

Mechanisms for food polyphenols to ameliorate insulin resistance and endothelial dysfunction: therapeutic implications for diabetes and its cardiovascular complications

Kashif M. Munir,¹ Sruti Chandrasekaran,¹ Feng Gao,² and Michael J. Quon¹

¹Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, Maryland; and ²Department of Physiology and Department of Cardiology, Xijing Hospital, The Fourth Military Medical University, Xi'an, China

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Munir KM, Chandrasekaran S, Gao F, Quon MJ. Mechanisms for food polyphenols to ameliorate insulin resistance and endothelial dysfunction: therapeutic implications for diabetes and its cardiovascular complications. *Am J Physiol Endocrinol Metab* 305: E679–E686, 2013. First published July 30, 2013; doi:10.1152/ajpendo.00377.2013.—The rising epidemic of diabetes is a pressing issue in clinical medicine worldwide from both healthcare and economic perspectives. This is fueled by overwhelming increases in the incidence and prevalence of obesity. Obesity and diabetes are characterized by both insulin resistance and endothelial dysfunction that lead to substantial increases in cardiovascular morbidity and mortality. Reciprocal relationships between insulin resistance and endothelial dysfunction tightly link metabolic diseases including obesity and diabetes with their cardiovascular complications. Therefore, therapeutic approaches that target either insulin resistance or endothelial dysfunction alone are likely to simultaneously improve both metabolic and cardiovascular pathophysiology and disease outcomes. Moreover, combination therapies with agents targeting distinct mechanisms are likely to have additive or synergistic benefits. Conventional therapies for diabetes and its cardiovascular complications that are both safe and effective are insufficient to meet rising demand. Large, robust, epidemiologic studies demonstrate beneficial metabolic and cardiovascular health effects for many functional foods containing various polyphenols. However, precise molecular mechanisms of action for food polyphenols are largely unknown. Moreover, translation of these insights into effective clinical therapies has not been fully realized. Nevertheless, some functional foods are likely sources for safe and effective therapies and preventative strategies for metabolic diseases and their cardiovascular complications. In this review, we emphasize recent progress in elucidating molecular, cellular, and physiological actions of polyphenols from green tea (EGCG), cocoa (ECG), and citrus fruits (hesperidin) that are related to improving metabolic and cardiovascular pathophysiology. We also discuss a rigorous comprehensive approach to studying functional foods that is essential for developing novel, effective, and safe medications derived from functional foods that will complement existing conventional drugs.

insulin resistance; endothelial dysfunction; functional foods; polyphenols

OBESITY, PREDIABETES, METABOLIC SYNDROME, and diabetes are characterized by insulin resistance, endothelial dysfunction and resultant cardiovascular complications (38, 39, 45).¹ Glucose intolerance and diabetes typically result from a combination of

insulin resistance and impaired insulin secretion (17, 18, 66). Cardiovascular complications of metabolic diseases are related primarily to vascular endothelial dysfunction. Reciprocal relationships between insulin resistance and endothelial dysfunction help to link obesity and diabetes with their cardiovascular complications (39, 55, 57).

Conventional therapies for diabetes and its cardiovascular complications that are both safe and effective are insufficient to meet rising demand. Thus, developing alternative approaches for treatment as well as for prevention are imperative. One promising alternative therapeutic area comprises functional foods. That is, foods with health benefits beyond their nutritive value alone. A number of well-performed large epidemiologic studies demonstrate that consumption of functional foods con-

¹ This article type is the topic of an Editorial by Peter D. Wagner (85a). The aim of this series is to provide practicing MDs and researchers an up-to-date physiological understanding of disease. Articles published in this series are intended to provide a thoughtful and lucid linkage of science to the patient. Go here for more information on this series and to browse the content (<http://www.the-aps.org/mm/Publications/Journals/PIM>).

Address for reprint requests and other correspondence: M. J. Quon, Division of Endocrinology, Diabetes & Nutrition, Univ. of Maryland, Baltimore, 660 W. Redwood St., HH 495, Baltimore, MD 21201 (e-mail: quonm@medicine.umaryland.edu).

taining bioactive polyphenols, including green tea, cocoa, and citrus fruits, are associated with dose-dependent improvement in metabolic and/or cardiovascular morbidity and mortality (7, 15, 28, 31, 32, 40, 46, 65, 90). In this review, we discuss the physiology of coordinated regulation of metabolic and cardiovascular actions of insulin, the pathophysiology of reciprocal relationships between insulin resistance and endothelial dysfunction, and a rigorous comprehensive approach to understanding molecular, cellular, and physiological mechanisms of actions for food polyphenols as potential therapeutic agents that simultaneously target improvement in metabolic and cardiovascular phenotypes. The scientific paradigm we outline here is a robust, efficient, and informative approach for understanding putative health benefits of functional foods. This approach is a useful means to discover and develop safe and effective novel therapies that complement existing conventional drugs for the treatment and prevention of diabetes and its cardiovascular complications.

Physiology of Insulin Action

Classical metabolic actions of insulin that help regulate glucose homeostasis include promotion of glucose uptake and utilization in skeletal muscle, suppression of hepatic glucose production, and inhibition of lipolysis in adipose tissue (18, 57). In recent years, it has become clear that a multitude of other biological actions of insulin in unconventional targets including vascular endothelium, brain, β -cell, kidney, gut, and other organs contribute importantly to both metabolic and cardiovascular homeostasis (55, 57, 58). Most, if not all, actions of insulin are initiated by the binding of insulin to specific cell surface insulin receptors, which are expressed on nearly every cell in the body (57). This initiates a complex signaling network that culminates in various biological actions of insulin in myriad tissues. For example, insulin signal transduction pathways promoting classical metabolic actions of insulin generally involve autophosphorylation of the insulin receptor tyrosine kinase that then phosphorylates insulin receptor substrates including IRS-1. Tyrosine phosphorylated IRS-1 binds and activates the lipid kinase phosphatidylinositol 3-kinase (PI3K) that then stimulates a serine kinase cascade (including PDK-1 and Akt) to promote translocation of the insulin-responsive glucose transporter GLUT4 from an intracellular compartment to the plasma membrane, where GLUT4 facilitates glucose entry into skeletal muscle and adipose tissue (61). In addition to PI3K-dependent insulin signaling, a MAPK-dependent branch of insulin signaling commences with tyrosine-phosphorylated IRS-1 or Shc binding to the SH2 domain of Grb-2, resulting in activation of the preassociated GTP exchange factor Sos (53, 61). This further activates the small GTP binding protein Ras, which then initiates a kinase phosphorylation cascade involving Raf, MAPK/extracellular signal-regulated kinase, and MAPK (61, 74). MAPK-dependent insulin signaling generally regulates effects of insulin to promote mitogenesis, growth, and differentiation.

Interestingly, in vascular endothelium, the PI3K branch of insulin signaling regulates activation of endothelial nitric oxide synthase (eNOS), leading to production of nitric oxide (NO), a potent vasodilator (50, 52, 95). In addition, PI3K-dependent insulin signaling in endothelium leading to phosphorylation of the transcription factor FOXO1 reduces synthesis and secretion

of the vasoconstrictor peptide endothelin-1 (ET-1) (11, 57, 67, 73). At the same time, insulin signaling through MAPK-dependent pathways in endothelium promotes ET-1 synthesis and secretion (55, 57, 67). Thus, insulin regulates opposing vasoactive actions in vascular endothelium through relatively distinct major branches of insulin signaling pathways.

Pathophysiology of Insulin Resistance and Endothelial Dysfunction

Insulin resistance is typically defined as decreased sensitivity or responsiveness to the metabolic actions of insulin to promote glucose uptake and utilization (56). Insulin-resistant states are characterized by pathway-selective impairment in PI3K-dependent insulin signaling pathways with intact or augmented MAPK-dependent insulin signaling in both metabolic and vascular tissues. Compensatory fasting hyperinsulinemia attempts to maintain euglycemia with the unintended consequence of overdriving unaffected or augmented MAPK-dependent signaling and actions that are prohypertensive and inflammatory (57). In vitro models of insulin resistance in vascular endothelium are characterized by blunted PI3K-dependent eNOS expression and NO production. Simultaneously, signaling through MAPK pathways are substantially enhanced, leading to increased expression of endothelial adhesion molecules that are proatherogenic as well as increased mitogenic responsiveness of vascular smooth muscle cells (VSMC) in response to growth factors including insulin and VEGF that increase VSMC proliferation, another proatherogenic event (51).

Insulin stimulates MAPK-dependent ET-1 synthesis and secretion as well as PI3K-dependent NO production from vascular endothelium in healthy subjects to maintain normal net vascular tone. Pathophysiological imbalance between PI3K and MAPK signaling in response to insulin in the vasculature results in endothelial dysfunction that contributes substantially to insulin-resistant metabolic tissues in humans (10). Indeed, clinical physiology studies consistently show that diminished insulin-stimulated vasodilation is correlated with reduced insulin-stimulated vascular blood flow in subjects with obesity and/or type 2 diabetes (4, 12, 25, 41, 80, 82, 87). Furthermore, there is reduced NO activity (PI3K-dependent pathway) and augmented response of insulin to stimulate ET-1 production (MAPK-dependent pathway) in overweight, obese, and type 2 diabetes subjects (9, 43, 62, 77). Thus, an imbalance with overdriven MAPK-dependent signaling together with impaired PI3K-dependent signaling in the vascular endothelium leads to endothelial derangements that secondarily contribute to metabolic insulin resistance. These important pathophysiological relationships help link metabolic diseases with their cardiovascular complications (57). Since many of these pathophysiologicals are present before the development of frank disease, it is possible that safe interventions including food polyphenols may also act as effective preventative measures.

Classic metabolic and cardiovascular diseases linked to the pathophysiology of insulin resistance include diabetes, metabolic syndrome, obesity, coronary heart disease, atherosclerosis, and hypertension. However, there are a host of other diseases associated with either or both insulin resistance and endothelial dysfunction. Some of these associations are at the level of epidemiological links, while others have putative pathophysiological mechanisms rooted in either insulin resis-

as well, similar to the use of calcium supplementation in addition to consumption of calcium-rich foods to promote bone health. In the same way, food polyphenols, if proven safe for the general population, may also be considered in a preventative light. Compounds derived from functional foods available over the counter at sources including GNC are generally presumed to be safer than conventional drugs that require prescriptions. However, this may or may not be actually true. The oversight of functional foods and nutritional supplements is much more lax than that of ethical drugs. For example, EGCG (epigallocatechin gallate), which can be purchased easily at GNC has potential liver toxicity if taken at too high a dose (44, 49).

Examples of Paradigmatic Studies of Functional Foods

Below, we review studies of plant food polyphenols from green tea (EGCG), cocoa [epicatechin gallate (ECG)], and citrus fruits (hesperidin) that support putative cardiometabolic benefits of these compounds derived from functional foods. We organize our discussions according to the research paradigm set out above. The health benefits from food polyphenols are often attributed to their antioxidant properties. However, it is extremely important to emphasize at the outset that there is absolutely no rigorous scientific evidence to support an antioxidant mechanism for therapeutic benefits of food polyphenols. Indeed, the polyphenols we discuss below are actually mildly prooxidant in *in vivo* settings. Thus, the mechanisms of action for food polyphenols to promote metabolic and cardiovascular health are unrelated to scavenging oxidants. Moreover, many of the health benefits of food polyphenols are initiated by low level prooxidant signals from polyphenols *in vivo* (e.g., H₂O₂) that stimulate beneficial cellular signal transduction pathways leading to improved physiological homeostasis.

As described above, data on bioavailability, pharmacokinetics, and active metabolites *in vivo* for food polyphenols tend to be quite limited, especially in humans. Ingestion of two cups of green tea in humans results in peak plasma levels of EGCG of 0.2–0.3 μ M. Drinking ten cups of green tea raises circulating levels of EGCG into the low micromolar range, where biological effects are observed in cell-based assays (93). Hesperidin, a food polyphenol derived from citrus, has an oral bioavailability of ~5% and is hydrolyzed *in vivo* and conjugated into glucuronides and sulfoglucuronides (hesperitin, the active metabolite) (42). Consumption of 1.5 liters of orange juice results in circulating hesperitin concentrations in the low micromolar range sufficient to produce bioactive effects in cells *in vitro* (42). Similarly, cocoa binds to salivary proteins and reaches the small intestine largely intact, where roughly 20% of ECG (the major cocoa polyphenol) is absorbed (21). In general, bioavailability of food polyphenols tends to be relatively poor. However, when taken as single purified compounds at feasible dosages, circulating concentrations of active food-derived polyphenolic compounds and metabolites in the low micromolar range result in therapeutic concentrations where physiological bioactivity is plausible.

Green tea. Large epidemiologic studies have shown that consumption of green tea is associated with decreased all-cause mortality and cardiovascular mortality, but not cancer mortality, in a dose-dependent fashion (40, 65). Similarly, epidemi-

ologic studies show a decrease in type 2 diabetes among drinkers of green tea (29). Green tea contains the polyphenol EGCG. EGCG inhibits gluconeogenesis in hepatocytes and stimulates glucose uptake in rat skeletal muscle cells by using a PI3K-dependent mechanism that mimics metabolic actions of insulin (33, 86). Also, EGCG directly and acutely stimulates production of NO from primary endothelial cells by using a signaling pathway that involves low-level generation of H₂O₂ and possibly other reactive oxygen species that then goes on to stimulate a signaling cascade including sequential activation of the src family kinase fyn, PI3K, Akt, and eNOS (37). Interestingly, this pathway shares features in common with insulin signaling pathways regulating activation of eNOS and NO production in endothelial cells (39, 85). This increased production of NO tends to lower peripheral vascular resistance and increase blood flow through both conduit arteries as well as recruiting nutritive capillaries in skeletal muscle. Thus, the NO-mediated vasodilator actions of EGCG have primary benefits for cardiovascular homeostasis and secondary metabolic benefits mediated by increased delivery of substrates and hormones to metabolic targets (39). EGCG also decreases ET-1 expression, in part through regulation of FOXO1. This reduces vasoconstrictor tone and may also directly increase bioavailability of NO to improve endothelial function and oppose atherogenesis (73). Human aortic endothelial cells treated with EGCG have increased expression of heme oxygenase-1 mRNA, protein, and activity (69). Increased heme oxygenase-1 may mediate the anti-inflammatory actions of EGCG, accounting in part for its cardiovascular and metabolic benefits. Finally, suppression of hepatic gluconeogenesis in isolated hepatocytes through EGCG-activated AMPK helps to explain the beneficial metabolic actions of EGCG in glucose homeostasis (13).

In spontaneously hypertensive (SHR) rats (genetic model of human metabolic syndrome with hypertension, endothelial dysfunction, insulin resistance, and overweight), we demonstrated that 3 wk of EGCG administration reduces insulin resistance, improves endothelial dysfunction, opposes hypertension, protects against cardiac ischemia/reperfusion injury, and raises plasma adiponectin levels (68). Antidiabetic or lipid lowering properties of green tea or EGCG have been demonstrated in rodent models of type 2 diabetes as well as obesity (26, 76, 84, 94). In diabetic *db/db* mice, EGCG increases the size and number of pancreatic islets and improves β -cell morphology, leading to better glucose tolerance comparable to rosiglitazone (63). Moreover, in human subjects who smoke or those with coronary artery disease, consumption of green tea significantly reverses their endothelial dysfunction (59, 88). Meta-analyses show green tea consumption lowers both total and LDL-cholesterol levels and improves endothelial function as measured by flow-mediated dilation of the brachial artery (36, 71, 97). Green tea also lowers blood pressure, improves insulin sensitivity, and lowers cholesterol in obese, hypertensive patients (5, 81). In addition to direct and indirect metabolic and cardiovascular actions of EGCG discussed above, EGCG may also possess anti-inflammatory properties that contribute to its salutary effects on glucose and vascular homeostasis (35).

Citrus. Citrus fruit consumption is associated in large epidemiologic and prospective studies with lower rates of coronary disease and ischemic stroke (15, 27, 31, 32, 90). Hesperidin, a predominant polyphenol found in citrus fruits, is one

candidate that may mediate beneficial metabolic and vascular effects observed with citrus fruit consumption. Our laboratory has demonstrated that treatment of bovine aortic endothelial cells (BAEC) with hesperetin (a metabolite of hesperidin) acutely stimulates sequential activation of Src, PI3K, Akt, AMPK, and eNOS to produce NO (75). Similarly to EGCG, this pathway shares features in common with insulin signaling pathways regulating activation of eNOS and NO production in endothelial cells (39, 85, 96). Hesperidin may also have anti-inflammatory properties that contribute to its salutary effects on vascular homeostasis. We demonstrated that pretreatment of BAEC with hesperetin protects against TNF α -stimulated increases in expression of VCAM-1 and adhesion of monocytes to endothelial cells (75).

In animal studies, intake of hesperidin decreases infarct size in male Wistar rats after middle cerebral artery occlusion (72). In SHR rats, hesperidin causes a dose-dependent decrease in systolic blood pressure and improves endothelial dysfunction (91, 92). In diabetic rodents, hesperidin therapy has hypoglycemic and hypolipidemic effects and can lower circulating plasma free fatty acids, triglycerides, and total cholesterol along with lowering hepatic triglyceride levels and decreasing fatty acid oxidation (2, 3, 34). Interestingly, hesperidin augments the effects of exercise on cholesterol and insulin sensitivity (16).

Hyperlipidemic human subjects treated with hesperidin have lower circulating triglyceride levels (47, 48). In our recent clinical study of patients with metabolic syndrome, we demonstrated that hesperidin treatment (vs. placebo) substantially improved brachial artery flow-mediated dilation (FMD) compared with placebo treatment (7.78 vs. 10.26%, $P = 0.02$). In addition, hesperidin therapy reduced total cholesterol and apoB and increased HDL (75). These results are in keeping with cellular studies of hesperetin actions that mimic the ability of insulin to inhibit assembly and secretion of apoB-100-containing lipoproteins from hepatoma cells (6). Consistent with the anti-inflammatory effects of hesperetin in BAEC, we also observed significant decreases in circulating hsCRP, SAA protein, and sE-selectin in human subjects with metabolic syndrome treated with hesperidin (500 mg/day for 3 wk vs. placebo, $P < 0.01$) (75) as well as a trend toward improvement in insulin sensitivity as assessed by the surrogate index QUICKI ($P = 0.06$) (75). It is important to note that in our clinical intervention trial the positive prospectively designated primary outcomes and secondary positive physiological outcomes were fully consistent with the mechanism of action worked out in preclinical cellular and animal studies and supported by circulating biomarker data obtained during the clinical intervention trial.

Cocoa. Cocoa is rich in monomeric (ECG and catechin) and oligomeric (procyanidin) flavanols (89). Epidemiologic evidence supports an inverse relationship between cocoa consumption and blood pressure, stroke, myocardial infarction, coronary heart disease, and cardiovascular and all-cause mortality (7, 8, 19, 20). In nondiabetic patients followed after acute myocardial infarction, chocolate consumption shows a strong dose-related inverse relationship to cardiac mortality (30).

Cocoa contains high levels of flavonoids, and in particular the polyphenol ECG, which enhances tyrosine phosphorylation in an insulin-like manner, activating the PI3K/Akt and AMPK pathways in the vascular endothelium to increase NO produc-

tion (14, 21). In human coronary artery endothelial cells, epicatechin phosphorylates and activates eNOS through Akt and a complex with HSP90 (70). Incubating human umbilical vein endothelial cells with cocoa extract inhibits angiotensin-converting enzyme activity and enhances NO production (64).

Male mice treated with epicatechin have improved exercise performance and resistance to muscle fatigue. Mice treated with epicatechin alone (without exercise) have structural and metabolic changes in skeletal and cardiac muscle similar to those of mice undergoing exercise (60). Epicatechin alone and in combination with exercise has an additive effect to stimulate myocardial angiogenesis (70). Obese, diabetic *db/db* mice have prolonged lifespan, along with a decrease in markers of inflammation and LDL-cholesterol when treated with epicatechin (79). It is important to note that these animal studies are observational in nature and need to be followed up by more mechanistic studies in the future.

In human studies, cocoa ingestion acutely improves NO-dependent flow-mediated vasodilation and may contribute to amelioration of insulin resistance, hypertension, and metabolic syndrome (22). Grassi et al. showed that consumption of flavanol-rich dark chocolate for 2 wk decreased daytime and nighttime blood pressure, reduced insulin resistance, and improved NO-dependent vasorelaxation in a placebo-controlled (white chocolate) crossover study (24). Heart transplant recipients have improved coronary function and vasodilation, with decreased platelet reactivity 2 h after consumption of flavanol-rich dark chocolate (23). A recent Australian study showed the benefits of dark chocolate as a cost-effective measure in reducing cardiovascular events in subjects with metabolic syndrome (98). Our laboratory has shown that consumption of polyphenol-rich cocoa (vs. polyphenol-depleted cocoa placebo control) for 2 wk improves endothelial function in patients with hypertension without changing blood pressure, insulin sensitivity, or circulating inflammatory markers (54). A recent meta-analysis of flavonoids from cocoa included 24 studies (covering 1,106 participants) that studied the short-term effects of flavonoids. In this analysis, short-term cocoa consumption showed benefits in cardiovascular health, blood pressure, LDL-cholesterol levels, and insulin resistance. Since they were all short-term studies, no effect on BMI or worsening of diabetes was evident (78). Similar results were found in a smaller meta-analysis (83). Conclusions from these particular meta-analyses need to be considered with extreme caution, since most of the studies included in these analyses did not have appropriate blinding, randomization, or placebo controls.

PERSPECTIVES/CONCLUSIONS

Insulin signal transduction occurs through two major relatively distinct PI3K-dependent and MAPK-dependent pathways that have divergent effects on metabolic and vascular biology. In normal physiology, signaling through the PI3K pathway predominates, leading to increased insulin-dependent glucose uptake in muscle and fat with simultaneous improvements in vascular endothelial function. In the pathophysiological state of insulin resistance, PI3K signaling is selectively impaired, allowing signaling through the MAPK pathway to predominate. This leads to enhanced mitogenic activity and diminished NO-mediated vasodilation, contributing to endothelial dysfunction and metabolic insulin resistance.

Rigorous scientific investigation of functional foods and nutritional supplements for potential therapeutic health benefits represents an exciting new approach for deriving effective, safe treatments for diabetes and its cardiovascular complications that may complement existing conventional therapies. Use of dietary supplements is a popular practice that many people use to try to improve their health. However, it is essential to scientifically study, understand, and validate the benefits of functional foods and their derivatives by using the research paradigm outlined above. Without such research, there is no solid foundation for recommending functional foods for health benefits or disease prevention. The food polyphenols discussed above are often touted for their potent antioxidant activity. However, as explained above, the mechanisms of action to promote metabolic and cardiovascular health for food polyphenols is unrelated to scavenging oxidants and is most likely due to low-level prooxidant-induced signal transduction pathways that are beneficial for maintaining metabolic and cardiovascular homeostasis. Indeed, the American Diabetes Association recommends against routine supplementation with antioxidants, not only because of lack of evidence of efficacy but also because of concerns related to the long-term safety of these antioxidants (1). A rigorous approach to identify potentially beneficial foods from epidemiologic data and then undertaking systematic study in cells, animals, and finally humans is necessary to provide convincing evidence of safe therapeutic potential. Polyphenols derived from functional foods offer a novel and important approach for adjunctive treatment of the pathophysiology of metabolic disorders associated with insulin resistance and their cardiovascular complications.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: K.M.M. and M.J.Q. conception and design of research; K.M.M. and M.J.Q. analyzed data; K.M.M. and M.J.Q. interpreted results of experiments; K.M.M. prepared figures; K.M.M., S.C., F.G., and M.J.Q. drafted manuscript; K.M.M., F.G., and M.J.Q. edited and revised manuscript; K.M.M., F.G., and M.J.Q. approved final version of manuscript.

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