin) for the treatment of diabetes mellitus (Rao et al., 2010). Numerous studies have been carried out to evaluate natural products, including plant materials, as alternative or complementary treatments for diabetes (Yin et al., 2008). Herbs that exhibit antidiabetic activity include *Aloe barbadensis*, *Eugenia jambolana*, *Gymnema sylvestre*, *Ocimum tenuiflorum*, *Trigonella foenum-graecum*, *Allum sativum*, *Galega officinalis, Panax ginseng*, *Momordica charantia* and *Saururus chinensis* Baill (Aggarwal et al., 2007; Grover et al., 2002; Hwang et al., 2007; Mentreddy, 2007; Yin et al., 2008).

Serviceberry (*Amelanchier alnifolia* Nutt., Rosaceae) (also called Saskatoon berry, sarvis berry, Juneberry, shadbush, or Okinoki by the Native Indians), found in the North Glacier forests of the Rocky Mountains in Montana, is a traditional food among Blackfeet Indians. Serviceberries are eaten raw, cooked, or dried (Kindscher, 1987; McClintock, 2004). Furthermore, a tea made from dried serviceberry twigs has been used by Blackfeet Indians for treating diabetes (Johnston, 1987). This traditional knowledge continues to be practiced to this day in a holistic approach in the management of diabetes. However, there have been very few

scientific studies to validate this traditional knowledge. Previously we have shown that serviceberry leaf extracts inhibit mammalian alpha-glucosidase activity and delay the absorption of carbohydrates in a mouse model of diet-induced obesity and hyperglycemia (Zhang et al., 2012). Another recent study by Burns Kraft et al. (2008) showed that *Amelanchier alnifolia* samples improved glucose uptake and lowered the expression of the inflammatory cytokine, IL-1β (Burns Kraft et al., 2008). In this study we examined the effects of serviceberry extracts on metabolic targets that modulate insulin action and glucose homeostasis. Using *in vitro* methods and cell culture, we demonstrate that serviceberry extracts increased glucose uptake in L6GLUT4myc skeletal muscle cells, potentially through activation of AMPK. Consistent with this, serviceberry extracts also activated AMPK in H4IIE rat hepatoma cells and suppressed phosphoenol pyruvate carboxykinase (PEPCK) and glucose-6 phosphatase (G6Pase) gluconeogenic gene expression.

Materials and Methods

Reagents and antibodies. Recombinant human insulin was purchased from Roche Diagnostics (Indianapolis, IL); dexamethasone, poly ($Glu^{80}Tyr^{20}$), from Sigma-Aldrich (St. Louis, MO); [^{3}H] 2-deoxy-D-glucose (2-DOG) from Perkin-Elmer (Boston, MA), and phospho-AMPKalpha (Thr172), AMPKalpha, phospho-Akt (Ser473), and Akt antibody were purchased from Cell Signaling Technology (Beverly, MA).

Plant material collection and preparation of extract: Fresh plants of serviceberry were collected from Browning, MT. The location, time, temperature and conditions when plant samples were harvested were recorded *(Table 1)*. Harvested plants were separated into different parts as shown in Table 1, air dried, powdered, and extracted with aqueous methanol [50 g powdered plant material, 200mL H_2O: MeOH (20:80)], by sonication for 30 min. Plant materials were filtered and extraction was repeated twice. Filtrates were combined, and methanol was removed using a Rotary evaporator. The aqueous extract was partitioned with ethyl acetate (25 mL, three times).The ethyl acetate layer was concentrated under vacuum and the aqueous layer was lyophilized. Dried ethyl acetate ('A' samples) and water ('B' samples) fractions were used for the initial screening of bioactivity (Figs. 1A and 1B). Samples 2B and 5B (leaves with few berry buds, and twigs, respectively) were lyophilized and further fractionated using medium pressure chromatography onto a 40+M, C18 column, and eluted in a gradient manner with 5% MeOH in water to 100% MeOH. Eluates from medium pressure chromatographic separation were monitored by their on-line UV profiles. Similar fractions were combined yielding 5 sub-fractions for 2B (17-1 through 17-5) and four for 5B (17-6 through 17-9) (Fig. 1C).

Cell culture. H4IIE rat hepatoma cells were purchased from American Type Culture Collection (Manassas, VA, USA). These were cultured in DMEM (1g/L glucose) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. L6 muscle cells stably expressing myc-tagged GLUT4 (L6-GLUT4myc cells) were obtained from Dr. Amira Klip, and cultured as described previously (Ueyama et al., 1999).

Glucose uptake assay. Serum-starved, confluent L6-GLUT4myc myoblasts were pre-incubated with various serviceberry extracts and sub-fractions for 30 minutes before insulin (100 nM) treatment was for 30 minutes. Glucose uptake was assayed using [^{3}H] 2-deoxy-D-glucose (2-DOG), as described earlier (Klip et al., 1982).

Insulin receptor tyrosine kinase activity (IR-TKA). Insulin receptors (IRs) were partially purified from rat liver, as described previously (Mathews et al., 2000). IRs were pre-incubated with serviceberry sub-fractions in assay buffer (25mM HEPES, pH 7.4, 0.1% Triton X-100, 0.05% BSA) for 30 minutes prior to insulin stimulation (100 nM) for 10 minutes. An exogenous substrate, poly ($Glu^{80}Tyr^{20}$) was added and phosphorylation was carried out as described (Mathews et al., 2000).

Quantitative real-time PCR. H4IIE cells were treated 100 nM insulin, and/or 5 μM dexamethasone, in the presence or absence of serviceberry sub-fractions for 24 hours. RNA was extracted using RNeasy Mini RNA isolation kit (QIAGEN, Valencia, CA). cDNA was synthesized by using iScript cDNA synthesis kit (BIO-RAD, Hercules, CA) and real-time PCR was performed using iQ SYBR Green Supermix (BIO-RAD) according to protocols

recommended by the manufacturers. Primer sequences for real-Time PCR were as follows: Phosphoenol pyruvate carboxy kinase (PEPCK) forward 5'-GGG TGC TAG ACT GGA TCT GC-3', PEPCK reverse 5'-GAG GGA GAA CAG CTG AGT GG-3'; glucose-6 phosphatase (G6Pase) forward 5'-GGG TGT AGA CCT CCT GTG GA-3', G6Pase reverse 5'-GAG CCA CTT GCT GAG TTT CC-3'; β-actin forward 5'- CCT CTA TGC CAA CAC AGT GC-3', β-actin reverse 5'-CAT CGT ACT CCT GCT TGC TG-3'. Reaction conditions were as follows: 95^{o}C, 3:0 min; 95 ^{o}C, 0:15 min, 60 ^{o}C, 0:30 min, 72 ^{o}C, 0:30 min, repeated 40 cycles; 55 ^{o}C, 0:10 min, repeated 80×. Expression levels were normalized to β-actin and gene expression was calculated as $2^{-\Delta\Delta C}{}_{T}$ and expressed as fold change, as described by earlier (Livak and Schmittgen, 2001). All assays were carried out in triplicate.

Western blot analyses of phospho-AMPK and phospho-Akt. To assay AMPK activation, confluent 6-well plates of H4IIE cells and differentiated L6 GLUT4myc cells were serum-starved for 30 minutes prior to incubation with serviceberry sub-fractions, AICAR (0.5 mM), or metformin (2 mM) in serum-free media for 30 min. For time-course study, serum-starved L6 cells were incubated with serviceberry sub-fractions for 0.5, 1, 3 or 8h. Vehicle used was DMSO (0.2%). For Akt activation assay, differentiated L6-GLUT4myc cells were serum-starved overnight, prior to treatment with serviceberry sub-fractions or 100 nM insulin for 10 min. Cells were washed three times with ice-cold PBS and lysed in 300 µl of cell lysis buffer [50 mM HEPES, pH7.4, 100 mM sodium pyrophosphate, 10 mM EDTA, 20 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, 0.01 mg/ml aprotinin, 0.01 mg/ml leupeptin, 1% Triton X-100, and protease inhibitor cocktail tablet (Roche Diagnostics, Indianapolis)]. Cell lysate proteins were separated by 4–20% SDS–PAGE precast gels (NuSep Inc., Austell, GA) followed

by Western blot analysis using antibodies against phospho-AMPKα (Thr172) or phospho-Akt (Ser473). The membranes were stripped and reblotted using AMPK or Akt antibodies, respectively, for loading equivalence. Chemiluminescence detection and image analysis were performed with UVP-Biochimie Bioimager and LabWorks software (UVP, Upland, CA).

Results

Serviceberry extracts increase glucose uptake in skeletal muscle cells. Serviceberry extracts were tested for their effects on glucose uptake in L6GLUT4myc skeletal muscle cells, both in the basal and insulin-stimulated conditions (*Fig. 1A,B*), by determining the incorporation of ^{3}H-labelled 2-deoxyglucose, a non-metabolizable glucose analog. The L6GLUT4myc cell line stably expresses the GLUT4 protein, and responds to insulin treatment with ~ 2-fold stimulation of glucose uptake (Ueyama et al., 1999; Wang et al., 1998). Incubation of serviceberry extracts 2B and 5B for 1 hour, following a 5-hour serum-starvation, stimulated glucose uptake at levels nearly similar to an insulin-stimulated (100 nM, 30 min) glucose uptake in L6GLUT4myc myoblasts (*Fig. 2B*). Further, samples 2B and 5B seemed to potentiate insulin-stimulated glucose uptake. A short-term incubation (20 minutes) with serviceberry extracts was not as effective in increasing glucose uptake (Supplemental data, *Fig. S1*). Next, serviceberry extracts 2B and 5B were sub-fractionated (*Fig. 1C*) and the sub-fractions were tested for their effects on glucose uptake. Compared to a 2-fold insulin-stimulation (100 nM, 30 min), incubation with sub-fraction 17-2 (40 µg/mL, 1 hour) demonstrated a ~ 1.7-fold increase in glucose uptake and sub-fraction 17-7 (40 µg/ml, 1 hour) showed a ~1.4-fold increase in glucose uptake in the absence of insulin (*Fig. 1D)*. Since samples 1A through 6A, and 1B through 6B were initial fractions, these extracts were used to screen for several assays including glucose uptake (basal and insulin-stimulated), insulin receptor activation, alpha-glucosidase inhibition (data not shown), and dipeptidyl peptidase-4 inhibition (Supplemental data, *Fig. S2*). The limited availability of serviceberry extracts dictated experimental set-up conditions, and the absence of error bars indicate that individual experiments were not replicated. However, it is important to note that sequential

experiments confirmed findings. This is evident through an increased glucose uptake for serviceberry extracts 2B, 5B, and the sub-fractions 17-2, and 17-7.

Mechanisms mediating glucose uptake in skeletal muscle cells. Since serviceberry extracts demonstrated an insulin-like or insulin-sensitizing effect on glucose uptake in skeletal muscle cells, we explored potential mechanisms that mediate these effects. We demonstrated that neither serviceberry extracts 2B and 5B, nor serviceberry sub-fractions had an effect on the activation of insulin receptor tyrosine kinase (*Fig. 2A*). Next, we examined the effect of serviceberry extracts on Akt activation in L6 myotubes. While insulin (100 nM) demonstrated a strong activation of Akt, serviceberry sub-fractions 17-2, and 17-7 did not phosphorylate Akt in L6 myotubes (*Fig. 2B*). These findings suggested that the effects of serviceberry extracts on increasing glucose uptake were not mediated through activation of the insulin receptor or signaling through the PI-3 kinase-Akt pathway, but through another mechanism. Since activation of AMPK has been shown to increase glucose uptake (Hayashi et al., 1998), independent of insulin signaling pathway, we examined serviceberry's effects on AMPK activation. We examined both a short-term treatment (up to 1 hour) and a long-term treatment (3 – 8 hours) on AMPK activation. In the short-term treatment, serviceberry extracts elicited only marginal effects on AMPK phosphorylation status (*Fig. 2C,D* & Supplemental data, *Fig. S3*). However, long-term treatment (up to 8 hours) of serviceberry sub-fractions 17-2 and 17-7 (50 μg/ml) significantly increased AMPK phosphorylation, in L6GLUT4myc myoblasts (*Fig. 2C,D*).

Serviceberry extracts activate AMPK and suppress dexamethasone-induced gluconeogenic gene expression in H4IIE rat hepatoma cells. Previous studies have clearly established a role for AMPK activation in the suppression of two key gluconeogenic genes phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) (Lochhead et al., 2000). Therefore, we tested the hypothesis that serviceberry extracts inhibit gluconeogenic gene expression via activation of AMPK in H4IIE hepatoma cells. We demonstrate that serviceberry sub-fractions 17-2 and 17-7 (50 µg/ml, 30 min) increase phosphorylation of AMPK (*Fig. 3A*), albeit to a lesser extent compared to 5-aminoimidazole-4-carboxamide riboside (AICAR, 0.5 mM) or metformin (2 mM). Next, we examined the effect of serviceberry sub-fractions on gluconeogenic gene expression. Treatment of H4IIE cells with dexamethasone increased G6Pase and PEPCK gene expression nearly 5-fold. As expected, insulin (100 nM) significantly suppressed G6Pase and PEPCK gene expression. Treatment with serviceberry sub-fractions 17-2 and 17-7 (50 µg/ml) decreased G6Pase and PEPCK gene expression to ~50% of basal *(Fig.3A,B)*. Additionally, serviceberry sub-fractions suppressed dexamethasone-induced G6Pase and PEPCK gene expression, in a dose-dependent manner. This suppression of gluconeogenic gene expression was similar to insulin-mediated suppression of G6Pase and PEPCK. Serviceberry sub-fractions and insulin (100 nM), synergistically suppressed dexamethasone-induced gene expression of both PEPCK and G6Pase.

Discussion

The Blackfeet Indians of Montana have traditionally used serviceberry (*Amelanchier alnifolia*) both for food and medicine, incorporating it in soups, stews, and pemmican, and making a tea from dried twigs for the management of diabetes. Earlier, we reported that serviceberry leaf extracts inhibited mammalian alpha glucosidase and lowered post-prandial glycemic response in a mouse model of diet-induced obesity and hyperglycemia (Zhang et al., 2012). Also, another earlier study demonstrated that water extracts of *A.alnifolia* increased glycogen accumulation in L6 rat skeletal muscle cells and reduced IL-1β gene expression (Burns Kraft et al., 2008). These studies suggest a potential benefit with regard to diabetes by improving blood glucose utilization and decreasing inflammatory markers. In this study, we have presented evidence showing that water extracts of serviceberry and its chromatographic sub-fractions increased glucose uptake in L6GLUT4myc rat skeletal muscle cells. This increase in glucose uptake was independent of insulin receptor activation or Akt phosphorylation, and may be attributed to activation of AMPK. Furthermore, we have shown that *A. anifolia* subfractions activated AMPK and suppressed dexamethasone-induced gluconeogenic gene expression in H4IIE rat hepatoma cells.

AMPK, a "metabolic master switch" that mediates cellular and whole-body energy homeostasis, has been implicated as a potential target of type 2 diabetes mellitus and obesity. Activation of AMPK in skeletal muscle has been shown to increase glucose uptake in skeletal muscle (Hardie, 2004; Horike et al., 2008; Mues et al., 2009). Exercise or muscle contraction is a prototypical AMPK activator (Hayashi et al., 1998). AICAR, an analog of AMP, activates AMPK and increases glucose uptake through the recruitment of GLUT4 to plasma membrane (Bergeron et al., 1999; Kurth-Kraczek et al., 1999). AMPK is a direct adenylate charge-sensing

protein kinase (Oakhill et al.) that is regulated by phosphorylation of AMPKα subunit on Thr172 by upstream kinases including Ca^{2+}/calmodulin-dependent kinase kinase β (CaMKKβ) (Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005) and liver kinase B1 (LKB1) (Shaw et al., 2005), which promotes AMP binding to AMPKγ subunit. Our studies demonstrate that long-term treatment of L6Glut4 myc rat skeletal muscle cells (up to 8 hours) with serviceberry extracts activated AMPK significantly compared to a short-term treatment (up to 1 hour). It may therefore be speculated that serviceberry extracts activate AMPK potentially through allosteric activation by AMP, rather than by activating upstream kinases such as CaMKKβ or LKB1.

Hepatic glucose production (HGP) is a major cause of fasting hyperglycemia in diabetic subjects. Activation of AMPK by AICAR treatment was shown to inhibit HGP in both normal and insulin-resistance obese rats (Bergeron et al., 2001). Further, activation of AMPK has been shown to phosphorylate and inactivate ACC, resulting in the inhibition of conversion of acetyl CoA to malonyl CoA for fatty acid synthesis, and increasing fatty acid oxidation (Gruzman et al., 2009). A large body of evidence from clinical studies and animal models suggest that metformin, currently the drug of first choice for the treatment of type 2 diabetes, decreases hepatic glucose production by inhibiting hepatic gluconeogenesis (Natali and Ferrannini, 2006) via activation of AMPK (Shaw et al., 2005; Zhou et al., 2001). Our findings that serviceberry subfractions inhibited hepatic gluconeogenic gene expression of PEPCK and G6Pase in H4IIE cells, were consistent with its effects on activation of AMPK. Interestingly, several polyphenols including resveratrol, apigenin, and S17834, a synthetic polyphenol, and epigallocatechin-3-gallate, a green tea polyphenol, have been shown to be potent activators of AMPK (Collins et al., 2007; Zang et al., 2006; Zhang et al., 2010), albeit by different mechanisms. Epigallocatechin-3-gallate (EGCG) activates AMPK mediated by Ca^{2+}/calmodulin-dependent protein kinase kinase

(CaMKK) in rat primary hepatocytes (Collins et al., 2007), whereas resveratrol activates AMPK through SIRT1/LKB1, but not CaMKK in HepG2 hepatoma (Hou et al., 2008).

Several bioactive components have been identified in *A. alnifolia* berries. *A. alnifolia* cultivars showed a high concentration of total polyphenols in berries (Bakowska-Barczak and Kolodziejczyk, 2008). These authors identified cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, and cyanidin-3-xyloside as four major anthocyanin compounds, confirming previous findings (Mazza, 1986; Mazza and Cottrell, 2008). Hydroxycinnamic acid derivatives, chlorogenic acid, flavonols (quercetin derivatives) and procyanidins consisting mainly of epicatechin units were also identified (Bakowska-Barczak and Kolodziejczyk, 2008; Hellstrom et al., 2007). *A. alnifolia* berries demonstrated high antioxidant activity that was associated with its high content of polyphenols (Bakowska-Barczak and Kolodziejczyk, 2008; Hu et al., 2005). Adhikari et al. isolated 6 components from ethyl acetate and methanol extracts of *A. alnifolia* berries that demonstrated inhibition of cyclooxygenase enzymes activity (Adhikari et al., 2006). Recently, Lavola et al. have reported bioactive polyphenols in leaves and stems of Saskatoon cultivars. *A. alnifolia* leaf components include quercetin- and kaempferol-derived glycosides (42% of the phenols), hydroxycinnamic acids (36%), catechins, and neolignans (Lavola et al., 2012). Quercetin-derived glycosides were also shown to enhance glucose uptake by 38–59% (50 mM; 18 h treatment) in the absence of insulin, through the activation of AMPK pathway (Eid et al., 2010). Chlorogenic acid and ferulic acid show a modest but significant increase in 2-deoxy-D-glucose transport into L6 myotubes, comparable to metformin and 2,4-thiazolodiendione, two common commercial oral hypoglycemic drugs (Prabhakar and Doble, 2009). Additional studies are warranted to identify the bioactive components in serviceberry leaves and stems that mediate its antidiabetic effects.

Overall, these studies have shown that an improvement of glucose uptake in skeletal muscle cells, and suppression of hepatic glucose production may explain the glucose lowering effects of serviceberry extracts. This novel mechanism of action of serviceberry extracts, along with its fruit's well-characterized antioxidant and anti-inflammatory properties (Bakowska-Barczak and Kolodziejczyk, 2008; Hu et al., 2005), suggest a potential complementary role for serviceberry extracts or its bioactive component(s) in the management of diabetes.

Acknowledgement

This study was funded by grants from the National Institute of Food and Agriculture (2009-38424-05766) and (ALA095-001). Technical help of Gloria Hervey is greatly appreciated.

References

Adhikari, D.P., Schutzki, R.E., DeWitt, D.L. and Nair, M.G., (2006). Effects of *Amelanchier* fruit isolates on cyclooxygenase enzymes and lipid peroxidation. Food chemistry 97, 56-64.

Aggarwal, B.B., Sundaram, C., Malani, N. and Ichikawa, H., (2007). Curcumin: the Indian solid gold. Adv Exp Med Biol 595, 1-75.

Bakowska-Barczak, A.M. and Kolodziejczyk, P., (2008). Evaluation of Saskatoon berry (*Amelanchier alnifolia* Nutt.) cultivars for their polyphenol content, antioxidant properties, and storage stability. J Agric Food Chem 56, 9933-9940.

Bergeron, R., Kjaer, M., Simonsen, L., Bulow, J., Skovgaard, D., Howlett, K. and Galbo, H., (2001). Splanchnic blood flow and hepatic glucose production in exercising humans: role of renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol 281, R1854-1861.

Bergeron, R., Russell, R.R., 3rd, Young, L.H., Ren, J.M., Marcucci, M., Lee, A. and Shulman, G.I., (1999). Effect of AMPK activation on muscle glucose metabolism in conscious rats. Am J Physiol 276, E938-944.

Berkes, F. and Farkas, C.S., (1978). Eastern James Bay Cree Indians: changing patterns of wild food use and nutrition. Ecology of Food and Nutrition 7, 155-172.

Burns Kraft, T.F., Dey, M., Rogers, R.B., Ribnicky, D.M., Gipp, D.M., Cefalu, W.T., Raskin, I. and Lila, M.A., (2008). Phytochemical composition and metabolic performance-enhancing activity of dietary berries traditionally used by Native North Americans. J Agric Food Chem 56, 654-660.

CDC, 2011. National Diabetes Fact sheet http://www.cdc.gov/diabetes/pubs/factsheet11.htm.

Collins, Q.F., Liu, H.Y., Pi, J., Liu, Z., Quon, M.J. and Cao, W., (2007). Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5'-AMP-activated protein kinase. J Biol Chem 282, 30143-30149.

Eid, H.M., Martineau, L.C., Saleem, A., Muhammad, A., Vallerand, D., Benhaddou-Andaloussi, A., Nistor, L., Afshar, A., Arnason, J.T. and Haddad, P.S., (2010). Stimulation of AMP-activated protein kinase and enhancement of basal glucose uptake in muscle cells by quercetin and quercetin glycosides, active principles of the antidiabetic medicinal plant Vaccinium vitis-idaea. Mol Nutr Food Res 54, 991-1003.

Gittelsohn, J. and Rowan, M., (2011). Preventing diabetes and obesity in American Indian communities: the potential of environmental interventions. Am J Clin Nutr 93, 1179S-1183S.

Grover, J.K., Yadav, S. and Vats, V., (2002). Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 81, 81-100.

Gruzman, A., Babai, G. and Sasson, S., (2009). Adenosine monophosphate-activated protein kinase (AMPK) as a new target for antidiabetic drugs: a review on metabolic, pharmacological and chemical considerations. The review of diabetic studies: RDS 6, 13.

Hardie, D.G., (2004). AMP-activated protein kinase: a master switch in glucose and lipid metabolism. Rev Endocr Metab Disord 5, 119-125.

Hawley, S.A., Pan, D.A., Mustard, K.J., Ross, L., Bain, J., Edelman, A.M., Frenguelli, B.G. and Hardie, D.G., (2005). Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. Cell Metab 2, 9-19.

Hayashi, T., Hirshman, M.F., Kurth, E.J., Winder, W.W. and Goodyear, L.J., (1998). Evidence for 5' AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. Diabetes 47, 1369-1373.

Hegele, R.A., (2001). Genes and environment in type 2 diabetes and atherosclerosis in aboriginal Canadians. Curr Atheroscler Rep 3, 216-221.

Hellstrom, J., Sinkkonen, J., Karonen, M. and Mattila, P., (2007). Isolation and structure elucidation of procyanidin oligomers from Saskatoon berries (*Amelanchier alnifolia*). J Agric Food Chem 55, 157-164.

Horike, N., Sakoda, H., Kushiyama, A., Ono, H., Fujishiro, M., Kamata, H., Nishiyama, K., Uchijima, Y., Kurihara, Y., Kurihara, H. and Asano, T., (2008). AMP-activated protein kinase activation increases phosphorylation of glycogen synthase kinase 3beta and thereby reduces cAMP-responsive element transcriptional activity and phosphoenolpyruvate carboxykinase C gene expression in the liver. J Biol Chem 283, 33902-33910.

Hou, X., Xu, S., Maitland-Toolan, K.A., Sato, K., Jiang, B., Ido, Y., Lan, F., Walsh, K., Wierzbicki, M. and Verbeuren, T.J., (2008). SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. Journal of Biological Chemistry 283, 20015-20026.

Hu, C., Kwok, B.H.L. and Kitts, D.D., (2005). Saskatoon berries (*Amelanchier alnifolia* Nutt.) scavenge free radicals and inhibit intracellular oxidation. Food research international 38, 1079-1085.

Hurley, R.L., Anderson, K.A., Franzone, J.M., Kemp, B.E., Means, A.R. and Witters, L.A., (2005). The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. J Biol Chem 280, 29060-29066.

Hwang, J.Y., Zhang, J., Kang, M.J., Lee, S.K., Kim, H.A., Kim, J.J. and Kim, J.I., (2007). Hypoglycemic and hypolipidemic effects of *Saururus chinensis* Baill in streptozotocin-induced diabetic rats. Nutr Res Pract 1, 100-104.

IDF, (2011). 5th Edition of the Diabetes Atlas released on World Diabetes Day http://www.idf.org/diabetesatlas/news/fifth-edition-release.

Johnston, A., (1987). Plants and the Blackfoot. Lethbridge Historical Society (Lethbridge, Alta.).

Kindscher, K., (1987). Edible Wild Plants of the Prairie - An Ethnobotanical Guide. University Press of Kansas, Lawrence, Kansas 66049.

Klip, A., Logan, W.J. and Li, G., (1982). Hexose transport in L6 muscle cells. Kinetic properties and the number of [3H]cytochalasin B binding sites. Biochim Biophys Acta 687, 265-280.

Knowler, W.C., Pettitt, D.J., Bennett, P.H. and Williams, R.C., (1983). Diabetes mellitus in the Pima Indians: genetic and evolutionary considerations. Am J Phys Anthropol 62, 107-114.

Kurth-Kraczek, E.J., Hirshman, M.F., Goodyear, L.J. and Winder, W.W., (1999). 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. Diabetes 48, 1667-1671.

Lavola, A., Karjalainen, R. and Julkunen-Tiitto, R., (2012). Bioactive Polyphenols in Leaves, Stems, and Berries of Saskatoon (*Amelanchier alnifolia* Nutt.) Cultivars. Journal of Agricultural and Food Chemistry 60, 1020-1027.

Livak, K.J. and Schmittgen, T.D., (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402-408.

Lochhead, P.A., Salt, I.P., Walker, K.S., Hardie, D.G. and Sutherland, C., (2000). 5-aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. Diabetes 49, 896-903.

Mathews, S.T., Chellam, N., Srinivas, P.R., Cintron, V.J., Leon, M.A., Goustin, A.S. and Grunberger, G., (2000). Alpha2-HSG, a specific inhibitor of insulin receptor autophosphorylation, interacts with the insulin receptor. Mol Cell Endocrinol 164, 87-98.

Mazza, G., (1986). Anthocyanins and Other Phenolic Compounds of Saskatoon Berries (*Amelanchier alnifolia* Nutt.). Journal of Food Science 51, 1260-1264.

Mazza, G. and Cottrell, T., (2008). Carotenoids and cyanogenic glucosides in saskatoon berries (*Amelanchier alnifolia* Nutt.). Journal of Food Composition and Analysis 21, 249-254.

McClintock, W., (2004). The Old North Trail: Or Life, Legends and Religions of the Blackfeet Indians. Kessinger Publishing.

Mentreddy, S.R., (2007). Medicinal plant species with potential antidiabetic properties. Journal of the Science of Food and Agriculture 87, 743-750.

Mues, C., Zhou, J., Manolopoulos, K.N., Korsten, P., Schmoll, D., Klotz, L.O., Bornstein, S.R., Klein, H.H. and Barthel, A., (2009). Regulation of glucose-6-phosphatase gene expression by insulin and metformin. Horm Metab Res 41, 730-735.

Natali, A. and Ferrannini, E., (2006). Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. Diabetologia 49, 434-441.

Neel, J.V., 1999. The "thrifty genotype" in 1998. Nutr Rev 57, S2-9.

Oakhill, J.S., Steel, R., Chen, Z.P., Scott, J.W., Ling, N., Tam, S. and Kemp, B.E., (2011). AMPK is a direct adenylate charge-regulated protein kinase. Science 332, 1433-1435.

Prabhakar, P.K. and Doble, M., (2009). Synergistic effect of phytochemicals in combination with hypoglycemic drugs on glucose uptake in myotubes. Phytomedicine 16, 1119-1126.

Rao, M.U., Sreenivasulu, M., Chengaiah, B., Reddy, K.J. and Chetty, C.M., (2010). Herbal Medicines for Diabetes Mellitus: A Review. Int J PharmTech Res 2, 1883-1892.

Shaw, R.J., Lamia, K.A., Vasquez, D., Koo, S.H., Bardeesy, N., Depinho, R.A., Montminy, M. and Cantley, L.C., (2005). The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 310, 1642-1646.

Skyler, J.S., (2004). Diabetes mellitus: pathogenesis and treatment strategies. J Med Chem 47, 4113-4117.

Ueyama, A., Yaworsky, K.L., Wang, Q., Ebina, Y. and Klip, A., (1999). GLUT-4myc ectopic expression in L6 myoblasts generates a GLUT-4-specific pool conferring insulin sensitivity. Am J Physiol 277, E572-578.

Wang, Q., Khayat, Z., Kishi, K., Ebina, Y. and Klip, A., (1998). GLUT4 translocation by insulin in intact muscle cells: detection by a fast and quantitative assay. FEBS Lett 427, 193-197.

Woods, A., Dickerson, K., Heath, R., Hong, S.P., Momcilovic, M., Johnstone, S.R., Carlson, M. and Carling, D., (2005). Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell Metab 2, 21-33.

Yin, J., Zhang, H. and Ye, J., (2008). Traditional chinese medicine in treatment of metabolic syndrome. Endocr Metab Immune Disord Drug Targets 8, 99-111.

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

Zhang, A.J., Rimando, A.M., Fish, W., S.R., M. and Mathews, S.T., (2012). Serviceberry (Amelanchier alnifolia) leaf extract inhibits mammalian α-glucosidase activity and

suppresses postprandial glycemic response in a mouse model of diet-induced obesity and hyperglycemia. J Ethnopharmacol, manuscript in review.

Zhang, Z.F., Li, Q., Liang, J., Dai, X.Q., Ding, Y., Wang, J.B. and Li, Y., (2010). Epigallocatechin-3-O-gallate (EGCG) protects the insulin sensitivity in rat L6 muscle cells exposed to dexamethasone condition. Phytomedicine 17, 14-18.

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. and Moller, D.E., (2001). Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 108, 1167-1174.

Figure legends

Fig 1. Serviceberry extracts increase glucose uptake in L6GLUT4myc skeletal muscle cells

L6GLUT4-myc rat myoblasts were treated with serviceberry plant extracts (1A-3A, 400 μg/ml; 4A-6A, 100 μg/ml, and 1B-6B, 400 μg/ml) in the presence (A) or absence of 100 nM insulin (B). *Amelanchier alnifolia* leaf extract 2B (40 and 100 μg/ml) and stem extract 5B (40 and 100 μg/ml), treated for 1 hour, showed increased [^{3}H] 2-deoxy-D-glucose uptake activity in L6GLUT4 skeletal muscle cells in the absence of insulin (B). *A. alnifolia* extracts 2B and 5B were further fractionated using medium pressure chromatography to yield 5 sub-fractions for 2B (17-1 through 17-5) and 4 sub-fractions for 5B (17-6 through 17-9) (C). Sub-fractions 17-2 (5 μg/mL) and 17-7 (5 μg/mL) demonstrated an increase in glucose uptake in L6GLUT4 skeletal muscle cells in the absence of insulin (D).

Fig 2. Serviceberry extracts increase AMPK phosphorylation (Thr172) in L6GLUT4myc skeletal muscle cells, without affecting insulin signal transduction

Partially-purified insulin receptors from rat liver were incubated with *A. alnifolia* extracts and sub-fractions (125 μg/ml) for 30 minutes and insulin receptor tyrosine kinase (IR-TK) activation was assayed by the incorporation of ^{32}P from ^{32}P [γ-ATP] onto poly (Glu,Tyr), an exogenous substrate (A). Differentiated L6GLUT4myc myotubes were treated with serviceberry leaf- (17-2) and stem sub-fraction (17-7) and cell lysates were immunoblotted with phospho-Akt (Ser473) and Akt (B) antibodies, or phospho-AMPK (Thr172) and AMPK-α antibodies (C,D). Data are expressed as fold-change over basal for IR-TK activity and AMPK activation.

Fig 3. Serviceberry extracts activate AMPK and suppresses dexamethasone-induced PEPCK and G6Pase expression in rat hepatoma cells.

H4IIE rat hepatoma cells were incubated with AICAR 0.5 mM, Metformin 2 mM or serviceberry sub-fractions 17-2 and 17-7 for 30 min. Cell lysates were immunoblotted for phospho-AMPK (Thr172) and AMPK-α (A). H4IIE rat hepatocytes were treated with dexamethasone (Dex) and serviceberry sub-fractions 17-2 (B) with or without insulin, and 17-7 (C) for 24 hours. Total RNA was isolated and gene expression of PEPCK and G6Pase were analyzed by real-time PCR. Data are expressed as fold change over basal relative to the housekeeping gene, β-actin.

Table 1: Location and conditions during plant sample collection

ID#	Plant part	Location/Sample collection conditions
1	Leaves	Birchcreek, Browning, dry rocky hillside; 1:30 PM, 62°F, patchy clouds
2	Leaves with few berry buds	East Glacier, hill side; 10:30 AM, 90°F, slightly cloudy.
3	Leaves and few buds	East Glacier Lodge (10 miles W of Browning) hillside; 10:30 AM, 62°F, slightly cloudy.
4	Leaves and berry buds	Cut Bank Creek (20 miles NW of Browning) at bridge off Highway 89 and Star School Rd.; 9:30 AM, 88°F, no clouds.
5	Twigs (cut)	Birchcreek area, 30 m of Browning. Dry rocky hillside, no wind. 1:30 PM, 62°F; Mission area 10m S of Browning; 3:00 PM, 67°F; humid.
6	Leaves	East Glacier, hillside; 10:30 AM 90°F, slightly cloudy Also collected from Cut Bank Creek (*see ID#4*)

Fig 1

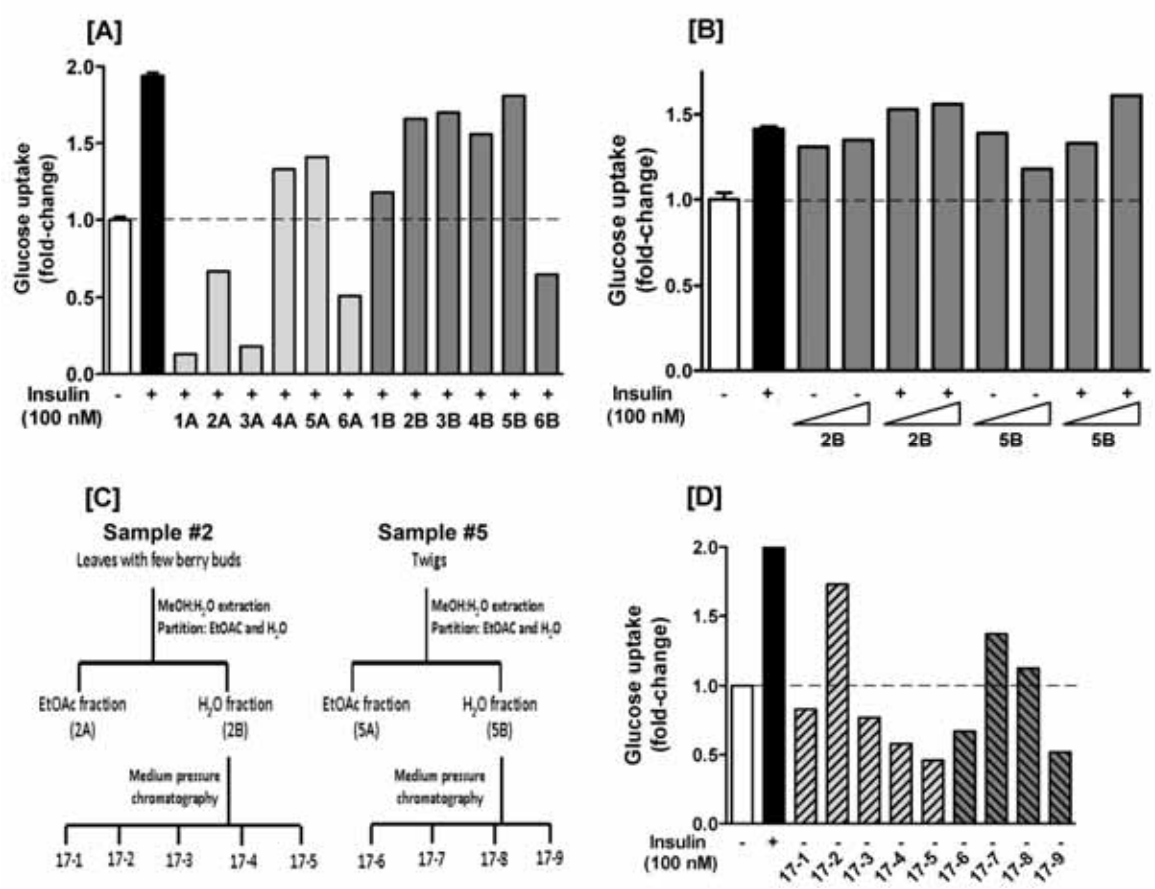

[A]
Glucose uptake (fold-change)
2.0
1.5
1.0
0.5
0.0
Insulin (100 nM) - + + + + + + + + + + + + +
1A 2A 3A 4A 5A 6A 1B 2B 3B 4B 5B 6B
[B]
Glucose uptake (fold-change)
1.5
1.0
0.5
0.0
Insulin (100 nM) - + - - + + - - + +
2B 2B 5B 5B
[C]
Sample #2
Leaves with few berry buds
MeOH:H2O extraction
Partition: EtOAC and H2O
EtOAc fraction (2A)
H2O fraction (2B)
Medium pressure chromatography
17-1 17-2 17-3 17-4 17-5
Sample #5
Twigs
MeOH:H2O extraction
Partition: EtOAC and H2O
EtOAc fraction (5A)
H2O fraction (5B)
Medium pressure chromatography
17-6 17-7 17-8 17-9
[D]
Glucose uptake (fold-change)
2.0
1.5
1.0
0.5
0.0
Insulin (100 nM) - + 17-1 17-2 17-3 17-4 17-5 17-6 17-7 17-8 17-9

Fig 2

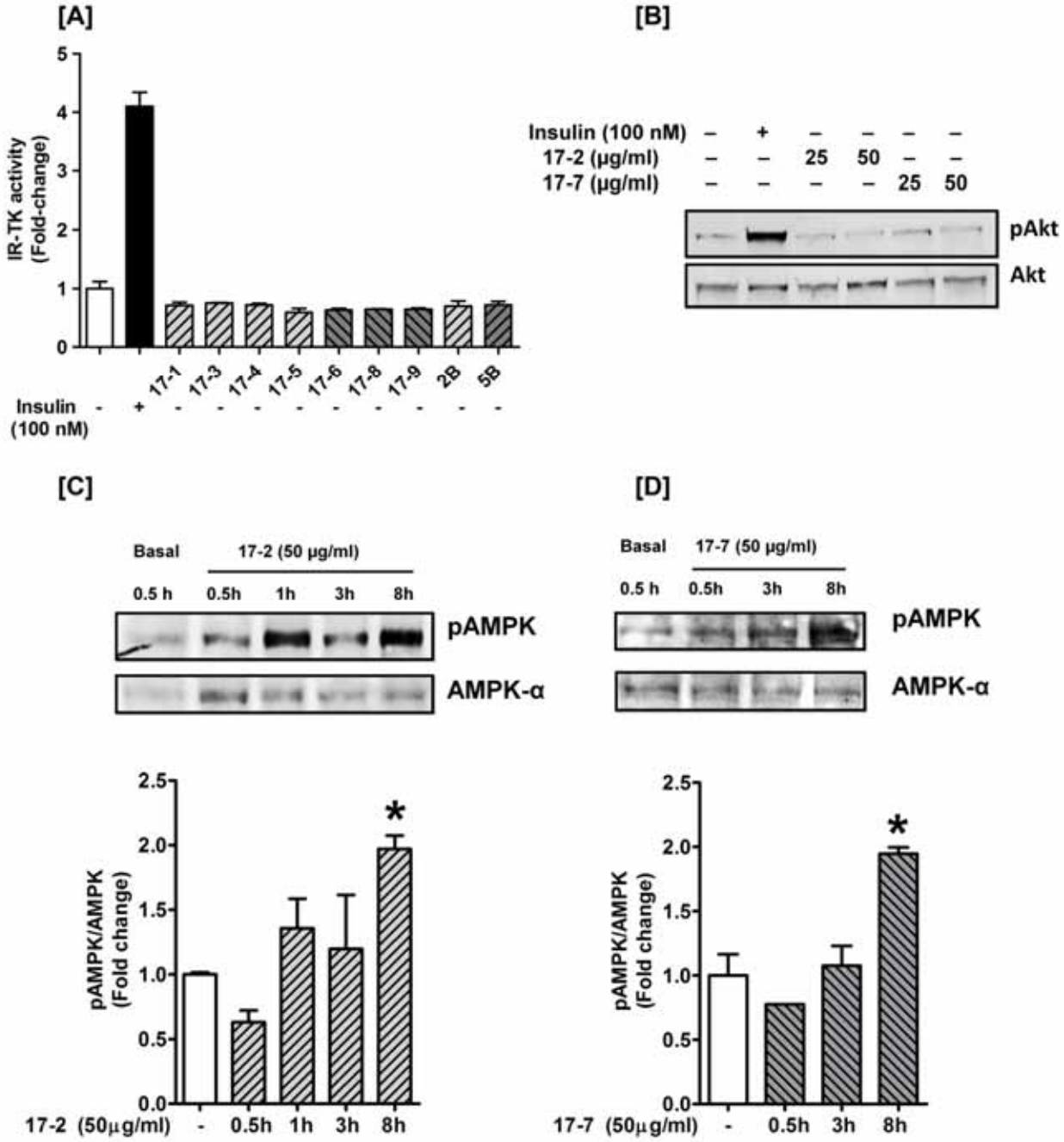

[A]
IR-TK activity (Fold-change)
Insulin (100 nM)
17-1
17-3
17-4
17-5
17-6
17-8
17-9
2B
5B
[B]
Insulin (100 nM)
17-2 (μg/ml)
17-7 (μg/ml)
pAkt
Akt
[C]
Basal
17-2 (50 μg/ml)
0.5 h
pAMPK
AMPK-α
pAMPK/AMPK (Fold change)
17-2 (50μg/ml)
[D]
Basal
17-7 (50 μg/ml)
17-7 (50μg/ml)

Fig 3

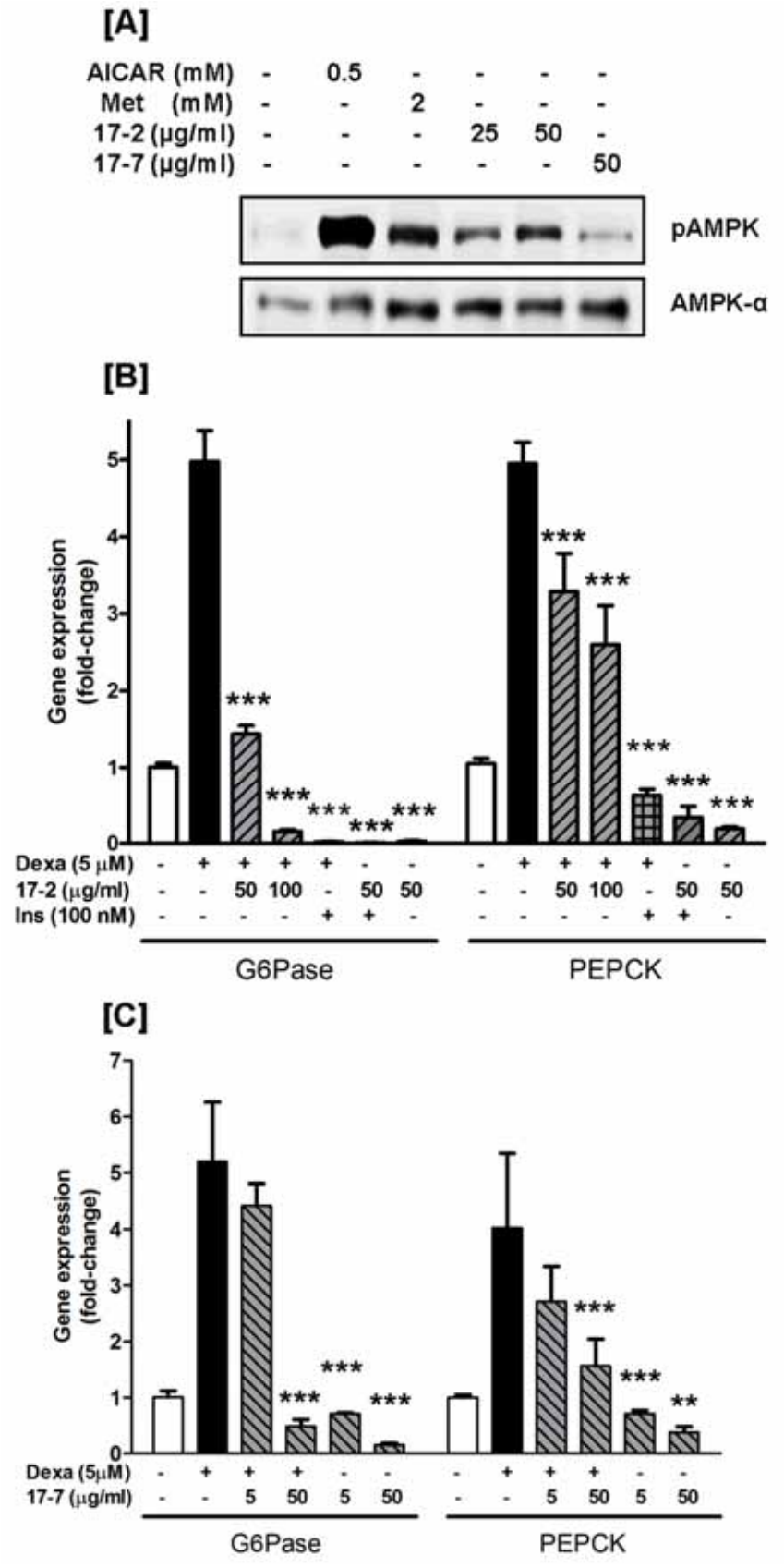

[A]
AICAR (mM) - 0.5 - - - -
Met (mM) - - 2 - - -
17-2 (μg/ml) - - - 25 50 -
17-7 (μg/ml) - - - - - 50
pAMPK
AMPK-α
[B]
Gene expression (fold-change)
Dexa (5 μM) - + + + + - - - + + + + - -
17-2 (μg/ml) - - 50 100 - 50 50 - - 50 100 - 50 50
Ins (100 nM) - - - - + + - - - - - + + -
G6Pase
PEPCK
[C]
Gene expression (fold-change)
Dexa (5μM) - + + + - - - + + + - -
17-7 (μg/ml) - - 5 50 5 50 - - 5 50 5 50
G6Pase
PEPCK

Figure Legends: Supplemental Data

Fig.S1: Confluent L6GLUT4myc cells, in 12-well plate, were serum depleted for 5 hours followed by treatment with *A.alnifolia* extracts for 20 minutes in the absence of insulin. [^{3}H]-2-deoxy-D-glucose uptake assay was measured as described in the Methods section. *A.alnifolia* extracts 2B and 5B showed an increase in glucose uptake compared to basal conditions.

Fig.S2: Dipeptidyl peptidase 4 (DPP-4) activity was quantified by incubating the substrate Gly-Pro-p-nitroanilide with porcine DPP-4 enzyme, in the presence or absence of 0.5 mg/ml of serviceberry extracts or sub-fractions at 37^{o}C for 15 minutes (A, B). The amount of p-nitroaniline (pNA) released from Gly-Pro-pNA was measured at 405nm. *A. alnifolia* extracts and sub-fractions were without effect on DPP-4 activity.

Fig. S3: Differentiated L6GLUT4myc myotubes were treated with 50 mg/ml serviceberry leaf-(17-2) and stem sub-fraction (17-7) for 30 minutes. Cell lysates were subjected to Western blot analyses using phospho-AMPK (Thr172) and AMPK-α antibodies. AICAR (0.5 mM) and metformin (2 mM) were used as positive controls. A representative Western blot is shown (A). Data are expressed as fold-change over basal for AMPK activation (B).

Fig.S1

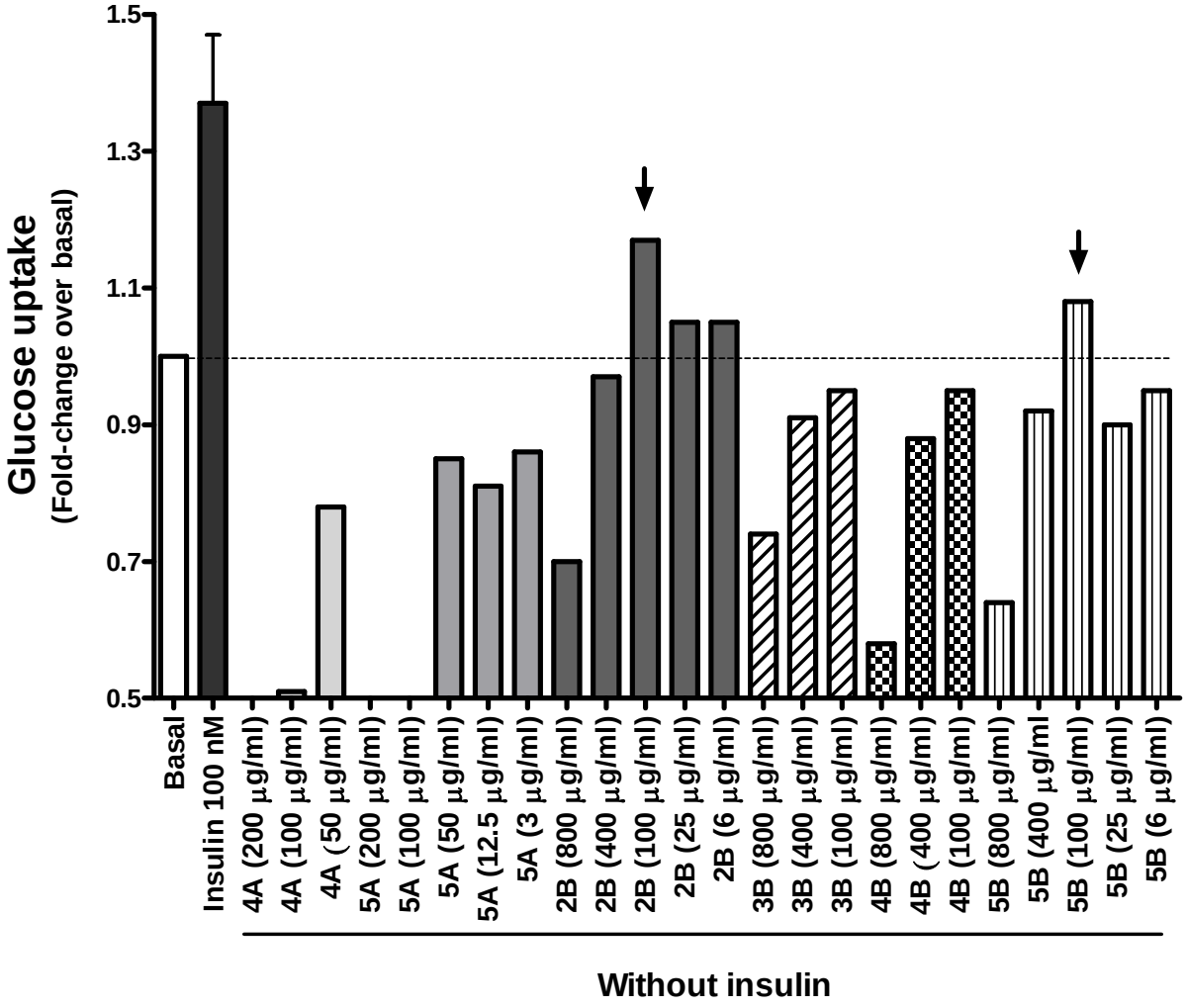

Glucose uptake
(Fold-change over basal)
1.5
1.3
1.1
0.9
0.7
0.5
Basal
Insulin 100 nM
4A (200 µg/ml)
4A (100 µg/ml)
4A (50 µg/ml)
5A (200 µg/ml)
5A (100 µg/ml)
5A (50 µg/ml)
5A (12.5 µg/ml)
5A (3 µg/ml)
2B (800 µg/ml)
2B (400 µg/ml)
2B (100 µg/ml)
2B (25 µg/ml)
2B (6 µg/ml)
3B (800 µg/ml)
3B (400 µg/ml)
3B (100 µg/ml)
4B (800 µg/ml)
4B (400 µg/ml)
4B (100 µg/ml)
5B (800 µg/ml)
5B (400 µg/ml
5B (100 µg/ml)
5B (25 µg/ml)
5B (6 µg/ml)
Without insulin

Fig.S2

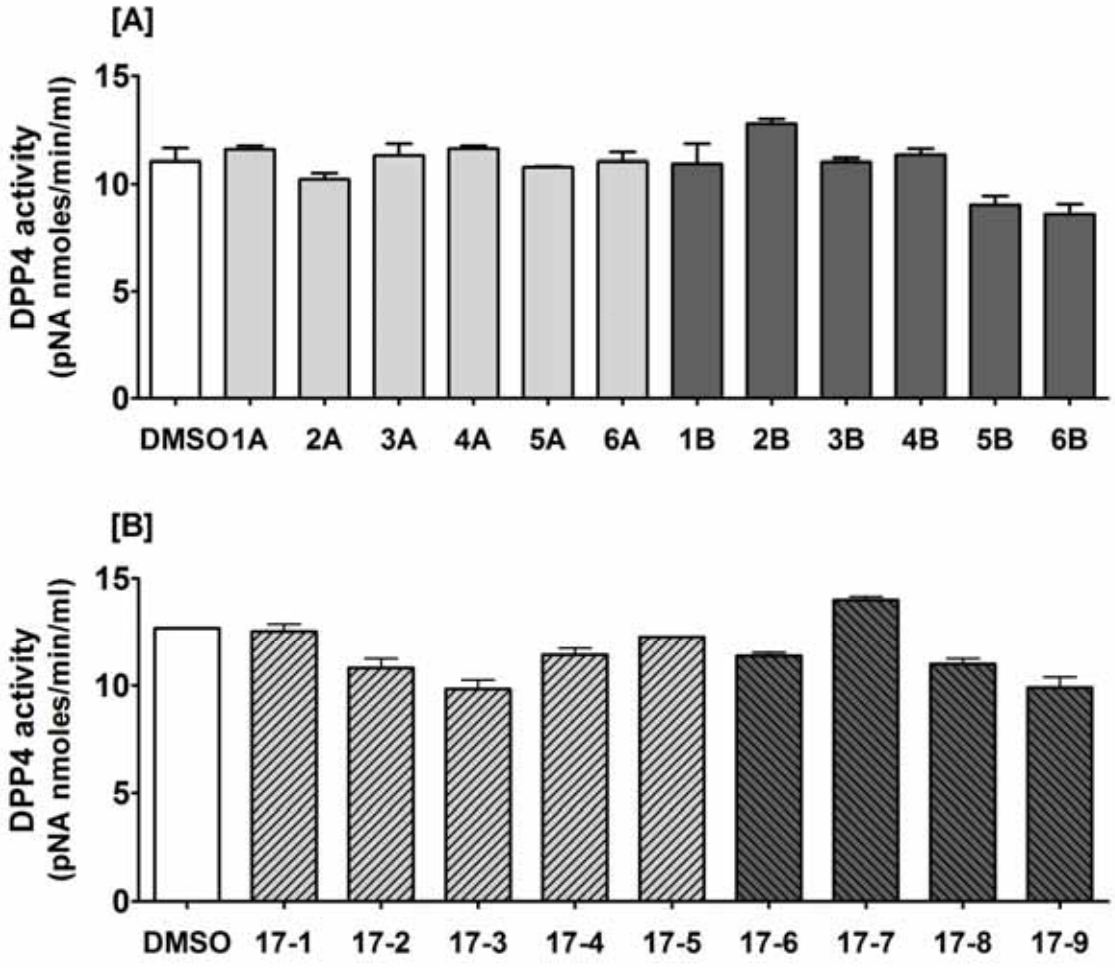
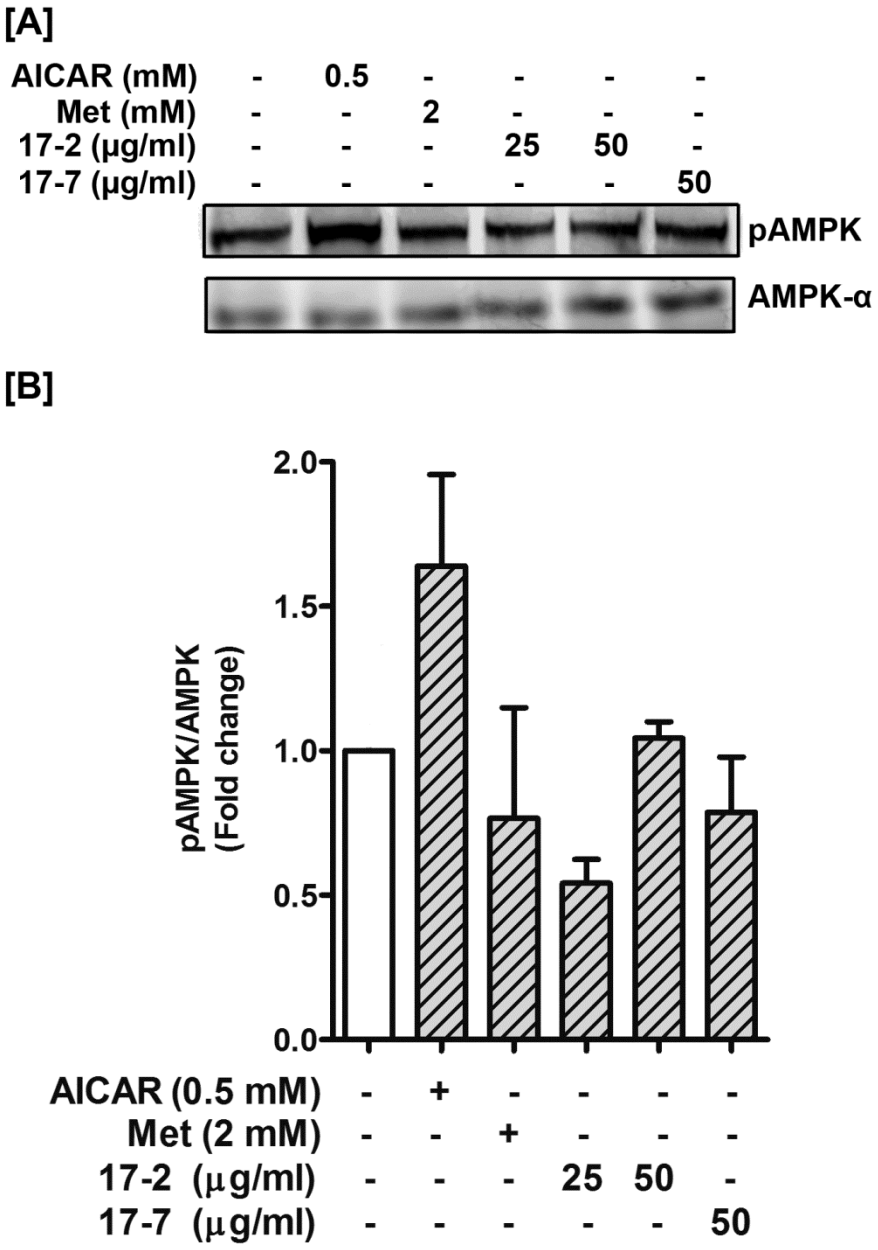

[A]
DPP4 activity
(pNA nmoles/min/ml)
15
10
5
0
DMSO 1A 2A 3A 4A 5A 6A 1B 2B 3B 4B 5B 6B
[B]
DPP4 activity
(pNA nmoles/min/ml)
15
10
5
0
DMSO 17-1 17-2 17-3 17-4 17-5 17-6 17-7 17-8 17-9

Fig.S3


[A]
AICAR (mM) - 0.5 - - - -
Met (mM) - - 2 - - -
17-2 (μg/ml) - - - 25 50 -
17-7 (μg/ml) - - - - - 50
pAMPK
AMPK-α
[B]
pAMPK/AMPK (Fold change)
2.0
1.5
1.0
0.5
0.0
AICAR (0.5 mM) - + - - - -
Met (2 mM) - - + - - -
17-2 (μg/ml) - - - 25 50 -
17-7 (μg/ml) - - - - - 50

Chapter 5: Curcumin induces transcriptional activation of PPARγ without inducing adipocyte differentiation

Abstract

Curcumin, the bioactive component of turmeric, has potent antioxidant and anti-inflammatory properties. Several studies demonstrate that curcumin lowers blood glucose and plasma cholesterol levels in animal models of diabetes. However, the molecular mechanisms related to the anti-diabetic effects of curcumin are not well understood. Here, we report curcumin's effects on the transcriptional activation of PPARγ. We observed that, compared to rosiglitazone, curcumin and THC induced a moderate transcriptional activation of PPARγ. Further, unlike rosiglitazone, curcumin did not induce differentiation of human subcutaneous preadipocytes. Curcumin treatment also inhibited rosiglitazone-induced adipocyte differentiation in human subcutaneous preadipocytes. Consistent with PPARγ activation, curcumintreatment increased gene expression and secretion of adiponectin from differentiated 3T3-L1 adipocytes and human subcutaneous adipocytes. These studies raise the possibility that curcuminoids may modulate insulin action and glucose metabolism by potentially functioning as a selective PPARγ modulator, inducing partial transcriptional activation and upregulation of PPARγ target genes, including adiponectin, but without the undesirable adipogenesis commonly observed with the TZD class of drugs.

Introduction

Curcumin,(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione or diferuloylmethane, a polyphenol isolated from the perennial plant *Curcuma longa*, exhibits cardioprotective (Kang et al., 2010; Morimoto et al., 2010), anti-cancer (Agrawal and Mishra, 2010; Bar-Sela et al., 2010), anti-inflammatory (Bereswill et al., 2010; Bisht et al., 2010), antioxidant (Aftab and Vieira, 2009; Agarwal et al., 2010), and neuroprotective (Ataie et al., 2010) activity. Several lines of evidence suggest that cucurmin may play a beneficial role in diabetes by lowering blood glucose levels in streptozotocin (STZ)- and alloxan-induced diabetic rats (Arun and Nalini, 2002; Murugan and Pari, 2007; Murugan et al., 2008; Peeyush et al., 2009), db/db mice (Seo et al., 2008), type 2 diabetic KK-Ay mice (Nishiyama et al., 2005), high-fat diet-induced obese C57BL/6J and leptin-deficient *ob/ob* mice (Weisberg et al., 2008). At the molecular level, curcumin has been shown to modulate a wide range of transcription factors, growth factors, inflammatory cytokines, enzymes, and kinases (Shehzad et al., 2011), including peroxisome proliferator-activiated receptor-γ, vascular endothelial growth factor, tumor necrosis factor α, cyclooxygenase-2 and AMP-activated protein kinase (Chan, 1995; Jacob et al., 2007; Kim et al., 2009; Tan et al., 2011; Wang et al., 2009).

PPARγ, one of the nuclear receptor superfamily of transcription factors (Green and Wahli, 1994), is highly expressed in adipose tissue and plays a crucial role in adipocyte differentiation (Lemberger et al., 1996). It is also the predominant molecular target for insulin-sensitizing thiazolinedione (TZD) drugs such as rosiglitazone and pioglitazone (Moller, 2001). Studies have shown that curcumin increases PPARγ gene expression and its transcriptional activation in hepatic stellate cells (Lin and Chen, 2008; Xu et al., 2003; Zheng and Chen, 2004). Curcumin was also shown to exert its anti-inflammatory effect by upregulation of PPARγ (Jacob et al.,

2007). Further, curcumin was shown to promote cholesterol efflux from adipocytes by potentially regulating PPARγ-LXRα-ABCA1 pathway (Dong et al., 2011). Whether curcumin is a ligand for PPARγ is debatable, with two groups demonstrating that curcurmin is a PPARγ ligand (Chen and Xu, 2005; Kuroda et al., 2005; Nishiyama et al., 2005), and another group showing otherwise (Narala et al., 2009). Since the TZD class of antidiabetes drugs, including rosiglitazone and pioglitazone, have serious side effects such as fluid retention, weight gain, congestive heart failure (Nesto et al., 2004) and loss of bone mineral density (Kahn et al., 2008) in type 2 diabetic patients, identification of novel compounds or bioactive components that that can selectively modulate PPARγ activation and target gene expression may be of significant merit.

The goal of this study was to evaluate the ability of curcumin to modulate PPARγ transcriptional activity, its target gene expression, and adipocyte differentiation in human subcutaneous adipocytes. We demonstrate that curcumin increases PPARγ transcriptional activity and upregulates adiponectin gene expression, without inducing adipocyte differentiation, suggesting that the anti-diabetic effects of curcuminoids could, at least in part, be mediated by regulation of PPARγ target gene expression.

Materials and Methods

Reagents

Curcumin (>90% purity) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Curcumin C3 complex® (ratio-defined mixture of curcumin, bisdemethoxycurcumin, and demethoxycurcumin) and tetrahydrocurcuminoids (THC: ratio-defined mixture of tetrahydrocurcumin, tetrahydrobisdemethoxycurcumin, and tetrahydrodemothoxycurcumin) were gifts from Sabinsa Corporation (Piscataway, NJ). Curcumin, C3 complex®, and THC were dissolved in DMSO. Troglitazone (Tro), rosiglitazone (Rosi), 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (PG), a natural PPARγ-agonist, and GW-9662, a PPARγ-antagonist, were purchased from Axxora LLC (San Diego, CA). Recombinant human insulin was purchased from Roche Diagnostics (Indianapolis, IL); 3-isobutyl-1-methylxanthine (IBMX) and dexamethasone were from Sigma-Aldrich Chemical (St. Louis, MO); adiponectin antibody was from Cell Signaling Technology (Danvers, MA); NIH3T3 and 3T3-L1 fibroblast cells were purchased from the American Type Culture Collection (Manassas, VA).

Cell culture and transfections

NIH-3T3 cells, seeded in 24-well cell culture plates, and cultured in growth medium consisting of DMEM, 10% fetal bovine serum, and 1% penicillin/streptomycin, were transfected with plasmids encoding the ligand-binding domain of PPARγ, RXRα, FATP-luciferase, and β-galactosidase, using Lipofectamine™ 2000 transfection reagent (Life Technologies, Grand Island, NY). Cells were exposed to curcuminoids or rosiglitazone for 24 h, lysed, and assayed for luciferase and β-galactosidase activity using the dual-light luciferase and β-galactosidase reporter gene assay system (Applied Biosystems, Foster City, CA) in a 96-well luminometer (Promega,

Madison, WI). Transfections were performed in triplicate. Data was normalized to luminescence from β-galactosidase expression and depicted as fold-change from DMSO treatment.

Human adipogenesis assay

Human adipocyte differentiation was assayed using human adipogenesis assay kit from Zen-Bio, Inc. (Research Triangle Park, NC). Briefly, subcutaneous human preadipocytes were obtained from Zen-Bio, Inc. and cultured in preadipocyte medium (Zen-Bio, catalog #PM-1) according to the manufacture's recommendation. To induce differentiation, confluent cells were cultured in initiation medium (Zen-Bio catalog #IM), containing insulin, dexamethasone, isobutylmethylxanthine, for 7 days, in the presence or absence of curcumin (1,5,20 μM), curcumin C3 complex® (1,5,20 μM), tetrahydrocurcuminoids (THC: 1,5,20 μM), PPARγ agonists troglitazone (5 μM), rosiglitazone (5 μM), 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (5 μM), and PPARγ antagonist GW9662 (5 μM). Following differentiation, triglyceride content of adipocytes was assayed by measuring the concentration of glycerol released based on the equation, 1 M triglyceride yields 1 M glycerol + free fatty acids.

3T3-L1 adipocyte differentiation and PPARγ gene expression

Differentiated 3T3-L1 adipocytes were treated with different concentrations of curcumin for 24 hours, after which cells were pelleted and subjected to RNA extraction using RNeasy Mini RNA isolation kit (QIAGEN, Valencia, CA). cDNA was synthesized using iScript cDNA synthesis kit (BIO-RAD, Hercules, CA) and real-time PCR was performed using iQ SYBR Green Supermix (BIO-RAD) according to protocols recommended by the manufacturer. The primer sequences for target genes are as follows: Adiponectin, forward 5'-AGA GAA GGG

AGA GAA AGG AGA TGC-3', reverse 5'-TGA GCG ATA CAC ATA AGC GGC-3'; CD36, forward 5'-GGT AGA GAT GGC CTT ACT TGG GAT TG-3', reverse 5'-GCC AGT GTA TAT GTA GGC TCA TCC ACT ACT-3'; FATP, forward 5'-ATG CTC GGC CCA TCT TCC TG-3', reverse 5'-GAA AGA AGA GCC TGT CTG AGG TCT GA-3'; LPL, forward 5'-TCT GTG CTA GGG AGA AAG TT-3', reverse 5'-TGT TTG TTT GTC CAG TGT CA-3'; and, β-Actin, forward 5′-ATC ACT ATT GGC AAC GAG CGG TTC-3′, reverse 5′-CGT CAC ACT TCA TGA TGG AAT TGA ATG-3′. Reaction conditions were as follows: 95 °C, 3:0 min; 95 °C, 0:15 min, 60 °C, 0:30 min, 72 °C, 0:30 min, repeated 40×; 55 °C, 0:10 min, repeated 80×. Expression levels were normalized to β-actin and gene expression was calculated as $2^{-\Delta\Delta C}{}_{T}$ and expressed as fold-change, as described by Livak and Schmittgen (Livak and Schmittgen, 2001). All assays were carried out in triplicate.

Western blot analyses of adiponectin

Confluent 6-well dishes of 3T3-L1 adipocyte cells were treated with curcuminoids, rosiglitazone (0.5 μM), in serum-free media for 24 hours. Cells were washed with ice-cold PBS three times and lysed in 300 μl of cell lysis buffer [50 mM HEPES, pH7.4, 100 mM sodium pyrophosphate, 100 mM EDTA, 20 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, 0.01 mg/ml aprotinin, 0.01 mg/ml leupeptin, 1% Triton X-100, and protease inhibitor cocktail tablet (Roche Diagnostics, Indianapolis)]. Cell lysate proteins were separated by 4–20% SDS–PAGE precast gels (NuSep Inc., Austell, GA) followed by Western blot analysis using antibodies against adiponectin. Chemiluminescence detection and image analysis were performed with UVP-Biochimie Bioimager and LabWorks software (UVP, Upland, CA).

Human adiponectin ELISA

Human primary subcutaneous adipocytes (Zen-Bio Inc, NC), were treated with curcumin (1,5,20 μM), curcumin C3 complex® (1,5,20 μM), tetrahydrocurcuminoids (1,5,20 μM) in the presence or absence of rosiglitazone (5 μM), for 3 days at 37˚C in a CO_2 incubator. Adiponectin secreted into the media was assayed using a human adiponectin ELISA kit from B-Bridge International, Inc., (San Jose, CA). All treatments were in triplicate.

Results

Curcuminoids increase transcriptional activity of PPARγ in NIH-3T3 cells

Whether curcumin induces PPARγ transcriptional activity is unclear; while earlier reports demonstrate that curcumin activates PPARγ, a recent report argues to the contrary. Here, we tested the ability of curcumin, C3, and THC, to activate PPARγ. NIH 3T3 cells, that are known to lack endogenous PPARγ, were co-transfected with a PPARγ-expression plasmid harboring the ligand binding domain of PPARγ (pTR100-PPARγ), a plasmid expressing its heterodimerizing partner RXRα, and the FATP-PPRE-luc plasmid harboring the peroxisome proliferator response expression element (PPRE) of fatty acid transport protein that drives the expression of the luciferase reporter gene. Transfected NIH 3T3 cells were treated with a range of concentrations (0.001-2.5 μM) of curcumin, C3, and THC, and rosiglitazone as a positive control (Fig. 1). Curcumin, C3, and THC increased transcriptional activation of PPARγ dose-dependently from 1 nM - 2.5 μM. Transcriptional activation of PPARγ at lower doses of curcumin, C3, and THC, was comparable to rosiglitazone. Calculated EC_{50} (μM) values indicate that curcuminoids have a moderately lower affinity (EC_{50}: 208 – 482 nM) compared to rosiglitazone (EC_{50}: 130 nM) (Fig. 1, inset). However, at the highest concentration, curcuminoids (2.5 μM) induced a maximum of ~20-fold transcriptional activity, compared to a ~90-fold increase in transcriptional activity by rosiglitazone at a similar concentration. These findings suggest that, compared to rosiglitazone, curcuminoids may only partially modulate transcriptional activation of PPARγ.

Curcuminoids do not induce adipocyte differentiation and prevent adipocyte differentiation in human primary subcutaneous preadipocytes

Human preadipocytes differentiated with insulin, dexamethasone, IBMX and a PPARγ agonist such as troglitazone (Tro), rosiglitazone (Rosi), or the natural PPARγ-agonist 15-deoxy-

$\Delta^{12,14}$-prostaglandin J_2 (PG), showed strong adipogenic activities, evidenced by increased lipid droplet formation in adipocytes (Fig.2A, inset – 10X magnification). The amount of triglyceride accumulation, an indicator of adipocyte differentiation, was used for quantitative comparison between treatments. Rosiglitazone, which demonstrated the highest transcriptional activation, showed highest triglyceride content compared to Tro and PG (Fig. 2A). GW9662, a PPARγ antagonist, suppressed Rosi- and PG-induced triglyceride accumulation. Treatment of human primary subcutaneous preadipocytes with curcumin, C3, or THC (1-20 μM), along with insulin, dexamethasone and IBMX, failed to induce adipocyte differentiation (Fig.2A). However, curcumin, C3, and THC, at lower doses (0.5 μM), demonstrated a ~2-fold increase in triglyceride content, which was suppressed by the PPARγ antagonist GW9662 (data not shown). Furthermore, curcumin, C3, and THC at 5 and 20 μM suppressed rosiglitazone-induced differentiation of human primary subcutaneous preadipocytes to adipocytes and decreased triglyceride accumulation (Fig. 2B). These results suggest that the partial transcriptional activation of PPARγ by curcuminoids, may not be sufficent to induce adipogenesis, and that curcuminoids may block rosiglitazone-induced differentiation of adipocytes in humans.

Curcumin increase adiponectin, CD36, and LPL gene expression in 3T3-L1 adipocytes

To further confirm that activation of PPARγ results in the induction of its target genes, we assayed gene expression of fatty acid translocase (CD36), fatty acid transport protein (FATP), and lipoprotein lipase (LPL) that promote fatty acid uptake and storage in adipocytes, and the expression of adiponectin. In differentiated 3T3-L1 adipocytes, curcumin (2.5 μM) induced ~1.8-fold increase in gene expression of adiponectin compared to ~2.5-fold increase with rosiglitazone treatment (Fig.3). Consistent with this, curcumin treatment also induced secretion

of adiponectin into the culture media of differentiated 3T3-L1 adipocytes (Fig.3, inset). Further, curcumin (2.5 μM) induced ~1.75-fold increase in CD36 gene expression compared to ~3-fold induction by rosiglitazone. Induction of LPL gene expression by curcumin was comparable to rosiglitazone. For the duration of the treatment (24 hours), FATP gene expression was not significantly upregulated by either rosiglitazone or curcumin treatment. These findings indicate the possibility, that similar to TZDs, curcuminoids have the potential to upregulate PPARγ target genes in adipocytes.

Curcuminoids increase adiponectin secretion in human primary subcutaneous adipocytes

Differentiated human primary subcutaneous adipocytes were treated with various concentrations of curcuminoids in the presence or absence of rosiglitazone, or GW9662, for 3 days, and adiponectin secreted into the culture media was assayed by ELISA. Curcumin (5 μM), C3 (5 μM), and THC (5, 20 μM) demonstrated an increasing trend in adiponectin secretion, though this was not statistically significant (Fig 4). Interestingly, curcumin and curcumin C3 complex showed a synergistic effect in significantly increasing adiponectin secretion compared to DMSO control and rosiglitazone.

Discussion

Natural and synthetic ligands for PPARγ bind to and transactivate the receptor with distinct affinities. Among these, the TZDs, high-affinity ligands for PPARγ, have been shown to cause weight gain in rodent model and human studies, limiting its usage (Demers et al., 2008). Since PPARγ ligand-binding domain has a large binding pocket, it can elicit numerous potential ligand-binding conformations. Compared to full agonists, such as rosiglitazone or troglitazone, partial agonists may have lower maximal activity. A selective modulator, on the other hand, is a ligand that, compared to a full agonist, differentially induces specific receptor effects (Higgins and Depaoli, 2010). Several small molecules, including metaglidasen (formerly MBX-102), INT131, and, endogenous ligands, including lipocalin 2, a cytokine, and decanoic acid, a 10-carbon fatty acid, have been identified as selective PPARγ modulators (Chandalia et al., 2009; Higgins and Mantzoros, 2008; Jin et al., 2011; Malapaka et al., 2012).

A growing body of evidence suggests that curcumin, the bioactive component of curry spice turmeric, upregulates PPARγ gene expression and induces its transcriptional activation. Curcumin has been reported to increase gene expression and activation of PPARγ, contributing to the inhibition of hepatic stellate cell growth (Lin and Chen, 2008; Xu et al., 2003; Zheng and Chen, 2004). Turmeric extracts, curcumin, demethoxycurcumin, bisdemethoxycurcumin, and ar-turmerone exhibited PPARγ ligand-binding activity in a GAL4-PPARγ chimera assay (Nishiyama et al., 2005). In RAW264.7 cells, and in a rat model of sepsis, curcumin treatment increased PPARγ expression, and reduced mortality (Siddiqui et al., 2006). While several studies demonstrate that curcumin's effects are abrogated by GW9662 and BADGE, antagonists of PPARγ (Rinwa et al., 2010; Wang et al., 2010), a recent study suggests that curcumin is not a

ligand for PPARγ. Therefore, these studies sought to examine the role of curcumin in PPARγ activation, adipocyte differentiation, and expression of target genes, including adiponectin.

Our studies demonstrate that curcumin, C3, and THC induced a moderate increase in PPARγ transcriptional activation (~20-fold increase compared to 90-fold increase with rosiglitazone). Interestingly, unlike rosiglitazone, troglitazone, and the natural ligand 15-deoxy-d 12,14 prostaglandin J2, curcumin, C3, or THC did not induce differentiation of human subcutaneous adipocyt*e*s. Additionally, curcumin treatment decreased rosiglitazone-induced adipocyte differentiation, suggesting an uncoupling of effects and/or a differentially-induced receptor effect. These findings are consistent with previous findings in 3T3-L1 cells (Ahn et al., 2010; Ejaz et al., 2009; Lee et al., 2009), that indicate suppression of early adipogenic transcription factors including CCAAT/enhancer binding protein α (CEBPα) and PPARγ (Kim et al., 2011), and activation of Wnt/beta-catenin signaling (Ahn et al., 2010). Previously, we have demonstrated that curcumin activates AMPK and suppresses gluconeogenic gene expression in hepatoma cells (Kim et al., 2009). Similarly, two recent studies have shown that curcumin activates AMPK in 3T3-L1 adipocytes, which downregulates PPARγ, leading to the inhibition of adipocyte differentiation (Lee et al., 2009). Our data is supported by animal studies that demonstrate reduced weight gain, adiposity, and increased lean mass in curcumin-supplemented high-fat fed and *ob/ob* mouse models of obesity (Ejaz et al., 2009; Weisberg et al., 2008).

Next, we examined curcumin's effects on PPARγ target gene expression. Unlike rosiglitazone, curcumin moderately induced CD36 and adiponectin gene expression in differentiated 3T3-L1 adipocytes; while LPL gene expression was similar for both rosiglitazone and curcumin treatment, suggesting a moderate increment of fatty acid uptake into adipocytes in response to curcumin. This is consistent with our data on human adipogenesis that showed

decreased triglyceride accumulation following curcumin-treatment. Further, curcumin significantly increased adiponectin secretion compared to rosiglitazone, in 3T3-L1 adipocytes. In differentiated subcutaneous human adipocytes, curcumin, C3, and THC-treatment showed an increasing trend in adiponectin secretion. Interestingly, curcumin- or C3-treatment potentiated rosiglitazone's effects on adiponectin secretion from adipocytes. This may indicate another target of curcumin, which can signal to increase adiponectin secretion. Whether activation of AMPK is involved in curcumin's role in potentiation of rosiglitazone-induced adiponectin secretion is speculation. Our findings that curcumin increases adiponectin gene expression and its secretion from adipocytes is consistent with the findings of Weisberg et al. (Weisberg et al., 2008), that demonstrate significantly higher serum adiponectin levels in both curcumin-treated, high-fat-fed and *ob/ob* mice.

GW9662 is a selective, irreversible PPARγ antagonist, which binds to the ligand-binding domain of PPARγ and covalently modifies Cys^{285}, resulting in an irreversible loss of ligand binding. In our studies, GW9662 demonstrated a varied activity; while it blocked THC-induced adipocyte differentiation, it was without effect on curcumin and C3's effects on adipocyte differentiation. GW9662 was without any effect on curcumin-, C3-, or THC-induced adiponectin secretion. It may be argued therefore, that curcuminoids are not PPARγ ligands. While this may be a possibility, it is evident from our studies that curcuminoids induce a dose-dependent increase in PPARγ activation and expression of its target genes. A similar, ligand-independent activation of PPARγ by insulin and C-peptide has been reported (Al-Rasheed et al., 2004) (Al-Rasheed et al, 2004). Post-translational modification of PPARγ may be linked with positive or negative regulation of ligand-independent transcriptional activity. ERK (MAP kinase)-mediated phosphorylation of Ser 82 of PPARγ 1 and Ser 112 of PPARγ 2 has been shown to be

accompanied by a reduction in transcriptional activity (Camp and Tafuri, 1997). Recent studies indicate that rosiglitazone and other anti-diabetic drugs inhibit the phosphorylation of PPARγ by protein kinase Cdk5 (cyclin-dependent kinase 5) on Ser 273, induced by high-fat feeding (Choi et al., 2010). Currently, curcumin's role in inhibiting PPARγ phosphorylation on Ser 273 is unknown.

In conclusion, we have presented evidence that curcuminoids induce transcriptional activation of PPARγ. Unlike rosiglitazone, curcuminoids elicited a moderate transcriptional activation, and inhibited adipogenesis in human subcutaneous adipocytes. Further, curcumin induced a moderate upregulation of PPARγ target genes, and enhanced adiponectin secretion from fat cells. These data are suggestive of a potential role for the bioactive component, curcumin, to function as a selective PPARγ modulator. Additional *in vivo* studies examining molecular mechanisms of PPARγ activation are warranted.

Reference

Aftab, N., and Vieira, A. (2009). Antioxidant activities of curcumin and combinations of this curcuminoid with other phytochemicals. Phytother Res *24*, 500-502.

Agarwal, R., Goel, S.K., and Behari, J.R. (2010). Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. J Appl Toxicol *30*, 457-468.

Agrawal, D.K., and Mishra, P.K. (2010). Curcumin and its analogues: potential anticancer agents. Med Res Rev *30*, 818-860.

Ahn, J., Lee, H., Kim, S., and Ha, T. (2010). Curcumin-induced suppression of adipogenic differentiation is accompanied by activation of Wnt/beta-catenin signaling. Am J Physiol Cell Physiol *298*, C1510-1516.

Al-Rasheed, N.M., Chana, R.S., Baines, R.J., Willars, G.B., and Brunskill, N.J. (2004). Ligand-independent activation of peroxisome proliferator-activated receptor-gamma by insulin and C-peptide in kidney proximal tubular cells: dependent on phosphatidylinositol 3-kinase activity. J Biol Chem *279*, 49747-49754.

Arun, N., and Nalini, N. (2002). Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. Plant Foods Hum Nutr *57*, 41-52.

Ataie, A., Sabetkasaei, M., Haghparast, A., Moghaddam, A.H., and Kazeminejad, B. (2010). Neuroprotective effects of the polyphenolic antioxidant agent, Curcumin, against homocysteine-induced cognitive impairment and oxidative stress in the rat. Pharmacol Biochem Behav *96*, 378-385.

Bar-Sela, G., Epelbaum, R., and Schaffer, M. (2010). Curcumin as an anti-cancer agent: review of the gap between basic and clinical applications. Curr Med Chem *17*, 190-197.

Bereswill, S., Munoz, M., Fischer, A., Plickert, R., Haag, L.M., Otto, B., Kuhl, A.A., Loddenkemper, C., Gobel, U.B., and Heimesaat, M.M. (2010). Anti-inflammatory effects of resveratrol, curcumin and simvastatin in acute small intestinal inflammation. PLoS One *5*, e15099.

Bisht, K., Wagner, K.H., and Bulmer, A.C. (2010). Curcumin, resveratrol and flavonoids as anti-inflammatory, cyto- and DNA-protective dietary compounds. Toxicology *278*, 88-100.

Camp, H.S., and Tafuri, S.R. (1997). Regulation of peroxisome proliferator-activated receptor gamma activity by mitogen-activated protein kinase. J Biol Chem *272*, 10811-10816.

Chan, M.M. (1995). Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochem Pharmacol *49*, 1551-1556.

Chandalia, A., Clarke, H.J., Clemens, L.E., Pandey, B., Vicena, V., Lee, P., Lavan, B.E., and Gregoire, F.M. (2009). MBX-102/JNJ39659100, a Novel Non-TZD Selective Partial

PPAR-gamma Agonist Lowers Triglyceride Independently of PPAR-alpha Activation. PPAR Res *2009*, 706852.

Chen, A., and Xu, J. (2005). Activation of PPAR{gamma} by curcumin inhibits Moser cell growth and mediates suppression of gene expression of cyclin D1 and EGFR. Am J Physiol Gastrointest Liver Physiol *288*, G447-456.

Choi, J.H., Banks, A.S., Estall, J.L., Kajimura, S., Bostrom, P., Laznik, D., Ruas, J.L., Chalmers, M.J., Kamenecka, T.M., Bluher, M.*, et al.* (2010). Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5. Nature *466*, 451-456.

Demers, A., Rodrigue-Way, A., and Tremblay, A. (2008). Hexarelin Signaling to PPARgamma in Metabolic Diseases. PPAR Res *2008*, 364784.

Dong, S.Z., Zhao, S.P., Wu, Z.H., Yang, J., Xie, X.Z., Yu, B.L., and Nie, S. (2011). Curcumin promotes cholesterol efflux from adipocytes related to PPARgamma-LXRalpha-ABCA1 passway. Mol Cell Biochem *358*, 281-285.

Ejaz, A., Wu, D., Kwan, P., and Meydani, M. (2009). Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. J Nutr *139*, 919-925.

Green, S., and Wahli, W. (1994). Peroxisome proliferator-activated receptors: finding the orphan a home. Mol Cell Endocrinol *100*, 149-153.

Higgins, L.S., and Depaoli, A.M. (2010). Selective peroxisome proliferator-activated receptor gamma (PPARgamma) modulation as a strategy for safer therapeutic PPARgamma activation. Am J Clin Nutr *91*, 267S-272S.

Higgins, L.S., and Mantzoros, C.S. (2008). The Development of INT131 as a Selective PPARgamma Modulator: Approach to a Safer Insulin Sensitizer. PPAR Res *2008*, 936906.

Jacob, A., Wu, R., Zhou, M., and Wang, P. (2007). Mechanism of the Anti-inflammatory Effect of Curcumin: PPAR-gamma Activation. PPAR Res *2007*, 89369.

Jin, D., Guo, H., Bu, S.Y., Zhang, Y., Hannaford, J., Mashek, D.G., and Chen, X. (2011). Lipocalin 2 is a selective modulator of peroxisome proliferator-activated receptor-gamma activation and function in lipid homeostasis and energy expenditure. FASEB J *25*, 754-764.

Kahn, S.E., Zinman, B., Lachin, J.M., Haffner, S.M., Herman, W.H., Holman, R.R., Kravitz, B.G., Yu, D., Heise, M.A., Aftring, R.P.*, et al.* (2008). Rosiglitazone-associated fractures in type 2 diabetes: an Analysis from A Diabetes Outcome Progression Trial (ADOPT). Diabetes Care *31*, 845-851.

Kang, B.Y., Khan, J.A., Ryu, S., Shekhar, R., Seung, K.B., and Mehta, J.L. (2010). Curcumin reduces angiotensin II-mediated cardiomyocyte growth via LOX-1 inhibition. J Cardiovasc Pharmacol *55*, 417-424.

Kim, C.Y., Le, T.T., Chen, C., Cheng, J.X., and Kim, K.H. (2011). Curcumin inhibits adipocyte differentiation through modulation of mitotic clonal expansion. J Nutr Biochem *22*, 910-920.

Kim, T., Davis, J., Zhang, A.J., He, X., and Mathews, S.T. (2009). Curcumin activates AMPK and suppresses gluconeogenic gene expression in hepatoma cells. Biochem Biophys Res Commun *388*, 377-382.

Kuroda, M., Mimaki, Y., Nishiyama, T., Mae, T., Kishida, H., Tsukagawa, M., Takahashi, K., Kawada, T., Nakagawa, K., and Kitahara, M. (2005). Hypoglycemic effects of turmeric (Curcuma longa L. rhizomes) on genetically diabetic KK-Ay mice. Biol Pharm Bull *28*, 937-939.

Lee, Y.K., Lee, W.S., Hwang, J.T., Kwon, D.Y., Surh, Y.J., and Park, O.J. (2009). Curcumin exerts antidifferentiation effect through AMPKalpha-PPAR-gamma in 3T3-L1 adipocytes and antiproliferatory effect through AMPKalpha-COX-2 in cancer cells. J Agric Food Chem *57*, 305-310.

Lemberger, T., Desvergne, B., and Wahli, W. (1996). Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu Rev Cell Dev Biol *12*, 335-363.

Lin, J., and Chen, A. (2008). Activation of peroxisome proliferator-activated receptor-gamma by curcumin blocks the signaling pathways for PDGF and EGF in hepatic stellate cells. Lab Invest *88*, 529-540.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25*, 402-408.

Malapaka, R.R., Khoo, S., Zhang, J., Choi, J.H., Zhou, X.E., Xu, Y., Gong, Y., Li, J., Yong, E.L., Chalmers, M.J.*, et al.* (2012). Identification and mechanism of 10-carbon fatty acid as modulating ligand of peroxisome proliferator-activated receptors. J Biol Chem *287*, 183-195.

Moller, D.E. (2001). New drug targets for type 2 diabetes and the metabolic syndrome. Nature *414*, 821-827.

Morimoto, T., Sunagawa, Y., Fujita, M., and Hasegawa, K. (2010). Novel heart failure therapy targeting transcriptional pathway in cardiomyocytes by a natural compound, curcumin. Circ J *74*, 1059-1066.

Murugan, P., and Pari, L. (2007). Influence of tetrahydrocurcumin on hepatic and renal functional markers and protein levels in experimental type 2 diabetic rats. Basic Clin Pharmacol Toxicol *101*, 241-245.

Murugan, P., Pari, L., and Rao, C.A. (2008). Effect of tetrahydrocurcumin on insulin receptor status in type 2 diabetic rats: studies on insulin binding to erythrocytes. J Biosci *33*, 63-72.

Narala, V.R., Smith, M.R., Adapala, R.K., Ranga, R., Panati, K., Moore, B.B., Leff, T., Reddy, V.D., Kondapi, A.K., and Reddy, R.C. (2009). Curcumin is not a ligand for peroxisome proliferator-activated receptor-纬. Gene therapy & molecular biology *13*, 20.

Nesto, R.W., Bell, D., Bonow, R.O., Fonseca, V., Grundy, S.M., Horton, E.S., Le Winter, M., Porte, D., Semenkovich, C.F., Smith, S.*, et al.* (2004). Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. Diabetes Care *27*, 256-263.

Nishiyama, T., Mae, T., Kishida, H., Tsukagawa, M., Mimaki, Y., Kuroda, M., Sashida, Y., Takahashi, K., Kawada, T., Nakagawa, K.*, et al.* (2005). Curcuminoids and sesquiterpenoids in turmeric (Curcuma longa L.) suppress an increase in blood glucose level in type 2 diabetic KK-Ay mice. J Agric Food Chem *53*, 959-963.

Peeyush, K.T., Gireesh, G., Jobin, M., and Paulose, C.S. (2009). Neuroprotective role of curcumin in the cerebellum of streptozotocin-induced diabetic rats. Life Sci *85*, 704-710.

Rinwa, P., Kaur, B., Jaggi, A.S., and Singh, N. (2010). Involvement of PPAR-gamma in curcumin-mediated beneficial effects in experimental dementia. Naunyn Schmiedebergs Arch Pharmacol *381*, 529-539.

Seo, K.I., Choi, M.S., Jung, U.J., Kim, H.J., Yeo, J., Jeon, S.M., and Lee, M.K. (2008). Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. Mol Nutr Food Res *52*, 995-1004.

Shehzad, A., Ha, T., Subhan, F., and Lee, Y.S. (2011). New mechanisms and the anti-inflammatory role of curcumin in obesity and obesity-related metabolic diseases. Eur J Nutr *50*, 151-161.

Siddiqui, A.M., Cui, X., Wu, R., Dong, W., Zhou, M., Hu, M., Simms, H.H., and Wang, P. (2006). The anti-inflammatory effect of curcumin in an experimental model of sepsis is mediated by up-regulation of peroxisome proliferator-activated receptor-gamma. Crit Care Med *34*, 1874-1882.

Tan, X., Poulose, E.M., Raveendran, V.V., Zhu, B.T., Stechschulte, D.J., and Dileepan, K.N. (2011). Regulation of the expression of cyclooxygenases and production of prostaglandin I and E in human coronary artery endothelial cells by curcumin. J Physiol Pharmacol *62*, 21-28.

Wang, H.M., Zhao, Y.X., Zhang, S., Liu, G.D., Kang, W.Y., Tang, H.D., Ding, J.Q., and Chen, S.D. (2010). PPARgamma agonist curcumin reduces the amyloid-beta-stimulated inflammatory responses in primary astrocytes. J Alzheimers Dis *20*, 1189-1199.

Wang, J.B., Qi, L.L., Zheng, S.D., Wang, H.Z., and Wu, T.X. (2009). Curcumin suppresses PPARdelta expression and related genes in HT-29 cells. World J Gastroenterol *15*, 1346-1352.

Weisberg, S.P., Leibel, R., and Tortoriello, D.V. (2008). Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology *149*, 3549-3558.

Xu, J., Fu, Y., and Chen, A. (2003). Activation of peroxisome proliferator-activated receptor-gamma contributes to the inhibitory effects of curcumin on rat hepatic stellate cell growth. Am J Physiol Gastrointest Liver Physiol *285*, G20-30.

Zheng, S., and Chen, A. (2004). Activation of PPARgamma is required for curcumin to induce apoptosis and to inhibit the expression of extracellular matrix genes in hepatic stellate cells in vitro. Biochem J *384*, 149-157.

Figure legends

Fig.1: Curcumin increases transcriptional activation of PPARγ. NIH3T3 cells were transfected with plasmids expressing the ligand-binding domain of PPARγ, FATP-Luc, RXRα, and β-galactosidase. Following treatment with curcuminoids or rosiglitazone for 24 hours, cells were lysed and luciferase activity indicating PPARγ transcriptional activation was measured in a luminometer. Data were normalized to luminescence from β-galactosidase expression and depicted as fold-change from DMSO treatment. Inset shows calculated EC50 values of curcuminoids compared to other PPARγ agonists.
* $p < 0.05$.

Fig.2: Curcuminoids fail to induce adipocyte differentiation, and prevent rosiglitazone-induced differentiation in human primary subcutaneous preadipocytes. (A) Curcumin, curcumin C3 complex®, and tetrahydrocurcuminoids (THC) were tested for their ability to induce differentiation of human primary subcutaneous preadipocytes (Zen-Bio Inc, NC). Troglitazone (Tro), rosiglitazone (Rosi), 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (PG), the natural PPARγ-agonist, and GW9662, a PPARγ-antagonist were used as controls. Adipocyte differentiation was induced using a cocktail containing IBMX, dexamethasone, and insulin (20× magnification, inset) for 7 days. (B) Curcumin (1,5,20 μM), curcumin C3 complex® (1,5,20 μM), and THC: 1,5,20 μM) were tested for their ability to prevent rosiglitazone-induced differentiation of human primary subcutaneous preadipocytes, as described above. Following differentiation, glycerol content of adipocytes (A, B) were analyzed by measuring the concentration of glycerol released based on the equation, 1M triglyceride yields 1M glycerol + free fatty acids. All treatments were in triplicate.
$^{*,\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#,}$***$p < 0.001$, *compared to DMSO, $^{\#}$compared to rosiglitazone.

Figure 3. Curcumin increases adiponectin, CD36, and LPL gene expression in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were incubated with rosiglitazone or curcumin for 24 hours. Gene expression was quantitated by real-time qPCR. Data are depicted as fold-change compared to β-actin, using the ΔΔCT method. Inset shows Western blot image of curcumin-induced secretion of adiponectin into the culture media of differentiated 3T3-L1 adipocytes.

Fig. 4: Curcuminoids increase adiponectin secretion in human primary subcutaneous adipocytes. Human primary subcutaneous adipocytes (Zen-Bio Inc, NC) were treated with curcumin (Cur: 1,5,20 μM), curcumin C3 complex® (C3: 1,5,20 μM), tetrahydrocurcuminoids (THC: 1,5,20 μM) in the presence or absence of rosiglitazone (5 μM), or GW9662 (5 μM) for 3 days at 37°C in a CO_2 incubator. Adiponectin secreted into the media was measured using a human adiponectin ELISA kit (B-Bridge International, Inc., CA). Treatments were performed in triplicate. $^{*,\#}p < 0.05$, $^{\#\#}p < 0.01$, ***$p < 0.001$, *compared to DMSO, $^{\#}$compared to rosiglitazone.

Fig.1

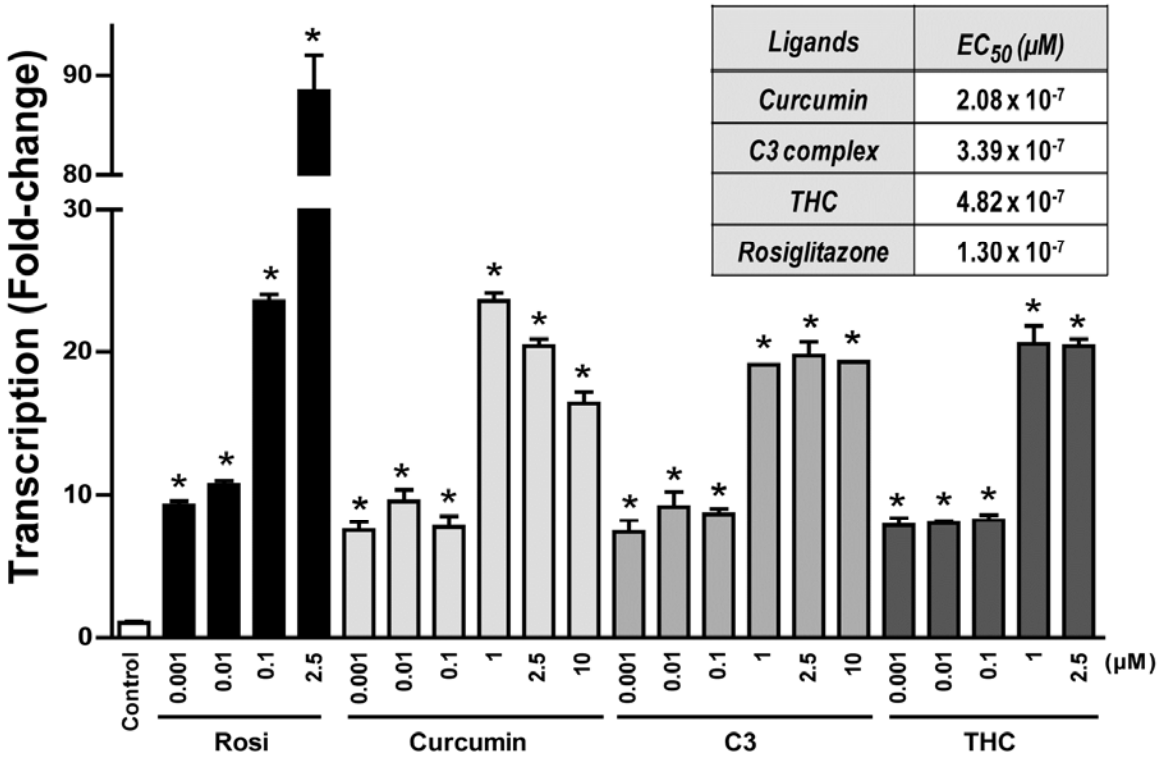

Transcription (Fold-change)
90
80
30
20
10
0
Control
0.001
0.01
0.1
2.5
1
10
(μM)
Rosi
Curcumin
C3
THC
Ligands
EC50 (μM)
Curcumin
2.08 x 10-7
C3 complex
3.39 x 10-7
THC
4.82 x 10-7
Rosiglitazone
1.30 x 10-7

Fig.2A

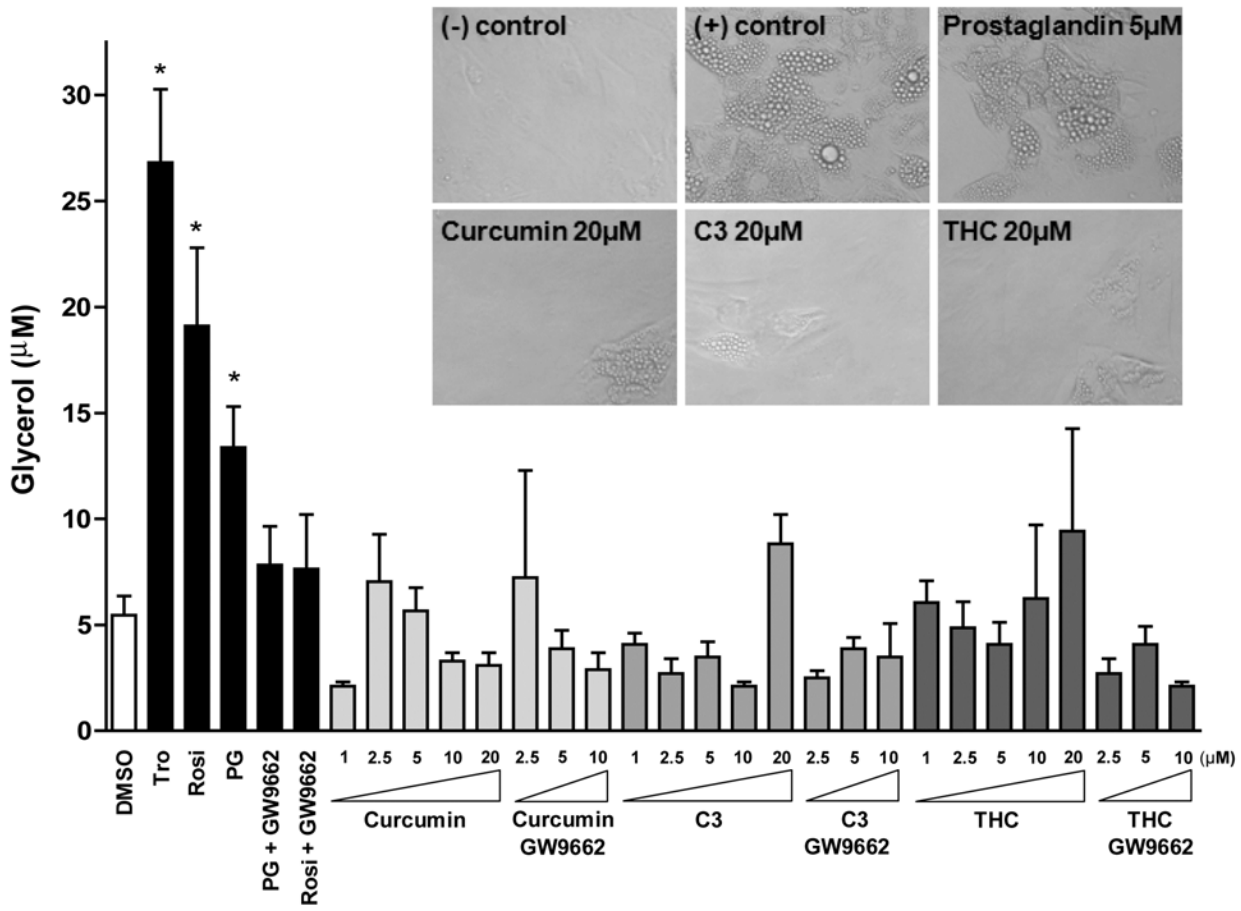
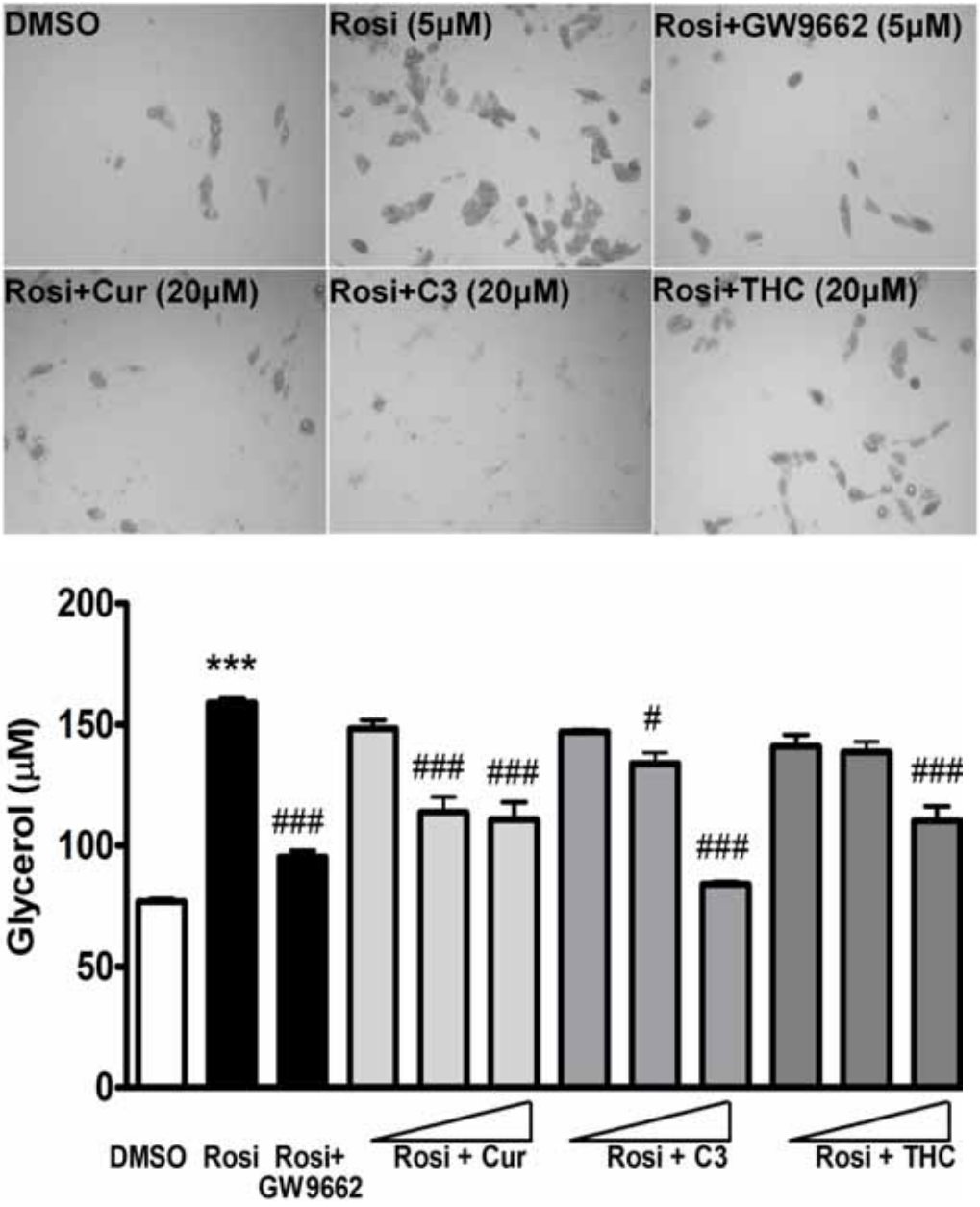

(-) control
(+) control
Prostaglandin 5μM
Curcumin 20μM
C3 20μM
THC 20μM
Glycerol (μM)
0
5
10
15
20
25
30
*
*
*
DMSO
Tro
Rosi
PG
PG + GW9662
Rosi + GW9662
1 2.5 5 10 20
Curcumin
2.5 5 10
Curcumin GW9662
1 2.5 5 10 20
C3
2.5 5 10
C3 GW9662
1 2.5 5 10 20
THC
2.5 5 10 (μM)
THC GW9662

Fig.2B


DMSO
Rosi (5μM)
Rosi+GW9662 (5μM)
Rosi+Cur (20μM)
Rosi+C3 (20μM)
Rosi+THC (20μM)
Glycerol (μM)
200
150
100
50
0
***
###
###
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DMSO
Rosi
Rosi+ GW9662
Rosi + Cur
Rosi + C3
Rosi + THC

Fig.3

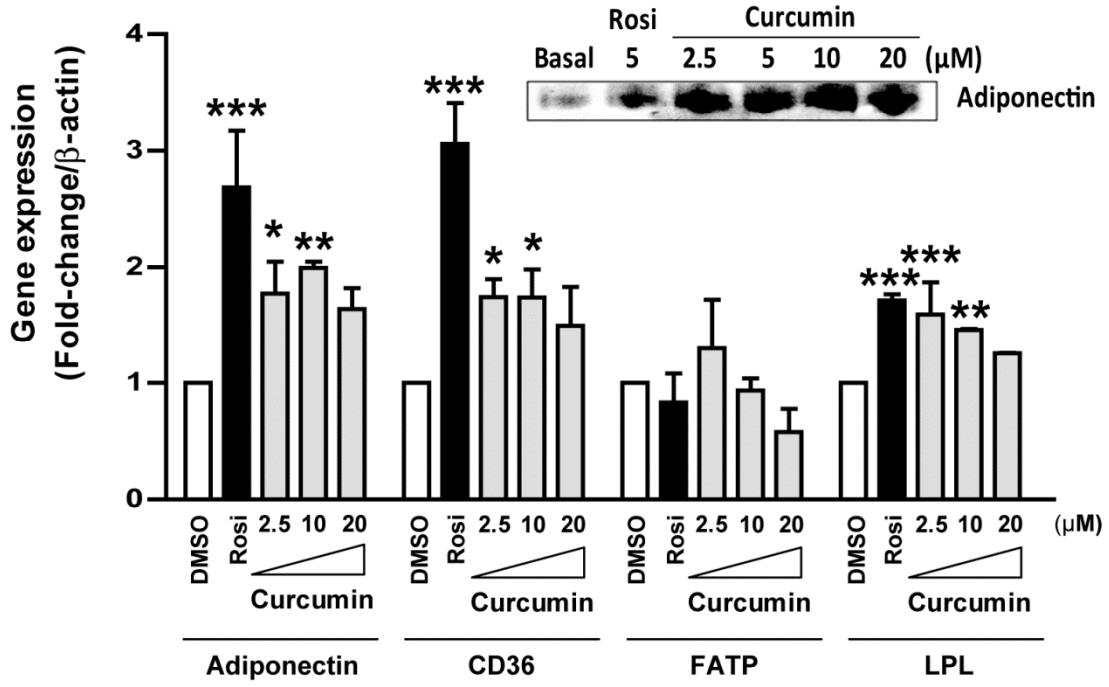
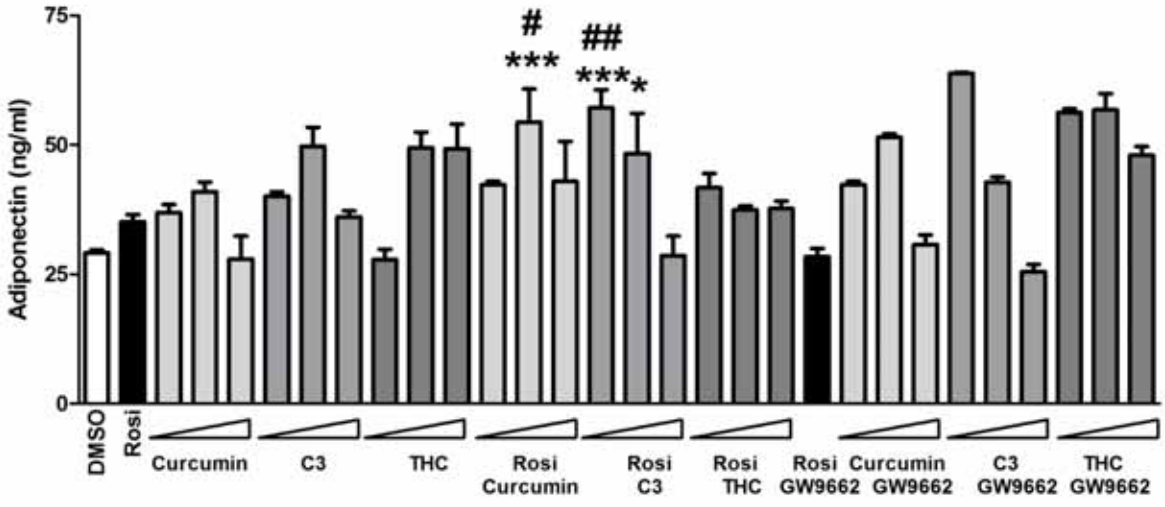

Rosi
Curcumin
Basal 5 2.5 5 10 20 (μM)
Adiponectin
Gene expression (Fold-change/β-actin)
DMSO Rosi 2.5 10 20 (μM)
Curcumin
Adiponectin
CD36
FATP
LPL

Fig.4


Adiponectin (ng/ml)
DMSO
Rosi
Curcumin
C3
THC
Rosi Curcumin
Rosi C3
Rosi THC
Rosi GW9662
Curcumin GW9662
C3 GW9662
THC GW9662

Chapter 6: Alpha-glucosidase Inhibitory effect of Resveratrol and Piceatannol

Abstract

Diet is an integral part in the management of diabetes. Dietary polyphenols have been shown to inhibit α-glucosidase, an enzyme target of anti-diabetic drugs acarbose, miglitol, and voglibose. Resveratrol, a polyphenol found in grapes and wine, has been reported to inhibit the activity of yeast α-glucosidase. This triggered our interest to synthesize analogs of resveratrol and determine their effect on mammalian α-glucosidase (EC 3.2.1.20) activity. Using either sucrose or maltose as substrate, resveratrol, piceatannol and 3'-hydroxypterostilbene (0.5 mg/ml) showed strong inhibition of mammalian α-glucosidase activity, while pinostilbene, *cis*- and *trans*-desoxyrhapontigenin showed moderate inhibitory activity. Compared to acarbose (IC_{50}: 3-13 μg/ml), piceatannol and resveratrol inhibited mammalian alpha glucosidase with an IC_{50} of 14-84 μg/ml and 111-120 μg/ml, respectively. 3'-Hydroxypterostilbene (IC_{50}: 105-302 μg/ml) was 23-35 fold less potent than acarbose. To investigate the *in vivo* effect of natural stilbenes on post-prandial blood glucose response, we administered resveratrol or piceatannol to high-fat fed C57Bl/6 mice, 60 min prior to sucrose-, starch-, or glucose-loading. Animals administered resveratrol (30 mg/kg BW) or piceatannol (14 mg/kg BW) prior to sucrose- or starch-loading, delayed the absorption of carbohydrates, resulting in significant lowering of post-prandial blood glucose concentrations, similar to the antidiabetic drug Acarbose™. Taken together, these studies demonstrate that the dietary polyphenols resveratrol and piceatannol lower post-prandial hyperglycemia and indicate that inhibition of intestinal alpha-glucosidase activity may be one potential mechanism contributing to its anti-diabetic properties.

Introduction

Diabetes, a group of metabolic chronic disease affecting over 25.8 million people in the United States and over 346 million people worldwide, is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2011; CDC, 2011; WHO, 2011). Normalization of blood glucose levels, especially postprandial hyperglycemia, is important to prevent the complications of diabetes, including retinopathy, nephropathy, neuropathy, coronary heart disease, stroke, and peripheral arteriopathy (Baron, 1998; Scheen, 2003). One class of drugs used in the treatment of diabetes is the α-glucosidase inhibitors. Alpha-glucosidase is a membrane-bound intestinal enzyme that hydrolyzes polysaccharides to glucose and other monosaccharides. Liberated glucose is then absorbed by the gut and contributes to postprandial hyperglycemia. Alpha-glucosidase inhibitors prevent or delay the digestion or absorption of carbohydrates and suppress postprandial hyperglycemia by inhibition of intestinal alpha-glucosidase, making such inhibitors useful in the management of type 2 diabetes (Bell, 2004). Acarbose and voglibose, alpha-glucosidase inhibitors, are currently used clinically in combination with either diet or other anti-diabetic agents to control blood glucose levels in patients with type 2 diabetes (Van de Laar et al., 2005).

trans-Resveratrol, piceatannol, and pterostilbene are naturally occurring stilbenes, a class of polyphenols, with strong antioxidant activities (Frombaum et al., 2012; Lee et al., 2010; Mikstacka et al., 2010; Rimando et al., 2002). Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) has been shown to have cardioprotective (Hung et al., 2004; Lekakis et al., 2005; Wang et al., 2005; Zern et al., 2003), cancer chemopreventive (Kraft et al., 2009; Patel et al., 2010), anti-inflammatory (Bishayee et al., 2010; Kang et al., 2009), antioxidant (Rizvi and Pandey, 2010), anti-amylodogenic (Riviere et al., 2007) and neuroprotective (Albani et al., 2010) effects.

Similarly, piceatannol, (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) has been reported to demonstrate cardioprotective (Kim et al., 2007), anti-cancer (Kuo and Hsu, 2008), anti-inflammatory (Son et al., 2010), antioxidant (Lee et al., 2010; Ovesna et al., 2006) and neuroprotective (Kim et al., 2007) activity. Pterostilbene, a natural methoxylated analog of resveratrol, has been shown to be a potent inhibitor of NFκB, AP-1, COX-2, and iNOS activities (Cichocki et al., 2008). Several reports have shown that stilbenes possess hypoglycemic activity, including resveratrol analogs from *Pterocarpus marsupium* and *Rheum undulatum*, and stilbene glycosides from rhubarb and *Rumex bucepahlophorus,* albeit, by various mechanisms (Choi et al., 2005; Kerem et al., 2006; Li et al., 2007; Manickam et al., 1997). Stilbenes have also been reported to inhibit α-glucosidase activity and lower postprandial glucose concentrations (Kubo, 1991; Lam et al., 2008). However, studies on α-glucosidase inhibitory properties of naturally occurring stilbenes such as pterostilbene, piceatannol and resveratrol are limited (Kerem et al., 2006; Wan et al., 2011).

In this study, we have investigated the ability of the natural stilbenes, pterostilbene, resveratrol and piceatannol, and several synthetic analogs to inhibit mammalian rat intestinal α-glucosidase activity *in vitro*. Further, the role of resveratrol and piceatannol in delaying carbohydrate absorption was examined in a mouse model of high fat diet-induced insulin resistance and hyperglycemia.

Materials and Methods

Synthesis of stilbenes: Synthesis of stilbenes #1-14 and #17-27 has been previously reported (Mizuno et al., 2008a; Mizuno et al., 2008b). Pterostilbene (#15) was synthesized according to Rimando et al., 2005 (Rimando et al., 2005). Resveratrol (#16) was obtained from Sigma-Aldrich. Stilbene #28 was synthesized following published procedures (Tolomeo et al., 2005). Stilbenes #29-32 were synthesized in the laboratory of Dr. Agnes Rimando, USDA-ARS, MS.

Reagents: Acarbose was purchased from LKT Laboratories (St. Paul, MN). Rat intestinal acetone powder, sucrose, maltose, and starch were purchased from Sigma Chemical Co. (St. Louis, MO); glucose color reagent was from Raichem, (Columbia, MD); and resveratrol and piceatannol used for animal study were purchased from Tocris Bioscience (Ellisville, MO). All other reagents were of analytical grade. Blood glucose concentrations were measured using Accu-Chek glucometer (Roche Diagnostics, Indianapolis, IN).

Yeast alpha-glucosidase inhibitory assay: Yeast alpha-glucosidase assay was assayed according to Watanabe, et al. (Watanabe et al., 1997). Stilbene compounds were incubated with 50 µl of yeast α-glucosidase enzyme solution (0.7 U/ml) (Sigma–Aldrich) in 0.1 M phosphate buffer, pH 7.0 for 5 min. This was followed by the addition of 50 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (Sigma) substrate solution and incubated for another 5 min at room temperature. Enzymatic inhibitory activity was quantitated by measuring the released p-nitrophenol at 405 nm, based on the following equation: α-glucosidase inhibitory activity (%) = $1-(S_2-S_1/C_2-C_1) \times 100$, where S = Sample, C = Control (DMSO), 1 = Initial A_{405}, and 2 = Final A_{405}. IC_{50} values were calculated based on percent inhibition obtained. All experiments were performed in triplicate.

Mammalian alpha-glucosidase inhibitory assay in vitro: Mammalian intestinal α-glucosidases is a complex consisting of three individual enzymes, namely sucrase, maltase, and isomaltase (Adachi et al., 2003). In this study, we examined the effect of natural stilbenes and their analogs on inhibition of mammalian intestinal α-glucosidase activity. Rat intestinal acetone powder (200 mg) was hand-homogenized using 10 mL of ice-cold 50 mM phosphate buffer. After the contents were centrifuged at 8,000 rpm for 25 min, the supernatant was applied to a Sephadex G-100 column and eluted with ice-cold 50 mM phosphate buffer. The activity of rat intestinal α-glucosidase extract was verified using p-nitrophenyl-α-D-glucopyranoside as substrate by comparing with pure yeast α-glucosidase (Sigma). An aliquot of enzyme extract was incubated in the presence or absence of stilbene compounds using either sucrose (56 mM) or maltose (5 mM) as substrate (Shinde et al., 2008). The amount of glucose liberated from the substrate was assayed by glucose oxidase method using a commercially available Autokit (Raichem, Columbia, MD). Acarbose, a widely used anti-diabetic drug and synthetic inhibitor of mammalian α-glucosidase, was used as positive control. The IC_{50} values of stilbene compounds for mammalian α-glucosidase inhibition were calculated from percent inhibition obtained from dose-response curves.

Alpha-glucosidase inhibitory activity in animals: Animal experimental protocols were approved by the Institutional Animal Care and Use Committee at Auburn University. Forty, male C57Bl/6 mice, 4 weeks old, were purchased from the Charles River Laboratories, Inc. (Wilmington, MA). These mice were fed a high-fat diet (D-12451, 45% kcals from fat), obtained from Research Diets, Inc. (New Brunswick, NJ) for a period of 8 weeks. After an overnight fast (food deprivation for at least 12 h, but with free access to water), mice were administered resveratrol (30 mg/kg BW) or piceatannol (14 mg/kg BW) orally, 60 min prior to an oral gavage of sucrose

(4 g/kg) or glucose (2 g/kg) or starch (3 g/kg). Blood samples were obtained at -60, 0, 15, 30, 60, and 120 min to assay glucose concentrations. Control animals received vehicle (water) instead of resveratrol or piceatannol. Acarbose (5 mg/kg) was used as positive control, and administered 60 minutes prior to sucrose (glucose or starch) loading.

Statistical analyses: Data are expressed as the mean ± standard error of the mean (SEM). Statistical significance for $AUC_{Glucose}$ between treated and control animals were determined using Student's t-test.

Results

Resveratrol and piceatannol inhibit mammalian alpha-glucosidase in vitro

In this study we investigated the role of natural stilbenes, including pterostilbene, resveratrol, and piceatannol, and synthetic stilbene analogs of resveratrol (Fig.1) in the inhibition of mammalian alpha-glucosidase activity, using sucrose (Fig.2A) or maltose (Fig.2B) as substrate. At concentrations (0.5 mg/ml) comparable to acarbose, resveratrol **(16)**, piceatannol **(29)**, and 3'-hydroxypterostilbene **(32)** demonstrated potent inhibition of mammalian alpha-glucosidase activity. The inhibitory effects of piceatannol **(29)** and 3-hydroxypterostilbene **(32)** on sucrose and maltase activity of α-glucosidase were similar to that of acarbose (~90% inhibition). Resveratrol inhibited sucrase activity by ~90% and maltase activity by ~60%. Further, *trans*-pinostilbene **(10)**, *trans*-desoxyrhapontigenin **(12)**, and *cis*-desoxyrhapontigenin **(13)** showed moderate α-glucosidase inhibitory activity, using either sucrose or maltose as substrate. Next, we generated dose response curves to calculate IC_{50} concentrations for alpha-glucosidase inhibition (*Fig.2C,D,E)*. As shown previously by Kerem et al (2006), we demonstrate that resveratrol was ~25 times more potent (IC_{50}: 9 μg/ml) than acarbose (IC_{50}: 247 μg/ml) in inhibiting yeast alpha-glucosidase (*Table 1*). Similarly, *trans*-pinostilene, trans-desoxyrhapontigenin, and piceatannol demonstrated lower IC_{50} for yeast α-glucosidase inhibition compared to acarbose. 3-Hydroxypterostilbene showed similar IC_{50} for yeast α-glucosidase inhibition as acarbose. Since mammalian alpha-glucosidase inhibition may be more biologically relevant than yeast alpha-glucosidase inhibition, and considering that acarbose is a potent inhibitor of mammalian alpha-glucosidase (Oki et al., 1999) we calculated IC_{50} concentrations of stilbenes for mammalian α-glucosidase. Piceatannol (IC_{50}: 14 μg/ml) demonstrated comparable α-glucosidase inhibition as acarbose (IC_{50}: 13 μg/ml), using sucrose as substrate, and much

lower potency using maltose as substrate (IC_{50}: 84 µg/ml vs. 3 µg/ml for acarbose). Compared to acarbose, resveratrol showed moderate inhibition of sucrase activity (IC_{50}: 120 µg/ml) and maltase activity (IC_{50}: 111 µg/ml). 3-Hydroxypterostilbene showed a similar IC_{50} (105 µg/ml) as resveratrol, using sucrose as substrate, but higher IC50 (300 µg/ml) using maltose as substrate. Similarly trans-pterostilbene and trans-desoxyhapontigenin demonstrated higher IC_{50} concentrations for mammalian α-glucosidase, compared to acarbose, using either sucrose or maltose as substrate.

Resveratrol and piceatannol inhibit alpha-glucosidase in vivo

Since piceatannol and resveratrol showed significant inhibitory effects on yeast and mammalian intestinal α-glucosidase activity, we investigated the effects of these compounds *in vivo*, on post-prandial glucose concentrations. We used the C57Bl/6 mouse model of diet-induced obesity for these studies. After a period of 8 weeks on high-fat diet (45% kcals from fat), these animals had significantly elevated fasting blood glucose levels (169.4 ± 14.8 mg/dl) compared to mice fed regular chow (132.8 132.8 ± 6.9 mg/dl). Resveratrol (30 mg/kg BW) and piceatannol (14 mg/kg BW) were administered orally, 60 min prior to an oral gavage of sucrose (4 g/kg). Animals administered resveratrol or piceatannol, prior to sucrose-loading, showed lower blood glucose concentrations compared to control animals *(Fig.3A)*. Area under the curve for glucose ($AUC_{Glucose}$) in animals administered resveratrol or piceatannol was significantly lower ($p = 0.0273$ and 0.0146, respectively) compared to $AUC_{Glucose}$ of control group, and was similar to acarbose group *(Fig.3B)*. Next, we examined the effect of resveratrol and piceatannol on starch loading in high-fat fed C57Bl/6 mice. Administration of resveratrol and piceatannol prior to starch-loading decreased post-prandial blood glucose levels as evidenced by significantly lower $AUC_{Glucose}$ levels compared to the control group ($p = 0.0026$ and 0.0039, respectively), and

this was similar to that of acarbose group (*Fig.3C,D*). To test whether the alpha-glucosidase inhibitory effect was the only contributor for the lowered postprandial glucose response effect of resveratrol or picteannol, high-fat fed mice were subject to glucose-loading *(Fig.3E,F)*. No significantly difference was observed between $AUC_{Glucose}$ of resveratrol and glucose ($p = 0.674$). While a decreasing trend in $AUC_{Glucose}$ was observed for piceatannol, this was not statistically significant compared to the glucose-loaded control group ($p = 0.109$) group (*Fig.2C*), suggesting that the hypoglycemic effect of resveratrol or piceatannol was primarily dependent on alpha-glucosidase inhibitory effect following an oral carbohydrate load.

Discussion

To our knowledge, this is the first report showing that resveratrol and piceatannol inhibit mammalian α-glucosidase activity *in vitro* and *in vivo*, leading to a decreased post-prandial blood glucose response in a high-fat fed mouse model of obesity, insulin resistance, and hyperglycemia. Further, these studies have compared α-glucosidase inhibitory properties of 28 synthetic analogs of resveratrol. Our studies suggest potential α-glucosidase inhibitory properties for trans-pterostilbene, 3'-hydroxystilbene, and trans-desoxyhapontigenin (in addition to resveratrol and piceatannol) based on inhibition of yeast α-glucosidase activity. Previously, it was shown that resveratrol demonstrated a potent inhibitory effect on yeast α-glucosidase activity (Kerem et al., 2006). However, there are several studies that show varied inhibitory effects of α-glucosidase inhibitors, depending on the source of the enzyme used. Voglibose, acarbose and glucono-1,5-lactone demonstrated potent inhibition of mammalian α-glucosidase, but were ineffective or less effective yeast α-glucosidase inhibitors (Oki et al., 1999). Contrary to this, (+)-catechin, green tea, and oolong tea inhibited yeast α-glucosidase, but were ineffective inhibitors of mammalian α-glucosidase activity (Oki et al., 1999). Similarly, other investigators Babu et al. (2004) and Shai et al. (2011) have shown that crude methanol extracts of several plants exerted potent inhibition of yeast α-glucosidase, but were less effective against mammalian α-glucosidase activity. This discrepancy may be attributed to structural differences (Lee and Lee, 2001). Since α-glucosidase from rat intestines closely mimics the mammalian system and biologically more relevant in the identification, design, and development of natural or synthetic bioactive compounds, we have used the mammalian enzyme source for our studies.

Our studies have shown that among the natural stilbenes and 28 synthetic analogs that were tested, piceatannol displayed potent mammalian α-glucosidase inhibitory properties *in vitro*

with an IC_{50} (14 μg/ml) that was comparable to acarbose (13 μg/ml), using sucrose as substrate. Resveratrol demonstrated moderate inhibition (IC_{50} = 120 μg/ml), and was 9 times less potent as acarbose, using sucrose as substrate. Pterostilbene did not show significant inhibition of either yeast or mammalian α-glucosidase activities. In comparison, studies by Zhang et al. show that grape-seed extract demonstrated an IC_{50} of 384 μg/ml, and oolong and green tea extracts demonstrated even higher IC_{50} (1.34 mg/ml and 735 μg/ml, respectively), against rat α-glucosidases (Oki et al., 1999; Zhang et al., 2011). Since piceatannol and resveratrol showed significant inhibition of mammalian rat intestinal α-glucosidase activity *in vitro*, these were tested for their ability to inhibit α-glucosidase activity in vivo in high-fat fed C57Bl/6 mice. Our findings, that both piceatannol and resveratrol delay the absorption of carbohydrates leading to a lowered post-prandial blood glucose response, confirm our in vitro findings. The dosages used for piceatannol (14 mg/kg BW) and resveratrol (30 mg/kg BW) were higher than that of acarbose (5 mg/kg BW). Additional dose-response studies are needed in animals to compare dosing efficacy. However, our studies show that, at the doses tested, both piceatannol and resveratrol lowered $AUC_{glucose}$ comparably as acarbose.

The antidiabetic effect of resveratrol has been reported in many studies (Szkudelski and Szkudelska, 2011). It has been shown that resveratrol improves glucose uptake in muscle and liver (Chi et al., 2007; Su et al., 2006), represses insulin secretion in pancreas (Szkudelski, 2006, 2008), decreases adipose tissue inflammation (Ikubo et al., 2009; Mader et al., 2010; Rotter et al., 2003), liver inflammation (Palsamy et al., 2010), mimics effect of caloric restriction (Barger et al., 2008) and enhances capacity for adaptive thermogenesis (Lagouge et al., 2006). Piceatannol (3,3',4,5'-tetrahydroxystilbene), which possesses an additional hydroxyl group on resveratrol (3,4',5-trihydroxystilbene), exerts higher free radical scavenging activity than

resveratrol (Fauconneau et al., 1997). Recently, piceatannol has been shown to inhibit adipogenesis in 3T3-L1 preadipocytes via modulation of mitotic clonal expansion and insulin signal transduction (Kwon et al., 2012).

In conclusion, we have presented novel evidence that the natural phenolic compounds piceatannol and resveratrol are potent inhibitors of mammalian α-glucosidase activity. Further, our studies showing that piceatannol and resveratrol lower post-prandial blood glucose contribute to the range of reported beneficial effects of stilbenes, and suggest a promising therapeutic potential for development of stilbene analogs in the control of hyperglycemia.

Acknowledgement

This study was funded by grants from Alabama Agricultural Experiment Station to STM.

References

ADA (2011). Diagnosis and classification of diabetes mellitus. Diabetes Care *34 Suppl 1*, S62-69.

Adachi, T., Mori, C., Sakurai, K., Shihara, N., Tsuda, K., and Yasuda, K. (2003). Morphological changes and increased sucrase and isomaltase activity in small intestines of insulin-deficient and type 2 diabetic rats. Endocrine Journal *50*, 271-279.

Albani, D., Polito, L., Signorini, A., and Forloni, G. (2010). Neuroprotective properties of resveratrol in different neurodegenerative disorders. BioFactors (Oxford, England).

Babu, K.S., Tiwari, A.K., Srinivas, P.V., Ali, A.Z., China Raju, B., and Rao, J.M. (2004). Yeast and mammalian alpha-glucosidase inhibitory constituents from Himalayan rhubarb Rheum emodi Wall.ex Meisson. Bioorganic & medicinal chemistry letters *14*, 3841-3845.

Barger, J.L., Kayo, T., Vann, J.M., Arias, E.B., Wang, J., Hacker, T.A., Wang, Y., Raederstorff, D., Morrow, J.D., Leeuwenburgh, C.*, et al.* (2008). A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. PLoS One *3*, e2264.

Baron, A.D. (1998). Postprandial hyperglycaemia and alpha-glucosidase inhibitors. Diabetes Res Clin Pract *40 Suppl*, S51-55.

Bell, D.S. (2004). Type 2 diabetes mellitus: what is the optimal treatment regimen? Am J Med *116 Suppl 5A*, 23S-29S.

Bishayee, A., Barnes, K.F., Bhatia, D., Darvesh, A.S., and Carroll, R.T. (2010). Resveratrol suppresses oxidative stress and inflammatory response in diethylnitrosamine-initiated rat hepatocarcinogenesis. Cancer prevention research (Philadelphia, Pa *3*, 753-763.

CDC (2011). National Diabetes Fact sheet.

Chi, T.C., Chen, W.P., Chi, T.L., Kuo, T.F., Lee, S.S., Cheng, J.T., and Su, M.J. (2007). Phosphatidylinositol-3-kinase is involved in the antihyperglycemic effect induced by resveratrol in streptozotocin-induced diabetic rats. Life sciences *80*, 1713-1720.

Choi, S.Z., Lee, S.O., Jang, K.U., Chung, S.H., Park, S.H., Kang, H.C., Yang, E.Y., Cho, H.J., and Lee, K.R. (2005). Antidiabetic stilbene and anthraquinone derivatives from Rheum undulatum. Arch Pharm Res *28*, 1027-1030.

Cichocki, M., Paluszczak, J., Szaefer, H., Piechowiak, A., Rimando, A.M., and Baer-Dubowska, W. (2008). Pterostilbene is equally potent as resveratrol in inhibiting 12-O-tetradecanoylphorbol-13-acetate activated NFkappaB, AP-1, COX-2, and iNOS in mouse epidermis. Molecular nutrition & food research *52 Suppl 1*, S62-70.

Fauconneau, B., Waffo-Teguo, P., Huguet, F., Barrier, L., Decendit, A., and Merillon, J.M. (1997). Comparative study of radical scavenger and antioxidant properties of phenolic compounds from Vitis vinifera cell cultures using in vitro tests. Life Sci *61*, 2103-2110.

Frombaum, M., Le Clanche, S., Bonnefont-Rousselot, D., and Borderie, D. (2012). Antioxidant effects of resveratrol and other stilbene derivatives on oxidative stress and *NO bioavailability: Potential benefits to cardiovascular diseases. Biochimie *94*, 269-276.

Hung, L.M., Su, M.J., and Chen, J.K. (2004). Resveratrol protects myocardial ischemia-reperfusion injury through both NO-dependent and NO-independent mechanisms. Free radical biology & medicine *36*, 774-781.

Ikubo, M., Wada, T., Fukui, K., Ishiki, M., Ishihara, H., Asano, T., Tsuneki, H., and Sasaoka, T. (2009). Impact of lipid phosphatases SHIP2 and PTEN on the time- and Akt-isoform-specific amelioration of TNF-alpha-induced insulin resistance in 3T3-L1 adipocytes. American journal of physiology *296*, E157-164.

Kang, O.H., Jang, H.J., Chae, H.S., Oh, Y.C., Choi, J.G., Lee, Y.S., Kim, J.H., Kim, Y.C., Sohn, D.H., Park, H.*, et al.* (2009). Anti-inflammatory mechanisms of resveratrol in activated HMC-1 cells: pivotal roles of NF-kappaB and MAPK. Pharmacol Res *59*, 330-337.

Kerem, Z., Bilkis, I., Flaishman, M.A., and Sivan, L. (2006). Antioxidant activity and inhibition of alpha-glucosidase by trans-resveratrol, piceid, and a novel trans-stilbene from the roots of Israeli Rumex bucephalophorus L. Journal of agricultural and food chemistry *54*, 1243-1247.

Kim, H.J., Lee, K.W., and Lee, H.J. (2007). Protective effects of piceatannol against beta-amyloid-induced neuronal cell death. Annals of the New York Academy of Sciences *1095*, 473-482.

Kraft, T.E., Parisotto, D., Schempp, C., and Efferth, T. (2009). Fighting cancer with red wine? Molecular mechanisms of resveratrol. Critical reviews in food science and nutrition *49*, 782-799.

Kubo, I.M.Y. (1991). Efficient isolation of glycosidase inhibitory stilbene glycosides from Rheum palmatum. Journal of natural products July/Aug *54*, 1115-1118.

Kuo, P.L., and Hsu, Y.L. (2008). The grape and wine constituent piceatannol inhibits proliferation of human bladder cancer cells via blocking cell cycle progression and inducing Fas/membrane bound Fas ligand-mediated apoptotic pathway. Molecular nutrition & food research *52*, 408-418.

Kwon, J.Y., Seo, S.G., Heo, Y.S., Yue, S., Cheng, J.X., Lee, K.W., and Kim, K.H. (2012). Piceatannol, a natural polyphenolic stilbene, inhibits adipogenesis via modulation of mitotic clonal expansion and insulin receptor-dependent insulin signaling in the early phase of differentiation. J Biol Chem.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P.*, et al.* (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell *127*, 1109-1122.

Lam, S.H., Chen, J.M., Kang, C.J., Chen, C.H., and Lee, S.S. (2008). alpha-Glucosidase inhibitors from the seeds of Syagrus romanzoffiana. Phytochemistry *69*, 1173-1178.

Lee, D.S., and Lee, S.H. (2001). Genistein, a soy isoflavone, is a potent alpha-glucosidase inhibitor. FEBS Letters *501*, 84-86.

Lee, H.H., Park, S.A., Almazari, I., Kim, E.H., Na, H.K., and Surh, Y.J. (2010). Piceatannol induces heme oxygenase-1 expression in human mammary epithelial cells through activation of ARE-driven Nrf2 signaling. Archives of biochemistry and biophysics *501*, 142-150.

Lekakis, J., Rallidis, L.S., Andreadou, I., Vamvakou, G., Kazantzoglou, G., Magiatis, P., Skaltsounis, A.L., and Kremastinos, D.T. (2005). Polyphenolic compounds from red grapes acutely improve endothelial function in patients with coronary heart disease. Eur J Cardiovasc Prev Rehabil *12*, 596-600.

Li, J.M., Che, C.T., Lau, C.B., Leung, P.S., and Cheng, C.H. (2007). Desoxyrhaponticin (3,5-dihydroxy-4'-methoxystilbene 3-O-beta-D-glucoside) inhibits glucose uptake in the intestine and kidney: In vitro and in vivo studies. J Pharmacol Exp Ther *320*, 38-46.

Mader, I., Wabitsch, M., Debatin, K.M., Fischer-Posovszky, P., and Fulda, S. (2010). Identification of a novel proapoptotic function of resveratrol in fat cells: SIRT1-independent sensitization to TRAIL-induced apoptosis. FASEB J *24*, 1997-2009.

Manickam, M., Ramanathan, M., Jahromi, M.A., Chansouria, J.P., and Ray, A.B. (1997). Antihyperglycemic activity of phenolics from Pterocarpus marsupium. J Nat Prod *60*, 609-610.

Mikstacka, R., Rimando, A.M., and Ignatowicz, E. (2010). Antioxidant effect of trans-resveratrol, pterostilbene, quercetin and their combinations in human erythrocytes in vitro. Plant Foods Hum Nutr *65*, 57-63.

Mizuno, C.S., Ma, G., Khan, S., Patny, A., Avery, M.A., and Rimando, A.M. (2008a). Design, synthesis, biological evaluation and docking studies of pterostilbene analogs inside PPARalpha. Bioorg Med Chem *16*, 3800-3808.

Mizuno, C.S., Schrader, K.K., and Rimando, A.M. (2008b). Algicidal activity of stilbene analogues. Journal of agricultural and food chemistry *56*, 9140-9145.

Oki, T., Matsui, T., and Osajima, Y. (1999). Inhibitory effect of alpha-glucosidase inhibitors varies according to its origin. Journal of agricultural and food chemistry *47*, 550-553.

Ovesna, Z., Kozics, K., Bader, Y., Saiko, P., Handler, N., Erker, T., and Szekeres, T. (2006). Antioxidant activity of resveratrol, piceatannol and 3,3',4,4',5,5'-hexahydroxy-trans-stilbene in three leukemia cell lines. Oncology reports *16*, 617-624.

Palsamy, P., Sivakumar, S., and Subramanian, S. (2010). Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats. Chem Biol Interact *186*, 200-210.

Patel, V.B., Misra, S., Patel, B.B., and Majumdar, A.P. (2010). Colorectal cancer: chemopreventive role of curcumin and resveratrol. Nutrition and cancer *62*, 958-967.

Rimando, A.M., Cuendet, M., Desmarchelier, C., Mehta, R.G., Pezzuto, J.M., and Duke, S.O. (2002). Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. Journal of agricultural and food chemistry *50*, 3453-3457.

Rimando, A.M., Nagmani, R., Feller, D.R., and Yokoyama, W. (2005). Pterostilbene, a new agonist for the peroxisome proliferator-activated receptor alpha-isoform, lowers plasma lipoproteins and cholesterol in hypercholesterolemic hamsters. Journal of agricultural and food chemistry *53*, 3403-3407.

Riviere, C., Richard, T., Quentin, L., Krisa, S., Merillon, J.M., and Monti, J.P. (2007). Inhibitory activity of stilbenes on Alzheimer's beta-amyloid fibrils in vitro. Bioorg Med Chem *15*, 1160-1167.

Rizvi, S.I., and Pandey, K.B. (2010). Activation of the erythrocyte plasma membrane redox system by resveratrol: a possible mechanism for antioxidant properties. Pharmacol Rep *62*, 726-732.

Rotter, V., Nagaev, I., and Smith, U. (2003). Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem *278*, 45777-45784.

Scheen, A.J. (2003). Is there a role for alpha-glucosidase inhibitors in the prevention of type 2 diabetes mellitus? Drugs *63*, 933-951.

Shai, L.J., Magano, S.R., Lebelo, S.L., and Mogale, A.M. (2011). Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. Journal of Medicinal Plants Research *5*, 2863-2867.

Shinde, J., Taldone, T., Barletta, M., Kunaparaju, N., Hu, B., Kumar, S., Placido, J., and Zito, S.W. (2008). Alpha-glucosidase inhibitory activity of Syzygium cumini (Linn.) Skeels seed kernel in vitro and in Goto-Kakizaki (GK) rats. Carbohydr Res *343*, 1278-1281.

Son, P.S., Park, S.A., Na, H.K., Jue, D.M., Kim, S., and Surh, Y.J. (2010). Piceatannol, a catechol-type polyphenol, inhibits phorbol ester-induced NF-{kappa}B activation and

cyclooxygenase-2 expression in human breast epithelial cells: cysteine 179 of IKK{beta} as a potential target. Carcinogenesis *31*, 1442-1449.

Su, H.C., Hung, L.M., and Chen, J.K. (2006). Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. American journal of physiology *290*, E1339-1346.

Szkudelski, T. (2006). Resveratrol inhibits insulin secretion from rat pancreatic islets. European journal of pharmacology *552*, 176-181.

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

Szkudelski, T., and Szkudelska, K. (2011). Anti-diabetic effects of resveratrol. Annals of the New York Academy of Sciences *1215*, 34-39.

Tolomeo, M., Grimaudo, S., Di Cristina, A., Roberti, M., Pizzirani, D., Meli, M., Dusonchet, L., Gebbia, N., Abbadessa, V., Crosta, L.*, et al.* (2005). Pterostilbene and 3'-hydroxypterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells. Int J Biochem Cell Biol *37*, 1709-1726.

Van de Laar, F.A., Lucassen, P.L., Akkermans, R.P., Van de Lisdonk, E.H., Rutten, G.E., and Van Weel, C. (2005). Alpha-glucosidase inhibitors for type 2 diabetes mellitus. Cochrane Database Syst Rev, CD003639.

Wan, X., Wang, X.B., Yang, M.H., Wang, J.S., and Kong, L.Y. (2011). Dimerization of piceatannol by Momordica charantia peroxidase and alpha-glucosidase inhibitory activity of the biotransformation products. Bioorg Med Chem *19*, 5085-5092.

Wang, Z., Zou, J., Cao, K., Hsieh, T.C., Huang, Y., and Wu, J.M. (2005). Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels. International journal of molecular medicine *16*, 533-540.

Watanabe, J., Kawabata, J., Kurihara, H., and Niki, R. (1997). Isolation and identification of alpha-glucosidase inhibitors from tochu-cha (Eucommia ulmoides). Biosci Biotechnol Biochem *61*, 177-178.

WHO (2011). Fact Sheets,Diabetes

Zern, T.L., West, K.L., and Fernandez, M.L. (2003). Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. The Journal of nutrition *133*, 2268-2272.

Zhang, L., Hogan, S., Li, J., Sun, S., Canning, C., Zheng, S.J., and Zhou, K. (2011). Grape skin extract inhibits mammalian intestinal alpha-glucosidase activity and suppresses postprandial glycemic response in streptozocin-treated mice. Food Chemistry *126*, 466-471.

Figure legends

Fig 1. Stilbenes: structure and chemical names

Natural stilbenes and resveratrol analogs were synthesized. These are listed as *trans*-stilbenes or (*E*)-stilbenes and *cis*-stilbenes or (*Z*)-stilbenes, along with the chemical names and structures. Compound 11 is 4-(3,5-dimethoxyphenethyl)phenol. Compounds 30, 31 and 32 are commonly known as pinosylvin, pinosylvin monomethyl ether and 3'-hydroxypterostilbene, respectively.

Fig 2. ***Resveratrol and piceatannol inhibit alpha-glucosidase in vitro.***

Natural stilbenes and resveratrol analogs were tested for inhibitory effect on mammalian alpha-glucosidase activity using either sucrose (A) or maltose (B) as substrate. Acarbose (Acar) was used as a positive control. Stilbenes 1-28 (0.77 μM final concentration), and 29-32 (0.5 mg/mL, final concentration) were dissolved in DMSO and incubated with alpha-glucosidase, purified from rat intestinal acetone powder, and either sucrose (56 mM) or maltose (5 mM) as substrate. The amount of glucose liberated from the substrate was measured by glucose oxidase method using a commercially available kit. Yeast and mammalian α-glucosidase IC_{50} concentrations were calculated from dose-response curves (C,D, E). Calculated IC_{50} concentrations are shown in Table 1

Fig 3. ***Resveratrol and piceatannol inhibit alpha-glucosidase in vivo***

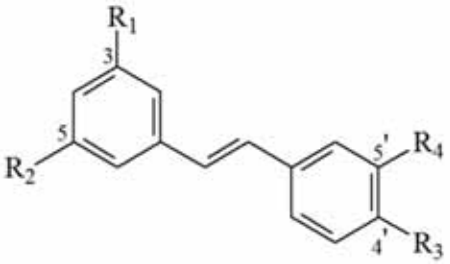
Male C57Bl/6 mice (4 weeks old) were fed a high-fat diet (D-12451, 45% kcals from fat) for a period of 8 weeks. Mice were administered resveratrol (30 mg/kg) or piceatannol (14 mg/kg), 60

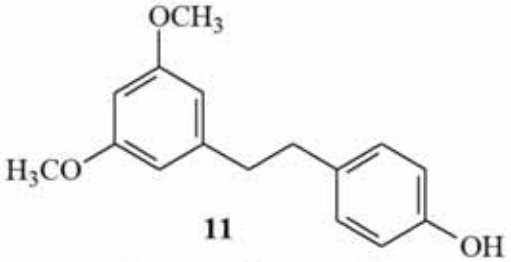
min prior to an oral gavage of (A) sucrose (4 g/kg) or (C) starch (3 g/kg) or (E) glucose (2 g/kg). Blood samples were obtained at -60, 0, 15, 30, 60, and 120 min to assay glucose concentrations. Control animals received vehicle (water) instead of resveratrol or piceatannol. Acarbose (5 mg/kg) was used as positive control, and administered 60 minutes prior to sucrose (glucose or starch) loading. Area under the curve for glucose ($AUC_{Glucose}$) was calculated. Data are shown as Mean $\pm$ SEM, n=10 mice/group, *$p < 0.05$, **$p < 0.01$.

Table 1: α-Glucosidase IC_{50} concentrations for resveratrol, piceatannol, and stilbenes

Compound	*Yeast α-glucosidase inhibition*		*Mammalian α-glucosidase inhibition*			
			Sucrase		*Maltase*	
	IC_{50} (mg/mL)	*Log IC_{50} ± SEM*	*IC_{50} (mg/mL)*	*Log IC_{50} ± SEM*	*IC_{50} (mg/mL)*	*Log IC_{50} ± SEM*
trans-Pterostilbene	0.028	-1.548 ± 0.098	0.501	-0.300 ± 0.077	0.518	-0.286 ± 0.234
trans-Desoxyhapontigenin	0.042	-1.375 ± 0.207	0.443	-0.353 ± 0.278	0.136	-0.867 ± 0.179
cis-Desoxyhapontigenin	5.481	0.739 ± 0.961	0.510	-0.297 ± 0.243	0.409	-0.388 ± 0.271
3'-Hydroxypterostilbene	0.230	-0.639 ± 0.284	0.105	-0.978 ± 0.099	0.302	-0.521 ± 0.191
Resveratrol	0.091	-2.042 ± 0.087	0.120	-0.927 ± 0.053	0.111	-0.953 ± 0.102
Piceatannol	0.060	-1.220 ± 0.071	0.014	-1.865 ± 0.095	0.084	-1.075 ± 0.061
Acarbose	0.247	-0.607 ± 0.139	0.013	-1.897 ± 0.092	0.003	-2.519 ± 0.068

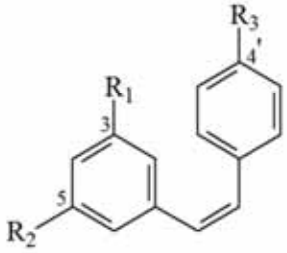
Fig.1





11

		R_1	R_2	R_3	R_4
1	(*E*)-1,3-dimethoxy-5-(4-nitrostyryl)benzene	OCH_3	OCH_3	NO_2	H
3	(*E*)-4-(3,5-dimethoxystyryl)aniline	OCH_3	OCH_3	NH_2	H
5	(*E*)-methyl 4-(3,5-dimethoxystyryl)benzoate	OCH_3	OCH_3	$COOCH_3$	H
7	(*E*)-4-(3,5-dimethoxystyryl)benzoic acid	OCH_3	OCH_3	COOH	H
8	(*E*)-1,3-dimethoxy-5-(4-methoxystyryl)benzene	OCH_3	OCH_3	OCH_3	H
10	*trans*-Pinostilbene	OCH_3	OH	OH	H
12	*trans*-Desoxyrhapontigenin	OH	OH	OCH_3	H
14	(*E*)-4-(3,5-dimethoxystyryl)phenyl dihydrogen phosphate	OCH_3	OCH_3	OPO_3H	H
15	*trans*-Pterostilbene	OCH_3	OCH_3	OH	H
16	*trans*-Resveratrol	OH	OH	OH	H
18	*trans* -Pterostilbene-4'-O-glucopyranoside	OCH_3	OCH_3	OGluc	H
19	(*E*)-1-(4-chlorostyryl)-3,5-dimethoxybenzene	OCH_3	OCH_3	Cl	H
21	(*E*)-1-(4-fluorostyryl)-3,5-dimethoxybenzene	OCH_3	OCH_3	F	H
23	(*E*)-1-(4-bromostyryl)-3,5-dimethoxybenzene	OCH_3	OCH_3	Br	H
25	(*E*)-1,3-dimethoxy-5-(4-(trifluoromethyl)styryl)benzene	OCH_3	OCH_3	CF_3	H
27	(*E*)-4-(3,5-dimethoxystyryl)benzenethiol	OCH_3	OCH_3	SH	H
28	(*E*)-4-(3,5-dimethoxystyryl)-2-methoxyphenol	OCH_3	OCH_3	OH	OCH_3
29	Piceatannol	OH	OH	OH	OH
30	5-(2-phenylethenyl)-1,3-benzenediol	OH	OH	H	H
31	3-methoxy-5-[(1*E*)-2-phenylethenyl]-phenol	OCH_3	OH	H	H
32	4-[(1*E*)-2-(3,5-dimethoxyphenyl)ethenyl]-1,2-benzenediol	OCH_3	OCH_3	OH	OH



		R_1	R_2	R_3
2	(*Z*)-1,3-dimethoxy-5-(4-nitrostyryl)benzene	OCH_3	OCH_3	NO_2
4	(*Z*)-4-(3,5-dimethoxystyryl)aniline	OCH_3	OCH_3	NH_2
6	(*Z*)-methyl 4-(3,5-dimethoxystyryl)benzoate	OCH_3	OCH_3	$COOCH_3$
9	(*Z*)-1,3-dimethoxy-5-(4-methoxystyryl)benzene	OCH_3	OCH_3	OCH_3
13	*cis*-Desoxyrhapontigenin	OH	OH	OCH_3
17	(*Z*)-4-(3,5-dimethoxystyryl)benzoic acid	OCH_3	OCH_3	COOH
20	(*Z*)-1-(4-chlorostyryl)-3,5-dimethoxybenzene	OCH_3	OCH_3	Cl
22	(*Z*)-1-(4-fluorostyryl)-3,5-dimethoxybenzene	OCH_3	OCH_3	F
24	(*Z*)-1-(4-bromostyryl)-3,5-dimethoxybenzene	OCH_3	OCH_3	Br
26	(*Z*)-1,3-dimethoxy-5-(4-(trifluoromethyl)styryl)benzene	OCH_3	OCH_3	CF_3

Fig.2

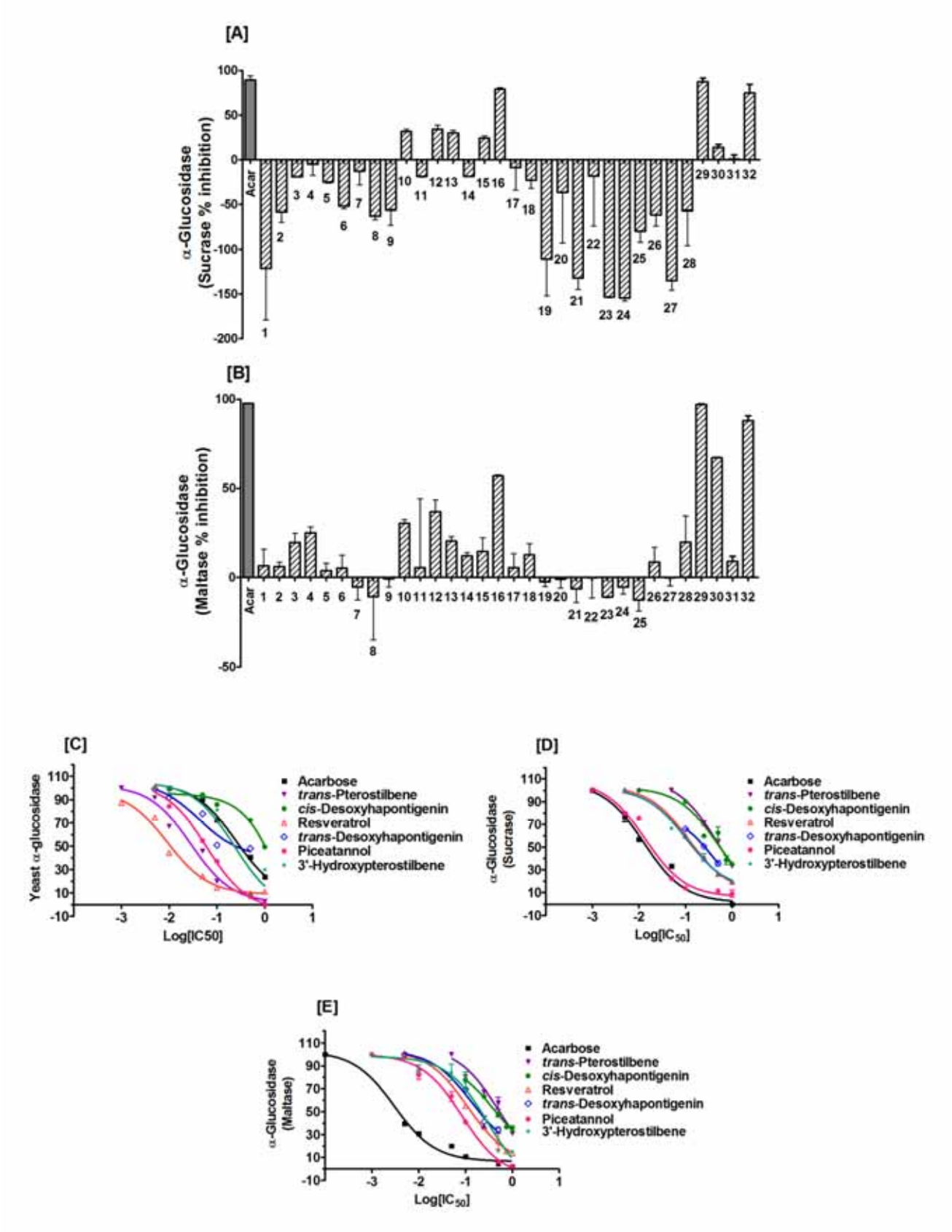
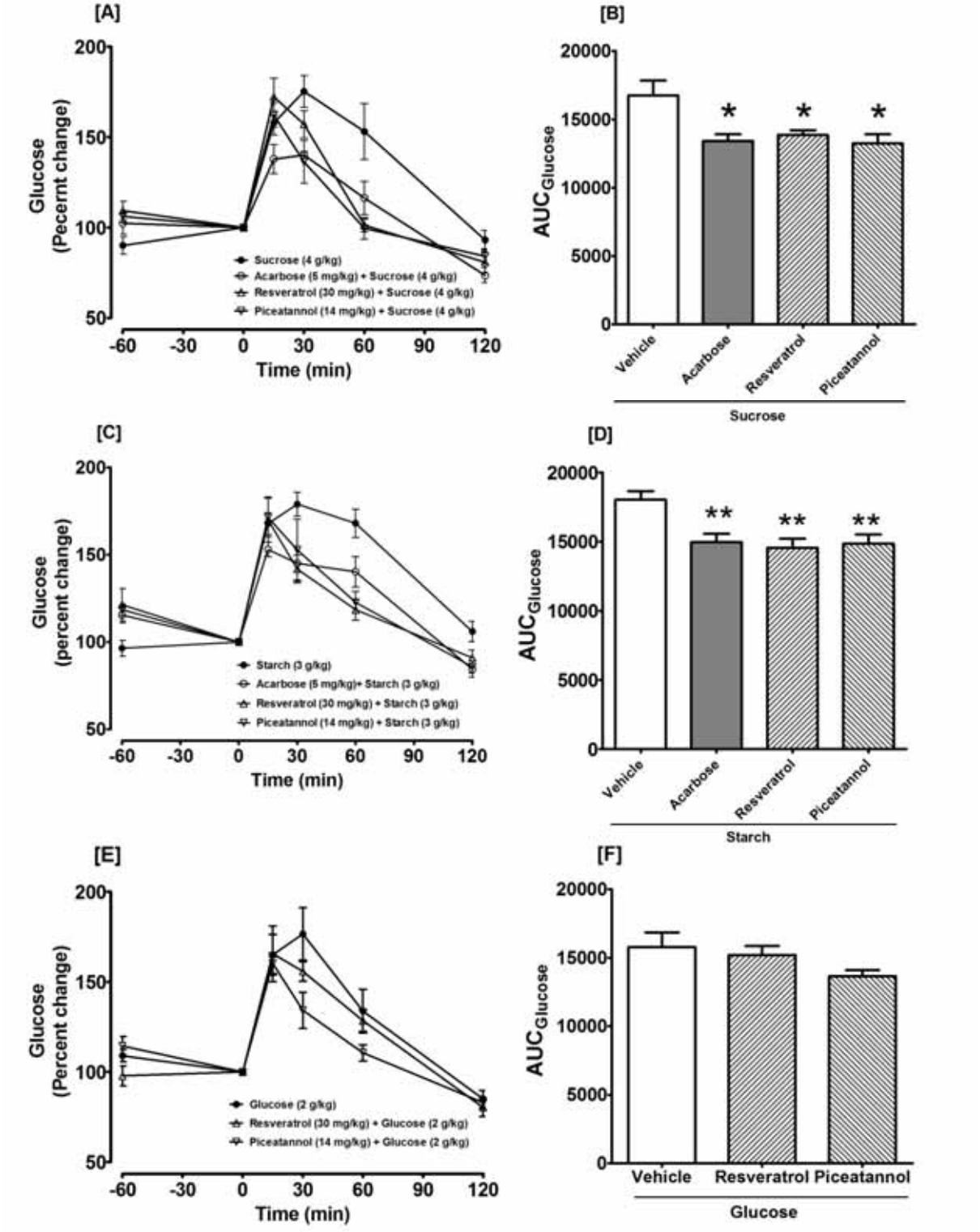

[A]
α-Glucosidase (Sucrase % inhibition)
Acar
[B]
α-Glucosidase (Maltase % inhibition)
Acar
[C]
Yeast α-glucosidase
Log[IC50]
[D]
α-Glucosidase (Sucrase)
Log[IC50]
[E]
α-Glucosidase (Maltase)
Log[IC50]
Acarbose
trans-Pterostilbene
cis-Desoxyhapontigenin
Resveratrol
trans-Desoxyhapontigenin
Piceatannol
3'-Hydroxypterostilbene

Fig 3


[A]
Glucose (Pecernt change)
Time (min)
Sucrose (4 g/kg)
Acarbose (5 mg/kg) + Sucrose (4 g/kg)
Resveratrol (30 mg/kg) + Sucrose (4 g/kg)
Piceatannol (14 mg/kg) + Sucrose (4 g/kg)
[B]
AUCGlucose
Vehicle
Acarbose
Resveratrol
Piceatannol
Sucrose
[C]
Glucose (percent change)
Time (min)
Starch (3 g/kg)
Acarbose (5 mg/kg)+ Starch (3 g/kg)
Resveratrol (30 mg/kg) + Starch (3 g/kg)
Piceatannol (14 mg/kg) + Starch (3 g/kg)
[D]
AUCGlucose
Vehicle
Acarbose
Resveratrol
Piceatannol
Starch
[E]
Glucose (Percent change)
Time (min)
Glucose (2 g/kg)
Resveratrol (30 mg/kg) + Glucose (2 g/kg)
Piceatannol (14 mg/kg) + Glucose (2 g/kg)
[F]
AUCGlucose
Vehicle
Resveratrol
Piceatannol
Glucose

Chapter 7: Summary and Conclusion

Recent U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) withdrawals, recalls, and restricted access of several medications, including antidiabetic medications, have heightened safety issues related to the use of some conventional drugs. On the other hand, interest in and use of plant extracts, herbal preparations, and natural products, including bioactive compounds, as prophylactic and therapeutic agents for many diseases have grown considerably in the past few decades. In 2007, almost 4 out of 10 adults had used complementary and alternative medicine (CAM) therapies in the past 12 months, with the most commonly used CAM therapy being nonvitamin, nonmineral, natural products (17.7%) (Barnes et al., 2008). Therefore, these studies focusing on characterizing the antidiabetic effects of serviceberry, curcumin and stilbenes offer a mechanistic basis to evaluate and assess potential use of these plant extracts and natural compounds in the management of diabetes.

Traditional knowledge of Native American Blackfeet Indians Tribe of Montana who consumed a tea made from serviceberry (*Amelanchier alnifolia*) twigs and leaves for the management of diabetes, led to our interest in serviceberry. We identified two potential mechanisms of action that may mediate the anti-diabetic activity of serviceberries. Aqueous extracts from serviceberry leaves and twigs increased glucose uptake into skeletal muscle cells. This increase in glucose uptake was mediated by activation of AMPK, and independent of signaling through the insulin receptor pathway. These extracts also increased AMPK activation in liver cells, and suppressed dexamethasone-induced gluconeogenic gene expression of PEPCK and G6Pase. We also identifiedpotent alpha-glucosidase inhibitory properties in aqueous leaf

extracts of serviceberry. Administration of subfractions of leaf extracts to high-fat fed insulin resistant, and hyperglycemic mice led to a significant decrease in postprandial blood glucose, which was similar to the antidiabetic drug, acarbose. Thus, these studies offer the first scientific evidence and validate the traditional use of serviceberry leaf and twig teas in the management of diabetes.

Curcumin, a polyphenol and major bioactive of turmeric, aptly called "the spice of life" has been used for centuries for a wide range of health benefits. Curcumin has powerful anti-inflammatory, anti-tumor, and anti-oxidant properties; is well tolerated in humans even at 12 g/day (Lao et al., 2006) and is currently in Phase III clinical trials for several forms of cancer. An increasing body of evidence indicates that curcumin can lower blood glucose levels and improve insulin action. However, the mechanisms mediating these are not clear. Our studies have shown that curcumin and its metabolite tetrahydrocurcumin induced a moderate transcriptional activation of PPARγ and its target genes, and enhanced adiponectin secretion from fat cells. Interestingly, unlike rosiglitazone, curcumin and THC did not induce adipogenesis in human subcutaneous adipocytes. These studies suggest that curcuminoids may function as a selective PPARγ modulator which preferentially improves insulin sensitivity, without the unwanted side-effects of weight-gain observed in the TZD class of drugs. Further, since troglitazone, rosiglitazone, and pioglitazones have been withdrawn or their access restricted, bioactives such as curcumin and tetrahydrocurcumin may serve as a safe and effect alternate therapy to improve insulin sensitivity.

Resveratrol, a natural stilbene and polyphenolic compound found in grapes, red wine, peanuts and berries has antioxidant, anti-inflammatory, and anti-atherogenic effects that can lower cardiovascular disease risk. In the last few years, rodent studies and experiments *in vitro*

have shown that resveratrol may be beneficial in preventing and treating some metabolic diseases, including diabetes (Szkudelska and Szkudelski, 2010). While a pivotal role has been ascribed to the activation of Sirt1 and AMPK, we were intrigued by a recent study that suggested that resveratrol may potentially inhibit yeast α-glucosidase (Kerem et al., 2006). Accordingly, we synthesized resveratrol, piceatannol (a natural stilbene), and 30 analogs of resveratrol and tested these for inhibitory properties against mammalian α-glucosidase *in vitro* and in animal studies. Resveratrol and piceatannol demonstrated potent inhibitory activities against mammalian α-glucosidase activity *in vitro*. Administration of these bioactives to high-fat fed C57Bl/6 mice before sucrose- or starch-loading led to a significantly decreased post-prandial glucose response, which was similar to the antidiabetic drug acarbose. These studies suggest a promising therapeutic potential of stilbenes such as resveratrol and piceatannol in controlling post-prandial hyperglycemia. Together with their potent antioxidant capacities, resveratrol and piceatannol can act in parallel in the gastrointestinal tract and delay the absorption of carbohydrates.

Future studies would include identification and characterization of the bioactive components from serviceberry that (a) activate AMPK and increase glucose uptake, and (b) inhibit intestinal α-glucosidase. Recent studies had identified several bioactives from serviceberry leaves and twigs, including polyphenols such as quercetin- and kaempferol-derived glycosides. It is a matter of debate as to whether an extract is better (equivalent of a combination therapy or multi-drug regimen) or a single active component (monotherapy). With an extract, the active ingredients are usually not completely isolated and are still along with other naturally occurring components of the plant. These other components can influence the efficacy of the active ingredient to give a synergistic effect. But without purification the undesirable substances such as chlorophyll, tannins, or resins, are still present in the extract. Additional studies are

needed to further characterize the PPARγ modulation effect of curcumin and tetrahydrocurcumin. Previously, work from our laboratory had shown that curcumin can activate AMPK in liver and muscle cells. How AMPK activation and PPARγ modulation work in concert in the adipocytes affecting adipokine synthesis and secretion would be of significant interest. Similarly, our novel findings on resveratrol and piceatannol's ability to inhibit α-glucosidase deserve further characterization to assess potential anti-diabetic effects.

In conclusion, our studies, while offering a mechanistic basis for the improvement of insulin action, suggest potential health benefits of serviceberry extracts, curcuminoids, and stilbenes, including resveratrol and piceatannol, in the management of diabetes.

References

Barnes PM, Bloom B, Nahin RL. Complementary and alternative medicine use among adults and children: United States, 2007. National health statistics reports; no 12. Hyattsville, MD: National Center for Health Statistics. 2008

Kerem, Z., Bilkis, I., Flaishman, M.A. and Sivan, L., (2006). Antioxidant activity and inhibition of alpha-glucosidase by trans-resveratrol, piceid, and a novel trans-stilbene from the roots of Israeli Rumex bucephalophorus L. J Agric Food Chem 54, 1243-1247.

Lao, C.D., Ruffin, M.T.t., Normolle, D., Heath, D.D., Murray, S.I., Bailey, J.M., Boggs, M.E., Crowell, J., Rock, C.L. and Brenner, D.E., (2006). Dose escalation of a curcuminoid formulation. BMC Complement Altern Med 6, 10.

Szkudelska, K. and Szkudelski, T., (2010). Resveratrol, obesity and diabetes. Eur J Pharmacol 635, 1-8.