

Review

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## **Impact of Bioflavonoids from Berryfruits on Biomarkers of Metabolic Syndrome**

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Running title: Berry bioflavonoids and metabolic syndrome biomarkers

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### **Abstract**

The phytochemical constituents which comprise many edible berry fruits have increasingly been linked to modulation of biomarkers associated with conditions of diabetes, overweight/obesity, and cardiovascular disease (CVD), all components of metabolic syndrome. While many wild berries have long been valued in traditional medicine as health protective, it is only recently that the ability of berry bioactives to affect particular clinical targets has been demonstrated. In addition to the widely recognized antioxidant power of berry extracts, both commercial berry varieties and wild species have been linked to hypoglycemic activity, inhibition of adipogenesis, amelioration of CVD risk factors, anti-inflammatory capacity, and ability to induce satiety/counteract overweight. In some cases, proanthocyanidin constituents or anthocyanin pigments have been shown to be the active agents, but in many other cases, interactions between co-occurring phytochemical constituents potentiate bioactivity of berry extracts.

### **Keywords**

Berry, bioactive flavonoid compounds, hyperglycemic response, insulin sensitivity

## Background

Metabolic syndrome is characterized by a constellation of physiological abnormalities that include Type 2 Diabetes Mellitus (T2DM), overweight and abdominal obesity, and cardiovascular disease (CVD). For T2DM, risk factors include elevated insulin levels (hyperinsulinemia), insulin resistance and glucose intolerance. For overweight and obesity, excessive abdominal adipose tissue is a hallmark. For CVD, classic risk factors include hypertension, elevated cholesterol levels, and dyslipidemia [1].

There is significant overlap between these co-occurring risk factors when any one of the three human conditions (diseases) is considered. In addition, a selection of nontraditional risk factors (e.g. inflammation response [iNOS or COX2 induction], elevated cell-surface protein gamma-glutamyltransferase (GGT), or abnormal blood coagulation response) are also relevant to the pathophysiological abnormalities grouped under the category 'metabolic syndrome' [2-5]. Clinical targets have included markers of lipid deposition (triglyceride levels or LDL), markers of adipogenesis (IL6/IL8, or opposing levels of adiponectin and leptin), classic markers of T2DM (lowered serum magnesium, which can indicate a prediabetic state, or fructosamine levels). Typical clinical targets of control include HbA1c, fasting glucose, LDL, total cholesterol and the total cholesterol/HDL ratio [3]. Because such a range of indicators are typically involved, it has been a challenge to clearly identify and classify the most appropriate biomarkers associated with metabolic syndrome, and to use the biomarkers to gauge the efficacy of dietary or botanical interventions against this disorder. In fact, there are a number of seemingly disparate, but actually relevant and interconnected biomarkers that can be used to diagnose this condition, or, to gauge the efficacy of a drug or dietary intervention in alleviating symptoms.

Accumulated *in vitro* research evidence, as well as recent evidence from animal studies and two new human clinical trials, all indicate that the phytochemical complexes comprising many berry fruits – in particular, the natural bioflavonoid compounds – are able to effectively modulate the biomarkers associated with metabolic syndrome. This brief review will overview the biologically-active constituents in berry fruits, and provide examples of how particular components or mixtures trigger changes in several of the key biomarkers associated with metabolic syndrome.

## Bioactive components in berries

Thanks to the growing body of research evidence on the health-relevant benefits of berries, the popular-press publicity that has followed, and subsequent increasing consumer demand, many berry fruits are now commercially-produced in both the Northern and Southern hemispheres, making them generally available year-round. The most popular and widely available dietary berries include strawberries (*Fragaria × ananassa*), blueberries (*Vaccinium corymbosum*, *V. angustifolium*, and *V. oxycoccus*), raspberries (*Rubus* spp.), and blackberries (*Rubus* spp.). Other

berries such as cranberries (*Vaccinium macrocarpon*), currants (*Ribes nigrum*), or gooseberries (*Ribes uva-crispa*) may only be available in fresh form on a seasonal basis. This is also the case for wildcrafted non-commercial berries which are not cultivated but are collected seasonally, or purchased from local farmer's markets, and may arguably be more health-protective than intensively-bred commercially-grown fruits [6-10]. In addition, significant publicity has increased consumer demand for 'exotic' berries which have been, based on a number of health-linked criteria such as total antioxidant capacity or anthocyanin concentrations, marketed as 'superfruits'; these include acai (*Euterpe oleracea*), goji (*Lycium barbarum*), mangosteen (*Garcinia mangostana*), maqui (*Aristotelia chilensis*), sea buckthorn (*Hippophae* spp.), and noni (*Morinda citrifolia*). While scientists and nutritionists agree that *some* of the recent hype has been more about marketing than science, current robust research evidence does suggest that these berry fruits have natural phytochemical profiles that uniquely contribute to human health and wellness [10].

One of the signature components of many berry fruits is the fiber content – insoluble fibers (cellulose and lignans) from berry skins contribute to digestive health; dietary soluble or viscous fibers (polysaccharides, gums, pectins, and inulins) which remain undigested until they reach the colon are excellent prebiotics [10]. Berry fruits are also a source of vitamins (including C, E, and B complex), amino acids and trace minerals, polysaccharides, and carotenoids. However, the unique, widely-diverse and intense complement of bioflavonoids are the most relevant to berry fruits' ability to exert influence on the biomarkers associated with metabolic syndrome [11-13]. These phytochemical constituents are typically concentrated in and just under the skins of the berries and in the seeds [13,14].

The bioactive flavonoid components in berries include anthocyanins, stilbenes, hydrolyzable tannins (gallo-and ellagitannins), flavan-3-ols, and flavonols [13-15]. Stilbenes (e.g. resveratrol) and ellagic acid/ellagitannins have recently been linked to health benefits in a range of human studies. The wealth of research evidence for human health benefits has centered on the anthocyanins, proanthocyanidins, and other flavonoids. Anthocyanins are the predominant group of flavonoids found in berries; they are water-soluble glycosides and acylglycosides, and are the red/blue pigments that give berries their bright attractive colors. Proanthocyanidins (condensed tannins) give berries their characteristic astringent tastes. Both anthocyanidins and proanthocyanidins have had extensive attention as health-protective natural components, due to their demonstrated antioxidant properties, and their strong association with reduced risk of several chronic diseases including CVD and cancer [16-20]. Anthocyanins and proanthocyanidins are also the two classes of berry components which are linked to positive changes in the biomarkers relevant to metabolic syndrome.

## **Influence of Berry Components on Metabolic Syndrome Biomarkers**

Most of the biomarkers noted below have cross-over significance for more than one of the component pathologies of metabolic syndrome; that is, T2DM, obesity/overweight, and CVD.

**Antioxidant capacity.** Antioxidant capacity is a somewhat ubiquitous biologically-relevant mechanism for natural products including those found in functional foods, and has direct or indirect roles in a plethora of human diseases, including metabolic syndrome. The antioxidant capacity can be gauged by various different assays. One of the most popular, the oxygen radical absorbance capacity (ORAC) assay [21], is versatile enough to measure this biomarker in plasma as well as in extracts from foods, but other popular laboratory assays including FRAP (ferric-reducing/antioxidant power), ABTS+ [2,2'-axinobis(3-ethylbenzothiazoline-6-sulphonic acid)], or DPPH (2,3,-diphenylpicrylhydrazyl) have been frequently used in berry research, with slight variations in measured results from each phytochemical class [11,18]. There have been mixed results for measurement of ORAC in plasma, leading to some controversy over whether absorption of some of these larger polyphenols occurs in the bloodstream. Berries and crude berry extracts *in general* have demonstrated remarkable properties to confront oxidative stress and maintain redox homeostasis both internally, when consumed as part of a diet or when applied topically to skin [11, 22]. Molan et al. [23] demonstrated a significant increase in the serum antioxidant potential of Sprague Dawley rats following six days of blueberry consumption, indicating the ability to elevate circulating antioxidant potentials *in vivo*. A broad range of berry polyphenolic compounds including phenolic acids, flavonoids, anthocyanins, proanthocyanidins and stilbenes have all demonstrated potent antioxidant capacities *in vivo* or *in vitro* [24-28] (Table 1).

**Hypoglycemic effects/inhibition of hyperglycemia.** Traditional medicinal uses of berries for diabetes-related symptoms are on record for Alaska Native and Native American tribal cultures [29]. Polyphenolic compounds from vegetative parts of the plant (stems and leaves) were shown to effectively inhibit accumulation of advanced glycation end-products (AGEs) characteristic of diabetic complications [30]. Extracts from blueberry vegetative plant tissues were found to enhance glucose uptake in cell-based *in vitro* assays [31], and fruit extracts after fermentation effectively potentiated glucose uptake by C2C12 myotubes and 3T3-L1 adipocytes both in the presence and absence of insulin [32]. In the same study, fermented blueberry extract increased phosphorylation of AMPK by 1.9 fold in C2C12 cells and 3.2 fold in 3T3-L1 cells, respectively, whereas non-fermented juice was ineffective. Activation of the AMPK pathway is involved in glucose transport. These studies demonstrated that blueberry extracts had insulin-like properties as evidenced by enhancement of insulin-dependent and insulin-independent glucose uptake. The ability of ingested blueberry extracts to alleviate symptoms of hyperglycemia was recently confirmed *in vivo*, using diabetic C57b1/6J mice [33]. In these experiments, a pharmaceutically-acceptable biocarrier (microemulsifier) was co-delivered to the animals to enhance bioavailability of the flavonoid-rich extracts. In particular, the anthocyanin-rich extracts from

the blueberry demonstrated hypoglycemic activity comparable to the anti-diabetic drug metformin.

**Table 1. Berry fruit modulation of biomarkers relevant to metabolic syndrome.**

<b>Biomarker</b>	<b>Berry Fruit</b>	<b>Relevant Pathologies</b>	<b>References</b>
Antioxidant capacity	Multiple	Multiple	Seeram 2008
Regulation of glucose uptake/improved insulin sensitivity	Blueberry Serviceberry Highbush Cranberry Buffaloberry Mossberry	Type II Diabetes	Vuong et al., 2007 Grace et al., 2009 Vuong et al., 2009 Kraft et al., 2008 Kellogg et al., 2010 Defuria et al., 2009 Stull et al., 2010
Inhibition of lipid (triglyceride) accumulation	Blueberry Maquiberry Mossberry	Type II Diabetes	Vuong et al., 2007 Schreckinger et al., 2010b Kellogg et al. 2010
Enzyme regulation	Serviceberry Highbush Cranberry Chokecherry Buffaloberry Blueberry Salmonberry	Type II Diabetes Obesity/overweight	Kraft et al., 2008 Schreckinger et al., 2010b Kellogg et al., 2010
Inhibition of pro-inflammatory gene expression/ anti-inflammatory effects	Chokecherry Buffaloberry Maquiberry Blueberry Blackberry	Type II Diabetes CVD	Kraft et al., 2008 Schreckinger et al., 2010b Dai et al., 2007 Defuria et al., 2009
Atherosclerosis/hypertension LDL levels	Multiple	CVD	Szajdek and Borowska, 2008 Heinonen et al, 1998 Smith et al., 2000 Kim et al., 2010 Seeram and Heber, 2006 Basu et al., 2010
Weight gain	Blueberry	Obesity/overweight	Molan et al., 2008 Vuong et al., 2009 Prior et al., 2010

When delphinidin-3-*O*-glucoside and malvidin-3-*O*-glucoside, the two major anthocyanin pigments of blueberry, were tested in pure form in the same model, only the latter anthocyanin was significantly hypoglycemic. Parallel studies with extracts from wild Alaskan berries (mossberry and two wild blueberry species) also significantly decreased blood glucose levels, particularly for phenolic and anthocyanin-rich fractions, but for these berry extracts, no biocarrier was required [6]. Similarly, administration of biotransformed blueberry juice gradually and significantly reduced high blood glucose levels in diabetic mice with maximum efficacy after 3 days [34].

Decreasing hyperglycemia, the cornerstone of diabetes treatment, is primarily accomplished by enhancing uptake of glucose from blood into muscle, and storing as glycogen. The polar constituents of wild berries (phenolic acids, anthocyanins and proanthocyanidins from serviceberry [*Amelanchier alnifolia*], highbush cranberry [*Viburnum trilobum*], and buffaloberry [*Shepherdia argentea*]) provoked significant glycogen accumulation in non-insulin stimulated cells [35]. Screening through a series of lipid metabolism biomarkers (fatty acid oxidation and energy expenditure) further showed that these berries had the ability to modulate lipid metabolism and energy expenditure consistent with amelioration of metabolic syndrome. In other studies, supplementing obese mice with whole blueberries lowered plasma glucose during a 90 minute intraperitoneal insulin tolerance test compared to mice fed a high fat diet alone, suggesting that the berry components improved high-fat diet-induced hyperglycemia [36]. Very recently, human clinical validation of blueberry's capacity to improve insulin sensitivity in obese human subjects was reported. In this unprecedented double-blind randomized placebo-controlled clinical trial, dietary supplementation with blueberry bioactives formulated in a smoothie beverage (equivalent to 2 servings of fruit, twice per day for 6 weeks) resulted in significantly improved insulin sensitivity in both male and female human volunteers [37].

**Inhibition of adipogenesis/triglyceride levels.** Proanthocyanidin-enriched fractions from a South American blueberry species (*Vaccinium floribundum*) provided strong inhibition of lipid accumulation by mature adipocytes as measured using an Oil Red O bioassay [26], and in parallel with these observations, it was the proanthocyanidin-rich subfractions from several wild Alaskan berry species that most effectively inhibited lipid accumulation [6]. Fermented blueberry juice dramatically inhibited triglyceride accumulation in 3T3-L1 adipocytes undergoing differentiation [32]. Accelerated triglyceride accumulation was used as a biomarker to indicate PPAR $\gamma$  stimulation, as activation of this nuclear receptor can increase expression of genes involved in glucose and lipid metabolism.

**Atherosclerosis/hypertension/LDL.** Chokeberry (*Aronia melanocarpua*) and blueberry juice constituents are noted for the ability to strengthen blood vessel walls, improve blood vessel elasticity, improve peripheral circulation, and inhibit LDL oxidation [13,18]. These properties

have been linked to the overall antioxidant capacity of the berry fruits, although in other cases it is evident that mechanisms other than antioxidant capacity are active [38]. Recent analysis of plasma lipid profiles in a hamster model fed high fat diets supplemented with blueberry pomace demonstrated significantly lowered LDL cholesterol and total cholesterol concentrations [39]. In this same study, genes related to cholesterol and bile acid metabolism were upregulated, suggesting that hepatic modulation of bile acid and cholesterol synthesis was the primary cause of the cholesterol-lowering effects. Screening of large populations of multiple berry species established a link between the antioxidant capacity of the berry types, and the ability of the extracts to inhibit LDL oxidation [40, 41]. A new human clinical trial has recently demonstrated that supplementing freeze-dried blueberry (50 g daily for 8 wk) selectively improved systolic and diastolic blood pressures (reducing CVD risks) as compared to the control, non-supplemented group [37].

**Pro-inflammatory gene expression.** Inhibited expression of LPS-induced pro-inflammatory genes (IL-1 $\beta$  or COX-2) in a mouse monocyte/macrophage cell line RAW 264.7 is a biomarker relevant to diabetes, obesity and CVD. Crude extracts of chokecherry (*Prunus virginiana*) and buffaloberry (*Shepherdia argentea*), two wild species traditionally used by Native Americans for medicine and subsistence foods, led to significant inhibition of IL-1 $\beta$  [35]. For two berries of South American origin, maqui berry (*Aristotelia chilensis*), and a wild blueberry species (*Vaccinium floribundum*), phenolic extracts significantly inhibited iNOS, PGE<sub>2</sub>, and COX-2 expression [26], with higher potency noted for semi-purified proanthocyanidin and anthocyanin fractions. Similar anti-inflammatory benefits have been recorded for blackberry [17,28].

**Inhibition or up-regulation of pathway enzymes.** One of the biomarkers for anti-diabetic drugs is inhibition of aldose reductase (alditol: NADP= 1-oxidoreductase), an enzyme involved in the etiology of diabetic microvascular complications. This enzyme catalyzes the first reaction in the polyol pathway which reduces glucose to sorbitol. Although aldose reductase has low affinity for hexoses such as glucose, there is increased sorbitol production in a diabetic hyperglycemic state because the substrate is elevated, resulting in hyperosmolarity and altered membrane permeability, thus aldose reductase inhibitors (ARIs) are clinically attractive biomarker targets. Flavonoids from berry fruits are a recognized natural source of ARIs [42], however recently non-polar constituents from wild berry fruits (including carotenoids) demonstrated potent ARI activity [35]. Other berry species have shown significant capacity to increase expression of Pref-1 enzyme, a transmembrane protein highly expressed in preadipocytes that effectively inhibits the initiation of adipogenesis; levels of this enzyme typically fall when adipogenesis commences. In particular, the proanthocyanidin components in extracts of *V. floribundum*, a blueberry species native to South America, were the most effective at increasing Pref-1 expression when added to a preadipocyte culture [26], with an efficacy

comparable to EGCG, a known adipogenesis inhibitor. Phenolic-rich extracts from wild Alaska native salmonberries also significantly increased Pref-1 expression in parallel tests, however, proanthocyanidins were not the most influential component in this case [6].

**Body weight gain.** The influence of berry consumption on satiety and/or weight gain appears to be specific to berry type. Molan et al. [23] concluded that while intake of a water extract from blueberry resulted in 6.2-8.6% reduction in food intake for Sprague Dawley rats (5.3-9.2% reduction in weight gain, depending on the blueberry cultivar used in treatment), this influence was unrelated to the antioxidant capacity of the berries. Further experimentation with these same berries suggested significant prebiotic activity (ability to enhance population size of lactobacilli and bifidobacteria) in vitro. Furthermore, blueberry consumption in animal trials enhanced gut microbial profiles, which is relevant due to the linkages between human intestinal microbiota and disease [43]. Incorporation of blueberry juice in drinking water significantly reduced both food intake and accumulated weight gain in KKA<sup>y</sup> mice over a 3 week period, whereas a simulated control replacement juice with the same sugar content as blueberries did not induce satiety, indicating that the phytochemicals in the blueberry juice were responsible for the observed effects [34]. Prior et al. [44,45] demonstrated that a low dose of the component anthocyanins in the drinking water of C57BL/6C mice was more effective than blueberry juice in preventing obesity, but showed that neither the whole fruit powder nor isolated anthocyanins from black raspberries had any satiety effect.

## Conclusions

While this brief review has taken note of the current evidence for bioactive components specifically in berry fruits, and their ability to modulate some of the key biomarkers associated with metabolic syndrome, it is by no means a comprehensive coverage of the literature. Many new exotic berry fruits with limited geographic distribution are only now being gauged in terms of health-protective properties, and other wild berries long used in tribal cultures for health have yet to be examined. Health protective benefits in berry fruits are conditioned by climatic and other variables in the production environment [6-8], and evidence suggests that combinations of interacting phytochemicals are more potent inhibitors than single chemical compounds [10,15,33]. The new and highly sensitive tools of metabolomics research and the currently enhanced capacity for detection of previously intractable or overlooked biomarkers continues to expand our understanding of how berry fruit components contribute to human health in general. It is clear however from the wealth of research presented to date, that the phytochemical constituents in berry fruits are potent agents for amelioration of metabolic disease symptoms, and the importance of potentiating interactions between key compounds are only now being fully recognized.



**Abbreviations used:** ABTS+: 2,2'-axinobis(3-ethylbenzothiazoline-6-sulphonic acid) antioxidant assay; AGEs: advanced glycation end-products; AMPK: 5' AMP-activated protein kinase, an enzyme integral to cellular energy homeostasis; ARIs: aldose reductase inhibitors; CVD: cardiovascular disease; DPPH: 2,3,-diphenylpicrylhydrazyl antioxidant assay; FRAP: ferric-reducing/antioxidant power assay; ORAC: oxygen radical absorbance capacity assay; T2DM: type-2 diabetes mellitus

### Conflict of interest

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