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## Vascular and Metabolic Actions of the Green Tea Polyphenol Epigallocatechin Gallate

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### Abstract

Epidemiological studies demonstrate robust correlations between green tea consumption and reduced risk of type 2 diabetes and its cardiovascular complications. However, underlying molecular, cellular, and physiological mechanisms remain incompletely understood. Health promoting actions of green tea are often attributed to epigallocatechin gallate (EGCG), the most abundant polyphenol in green tea. Insulin resistance and endothelial dysfunction play key roles in the pathogenesis of type 2 diabetes and its cardiovascular complications. Metabolic insulin resistance results from impaired insulin-mediated glucose disposal in skeletal muscle and adipose tissue, and blunted insulin-mediated suppression of hepatic glucose output that is often associated with endothelial/vascular dysfunction. This endothelial dysfunction is itself caused, in part, by impaired insulin signaling in vascular endothelium resulting in reduced insulin-stimulated production of NO in arteries, and arterioles that regulate nutritive capillaries. In this review, we discuss the considerable body of literature supporting insulin-mimetic actions of EGCG that oppose endothelial dysfunction and ameliorate metabolic insulin resistance in skeletal muscle and liver. We conclude that EGCG is a promising therapeutic to combat cardiovascular complications associated with the metabolic diseases characterized by reciprocal relationships between insulin resistance and endothelial dysfunction that include obesity, metabolic syndrome and type 2 diabetes. There is a strong rationale for well-powered randomized placebo controlled intervention trials to be carried out in insulin resistant and diabetic populations.

### Keywords

EGCG; endothelial function; green tea; insulin action; insulin sensitivity; metabolism; muscle blood flow; type 2 diabetes

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#**Author's Profile:** Dr. Keske is a Senior Research Fellow at the Menzies Research Institute Tasmania, University of Tasmania. Her research interests have focused on the control of microvascular blood flow in skeletal muscle. Her current research investigates interventions to prevent or reverse insulin resistance by regulating microvascular blood flow within skeletal muscle.

### CONFLICT OF INTEREST

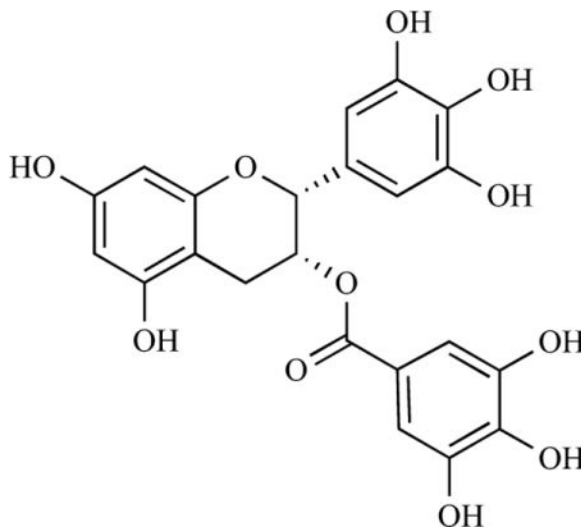
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## 1. INTRODUCTION

Accumulating laboratory and clinical studies suggest that polyphenol-rich plants have health-promoting effects with respect to cardiovascular and metabolic health [1–9], cancer prevention [10–13], and neurodegenerative diseases [14, 15]. Within polyphenol-rich plants, green tea and its flavan-3-ols are among the most extensively studied for their putative health benefits.

Tea comes from the *Camellia sinensis* plant. Tea leaves are categorized into different classes based on the degree of fermentation (or leaf oxidation) during processing. During fermentation, flavan-3-ols, the bioactive polyphenols in tea leaves, undergo polyphenol oxidase-dependent oxidative polymerisation, resulting in the formation of theaflavins and thearubigins [16]. Green tea is unfermented and contains the highest concentration of flavan-3-ols. Oolong tea is a partially fermented product and therefore contains a mixture of flavan-3-ols, theaflavins, and thearubigins. Black tea is the most fermented tea, and as a result, contains abundant theaflavins and thearubigins, and limited or no flavan-3-ols. There are five major types of flavan-3-ols in green tea including catechin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG).

EGCG is a polyphenol belonging to the catechin family, a group of polyphenolic compounds (see chemical structure). Catechins are found in a variety of foods including fruits, vegetables, chocolate, wine, and tea. However, EGCG is predominantly found in tea and is the most abundant polyphenol accounting for as much as 50% of green tea polyphenols [16, 17]. In recent years, extensive research has investigated potential health-promoting effects as well as underlying molecular mechanisms of green tea or purified EGCG relevant to cardiovascular and metabolic diseases [2, 4, 7, 9, 18–21] including type 2 diabetes.



Insulin resistance plays a key role in the pathogenesis of type 2 diabetes and precedes the onset of diabetes. Insulin resistance is characterized by impaired insulin-mediated glucose disposal in skeletal muscle and is often associated with endothelial dysfunction. The

conventional treatments available for type 2 diabetes are not sufficient to adequately control the disease which is increasing in prevalence and incidence as a result of the obesity epidemic in the developed world. Therefore, novel complementary therapeutic approaches including green tea and purified EGCG that oppose endothelial dysfunction and ameliorate metabolic insulin resistance in skeletal muscle and liver may augment current conventional treatments. There is growing public interest in the use of complementary and alternative approaches (such as green tea and its flavan-3-ols), for treating insulin resistance and type 2 diabetes because of unmet medical needs and the inherent safety benefits with functional foods including green tea.

## 2. EPIDEMIOLOGICAL STUDIES

Epidemiological studies show a positive relationship between tea consumption and reduced risk for type 2 diabetes [5–9, 22]. In 2009, two meta-analyses by Huxley *et al.* [23] and Jing *et al.* [24] report that, compared to non-tea drinkers, tea (green and black tea) consumption of >3 cups per day is associated with a 17 – 35 % lower risk of type 2 diabetes. Furthermore, in a British cohort [6] tea (green and black tea) consumption of >3 cups per day was associated with a 34% lower risk of diabetes. Whether theaflavins and thearubigins have similar bioactivities with EGCG, relevant to cardiovascular and metabolic diseases, is not known. Among Japanese adults, Iso *et al.* [9] report an inverse and dose-dependent association between green tea consumption and risk for type 2 diabetes. This study suggested that high green tea consumption (  $> 6$  cups.d<sup>-1</sup>) lowers risk for developing type 2 diabetes by 33% compared to those who drink < 1 cup per week. More recently, a meta-analysis by Zheng *et al.* [25] demonstrates green tea catechin treatment for 12 weeks, but not shorter term (< 12 weeks), is associated with lower fasting blood glucose levels. Large epidemiologic studies have also shown green tea consumption is associated with decreased cardiovascular and all-cause mortality [26, 27]. In this same study there was no effect on cancer risk demonstrating the specificity of green tea for metabolic and cardiovascular disease [26]. However, not all studies report these positive associations. Studies in Japanese [28] and Singapore Chinese [29] populations failed to uncover an association between green tea consumption and reduced risk of type 2 diabetes.

## 3. CELLULAR ACTIONS OF EGCG

The classical metabolic actions of insulin on glucose homeostasis include glucose uptake by skeletal muscle and adipose tissue, and suppression of hepatic glucose production. There is evidence from *in vitro* studies that EGCG has insulin-mimetic metabolic actions on myocytes [30–32], adipocytes [33–36] and hepatocytes [37, 38] (see Fig. 1)

Skeletal muscle is the major site for insulin-mediated glucose utilization and thereby contributes to postprandial blood glucose levels. In isolated myocytes, green tea or EGCG stimulates GLUT4 translocation and results in increased glucose uptake [30, 31, 39]. Similar to insulin, EGCG has been reported to stimulate muscle glucose uptake via the PI3-K/Akt signaling pathway in cultured myotubes [31, 32, 40]. Although it has been proposed that EGCG has insulin-mimetic actions on glucose uptake in myocytes [30–32, 39, 40], there is no evidence of EGCG directly activating the insulin receptor tyrosine kinase [31]. In

addition to the insulin-mimetic pathway, EGCG can also stimulate muscle glucose uptake by alternative pathways such as the adenosine monophosphate-activated protein kinase (AMPK, with doses > 20  $\mu\text{M}$ ) [40]. Therefore, it is likely that EGCG-mediated muscle glucose uptake *in vitro* involves multiple signaling pathways some of which are insulin-mimetic.

Chronic green tea supplementation (4–12 weeks) increases glucose uptake [33–35], and promotes GLUT4 translocation [34, 35] in isolated adipocytes, however the molecular pathway leading to glucose uptake is unknown. Interestingly, green tea catechins (especially EGCG) augment insulin-stimulated glucose metabolism in adipose cells using an *in vitro* assay that assesses insulin-dependent breakdown of glucose to  $\text{CO}_2$  [36].

EGCG suppresses gluconeogenesis in cultured hepatocytes. At high doses (> 25  $\mu\text{M}$ ), EGCG suppresses hepatic gluconeogenesis through the same pathway as insulin, wherein EGCG promotes signaling through IRS-1/PI3-K/Akt, resulting in inhibition of PEPCK and G6Pase gluconeogenic enzyme activity [38]. However, we have demonstrated in isolated hepatocytes that EGCG, at relatively low concentrations (1  $\mu\text{M}$ ), inhibits glucose production via inhibition of gluconeogenesis and expression of key gluconeogenic genes [37]. This involves activation of AMPK through reactive oxygen species and the  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase kinase  $\beta$  (CaMKK  $\beta$ ) pathway [37].

The microsomal enzyme 11 $\beta$ -hydroxysteroiddehydrogenase type 1 (11 $\beta$ -HSD1) catalyzes the interconversion of cortisone to cortisol. Strong evidence exists for an important etiological role of 11 $\beta$ -HSD1 in various metabolic disorders including insulin resistance, type 2 diabetes, hypertension, dyslipidemia and obesity. EGCG strongly inhibits 11 $\beta$ -HSD1 activity [41] thus implicating another potential mechanism for EGCG to ameliorate metabolic diseases.

In summary, the glucose lowering effects of EGCG may involve pathways that directly suppress hepatic glucose production while simultaneously stimulating glucose uptake in skeletal muscle and adipose tissue. However, the direct metabolic or cellular actions of EGCG on glucose metabolism have only been assessed *in vitro* and the effect of EGCG *in vivo* or in a vascularly intact model needs to be confirmed. In addition to these classical metabolic actions, EGCG also has vascular actions that may directly contribute to muscle glucose uptake (detailed below).

## 4. VASCULAR ACTIONS OF EGCG

### 4.1. Endothelial Cell Culture

EGCG stimulates nitric oxide (NO) production from vascular endothelial cells similar to insulin (see Figs. 2 and 3). Both insulin- and EGCG-mediated NO production is dependent on the activation of PI3-K, since wortmannin blocks NO production by both insulin and EGCG [20]. Furthermore, like insulin, EGCG requires the activation of Akt and endothelial nitric oxide synthase (eNOS) for NO production. Fig. (2) shows the time-course for EGCG on activation of Akt and eNOS. Thus, low micromolar concentrations of EGCG (achievable with consumption of 5 cups of green tea) can stimulate Akt and eNOS within 15 minutes in bovine aortic endothelial cells. Interestingly, these results also highlight that EGCG-

mediated signaling pathways share features in common with the insulin signaling pathway that lead to activation of eNOS and NO production in endothelial cells [42–44]. However, EGCG does not activate the insulin receptor or VEGF receptor tyrosine kinase, suggesting that the insulin and EGCG pathways converge at a point downstream from the insulin receptor.

It has been proposed that the laminin receptor is a specific cell surface receptor for EGCG that mediates some of its biological actions [45]. However, we have shown that EGCG-mediated activation of the laminin receptor does not play a significant role in the vascular endothelium relating to NO production [20]. Instead, EGCG-mediated signaling requires low level production of reactive oxygen species such as H<sub>2</sub>O<sub>2</sub> [20]. EGCG-mediated production of intracellular H<sub>2</sub>O<sub>2</sub> can be abrogated by N-acetylcysteine, a scavenger of reactive oxygen species [20]. Reactive oxygen species activate *Fyn*, a member of the *Src* family tyrosine kinases that is required for activation of PI3-K, eNOS and NO production in vascular endothelial cells. Again, pre-treatment with N-acetylcysteine blocks EGCG-mediated activation of *Fyn* [20]. The importance of these overlapping pathways highlight the possibility that EGCG may have insulin-mimetic and/or insulin-potentiating effects in the vascular system.

Insulin stimulates production of both NO and endothelin-1 (ET-1) from the endothelium. Insulin stimulates NO production (vasodilator) via a PI3-K dependent pathway and ET-1 production (vasoconstrictor) via a MAP kinase dependent pathway [46]. These agents have opposing vasoactive actions that are in balance under normal healthy conditions. In the presence of insulin, NO production dominates favoring vasodilation. Disruption of the balance between NO and ET-1 production is believed to contribute to the development of hypertension, type 2 diabetes and atherosclerosis [47]. Importantly, EGCG-stimulated phosphorylation of FoxO1, downstream from PI3-K/Akt inhibits both insulin and EGCG-stimulated synthesis and secretion of ET-1 [48, 49]. These data provide a second mechanism that involves PI3-K-dependent pathways to impair ET-1 secretion and favor vasodilator conditions. During insulin resistance, the PI3-K pathway is impaired [50] and the MAP kinase pathway is upregulated [51]. Ultimately this decreases NO production, while increasing ET-1 production. Thus, EGCG, which promotes NO while simultaneously inhibiting ET-1 production, may have a significant advantage as a therapeutic agent in the treatment of insulin resistance. The NO-favoring actions of EGCG have a primary benefit in improving cardiovascular homeostasis, while also improving the metabolic actions of insulin by allowing greater hormone and substrate access to metabolic targets including skeletal muscle. Fig. (3) details the proposed molecular pathways EGCG uses to promote vasodilation.

#### 4.2. Isolated Vessels

Studies have shown that EGCG is a potent vasodilator in isolated aortic rings [52, 53], bovine ophthalmic arteries [54], coronary artery rings [55], and mesenteric vascular beds [4]. Green tea catechins and EGCG have been reported to improve endothelial function in the spontaneous hypertensive rat [4, 56], pre-diabetic OLETF rat [57, 58] and the high fat-fed mouse [59]. These actions are PI3-K- and *Fyn*-dependent.

### 4.3. Skeletal Muscle

Our research implicates vascular dysfunction in skeletal muscle as one major cause of muscle insulin resistance. Insulin stimulates both total blood flow to skeletal muscle [60, 61] and increases microvascular perfusion of myocytes [62–67]. However, insulin's macrovascular and microvascular actions are temporally distinct events, and insulin-mediated glucose uptake is significantly altered by changes in microvascular rather than macrovascular responses [63, 68].

Systemic and local hindleg infusion of the NOS inhibitor L-NAME blocks most, if not all, of insulin-mediated microvascular perfusion in muscle and inhibits 30–40% of insulin-mediated muscle glucose uptake [62, 63, 69]. Therefore, insulin-mediated microvascular recruitment in muscle is, at least in part, NOS-dependent and plays an integral role in regulating muscle glucose uptake. We have demonstrated that insulin, whether infused intravenously or secreted from the pancreas following a mixed meal, stimulates microvascular blood flow in skeletal muscle in both experimental animals [62–65, 67–73] and human subjects [74–76]. This action of insulin to enhance microvascular recruitment in skeletal muscle facilitates delivery of glucose and insulin to the myocytes and enhances glucose disposal. Previously, we have demonstrated that insulin resistant rats [68, 70, 73] and humans [74, 75] have impaired insulin-mediated microvascular and metabolic responses in muscle, suggesting that the loss of microvascular insulin action may contribute to insulin resistance. We recently reported that high fat-induced insulin resistance can originate from impaired microvascular insulin responses and that this microvascular defect precedes the development of myocyte insulin resistance [68]. As mentioned previously, an imbalance between the vasodilator NO, and the vasoconstrictor ET-1, contributes to endothelial dysfunction. We have shown that acute ET-1 infusion inhibits insulin-mediated microvascular recruitment and muscle glucose uptake *in vivo* [77]. This further highlights that disruption of the balance between NO and ET-1 results in reduced insulin-stimulated muscle glucose uptake, indicative of muscle insulin resistance.

The above findings demonstrate a strong link between insulin resistance and endothelial dysfunction, positioning insulin's microvascular action as a critical factor in the development of insulin resistance and type 2 diabetes. Finding novel treatments that have insulin-mimetic and/or insulin-potentiating actions on these vascular targets is a novel approach for treating insulin resistance, endothelial dysfunction as well as type 2 diabetes and its cardiovascular complications.

Serotonin infusion induces muscle insulin resistance in both the perfused rat hindlimb preparation [78] and *in vivo* [67] via vascular actions. We have preliminary data (unpublished) demonstrating that EGCG vasodilates in the presence of serotonin in the constant flow perfused rat hindlimb. Thus, EGCG can oppose vasoconstriction associated with insulin resistance and impaired muscle nutrient exchange. Importantly, we have unpublished data demonstrating EGCG-mediated vasodilation in this preparation is NOS-dependent at low doses ( $< 10 \mu\text{M}$ ), and at least in part NOS-independent at high doses (100  $\mu\text{M}$ ).

The dependency of EGCG-induced vasodilation on NOS varies with the dose/concentration of EGCG. EGCG-mediated vasodilation in rat thoracic aorta is NOS dependent at low concentrations (0.01 – 10  $\mu\text{M}$ ), but not at higher doses of EGCG [52]. Some [52, 53], but not all [20, 54] studies have reported that high concentration of EGCG (100  $\mu\text{M}$ ) can induce vasodilation through different pathways by acting directly on vascular smooth muscle cells [52] or by activating the cAMP-dependent protein kinase pathway [53]. EGCG has also been shown to increase PGI<sub>2</sub> production in bovine aortic endothelial cells [79]. Together, these data are supportive of our unpublished studies that high dose EGCG-induced vasodilation involves NO-independent mechanisms.

As described above, insulin has important microvascular actions in skeletal muscle. Acute infusion of EGCG in rats *in vivo* (to raise the plasma EGCG level to 10  $\mu\text{M}$ ) does not alter femoral artery blood flow, but stimulates microvascular recruitment in muscle (unpublished observations). This suggests that these differential vascular actions of EGCG (macro- vs. micro-vascular) may be concentration-dependent. The magnitude of the increase in microvascular recruitment was similar in effect to raising plasma insulin concentrations to 1.5 nM. However, the microvascular actions of EGCG and insulin did not appear to be additive, i.e. EGCG has insulin-mimetic but not synergistic actions in muscle microvasculature. If similar effects are observed in human studies, this would suggest that daily chronic green tea consumption may promote enhanced microvascular function and oppose endothelial dysfunction.

## 5. ANIMAL STUDIES

Most of the compelling evidence for the anti-diabetic actions of green tea have come from animal studies including normal healthy [30, 33, 39], insulin resistant [2, 34, 39, 57–59, 80–82], type 2 diabetic [83–85] and hypertensive [4] rodent models (detailed in Table 1).

In healthy rats, green tea treatment for 3 weeks (raising plasma EGCG levels to ~40 nM) significantly reduces adiposity and circulating lipids; while increasing (~25%) muscle glucose uptake and GLUT-4 translocation *in vitro* [30]. In rats, Wu and colleagues [33] showed that 12 weeks of green tea treatment (containing mixed catechins 56 mg.d<sup>-1</sup>; EGCG 38 mg.d<sup>-1</sup>) significantly lowered fasting plasma glucose, insulin, and circulating lipids while concurrently improving glucose tolerance and insulin sensitivity. In mice, green tea (EGCG 610 mg.L<sup>-1</sup> in drinking water) supplementation for 14 weeks lowers fasting blood glucose levels. However, no concomitant changes in serum lipid levels were observed [39]. Interestingly, glucose tolerance and muscle glucose uptake in these mice were not altered by green tea therapy while adipose tissue glucose uptake was significantly impaired [39]. Thus, the effects of green tea or EGCG in healthy animals are inconclusive due to a limited number of studies. It should be noted, however, that it is extremely difficult to demonstrate improvement in these parameters, even with conventional drugs, if the animal start out with values in the normal range. The more compelling work indicating positive benefits of EGCG has been conducted in insulin resistant or diabetic animals.

Green tea, green tea extract or EGCG treatment have been reported to ameliorate diet-induced insulin resistance in a number of rodents studies [2, 34, 39, 59, 80–82] and also

studies involving genetic rodent models of insulin resistance and type 2 diabetes [4, 57, 58, 83–85]. Green tea (EGCG 1 g.L<sup>-1</sup> in drinking water) treatment for 12 weeks improves glucose tolerance, GLUT4 content and glucose uptake in adipocytes *in vitro* from fructose-fed rats [34]. Green tea treatment (EGCG 150 or 300 mg.kg<sup>-1</sup>.d<sup>-1</sup>) of fructose-fed hamsters dose-dependently improves glucose tolerance, increases serum adiponectin levels and reduces fasting serum insulin levels [80]. Similarly, Bose *et al.* [81] reported that 4 weeks of EGCG (3.2 g.kg<sup>-1</sup> diet) treatment to high fat-fed mice, significantly reduced fasting blood glucose and plasma insulin levels. However, the food intake of the mice was not reported, and therefore it is unclear how much food (or EGCG) was consumed by the mice each day. Chronic green tea treatment in high fat-fed mice for 14 weeks (EGCG 610 mg.L<sup>-1</sup>) [39] significantly improves glucose tolerance, GLUT4 translocation in muscle, and *in vitro* muscle glucose uptake, and reduces fasting plasma glucose. Similarly, EGCG treatment for 16 weeks (3.2 g.kg<sup>-1</sup> diet) [81] or 22 weeks (EGCG 2 mg.kg<sup>-1</sup>.d<sup>-1</sup>) [82] in high fat-fed mice reduces fasting plasma glucose, insulin, and the homeostasis model assessment of insulin resistance (HOMA-IR; a surrogate index of insulin resistance). In addition EGCG (50 mg.kg<sup>-1</sup>.d<sup>-1</sup>) treatment for 10 weeks in high fat-fed mice [59] lowers fasting serum glucose and insulin, thus improving quantitative insulin sensitivity check index (QUICKI, a surrogate index of insulin sensitivity) [86].

The *db/db* mouse is a commonly used genetic model of type 2 diabetes. One study showed that *db/db* mice treated with EGCG (at 100 mg.kg<sup>-1</sup>.d<sup>-1</sup> but not at 30 mg.kg<sup>-1</sup>.d<sup>-1</sup>) for 2 weeks improves glucose tolerance [84]. Wolfram *et al.* [84] also showed that EGCG treatment for 7 weeks (EGCG 0.25 – 1 g.kg<sup>-1</sup> diet) improves glucose tolerance and reduces blood glucose in this diabetic mouse model in a dose-dependent manner. Another study [85] reports that *db/db* mice treated with EGCG (1 g.kg<sup>-1</sup> diet) for 10 weeks significantly improves glucose tolerance and reduces fasting blood glucose with an effect size comparable to the anti-diabetic insulin sensitizer rosiglitazone. Interestingly, this study also shows EGCG intake reduces the number of pathologically altered islets of Langerhans, while increasing the number and the size of islets, thereby increasing beta cell mass and presumably insulin secretory capacity [85]. These effects correlated with a reduction in islet endoplasmic reticulum stress [85]. Acute green tea treatment (mixed catechins 22 mg.kg<sup>-1</sup>, EGCG 17 mg.kg<sup>-1</sup>) lowers fasting blood glucose levels in *db/db* mice, but not wild type mice [83]. In pre-diabetic OLETF rats, treatment with green tea catechins (25 – 30 mg.kg<sup>-1</sup>.d<sup>-1</sup>) for 12 weeks lowers blood pressure and fasting plasma glucose and insulin levels [57, 58]. EGCG treatment (200 mg.kg<sup>-1</sup>.d<sup>-1</sup>) for 3 weeks prevents development of insulin resistance and reduces systolic blood pressure significantly in spontaneously hypertensive rats [4].

The similar metabolic effects of green tea and EGCG in the above studies suggest that improvements in insulin sensitivity by green tea treatment might be attributable to the actions of EGCG, given that EGCG makes up such a large percentage of green tea polyphenols. However, there are multiple components in green tea that may have synergistic actions.



## 6. CLINICAL INVESTIGATIONS

Numerous clinical studies have been undertaken to investigate the effects of green tea or green tea extracts on glucose tolerance, insulin sensitivity, glucose homeostasis and other cardiometabolic outcomes in people ranging from normal and healthy to insulin resistant individuals and those with type 2 diabetes [18, 83, 87–107] (detailed in Table 1). The effect of green tea/EGCG consumption in healthy subjects appears to be heterogeneous with some reporting improved insulin sensitivity [105] and glucose tolerance [83], while others show no relationship [92, 98]. Again, as with the animal studies, determining differences in these metabolic parameters in healthy people is challenging even with conventional therapeutic agents.

In postmenopausal women with impaired glucose tolerance, EGCG treatment (300 mg.d<sup>-1</sup>) for 12 weeks reduces fasting plasma glucose by 5% [93]. Green tea extract treatment (EGCG 208 mg.d<sup>-1</sup>) for 12 weeks significantly reduces fasting blood glucose and insulin [107] levels in obese insulin resistant subjects. In contrast to these aforementioned studies, EGCG treatment (800 mg.d<sup>-1</sup>) for 8 weeks had no effects on glucose tolerance, fasting glucose and insulin levels in overweight and obese men [88].

In patients with type 2 diabetes, green tea extract (EGCG 860 mg.d<sup>-1</sup> for 16 weeks) significantly reduces HOMA-IR, HbA1c, and fasting insulin levels [97]. Conversely, Ryu *et al.* [104] showed that metabolic markers including blood lipids, glucose, insulin, and adiponectin levels were not altered following 4 weeks of green tea treatment (polyphenol content not reported). Similarly, another study [95] shows green tea treatment (540 mg.d<sup>-1</sup> polyphenols, EGCG content unknown) for 2 months has no apparent effect on metabolic markers such as fasting serum glucose and insulin, HbA1c, and HOMA-IR.

Some studies have assessed the metabolic effects of oolong tea in patients with type 2 diabetes. As previously mentioned, oolong tea is partially fermented and contains moderate amounts of EGCG. Shimada *et al.* [108] reported that oolong tea treatment for 4 weeks (45 mg.day<sup>-1</sup> of EGCG) significantly increases plasma adiponectin levels by 9.9% and lowers HbA1c levels by 3.3% in patients with various coronary risk factors. Additionally, there was a slight, but not significant, decrease in the fasting plasma glucose levels. Hosoda *et al.* [109] used a higher dose of oolong tea treatment (EGCG 390 mg.d<sup>-1</sup>) for 4 weeks and reported lower fasting plasma glucose levels in people with type 2 diabetes. The mechanism of the anti-hyperglycemic effects of the oolong tea is unclear. However, oolong tea appears to have a concentration-dependent effect on glycemia and this tea contains moderate amounts of EGCG.

Although these clinical studies are not conclusive, they suggest a relationship between green tea or EGCG consumption and reduced risk of type 2 diabetes. The potential of complementary alternative medicines as adjuncts to conventional therapy deserves further investigation by direct interventional studies that include biomarker and pharmacokinetic analyses as well as study outcomes that are clinically meaningful.

## CONCLUSION

Animal and cellular studies provide strong evidence that EGCG has beneficial actions to improve and/or augment insulin sensitivity. The molecular and cellular mechanisms, although not fully resolved, are under active investigation and likely involve signaling pathways that are shared with insulin as well as insulin-independent pathways (Figs. 1 and 3). In the vascular system, EGCG stimulates NO production with resulting vasodilation and microvascular recruitment, and inhibits vasoconstriction by opposing ET-1 release and inhibiting serotonin mediated vasoconstriction. Ultimately, these favourable actions of EGCG in skeletal muscle may contribute to ameliorating insulin resistance by improving microvascular delivery of hormones and nutrients to relevant target tissues regulating glucose homeostasis.

Along with robust epidemiological studies related to tea consumption and cardiometabolic health, molecular, cellular, and physiological studies in animals and humans provide a strong rationale for well-powered randomized placebo controlled intervention trials to be carried out in insulin resistant and diabetic populations. These studies should evaluate the efficacy of EGCG as an insulin sensitizer and adjunct functional food treatment for diabetes and its cardiovascular complications. It is important that these future clinical studies of EGCG intervention include formal pharmacokinetic and pharmacodynamic aspects that are often overlooked in human studies of functional foods and nutritional supplements [21]. As for example, in recent clinical intervention studies of glucosamine [110], cocoa [111], and vitamin C [112].

Randomized controlled trials of EGCG in combination with current anti-diabetic drugs are also worthwhile as the vascular actions of EGCG may provide additional benefits in terms of overcoming cardiovascular pathophysiology associated with insulin resistance and type 2 diabetes. These combination studies are also important because EGCG or green tea may be most effective as an adjunctive rather than a primary therapy.

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## Biography

Michelle A. Keske



## LIST OF ABBREVIATIONS

|                                  |                                                                      |
|----------------------------------|----------------------------------------------------------------------|
| <b>AKT</b>                       | Protein kinase B                                                     |
| <b>AMPK</b>                      | Adenosine monophosphate-activated protein kinase                     |
| <b>CaMKK <math>\beta</math></b>  | Ca <sup>2+</sup> /calmodulin-dependent protein kinase kinase $\beta$ |
| <b>DAF2-DA</b>                   | 4,5-diaminofluoresceine diacetate                                    |
| <b>EGCG</b>                      | Epigallocatechin gallate                                             |
| <b>ET-1</b>                      | Endothelin-1                                                         |
| <b>GLUT-4</b>                    | Glucose transporter 4                                                |
| <b>HOMA-IR</b>                   | Homeostasis model assessment of insulin resistance                   |
| <b>PGI<sub>2</sub></b>           | Prostaglandin I <sub>2</sub>                                         |
| <b>QUICKI</b>                    | Quantitative insulin sensitivity check index                         |
| <b>HbA1c</b>                     | Hemoglobin A1c                                                       |
| <b>L-NAME</b>                    | L-NG-Nitroarginine Methyl Ester                                      |
| <b>NOS</b>                       | Nitric oxide synthase                                                |
| <b>NO</b>                        | Nitric oxide                                                         |
| <b>OLETF</b>                     | Otsuka Long Evans Tokushima Fatty                                    |
| <b>PI3-K</b>                     | Phosphoinositide 3-kinase                                            |
| <b>11<math>\beta</math>-HSD1</b> | 11 $\beta$ -hydroxysteroiddehydrogenase type 1                       |
| <b>VEGF</b>                      | Vascular endothelial growth factor                                   |

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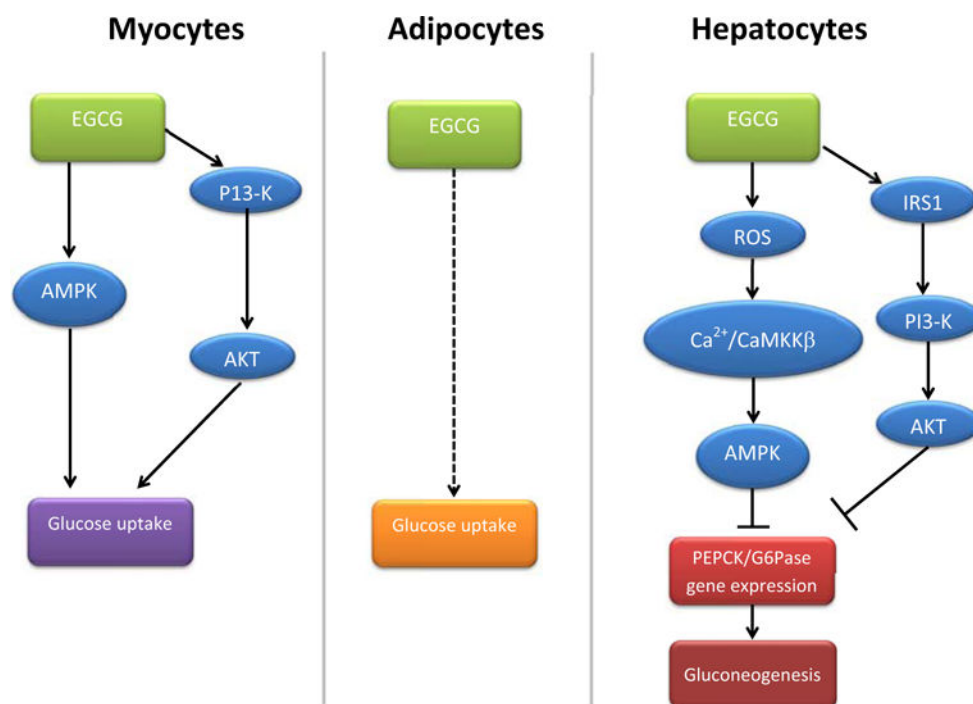
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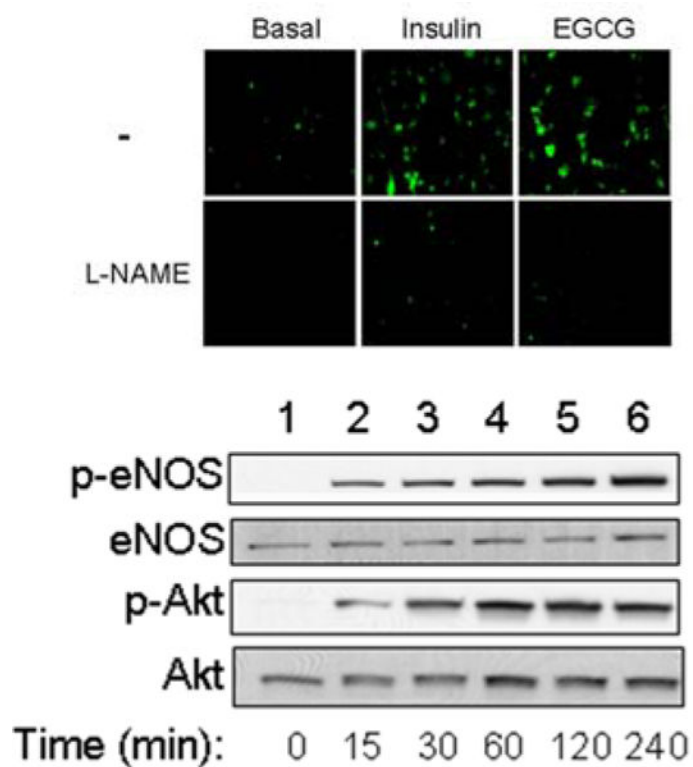
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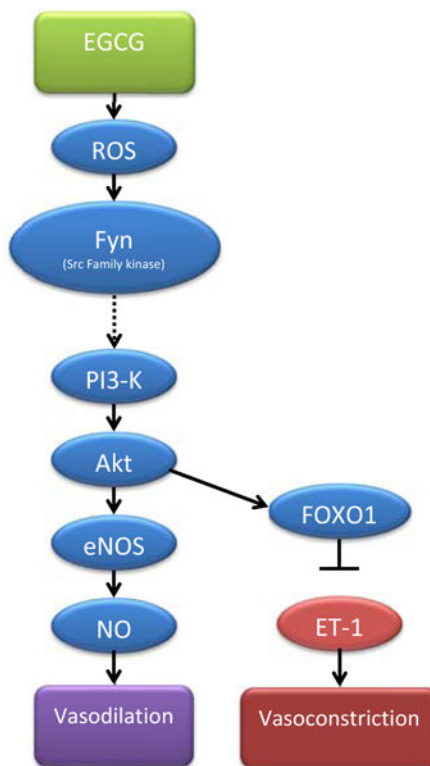


**Fig. 1. Schematic of proposed metabolic actions of EGCG in myocytes, adipocytes and hepatocytes**

Arrows indicate activation  $\perp$  while indicate inhibition. Dotted line represents an unknown pathway.



**Fig. 2. Stimulation of NO from endothelial cells via a PI3-K/Akt/eNOS pathway**  
Bovine aortic endothelial cells were loaded with 4,5-diaminofluoresceine diacetate (DAF2-DA). In the presence of NO, DAF2-DA emits green fluorescence. Both insulin (100 nM, 5 min) and EGCG (50  $\mu$ M, 5 min) stimulates NO production in endothelial cells. This effect was inhibited by the presence of the NOS inhibitor L-NAME [20].



**Fig. 3. Schematic of proposed vasoactive pathways of EGCG in the vasculature**  
Arrows indicate activation while  $\perp$  indicate inhibition.

**Table 1**

Summary of acute and chronic effects of green tea and EGCG in humans and animals.

|                       | Treatment                          | Subjects                       | Effects                        | References                     |
|-----------------------|------------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Human                 | Acute                              | Healthy                        | ↑GTT                           | [83]                           |
|                       |                                    |                                | ↑ Insulin sensitivity          | [105]                          |
|                       |                                    |                                | ↔ GTT                          | [105]                          |
|                       | Chronic (a3wks)                    | Healthy                        | ↔ Fasting blood/plasma glucose | [83, 105]                      |
|                       |                                    |                                | ↔ Fasting serum insulin        | [105]                          |
|                       |                                    |                                | ↑ Post-prandial plasma glucose | [98]                           |
| Chronic (a3wks)       | Overweight/Obese/Insulin Resistant | ↔ Post-prandial serum insulin  | [98]                           |                                |
|                       |                                    | ↔ Fasting plasma glucose       | [92]                           |                                |
|                       |                                    | ↓ Fasting plasma/serum glucose | [93, 107]                      |                                |
| Chronic (a3wks)       | Type 2 diabetes                    | ↓ Fasting serum insulin        | [107]                          |                                |
|                       |                                    | ↓ HOMA-IR                      | [107]                          |                                |
|                       |                                    | ↔ HOMA-IR                      | [88]                           |                                |
| Chronic (a3wks)       | Type 2 diabetes                    | ↔ GTT                          | [88]                           |                                |
|                       |                                    | ↔ Fasting blood/plasma glucose | [87–89, 94, 99, 100, 102, 106] |                                |
|                       |                                    | ↔ Fasting plasma insulin       | [88, 89, 94, 99, 102]          |                                |
| Chronic (a3wks)       | Type 2 diabetes                    | ↓ Fasting insulin              | [97]                           |                                |
|                       |                                    | ↓ HOMA-IR                      | [97]                           |                                |
|                       |                                    | ↓ HbA1c                        | [97, 108]*                     |                                |
| Chronic (a3wks)       | Type 2 diabetes                    | ↓ Fasting plasmagluose         | [109]*                         |                                |
|                       |                                    | ↔ Fasting blood/plasma glucose | [95, 101, 104]                 |                                |
|                       |                                    | ↔ Fasting plasma insulin       | [95, 104]                      |                                |
| Chronic (a3wks)       | Type 2 diabetes                    | ↔ HOMA-IR                      | [95, 104]                      |                                |
|                       |                                    | ↔ HbA1c                        | [95]                           |                                |
|                       |                                    | Animal                         | Acute ( 2hrs)                  | Healthy                        |
| ↓ GTT                 | [113]                              |                                |                                |                                |
| ↓ Insulin sensitivity | [113]                              |                                |                                |                                |
| Acute ( 2hrs)         | Insulin resistant                  |                                | ↑ GTT                          | [113]                          |
|                       |                                    |                                | ↑ Insulin sensitivity          | [113]                          |
|                       |                                    |                                | ↓ Plasma glucose               | [113]                          |
| Acute ( 2hrs)         | Type 2 diabetes                    | ↓ Plasma insulin               | [113]                          |                                |
|                       |                                    | Healthy                        | ↓ Blood glucose                | [83]                           |
|                       |                                    |                                | Healthy                        | ↓ Fasting blood/plasma glucose |
| Chronic ( 2wks)       | Insulin resistant                  |                                |                                | ↓ Fasting plasma insulin       |
|                       |                                    | ↔ GTT                          |                                | [33, 39]                       |
|                       |                                    | ↑ insulin sensitivity          | [33]                           |                                |
| Chronic ( 2wks)       | Insulin resistant                  | ↓ Fasting blood/plasma glucose | [39, 57, 58, 81, 82]           |                                |
|                       |                                    | ↓ Fasting plasma/serum insulin | [57–59, 80–82]                 |                                |
|                       |                                    | ↑ GTT                          | [2, 34, 39, 80]                |                                |
| Chronic ( 2wks)       | Type 2 diabetes                    | ↔ GTT                          | [82]                           |                                |
|                       |                                    | ↑ QUICKI                       | [4, 59]                        |                                |
|                       |                                    | ↓ HOMA-IR                      | [81, 82]                       |                                |
| Chronic ( 2wks)       | Type 2 diabetes                    | ↑ Endothelial function         | [4, 57–59]                     |                                |
|                       |                                    | Type 2 diabetes                | ↓ Fasting blood glucose        | [84, 85]                       |
|                       |                                    |                                | ↑ GTT                          | [84, 85]                       |

GTT: glucose tolerance test;

↑ : improve;

↓ : reduce;

↔ : no effects;

\* :oolong tea.

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