HHS Public Access

Author manuscript

Microcirculation. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Microcirculation. 2015 July ; 22(5): 391–399. doi:10.1111/micc.12208.

Peripheral microvascular vasodilatory response to estradiol and genistein in women with insulin resistance

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Abstract

Objective—Estradiol enhances vasodilation in healthy women, but vascular effects of the phytoestrogen genistein are still under investigation. Insulin resistance (IR) compromises microvascular function. We therefore examined the interaction of estradiol, genistein, and IR on microvascular vasodilatory responsiveness.

Methods—We hypothesized that estradiol and genistein increase microvascular vasodilation in healthy women (control, n=8, 23±2 yr, BMI 25.9±2.9 kg/m^2) but not in women with IR (n=7, 20±1 yr, BMI 27.3±3.0 kg/m^2). We used the cutaneous circulation as a model of microvascular vasodilatory function. We determined cutaneous vascular conductance (CVC) with laser Doppler flowmetry and beat-to-beat blood pressure during local cutaneous heating (42°C) with estradiol or genistein microdialysis perfusions. Because heat induced vasodilation is primarily an NO mediated response, we examined microvascular vasodilation with and without L-NMMA.

Results—In control women, estradiol enhanced CVC (94.4±2.6 % vs. saline 81.6±4.2 % CVCmax, P<0.05), which was reversed with L-NMMA (80.9±7.8 % CVCmax, P<0.05), but genistein did not affect vasodilation. Neither estradiol nor genistein altered CVC in IR, although L-NMMA attenuated CVC during genistein.

Conclusions—Our study does not support improved microvascular responsiveness during genistein exposure in healthy young women, and demonstrates that neither estradiol nor genistein improve microvascular vasodilatory responsiveness in women with IR.

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Author Contributions

MMW and NSS performed the experiments; NSS analyzed the data; MMW and NSS interpreted the results of the experiments; MMW prepared the figures; HST and NSS contributed to the conception and design of the research; MMW drafted the manuscript; MMW, HST, and NSS edited and revised the manuscript; MMW, HST, and NSS approved the final version of the manuscript.

DISCLOSURES: The authors report no conflicts of interest.

Key terms

cutaneous microdialysis; vascular reactivity; phytoestrogens; nitric oxide; estrogen

INTRODUCTION

Estrogens increase blood flow in many vascular beds. In particular, 17β-estradiol (E_2) enhances vasodilation by activating endothelial nitric oxide synthase (eNOS) to rapidly produce nitric oxide (NO), as elegantly reviewed by Kim et al (33). Recently, the cardiovascular benefits of estradiol have been subject to considerable debate (2, 48). Despite mechanistic studies supporting beneficial cardiovascular effects of estradiol, large-scale clinical trials have demonstrated estrogen-associated increases in the risk of thrombolytic events in women over 60 and in women with underlying or established cardiovascular disease (2, 48). Thus, many women cannot or do not wish to take estrogens and seek alternatives such as phytoestrogens.

Phytoestrogens are plant-derived compounds that can induce physiological effects similar to those of estrogens. Phytoestrogens are used to treat some menopausal symptoms, including vasomotor symptoms, and do not appear to increase the risk of carcinogenic outcomes associated with unopposed estrogens. Indeed, certain phytoestrogens may even provide protection against some gynecological cancers (44). The most commonly used phytoestrogen is genistein (GEN). As with estrogens, GEN may enhance vasodilation, improve endothelial function, and lower the risk of cardiovascular disease (8, 15). For example, in women with coronary heart disease, GEN elicits greater vasodilation in isolated subcutaneous vessels compared to E_2 (10). In healthy men, GEN causes NO-dependent vasodilation in forearm resistance vessels comparable in magnitude to the vasodilatory actions of E_2 (59). Therefore, the beneficial cardiovascular effects of GEN may be similar to, or even more favorable than, those of E_2 (10).

The American Diabetes Association indicates that approximately 35% of Americans are pre-diabetic including individuals with insulin resistance (IR). Moreover, independent of progression to diabetes, IR increases cardiovascular disease risk in both men and women. The primary physiological action of insulin is to facilitate glucose uptake by tissues, but insulin also modulates the sympathetic nervous system and mediates vasodilation by increasing NO release from endothelial cells (55). As such, IR is often associated with endothelial dysfunction and peripheral vascular disease, thereby increasing hypertension and cardiovascular disease risks. The endothelial dysfunction associated with IR is evident in the peripheral microcirculation (45) and related in part to compromised NO bioavailability and eNOS signaling (46, 55). Given the extent to which E_2-mediated vasodilation depends on NO and eNOS signaling, the vasodilatory actions of estrogens and phytoestrogens may be compromised in women with IR.

The cutaneous circulation is an easily accessible vascular bed, and is frequently used as a model to examine changes in peripheral microcirculatory function in humans (22, 35) because the same systems that contribute to regulating vascular smooth muscle, vascular tone, and endothelial function [i.e. NO and prostaglandins (11, 57)] operate in the skin

vasculature (12, 13, 21, 22, 28–30, 40). In addition, local heating of the skin induces vasodilation that is largely mediated by the endothelium and may be more sensitive in detecting microvascular dysfunction compared to iontophoresis of acetylcholine in patients with CVD (1, 23, 32). Cutaneous microdialysis (described below) is a minimally invasive technique that can explore mechanisms regulating vascular function *in vivo* and in humans, permitting local drug delivery without whole body exposure (22). This paradigm is of particular interest because vascular dysfunction is a systemic process that often begins in the microcirculation, and the mechanisms and degree of dysfunction in the cutaneous microvasculature reflect those in other microvascular beds (1, 9, 23, 32). Impaired vasomotor function in the microvascular circulation is a key feature of cardiovascular disease risk (1, 23, 32) associated with insulin resistance and hypertension (26, 45). The purpose of this study was to determine if either E_2 or GEN exposure enhances microvascular vasodilatory responses in women with IR. We hypothesized that E_2 and GEN enhance local heating-induced cutaneous microvascular vasodilation in healthy controls but not in women with IR.

MATERIALS and METHODS

Subjects and study design

Fifteen women participated in this study. Women were between 18–30 years of age, non-smoking, and taking no medication as indicated by a standard medical history. All of the women were sedentary or moderately active. Women completed two experimental visits: 1) oral glucose tolerance test (OGTT) to determine insulin resistance, and 2) microvascular vasodilatory assessment to determine microvascular responsiveness. We compared women with and without IR (as determined by the OGTT, see below). We focused on this IR group and not diabetics because IR can precede diabetes and is an important interventional state. All women gave written informed consent to participate in the study, which conformed to the guidelines contained in the Declaration of Helsinki and had prior approval by the Human Investigation Committee of Yale School of Medicine.

Oral glucose tolerance test (OGTT)

In order to assess insulin resistance, all women underwent a three-hour oral glucose tolerance test (OGTT). Women reported to the laboratory in the morning after an overnight fast. They provided a urine sample to determine hydration status and underwent an over-the-counter pregnancy test. Women were seated in a semi-recumbent position in a modified dental chair, and an IV catheter was placed in the left arm. After a 30-minute rest period, a blood sample was taken for fasting measures of plasma glucose and insulin. Women then consumed a 75 mg glucose beverage (Orangedex; Custom Laboratories, Baltimore, MD), and venous blood samples were drawn every 30 minutes to analyze plasma glucose and insulin concentrations. The area under the curve (AUC) for insulin concentration during the 180-minute period was used to determine insulin resistance (Table 1). Women were placed into control versus IR groups based on the spread of the data, with half above and half below the median of the area under the curve for insulin (see table below). Importantly, the responses of the women in each group were consistent with insulin and glucose responses typically associated with their respective groups (7).

Microvascular vasodilatory assessment

In the present investigation, we used the cutaneous circulation as a model to explore the impact of E_2 and GEN on microvascular vasodilatory responsiveness. To control for the variability in endogenous E_2 exposure across the menstrual cycle, all experiments were performed during the early follicular phase (days 1–6). No women were taking hormonal treatments of any kind. Studies were conducted in the morning after an overnight fast in an environmental chamber (T_a = 28°C). Women were seated in a semi-recumbent position (modified dental chair) with an IV catheter placed in the left arm to obtain a blood sample for the analysis of serum concentrations of E_2 (S[E_2]) and progesterone (S[P_4]). Following the blood collection, under sterile conditions, five microdialysis fibers each with a 30kDa cutoff (Basi, Inc. West Lafayette, IN) were placed just below the surface of the skin on the dorsal side of the right forearm as previously described (60, 61). After fiber insertion, we perfused 0.9% saline through all five fibers (2 μL/min) for at least 90 minutes following fiber insertion to permit trauma recovery. Red blood cell flux was then measured using laser Doppler flowmetry (Doppler Monitor, PF 5020 LDPM Unit, Perimed AB, Stockholm, Sweden) and used as an index of skin blood flow (SkBF). We measured beat-by-beat blood pressure on the left middle finger throughout the experiment (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands).

We measured resting baseline SkBF for 10 minutes with skin temperature clamped at 32°C. Following the baseline measurement, we perfused the following substances through five separate fibers: 0.9% saline; E_2 (75 nM); E_2 in combination with the nitric oxide synthase (NOS) inhibitor N^G-monomethyl-L-arginine (L-NMMA, 10 mM); GEN (200 nM); and GEN in combination with L-NMMA (10 mM) at 5μL/min for 45 minutes. All compounds were purchased from Sigma-Aldrich and perfusion solution prepared by the Investigational Drug Service at Yale-New Haven Hospital. Because E_2 and GEN are not water soluble, 0.01M of each compound was first dissolved in 100% ethanol. This solution was then diluted in normal saline for microdialysis perfusions at the desired concentrations (see below). The final concentration of ethanol is very small and had no effect on vasodilatory function; separate pilot studies in our laboratory indicated similar basal CVC (ethanol 0.29 vs. saline 0.24 au/mmHg) and vasodilatory responses to local heating (ethanol 1.1 vs. saline 1.7 au/mmHg) during perfusions of 0.01M ethanol in saline compared to saline alone. We attribute the differences in heating response to site-to-site variation in SkBF (a function of anatomical differences in blood vessel density at each skin site), but in any event, these data indicate that ethanol did not induce a vasodilation in these small amounts. The doses of E_2 (molecular weight = 272.38) and GEN (molecular weight = 270.2) were determined from pilot studies conducted in our laboratory on a subset of women using dose-response curves (Prism 5.0, Graphpad Inc, San Diego, California) generated with five incremental doses of E_2 (10, 30, 50, 75, 100 nM) and five incremental doses of GEN (10, 30, 60, 300, 600 nM). In order to induce vasodilation and determine the impact of E_2 and GEN these experiments were conducted during local heating. Infused E_2 and GEN concentrations were log transformed, and % CVCmax was normalized within each probe with the largest value of the data set at 100% and lowest value of the data set at 0% (vasodilation), and then plotted using nonlinear regression with a variable slope (62). Normalizing the % CVCmax to a maximum value of 100% (highest concentration of E_2 or GEN) and the minimum value 0%

(lowest concentration of E_2 or GEN) enabled the comparison of the dose-response curves on a similar scale and is useful when comparing curve position (62). Dose-response curves are presented in Figure 1.

Following these initial 45-minute hormone perfusions we increased the temperature of all Doppler probes to 42°C for 30–45 minutes until we achieved a plateau in SkBF (60). Local heating of the skin induces microvascular dilation that is largely endothelial-dependent (22, 31, 40). This plateau was measured for five minutes, after which we perfused sodium nitroprusside (SNP; 28 nM, an endothelial independent vasodilator) at a rate of 10 μL/min while increasing the temperature of the Doppler probes to 43°C to elicit maximal vasodilation (60).

Blood analysis—An aliquot was transferred to a tube without anticoagulant for the determination of S[E_2], and S[P_4]. The samples were centrifuged, frozen immediately and stored at −80°C until analysis. Serum [E_2] and S[P_4] were measured using competitive binding radioimmunoassay methods. Intra- and inter-assay coefficients of variation for the mid-range standards for S[E_2] (180±13.1 pg•ml^{-1}) were 2.7% and 4.3% (Siemens Healthcare Diagnostics, Los Angeles, CA, USA), for S[P_4] (3.5±0.2 ng•ml^{-1}) were 2.4% and 2.6% (Siemens Healthcare Diagnostics). Glucose samples were analyzed in duplicate immediately after withdrawal using a glucose/lactate analyzer (Yellow Springs Instruments, Yellow Springs OH). Insulin was measured in duplicate by competitive binding radioimmunoassay (Siemens Healthcare Diagnostics) with intra- and inter-assay coefficients of variation for the midrange standard (34.6μlU/ml) of 2.43% and 3.26%, respectively.

Data analysis and Statistics

Microvascular SkBF data were collected at 1000 Hz using Powerlab (ADInstruments, Bella Vista, NSW, Australia), and expressed as cutaneous vascular conductance (CVC), calculated by dividing SkBF by mean arterial blood pressure (Finometer) over the five-minute plateau period. We compared the plateau phase of local heating (to 42°C) across hormonal exposure between controls and women with IR using a 2-way ANOVA. In order to account for site to site variation in SkBF (a function of anatomical differences in blood vessel density at each skin site), all data are expressed as a percent of maximal CVC (%CVCmax) achieved during the perfusion of SNP concomitant with local heating to 43°C.

Sample size calculation—Sample size calculations were based on our primary outcome variable of interest, %CVCmax. The desired statistical test is two-sided and we assumed an α level of 0.01 to account for multiple comparisons. We used an effect size of 1.935 (6 subjects/group) (52). Given 6 per group and alpha=0.01, this effect size allowed us 80% power (0.843) for ANOVA to differentiate these changes from chance (14) (© G-Power 3.1).

RESULTS

Based on the AUC during the 180 minute OGTT, 8 women were classified as controls and 7 women as insulin resistant (Table 1). Fasting serum insulin concentrations and the AUC for

insulin was elevated in the IR group (by design). All other baseline subject characteristics were similar between groups (Table 1).

On the day of microdialysis testing blood analysis of ovarian hormones confirmed that women were in the early follicular phase of the menstrual cycle, (S[E_2], Control: 39.2±5.7 pg/ml, IR: 34.2±4.7 pg/ml; S[P_4], Control: 1.9± 0.9 ng/ml, IR 2.2 ± 1.3 ng/ml) with no differences between the groups in either hormone concentration. The SkBF increased during heating with saline perfusion in both groups of women, and this increase was higher in IR (P=0.04), indicating that overall microvascular responsiveness was preserved in women with IR. In controls only, E_2 perfusion enhanced microvascular dilation induced by local heating (Figure 2), and L-NMMA reversed this E_2 effect. In contrast, GEN had no impact on microvascular dilation in controls (Figure 2). In women with IR, neither E_2 nor GEN increased microvascular dilation, although NO blockade attenuated the heating-induced vasodilation during GEN perfusion (Figure 3). Mean arterial blood pressure was similar between groups as was maximal vasodilation achieved with SNP combined with heating (data not shown).

DISCUSSION

This is the first investigation to examine the direct effects of E_2 and GEN exposure on peripheral cutaneous microvascular vasodilatory function in humans, both with and without IR. Our most important findings were that although E_2 enhanced microvascular dilation in healthy young women, E_2 had no vasodilatory effect in the skin microvasculature of young women with IR. Furthermore, GEN exposure did not impact microvascular responses in healthy controls or women with IR. These findings have important clinical meaning because women use both E_2 and GEN with the expectation that these substances will improve cardiovascular health, or they will at least attenuate cardiovascular decline with aging. Our study does not support improved microvascular responsiveness during GEN exposure in healthy young women, and demonstrates that neither E_2 nor GEN improve microvascular vasodilatory responsiveness in women with IR. The latter is a particularly important clinical finding because women with IR are at especially high risk for endothelial dysfunction and peripheral microvascular disease. Thus, E_2-mediated vasodilation in women may depend on insulin resistance, and GEN appears to have little impact on peripheral microvascular function. Finally, our findings confirmed that NO is an important mediator of the E_2 effects on the endothelium in the microvasculature. These findings extend earlier data indicating that E_2 exposure improves conduit arterial function in both young and postmenopausal women (6, 39). The poor E_2-mediated vascular responsiveness in women with IR suggests either lower NO bioavailability, functional changes in estrogen receptor (ER) expression, or changes in downstream mechanisms associated with NO-mediated vasodilation in IR. Our data cannot distinguish which of the mechanisms play the most important role in the impaired response to E_2 in women with IR, but certainly suggest areas for future research both on physiological and molecular levels.

Estradiol binds primarily to the ERα receptor in the vasculature, stimulating eNOS and NO activation and leading to rapid vasodilation (19). Furthermore, estrogens modulate insulin sensitivity, likely through ERα receptors (47, 54). Indeed, a reduction of NO bioavailability

contributes to impaired endothelial function in men with IR (27, 41). Our young women with IR did not appear to exhibit impaired endothelial function, but inhibiting NO during E_2 perfusions had minimal effect on vasodilation. Therefore, it is possible that young women with IR have lower NO bioavailability, but that compensatory mechanisms are up-regulated to maintain endothelial function. However, we would still expect to see an increase in vasodilation during E_2 perfusion, whether it is NO or non-NO mediated. Thus, consistent with previous findings (37), we propose that low ERα receptor signaling may contribute to the increased risk of IR and metabolic syndrome (37). In support of this hypothesis, ERα knockout mice exhibit endothelial dysfunction (49), glucose intolerance and IR (18) compared to wild type mice, whereas glucose tolerance and insulin sensitivity are unchanged in ERβ knockouts (5). Furthermore, E_2 can promote re-endothelialization via the ERα receptor but not the ERβ receptor in mice (3). Given the lack of response to direct perfusion of E_2 in women with IR, we propose that there is a functional down-regulation of either ERα expression or transcriptional activity associated with IR (42). Future studies can determine whether IR *per se* modulates ER expression in various tissues, or whether a change in estrogen status (either hyper- or hypo- estrogenic) drives the onset of IR or the comprised cardiovascular function associated with IR.

The vasodilatory effects of GEN are still unclear. Chronic GEN administration (6 months, oral) improved flow-mediated dilation, blood lipids and cholesterol profiles as well as insulin sensitivity in postmenopausal women (24, 53, 58). However, GEN administration of either 8-weeks (50) or 2-weeks (17) did not improve endothelial function in postmenopausal women. Our data are the first to examine the effects of acute GEN exposure on microvascular function in young women, and indicate that GEN has little impact on microvascular dilation in young women with or without IR. These findings are in contrast to those in middle-aged or older adults, where intra-arterial infusions of GEN enhanced endothelial mediated dilation in middle-aged men and premenopausal women as measured by plethysmography (59). We speculate that GEN may attenuate age-related declines in endothelial function, but has little impact in young adults. Interestingly, we did observe a significant reduction in vasodilation with NO blockade during GEN exposure only in IR women that was not observed during E_2 perfusion, suggesting GEN may alter the mechanisms for NO mediated dilation. Because GEN may have a greater binding affinity for ERβ compared to 17β-estradiol (E_2) (34), we propose that either greater eNOS activation or NO bioavailability associated with GEN in women with IR may be related to alterations in ER expression. Consistent with these proposed mechanisms, GEN elicited greater vasodilation compared to E_2 in postmenopausal women with underlying cardiovascular disease, and this enhanced vasodilation is associated with upregulation of ERβ receptors in the vasculature of these vessels (10). Changes in the ERα/β ratio may also explain the lack of vasodilation during E_2 perfusion in our IR group. Thus, GEN may be more efficacious compared to E_2 to improve cardiovascular function in women with underlying disease because of its' affinity for the ERβ receptors in the vasculature (10). The contrasting responses to E_2 (ERα) and GEN (ERβ) suggest that selective targeting of ERs may have important therapeutic implications for women's cardiovascular health (10).

Insulin is an important vasoactive substance that can modulate both NO and ET-1 (55). Indeed, prior studies demonstrate impaired endothelial function in adults with IR, and endothelial function has important implications for blood pressure regulation and hypertension. Recently, impaired microvascular endothelial function in the cutaneous circulation has been demonstrated in women with IR, when adjusting differences in waist circumference between the two groups (45). We were surprised that heat-induced microvascular vasodilation in our group of women with IR was not attenuated, and in fact, was actually higher compared to control women. Because of redundant mechanisms mediating the local heating response, it is possible that additional mechanisms (such as potassium channels) or other compensatory mechanisms are up-regulated in women with insulin resistance, contributing to this preserved blood flow response. For example, acetylcholine-induced vasodilation was similar in control and hypercholesterolemic rabbits, but blockade of calcium-dependent potassium channels significantly reduced vasodilation in the hypercholesterolemic group only (43), suggesting a compensatory upregulation of additional non-NO mechanisms to preserve endothelial function. Similarly, microvascular vasodilation to acetylcholine and sodium nitroprusside were preserved in young adults with obesity or metabolic syndrome, whereas vasodilatory responsiveness to prostacyclin were impaired (36). Our findings of preserved microvascular function are consistent with these (36), although the reason for the contrasting findings among other studies is not clear from our data. It is possible that microvascular effects of these risk factors are additive (obesity and IR) to impair vasodilation. Our study was not powered to look at the independent contributions of CVD risk factors on impaired microvascular function, and was primarily focused on examining the effects of IR *per se*. Future studies can explore the link between additional risk factors such as family history of disease and birth weight, along with examining the duration of IR.

Limitations

The women in our study were instrumented with five microdialysis fibers, and it would be stressful to the subject to insert more within each session. Thus, we were unable to include perfusion of L-NMMA alone, and recognize this as a limitation to the interpretation of our findings. Because there are redundant mechanisms involved in mediating the vasodilatory response to local skin heating, it is it likely that other non-NO mechanisms may be involved in mediating the responses we observed. Although NO plays a primary role in the vasodilatory response to local heating, it is plausible that E_2 may have NO independent actions, which we cannot rule out in the absence of a separate L-NMMA site. For example, blockade of NO in addition to calcium-activated potassium channels almost completely abolished the vasodilation during local skin heating (4) indicating a role for potassium channels in mediating cutaneous vasodilation; E_2-associated relaxation is partially mediated through potassium channels in isolated mesenteric artery rings (56). Future studies can address the role of potassium channels in mediating E_2 associated vasodilation in the skin microcirculation. Moreover, we recognize that both norepinephrine and neuropeptide Y play a role in mediating cutaneous vasodilation during local skin heating (20), and that both pathways are modulated by E_2 (25, 51). Finally, we cannot rule out a potential role of oxidative stress, as reactive oxygen species are also involved in the local heating response (38). Because oxidative stress can be apparent before any signs of endothelial dysfunction in

adults with insulin resistance (16), it is possible that although vasodilatory function was preserved in women with IR, oxidative stress is still present. However, since E_2 is generally known to attenuate oxidative stress, this cannot explain the lack of response observed during E_2 perfusions in the women with IR, which may suggest an issue with estrogen receptors as highlighted above. The lack if E_2 vasodilation observed in women with IR may also be due to a 'ceiling effect' since the vasodilation during saline alone was augmented. However, we still would have expected to see an increase in vasodilation with E_2; 7 of the 8 women in the control group demonstrated a 10–15% increase in vasodilation during E_2 whereas only 3 women in the IR group had an increase in vasodilation (~6%) and 4 women had no change with E_2. Although this is the first study to examine the influence of reproductive hormones on vascular function in young women with IR, identifying the precise mechanisms involved remain an important and interesting area for future investigation.

In summary, our data are the first to show direct E_2 perfusion increased cutaneous microvascular dilation in healthy women, but not in women with IR. Further, our data show that GEN did not influence microvascular dilation in young women with or without IR. Although women use phytoestrogens assuming a level of cardiovascular benefit, our data do not support a role for GEN in improving microvascular responsiveness in young women, and suggest little impact on endothelial function. Finally, IR attenuated microvascular responsiveness to E_2, suggesting women with IR may not receive the same cardiovascular benefits as those presumed for healthy women taking estrogen.

Perspectives

In women, the prevalence of both cardiovascular disease and IR increase with age, particularly after menopause. Our data show little impact of E_2 or GEN on microvascular vasodilation in younger women with IR, but clearly similar studies need to be conducted in postmenopausal women. These data support the practice of determining insulin sensitivity in women before prescribing estrogens or phytoestrogens to improve cardiovascular health.

Acknowledgements

We gratefully acknowledge Cheryl Leone, MS for technical assistance, Osama Abdelghany, PharmD, BCOP, for microdialysis drug guidance and preparations, and the subjects for their time.

Grants

This research was supported by National Heart, Lung, and Blood Institute Grant R21-HL-109822 (NSS).

ABBREVIATIONS

E_2	Estradiol
GEN	Genistein
L-NMMA	N^G-monomethyl-L-arginine
CVC	Cutaneous vascular conductance
IR	Insulin resistance

C	Control women
BMI	Body mass index
NO	Nitric oxide
eNOS	endothelial nitric oxide synthase
OGTT	Oral glucose tolerance test
AUC	Area under the curve
$S_{[E2]}$	serum estradiol
$S_{[P4]}$	serum progesterone
SkBF	Skin Blood Flow
SNP	sodium nitroprusside
ER	estrogen receptor
CVD	cardiovascular disease

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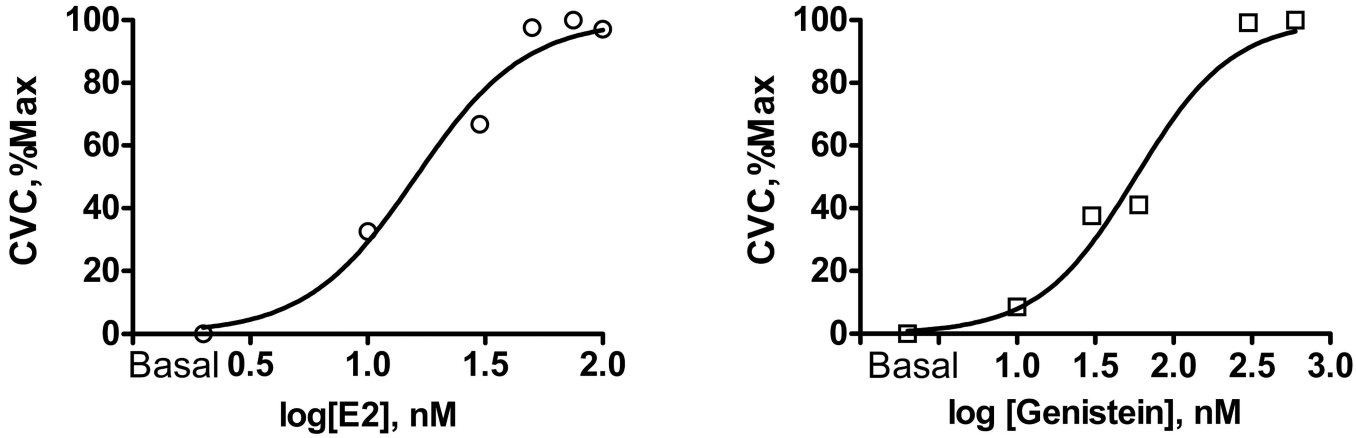

Figure 1.

Dose-response curves generated with five incremental doses of estradiol (E_2; Left: 10, 30, 50, 75, 100 nM) and five incremental doses of genistein (GEN; Right: 10, 30, 60, 300, 600 nM).

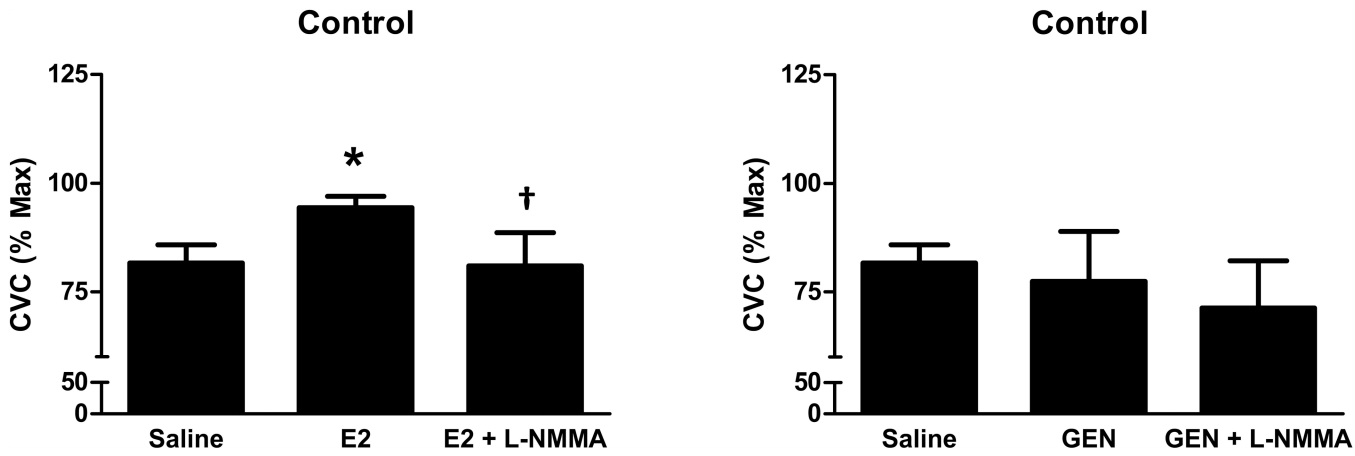

Figure 2.

Microvascular vasodilatory responses to estradiol (E_2; left) and genistein (GEN, right) in healthy control women. Data are presented as mean ± SEM. * Different from Saline. †Different from E_2. Differences were considered significant at $P < 0.05$.

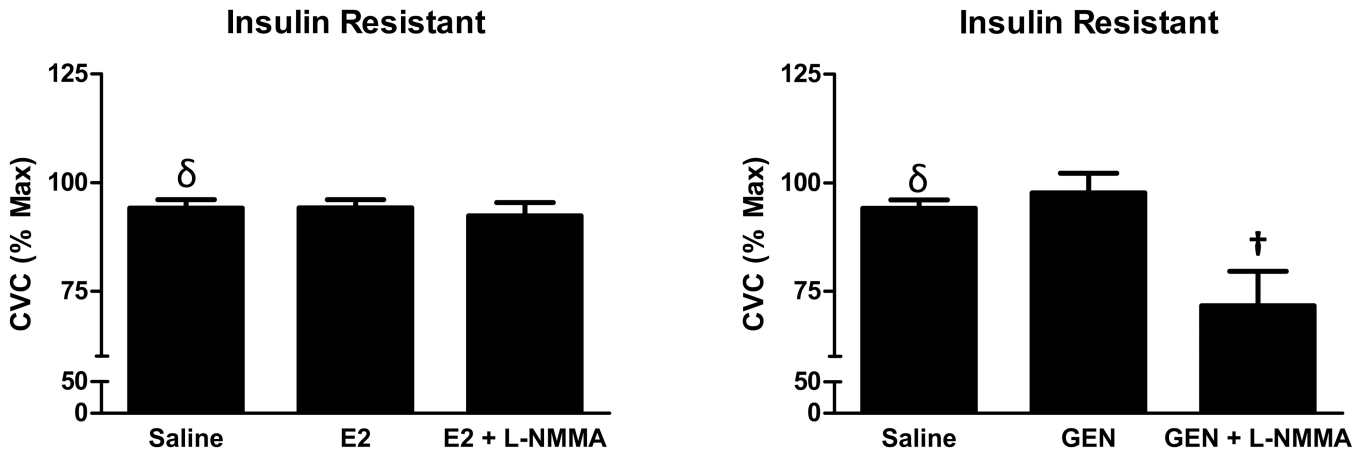

Figure 3.

Microvascular vasodilatory responses to estradiol (E_2; left) and genistein (GEN, right) in women with insulin resistance. Data are presented as mean ± SEM. δ Different from Controls. †Different from GEN. Differences were considered significant at $P < 0.05$.

Table 1

Subject Characteristics

	CONTROL	IR
Age, yr	23 ± 2	20 ± 1
Weight, kg	60.4 ± 4.5	76.9 ± 7.7
Height, cm	1.56 ± 0.08	1.69 ± 0.03
BMI, kg/m^2	25.9 ± 2.9	27.3 ± 2.9
P[Gl], mg/100 ml	81.2 ± 2.3	85.3 ± 3.6
P[Ins], μU/ml	4.8 ± 1.1	10.0 ± 1.9*
Glucose, mg/100 ml AUC	20,331 ± 727	20,167 ± 1,028
Insulin, μU/ml AUC	5,900 ± 457	10,933 ± 1,253*

Subject characteristics in Control and women with insulin resistance (IR) taken at rest on the day of the oral glucose tolerance test (OGTT). Fasting plasma concentrations of glucose (P[Gl]) and insulin (P[Ins]), and area under the curve (AUC) during the OGTT.

* Different from Control. Differences were considered significant at $P < 0.05$.