Botanicals and the metabolic syndrome¹–⁴


ABSTRACT

Metabolic syndrome describes the human condition characterized by the presence of coexisting traditional risk factors for cardiovascular disease, such as hypertension, dyslipidemia, glucose intolerance, and obesity, in addition to nontraditional cardiovascular disease risk factors, such as inflammatory processes and abnormalities of the blood coagulation system. Although the specific etiology for metabolic syndrome is not known, insulin resistance—a clinical state in which a normal or elevated insulin concentration reflects an impaired biological response—is present and is considered a key pathophysiologic abnormality. As such, metabolic syndrome can be considered to be a prediabetic state and contributes greatly to increased morbidity and mortality in humans. Given the public health significance of metabolic syndrome, successful strategies are direly needed to intervene in its development. As such, nutritional supplementation with botanicals that effectively address pathogenic mechanisms, combined with the acceptance and widespread use of botanical supplements by the general public, represents an attractive, novel, and potentially effective approach to the problem. Thus, the overall goal of our botanical research center is to comprehensively evaluate botanicals that effectively address pathogenic mechanisms leading to the development of insulin resistance and metabolic syndrome. Currently, each of the 3 research projects evaluates a specific botanical [Russian tarragon (Artemisia dracunculus L), shilianghua (Sinocrassula indica), and grape (Vitis vinifera) anthocyanins] and assesses the effect on pathogenic mechanisms leading to the development of insulin resistance. With the completion of our research, we anticipate a better understanding of the cellular mechanisms by which insulin resistance develops and the role of botanicals in modulating the progression to metabolic syndrome.

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KEY WORDS  Artemisia dracunculus L, shilianghua, anthocyanins, insulin, insulin resistance, botanicals

STATEMENT OF THE PROBLEM

The prevalence of diabetes is increasing at alarming rates worldwide, and the global projections for the disease are staggering. For example, in 2007 it was estimated that there were ≈246 million persons in the world with diabetes and that the number would increase significantly to ≈333 million by 2025 (1). No region of the world will remain unaffected by this problem. Currently, in the United States, it is estimated that ≈73 million persons, 35% of all adults, have either diabetes (fasting blood glucose ≥126 mg/dL) or prediabetes, which is defined as having a condition termed impaired fasting glucose, ie, a fasting blood glucose concentration of 100–125 mg/dL (2).

Most persons with diabetes, both worldwide and in the United States, have type 2 diabetes, a condition associated with both insulin resistance and declining pancreatic function that results in absolute or relative insulin deficiency (Figure 1) (3–6). The importance of insulin resistance as part of the natural history of diabetes is its association with metabolic syndrome: the human condition characterized by the presence of coexisting traditional risk factors for cardiovascular disease, such as hypertension, dyslipidemia, glucose intolerance, obesity, and insulin resistance, in addition to nontraditional cardiovascular disease risk factors, such as inflammatory processes and abnormalities of the blood coagulation system (7–11). Although the specific etiology for metabolic syndrome is not known, obesity and insulin resistance are generally present (10).

The public health significance of metabolic syndrome relates to its contribution to increased morbidity and mortality in humans. First, metabolic syndrome can be considered to be a prediabetic state because it and the associated insulin resistance figure prominently in the natural history of type 2 diabetes (Figure 1) (3, 4, 6). Second, coexisting cardiovascular disease risk factors are highly associated with the presence of insulin resistance, and cardiovascular disease appears to be accelerated as part of metabolic syndrome (7–11). Given the cardiovascular disease significance of metabolic syndrome, that metabolic syndrome may be 3–4 times as common as diabetes, and that obesity and other components of metabolic syndrome (ie, dyslipidemia and diabetes) have become global health epidemics, metabolic syndrome represents a serious public health concern.

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Successful strategies are needed to intervene in the development of metabolic syndrome. Although lifestyle interventions, eg, weight loss and exercise, will greatly improve insulin sensitivity and can delay the progression to type 2 diabetes, long-term maintenance of lifestyle changes is poor. Thus, nutritional supplementation with botanicals that effectively address pathogenic mechanisms, combined with the acceptance and widespread use of supplements by the general public, represents an attractive, novel, and potentially effective approach to the problem. Thus, the major scientific goal of the center is to provide a comprehensive evaluation of botanicals in addressing the pathophysiologic mechanisms that lead to the development of insulin resistance and the metabolic syndrome.

CENTER FOR THE STUDY OF BOTANICALS AND METABOLIC SYNDROME

The specific aims of our botanical research center are to 1) promote a collaborative and interactive research environment to establish an internationally recognized center of excellence in the area of botanicals and mechanisms of metabolic disease; 2) identify and further study botanicals with potential efficacy in metabolic syndrome, identify their bioactive constituents, standardize and optimize those botanicals, provide necessary preclinical and mechanisms of action data, and translate the foregoing findings into clinical studies in humans (the overarching aim is to find eventual applications for human health in the area of prevention of metabolic syndrome); and 3) expand the critical mass of investigators addressing botanical research by identifying, recruiting, and mentoring promising young investigators.

Our center has 3 specific research projects and 3 cores: the Animal Research Core, the Botanical Research Core, and the Administrative Core. Each research project evaluates a specific botanical and assesses the effect on pathogenic mechanisms leading to the development of insulin resistance. To accomplish our goals, our botanical center has significant contributions from 3 major academic institutions and each provides a unique strength. The botanical research center, the research projects, and the Animal Research Core are based at the Pennington Biomedical Research Center, an institution that has achieved an international reputation within the research community, particularly in nutrition and obesity. The Biotechnology Center for Agriculture and the Environment (Biotech Center) at Rutgers is considered one of the world’s leaders in botanicals research and discovery and is where the Botanical Research Core is based. The Louisiana State University Agricultural Center provides expertise in botanical preparation and characterization and contributes effort to both the Botanical Research Core in addition to expertise for specific projects. Our center also collaborates with several other universities, including Pennsylvania State University and the University of Arkansas.

The research plan for our center proposes to evaluate the pathophysiologic mechanisms contributing to the development of insulin resistance and metabolic syndrome and to assess the effects of botanicals in restoring metabolic balance. In particular, overwhelming evidence indicates that alterations in skeletal muscle and adipose tissue function can lead to the development of insulin resistance in skeletal muscle (12–15). As therefore proposed, susceptible persons in an obesogenic environment may develop metabolic abnormalities regulating adipocyte (eg, adipocytokine secretion, adipocyte differentiation) or skeletal muscle (eg, ectopic fat) metabolism, which can then lead to the development of insulin resistance. On the basis of these molecular and physiologic insights into pathogenic mechanisms, we are currently investigating the ability of botanicals to affect the proposed cellular pathways modulating insulin signaling, skeletal muscle lipid metabolism (eg, fatty acid uptake, fatty acid oxidation, and intramuscular lipid accumulation), and aspects of adipogenesis (Figure 2).

BOTANICALS UNDER STUDY

The botanicals chosen for initial study are Russian tarragon (Artemisia dracunculus L) for project 1, shilianhua (Sinocrassula indica) for project 2, and grape (Vitis vinifera) anthocyanins for project 3. These botanicals were selected on the basis of preliminary data suggesting effects on insulin resistance, such as modulation of insulin signaling pathways and pathways related to ectopic fat accumulation.

A. dracunculus L

An ethanolic extract from shoots of A. dracunculus L is being tested for its ability to enhance insulin action. A. dracunculus L, a wild species known as Russian tarragon, is a close relative of common cooking tarragon and has a history of medicinal use, including in diabetes (16–20). Artemisia extracts have anticoagulatory and antihyperlipidemic activities in rats (21). Reductions in serum cholesterol of 15% and in serum triacylglycerols of 25% were observed in rats treated with extract and maintained on a hyperlipidemic diet. An herba-alba extract was an effective treatment for diabetes in a study done in 15 patients (22).

The extract from A. dracunculus L was originally identified at Rutgers University Biotech Center from a screening of extracts that had the most robust effect in decreasing insulin resistance and the most potential in preventing the development of metabolic syndrome. The ethanolic extract of A. dracunculus L is produced from plants grown hydroponically in greenhouses maintained under uniform and strictly controlled conditions for plants with consistent phytochemical content. The preparation is produced from fresh herbs to preserve the active components.

The extract of A. dracunculus L was characterized through the isolation of active components by activity-guided fractionation.
with in vitro bioassays and confirmation in vivo (23). The extract is standardized by the presence of its active compounds as well as by its bioactivity through the use of in vitro bioassays to ensure consistency. Because safety information on *A. dracunculus* and its extract is limited to historical use, our center investigators provided a comprehensive examination in a series of toxicologic animal studies (24). No noteworthy signs of toxicity were noted on feeding or body weight, results on a functional observational battery, or motor activity. Furthermore, gross necropsy and clinical chemistry showed no effects on organ mass or blood chemistry, and microscopic examinations found no lesions associated with treatment. Therefore, the extract appears to be safe and nontoxic. In several animal models, our group has confirmed in vivo effects on carbohydrate metabolism. Specifically, in genetically diabetic KK-A^1^ mice, treatment (by gavage with 500 mg · kg body wt^−1^ · d^−1^ for 7 d) significantly improved hyperglycemia by 24% compared with decreases of 28% and 41% with the known antidiabetic drugs metformin and troglitazone, respectively (25). In addition, we showed that hyperinsulinemia can be improved; the extract reduced insulin concentrations by 33% as compared with 48% with troglitazone and 52% with metformin (25). These studies also noted significantly improved insulin sensitivity with use of the extract (Figure 3) (25). In streptozotocin-induced diabetic mice, the extract of *A. dracunculus* L significantly lowered blood glucose concentrations by 20% relative to the control.

The mechanisms by which an extract of *A. dracunculus* L improves carbohydrate metabolism in vivo is under active investigation by our group. Thus far, center investigators have reported that a contributing mechanism may be secondary to an
effect on phosphoenolpyruvate carboxykinase gene expression, because mRNA expression was reduced by 28% in animal models given the extract as opposed to control conditions (25). The extract was also shown to increase the binding of glucagon-like peptide to its receptor in vitro (25).

With particular references to the insulin receptor signaling cascade, we have observed that the extract of *A. dracunculus* L increases glucose uptake and enhances cellular signaling as shown by increases in Akt-phosphorylation (Figure 4), phosphoinositol-3 kinase activity, and glycogen synthesis in cell culture systems. Current investigations are focusing on how the extract modulates gene expression for pathways involved in insulin action and potential effects on skeletal muscle lipid oxidation.

**Shilianhua**

Shilianhua is an herb in the Crassulaceae family. The plant typically blooms in June through August in the northern hemisphere. Shilianhua was collected from the vast arid and mountainous areas nearby the Xingyi city of Guizhou Province in China. As a shrub, shilianhua grows in the southwestern part of China, including Yunnan, Guangxi, and Guizhou Provinces. The shilianhua plant was originally found in the Bama county of Guangxi province, China, which is famous for longevity. In the Bama county, the local residents have used shilianhua as a medicinal herb for hundreds of years. Its morphology suggests that the shilianhua used in this study is a local variety of *Sinocrassula indica*. Our shilianhua sample was certified by a taxonomist at the Institute of Medicinal Plant Development, the Chinese Academy of Medical Sciences in Beijing, an authority in the identification and characterization of traditional Chinese herbs and medicinal plants. The extract used to provide the preliminary data for this study was prepared and characterized by the Botanical Research Core.

The crude extract was first extracted from the dried plant with water. The fresh aerial part of the shilianhua was air-dried under shade to reduce its moisture content to ≈8% by wt. The dry material was ground into a powder, and crude shilianhua extract was made by soaking the shilianhua powder in deionized water at a 1:8 wt:vol ratio for 60 min at room temperature. The water-soluble extract was separated from the solids (structural components of fibers, cellulose, semi-cellulose, debris of cells) by centrifugation and then filtration. The liquid product was then concentrated by freeze-drying into a crude extract powder. The crude extract accounted for 29.8% by wt of the raw herb. This extract was used in this study for analysis of the bioactivity of shilianhua. The components in the extract were analyzed by HPLC, and 9 major components were identified according to the peaks in the HPLC profile. This HPLC profile is used to control the quality of the shilianhua extract in this study.

Shilianhua has been claimed to reduce blood glucose in a commercial product. However, there is a paucity of scientific literature to support this claim. As such, the major goal of this particular project was to test the bioactivity of shilianhua through well-designed experiments. In animal studies, our center investigators tested the extract of shilianhua in the regulation of insulin sensitivity in a mouse model of dietary induced obesity, ie, C57BL/6J. The shilianhua extract was incorporated into a high-fat diet (58% fat calories; D12331, Research Diets, New Brunswick, NJ) at 0.4% corresponding to an estimated 500–700 mg/kg body wt daily. Glucose metabolism was monitored during the course of diet feeding up to 4 mo. In this mouse model, body weight and fasting plasma glucose increased in a time-dependent manner, but no significant change was observed in body weight or glucose with the shilianhua supplement (data not shown).

On the basis of additional in vitro experiments using bioactivity-guided assays, it was determined that an alcoholic extract of shilianhua was ≈5 times more effective than the water extract at inhibiting the inflammatory responses in our cell cultures. Thus, the ethanolic extract was also tested at 0.4% dietary incorporation, so the estimated dosage was 500–700 mg/kg as above. With this preparation, we showed that fasting glucose and insulin were reduced by the shilianhua supplement (Figure 5, A and B). Insulin sensitivity was examined with the insulin tolerance test and the glucose tolerance test. In the insulin tolerance test, no significant effect was observed with the shilianhua extract (Figure 5C). However, in the glucose tolerance test, glucose concentrations were lower in the shilianhua group at every time point, although the difference was not statistically significant (Figure 5D). These data suggest that the organic extract of shilianhua may enhance glucose metabolism and insulin sensitivity, but the effect is weak. The factors that have contributed to a negative effect thus far for shilianhua are several. First, the dosages used thus far may be insufficient, or the bioavailability of shilianhua may be limited. These factors are being actively investigated by our group. Thus far, however, it remains to be tested whether shilianhua has promise as a nutritional supplement to improve insulin sensitivity.

**Anthocyanins**

Anthocyanins are the polyphenolic compounds that provide color in berry fruits. The most recent analysis of the National Health and Nutrition Examination Survey 2001–2002 data suggest a daily intake of 12.5 mg/d in the United States (26). Their antioxidant and anti-inflammatory properties have been studied in relation to cardiovascular disease and maintenance of brain function with aging (27–32). Anthocyanins show promise in preventing obesity and ameliorating hyperglycemia in mice (33). Mice fed a high-fat diet containing an anthocyanin preparation (purple corn color; 11 g/kg diet) rich in the anthocyanin cyanidin-3-O-D-glucoside (2 g/kg diet) for 12 wk had reduced adipose depot weights and liver triacylglycerol, serum insulin, and glucose concentrations. In addition, mRNA levels of genes involved in fatty acid synthesis were reduced.

**FIGURE 4.** The effect of free fatty acids (FFAs) to attenuate Akt phosphorylation and the role of *Artemisia dracunculus* L to modulate the effect. In this experiment, 3T3 adipocytes were incubated with or without insulin at a concentration of 20 or 200 nmol/L under each of the following conditions: control conditions only (lanes 1–3), *A. dracunculus* L only (lanes 4–6), FFA only (lanes 7–9), and both FFA and *A. dracunculus* L. Note the decrease in Akt phosphorylation after insulin in the presence of the FFA, indicating attenuation in insulin signaling, and the increase after the addition of the *A. dracunculus* L extract.
Anthocyanins used for preliminary studies were derived from the highly pigmented (both skins and flesh pigmented) wine grape A-1575 (provided by the University of Arkansas). The anthocyanins were isolated by solid-phase extraction from grape skins and contained \( \approx 70\% \) anthocyanins, mostly as glucosides as confirmed by HPLC analysis. Specifically, the anthocyanin composition of the extract derived from the A-1575 grape contained delphinidin (130 mg/g), cyanidin (30 mg/g), petunidin (154 mg/g), peonidin (75 mg/g), and malvidin (338 mg/g). Anthocyanins are present mostly as glucoside derivatives, including glucosides, acetylglucosides, and \((p\text{-coumaroyl})\text{glucoside.}\n
In our preliminary study, C57Bl/6 mice were fed a high-fat proatherogenic diet for 6 wk to increase oxidative stress. During this time, a semipurified anthocyanin extract was incorporated at 0.1 mg/mL in the drinking water (containing 1% ethanol) of one group of mice. A control group received drinking water with ethanol. To ensure stability of our anthocyanin preparation, brown water bottles were used, and the preparation was changed every 2 d.

Similar to the study described above (33), in our short-term study, we observed significant reductions in liver weights with anthocyanin treatment and a trend for a reduction in liver triacylglycerol (\( \approx 10\% \)). However, no significant differences were observed in glucose, cholesterol, triacylglycerol, or inflammatory cytokine concentrations.

Livers were extracted for both proteomic and microarray analysis. Two-dimensional gel electrophoretic analysis of both soluble and membrane-associated proteins identified several features that differed between the control and the anthocyanin-treated mice (Table 1). These proteins could be categorized by metabolic pathway into those involved in lipid binding, stress response, protein and amino acid metabolism, energy production, and cellular regulation.

Microarray analysis of liver mRNA confirmed and expanded the findings made by our proteomic analysis. Of particular interest, statistical analysis (MappFinder, University of California, San Francisco, CA) showed significant reductions in liver expression of several genes involved in lipid metabolism, stress response, and cellular regulation.

### Table 1

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<th>Proteins affected by anthocyanin treatment</th>
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<tr>
<td>Lipid binding</td>
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<td>Phosphatidylethanolamine binding protein</td>
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<td>Sterol carrier protein 2</td>
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<td>Fatty acid binding protein 1</td>
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<td>Energy production</td>
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<td>Pyruvate dehydrogenase</td>
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<td>Ketohexokinase</td>
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<td>Dihydrolipoyl dehydrogenase</td>
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<td>Guanidinoacetate N-methyltransferase</td>
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<td>3-Hydroxylacyl-CoA dehydrogenase</td>
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<td>Stress</td>
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<td>Heat shock cognate 70</td>
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<td>Peroxisin-1</td>
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<td>Cellular regulation</td>
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<td>Senescence marker prot-30</td>
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<td>Pkc inhibitor protein-1</td>
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<td>Prohibitin</td>
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<td>Protein and amino acid</td>
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<td>Glutamate dehydrogenase</td>
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<td>Peptidyl-prolyl cis-trans isomerase A</td>
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<td>Argininosuccinate synthase</td>
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<td>Uricase</td>
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San Francisco) identified pathways involved in energy derivation by oxidation of organic compounds ($z = 2.79$); main pathways of carbohydrate metabolism ($z = 2.4$); tricarboxylic acid cycle ($z = 2.59$); oxidative phosphorylation, ubiquinone to cytochrome $c$ ($z = 4.74$); and lipid binding ($z = 2.69$) as being affected by anthocyanin supplementation. Expression levels of genes involved in gluconeogenesis were decreased, whereas those involved in glycerogen synthesis were increased. Finally, in the liver, expression levels of genes involved in fatty acid and sterol binding, fatty acid oxidation, and fatty acid and sterol synthesis were decreased, whereas in the heart, expression levels for genes involved in fatty acid uptake and oxidation were increased. The general findings of our genomics and proteomics findings, combined with the physiologic changes in mice, suggested that anthocyanins elicited overall improvement in insulin sensitivity in the liver, reduced ectopic fat deposition, and improved fatty acid uptake and oxidation in peripheral tissues.

Our preliminary data also indicated that in tissue culture of differentiated 3T3-L1 adipocytes, anthocyanins increased insulin signaling, glycogen accumulation, and adiponectin secretion in the presence of free fatty acids (Figure 6). We therefore have additionally proposed that anthocyanins may affect the development of insulin resistance by modulating adipocyte endocrine factors. Thus, the finding of increased adiponectin secretion by adipocytes treated with anthocyanins by us and others (34) demonstrates a potential mechanism for the enhanced insulin sensitivity seen with anthocyanin treatment.

**REFERENCES**


