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Epigallocatechin-3-gallate (EGCG), A Green Tea Polyphenol, Suppresses Hepatic Gluconeogenesis through 5'-AMP-activated Protein Kinase

Q. F. Collins, H.-Y. Liu, J. Pi, Z. Liu, M. J. Quon and W. Cao
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EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR

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Departments of ¹Pharmacology and Human Physiology and ²Emergency and Organ Transplantation, Medical School, University of Bari, 70124 Bari, Italy; and ³Diabetes Unit, National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, Maryland

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Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, Kim J-a, Quon MJ, Montagnani M. EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. *Am J Physiol Endocrinol Metab* 292: E1378–E1387, 2007. First published January 16, 2007; doi:10.1152/ajpendo.00698.2006.—Epigallocatechin gallate (EGCG), a bioactive polyphenol in green tea, may augment metabolic and vascular actions of insulin. Therefore, we investigated effects of EGCG treatment to simultaneously improve cardiovascular and metabolic function in spontaneously hypertensive rats (SHR; model of metabolic syndrome with hypertension, insulin resistance, and overweight). In acute studies, EGCG (1–100 μM) elicited dose-dependent vasodilation in mesenteric vascular beds (MVB) isolated from SHR *ex vivo* that was inhibitable by *N*^o-nitro-L-arginine methyl ester (L-NAME; nitric oxide synthase antagonist) or wortmannin [phosphatidylinositol (PI) 3-kinase inhibitor]. In chronic studies, 9-wk-old SHR were treated by gavage for 3 wk with EGCG (200 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$), enalapril (30 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$), or vehicle. A separate group of SHR receiving L-NAME (80 mg/l in drinking water) was treated for 3 wk with either EGCG or vehicle. Vasodilator actions of insulin were significantly improved in MVB from EGCG- or enalapril-treated SHR (when compared with vehicle-treated SHR). Both EGCG and enalapril therapy significantly lowered systolic blood pressure (SBP) in SHR. EGCG therapy of SHR significantly reduced infarct size and improved cardiac function in Langendorff-perfused hearts exposed to ischemia-reperfusion (I/R) injury. In SHR given L-NAME, beneficial effects of EGCG on SBP and I/R were not observed. Both enalapril and EGCG treatment of SHR improved insulin sensitivity and raised plasma adiponectin levels. We conclude that acute actions of EGCG to stimulate production of nitric oxide from endothelium using PI 3-kinase-dependent pathways may explain, in part, beneficial effects of EGCG therapy to simultaneously improve metabolic and cardiovascular pathophysiology in SHR. These findings may be relevant to understanding potential benefits of green tea consumption in patients with the metabolic syndrome.

epigallocatechin gallate; insulin resistance; endothelial dysfunction; nitric oxide; metabolic syndrome; spontaneously hypertensive rats; ischemia-reperfusion

CARDIOVASCULAR COMPLICATIONS are a major cause of morbidity and mortality in patients with diabetes, obesity, and the metabolic syndrome (8, 16). Under healthy conditions, vascular

actions of insulin to stimulate production of nitric oxide (NO) from endothelium lead to vasodilation and increased blood flow that enhance glucose uptake in skeletal muscle (5). However, in diabetes, obesity, and the metabolic syndrome, reciprocal relationships between insulin resistance and endothelial dysfunction may increase susceptibility of patients to cardiovascular complications, including hypertension, accelerated atherosclerosis, and coronary heart disease (27). Thus therapeutic interventions aimed at improving insulin resistance and metabolic homeostasis may simultaneously improve endothelial dysfunction and associated cardiovascular abnormalities. At the same time, treatments designed to improve endothelial dysfunction may simultaneously increase insulin sensitivity, leading to beneficial effects in metabolic diseases. Indeed, treatment of diabetic, hypertensive patients with insulin sensitizers (thiazolidinediones) decreases both insulin resistance and blood pressure (46), whereas treatment of hypertensive or hypercholesterolemic patients with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) improves endothelial dysfunction and lowers blood pressure while simultaneously increasing insulin sensitivity and decreasing the incidence of diabetes (29–32, 58).

Epigallocatechin gallate (EGCG) is a polyphenol that makes up ~30% of the solids in green tea (57). In some epidemiological studies, green tea consumption is associated with reduced risk for cardiovascular disease (18, 43). However, the mechanisms underlying this reduction in risk are unknown. EGCG inhibits human platelet aggregation *in vitro* (23). In addition, acute treatment with EGCG reduces STAT1 phosphorylation and apoptosis in response to hypoxia in cultured cardiac myocytes and improves cardiac function in isolated rat hearts subjected to ischemia-reperfusion (I/R) injury (52). In vascular endothelial cells, EGCG acutely activates endothelial nitric oxide synthase (eNOS) and increases production of NO via a phosphatidylinositol (PI) 3-kinase/Akt-dependent pathway (37). EGCG also mimics metabolic actions of insulin to decrease hepatic glucose production (54) and increase fatty acid oxidation in skeletal muscle (42). Thus beneficial effects of green tea consumption to reduce risk for cardiovascular disease may depend, in part, on the ability of EGCG to mimic or augment both metabolic and vasodilator actions of insulin, leading to simultaneous improvement in both insulin sensitiv-

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ity and endothelial function. Spontaneously hypertensive rats (SHR) are a genetic model of hypertension with features of the human metabolic syndrome, including fasting hyperinsulinemia, insulin resistance, and overweight (47). In the present study, we evaluated acute vasodilator actions of EGCG in mesenteric vascular beds (MVB) isolated from SHR *ex vivo* as well as chronic effects of daily oral treatment with EGCG for 3 wk to simultaneously improve cardiovascular and metabolic parameters in SHR. Our results provide novel insight into mechanisms of action for green tea consumption as a potential adjunct to conventional therapies for the metabolic syndrome.

METHODS

Animal experiments. Experimental protocols were reviewed and approved by the Department Internal Committee (Article 7 of Law Decree 116/92). All procedures in animals were performed in accordance with Guidelines and Authorization for the Use of Laboratory Animals (Italian Government, Ministry of Health). Male SHR (SHR/NHsd, haplotype RT1^b) and age-matched normotensive Wistar-Kyoto (WKY) control rats were obtained from Harlan Italy (Milan) and used in all studies. Animals (8 wk old) were housed, handled, and trained for 1 wk to minimize stress associated with blood pressure measurements. SHR (9 wk old) were randomized into three groups and treated daily for 3 wk by gavage with vehicle alone, enalapril (30 mg·kg⁻¹·day⁻¹; see Ref. 17), or EGCG (200 mg·kg⁻¹·day⁻¹; see Ref. 11). WKY controls (9 wk old) were given vehicle alone for 3 wk. In a separate set of experiments, 9-wk-old SHR were administered *N*^ω-nitro-L-arginine methyl ester (L-NAME, 80 mg/l) in drinking water (6) and treated daily for 3 wk by gavage with vehicle alone or EGCG (200 mg·kg⁻¹·day⁻¹). Doses of drugs were chosen based on previous studies in the published literature.

In all groups of animals, systolic blood pressure (SBP) was measured noninvasively using a tail cuff (Leticia 5100; PanLab, Barcelona, Spain) according to standard procedures described previously (7). SBP values reported are the average of three sequential blood pressure measurements that were within 10 mmHg of each other. During the course of treatment, SBP was monitored every 3 days, the last time 24 h before death. Body weight and food intake were measured daily. Blood samples were obtained by cardiac puncture from rats fasted overnight and killed with ether. Serum concentrations of insulin (Linco Research, St. Charles, MO) and adiponectin (B-Bridge, Sunnyvale, CA) were measured by ELISA. Plasma glucose concentrations were determined with a diagnostic glucometer (Accu-Chek Active; Roche Diagnostics). Insulin sensitivity was assessed using the quantitative insulin sensitivity check index (QUICKI = 1/[log(insulin) + log(glucose)]) (25). For QUICKI analysis, triplicate values of six independent measures for fasting glucose and fasting insulin values were obtained from each group of animals.

Drugs. Drugs were obtained from the indicated sources: insulin from Novo Nordisk; norepinephrine (NE), ACh, enalapril, EGCG, wortmannin, and L-NAME from Sigma-Aldrich. Stock solutions of NE (100 mM), ACh (10 mM), and L-NAME (100 mM) were prepared with distilled water. Stock solution of wortmannin (100 μM) was prepared in DMSO. Final dilutions of these drugs were prepared in modified Krebs-Henseleit solution immediately before use. Stock solutions of enalapril in methanol (5%) and EGCG in DMSO (10%) were prepared. Final dilutions of these drugs were prepared in drinking water immediately before intragastric administration (~4× dilution). For *in vivo* treatment, L-NAME (80 mg/l) was diluted in drinking water (6). Vehicle-treated WKY and SHR received the same amount of methanol or DMSO as drug-treated animals.

Evaluation of vascular function *ex vivo*. MVB were isolated and removed from rats after 3-wk vehicle or drug therapy as described (47). Briefly, MVB mounted in a temperature-controlled moist chamber (type 834/1; Hugo Sachs Elektronik, March-Hungstetten, Ger-

many) were perfused with modified Krebs-Henseleit solution continuously gassed with a mixture of 95% O₂ and 5% CO₂ (pH 7.4). A constant flow rate of 5 ml/min through the MVB was maintained using a peristaltic pump (ISM 833; Hugo Sachs Elektronik). Drug solutions were infused in the perfusate proximal to the arterial cannula using another peristaltic pump. After an equilibration period (30–40 min), changes in perfusion pressure (PP) were measured with a Pressure Transducer System (SP 844; Capto, Horten, Norway) and recorded continuously using data acquisition and analysis equipment (PowerLab System; ADInstruments, Castle Hill, Australia).

Vasodilator responses in MVB. A steady-state PP of ~120 mmHg was obtained 30–40 min after initial administration of NE and was maintained by continuous NE infusion (10 and 3 μM in WKY and SHR, respectively). Dose-response curves measuring vasodilation (decrease in PP) in response to insulin or EGCG were obtained by adding increasing concentrations of insulin (0.1 nM, 3 μM/4 min perfusion) or EGCG (1–100 μM/4 min perfusion) to the perfusate. For all vasodilation experiments, data from each curve were normalized to PP obtained in WKY rats treated with a maximally stimulating dose of ACh (1 μM, 100% representing initial steady-state PP and 0% representing maximal reduction in response to ACh). In some experiments, EGCG-induced relaxation was measured before and after 20-min treatment with eNOS inhibitor L-NAME (100 μM) or PI 3-kinase inhibitor wortmannin (100 nM).

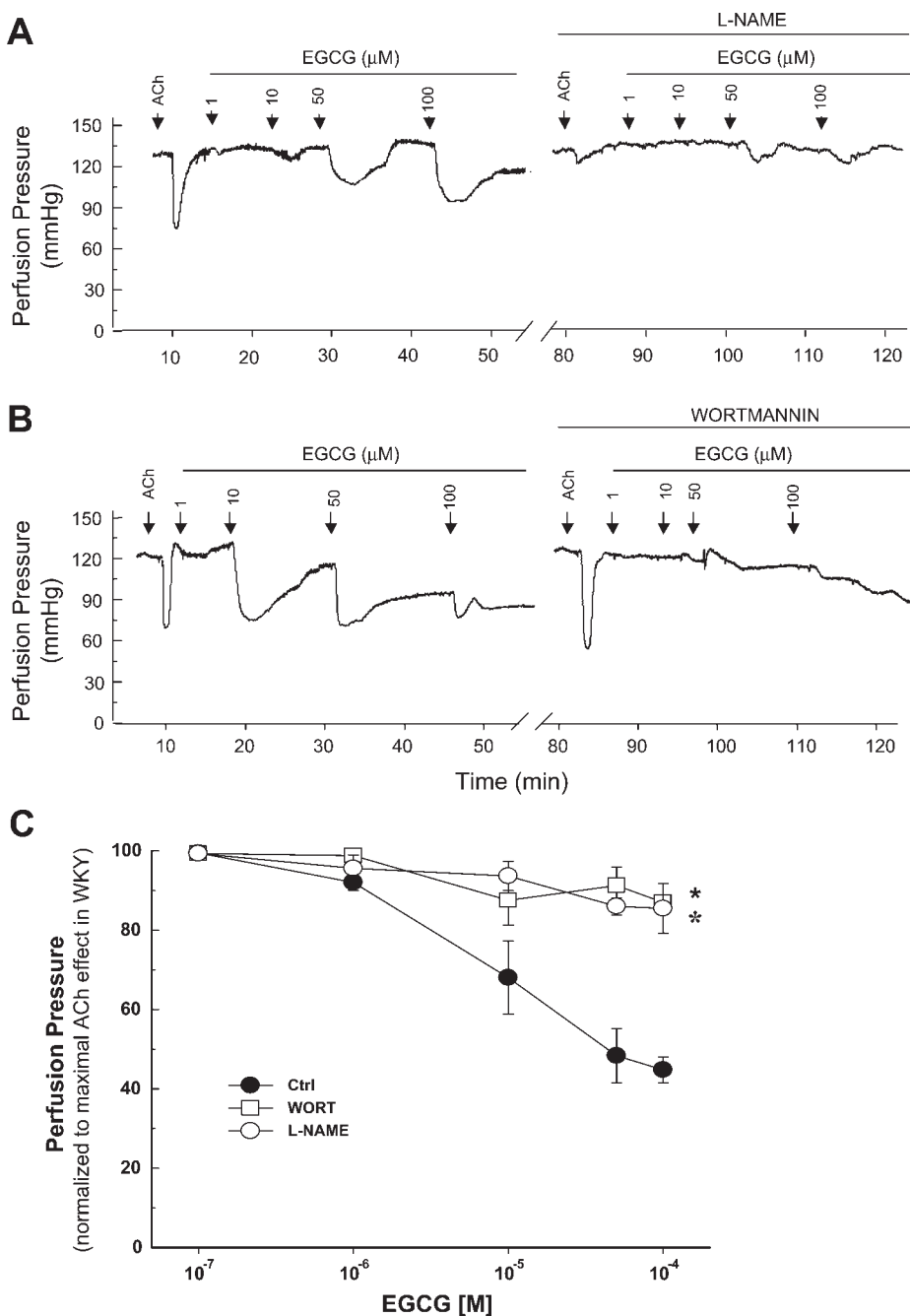
Evaluation of myocardial function in isolated hearts. Hearts from SHR treated for 3 wk with vehicle or EGCG were isolated and perfused according to the Langendorff technique as previously described (49). Briefly, excised hearts were placed in ice-cold buffer during aortic cannulation, and then hearts were perfused with modified Krebs-Henseleit solution continuously gassed with a mixture of 95% O₂ and 5% CO₂ (pH 7.4) at 37°C. PP was kept constant at 80 mmHg. Isovolumetric recordings of left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressures were obtained from a balloon catheter inserted in the left ventricle through the left atrium (LVEDP set to 5–10 mmHg at the beginning of the stabilization period). Coronary flow was measured by timed collections of the coronary effluent. Left ventricular developed pressure (LVDP) was calculated as LVDP = LVSP – LVEDP. After 20-min stabilization, inflow tubing to the hearts was clamped for 30 min to obtain global ischemia. Hearts were then reperfused for 120 min. Only hearts with LVSP between 60 and 160 mmHg, LVEDP 5 and 10 mmHg, and coronary flow 8 and 16 ml/min were studied under this protocol. LVSP, LVEDP, LVDP, and coronary flow were recorded two times before ischemia and after 5, 15, 30, 45, and 60 min of reperfusion. To measure infarct size after 120-min reperfusion, each heart was sliced in four sections (~2 mm thick), incubated in freshly prepared 1% 2,3,5-triphenyltetrazolium chloride (TTC) for 30 min at 37°C, and then stored in 10% formaldehyde for 48 h. Infarct size was calculated as the average of values measured on all four slices for each heart by standard planometric analysis (ImageJ 1.32j software; National Institutes of Health). The volume of infarcted tissue (TTC negative) was compared with the total volume of the left ventricle.

Statistical analysis. Results were expressed as means ± SE of *n* experiments (*n* = no. of rats). Student's *t*-tests (paired or unpaired) and two-way ANOVA for repeated measures with Bonferroni post hoc correction test were used as appropriate. Values of *P* < 0.05 were considered to indicate statistical significance.

RESULTS

Acute vasodilator actions of EGCG. To directly evaluate acute vasodilator actions of EGCG, we examined EGCG-mediated vasorelaxation in perfused MVB isolated *ex vivo* from 12-wk-old SHR (Fig. 1). Vessels were first precontracted with NE (3 μM) to a PP of ~120 mmHg and then exposed to a supramaximal dose of the vasodilator ACh to verify endo-

Fig. 1. Epigallocatechin gallate (EGCG) stimulates acute nitric oxide (NO)-dependent vasorelaxation mediated by phosphatidylinositol (PI) 3-kinase in mesenteric vascular beds (MVB) isolated from spontaneously hypertensive rats (SHR). MVB were isolated ex vivo from 12-wk-old SHR and perfused with norepinephrine (NE, 3 μ M) to maintain steady-state perfusion pressure of 120 mmHg. Representative tracings are shown from experiments that were repeated independently at least 3 times. *A*: tracings of vasodilator responses to ACh (1 μ M) and EGCG (1–100 μ M) in the absence (*left*) and presence (*right*) of pretreatment with *N*^o-nitro-L-arginine methyl ester [L-NAME; nitric oxide synthase (NOS) inhibitor, 100 μ M/20 min]. Arrows indicate the beginning of 30-s (ACh) and 4-min (EGCG) drug perfusions, respectively. *B*: tracings of vasodilator responses to ACh and EGCG in the absence (*left*) and presence (*right*) of pretreatment with wortmannin (PI 3-kinase inhibitor, 100 nM/20 min). *C*: dose-response curves for EGCG-induced relaxation were obtained from MVB of SHR under basal conditions (●) and after pretreatment with L-NAME (○) or wortmannin (□). Results are means \pm SE of 8 [control (Ctrl)], 4 (L-NAME), and 3 (wortmannin) independent experiments. Data from each curve were normalized by defining 100% as the initial steady-state perfusion pressure and 0% as the maximal reduction in perfusion pressure obtained in Wistar-Kyoto (WKY) rats treated with a maximally stimulating dose of ACh. **P* < 0.001 vs. Ctrl. Statistical comparisons between dose-response curves were performed using 2-way ANOVA for repeated measures.



thelial integrity. Subsequently, MVB were perfused with increasing concentrations of EGCG (1–100 μ M), resulting in a rapid, reversible, and dose-dependent reduction of PP (Fig. 1, *left*). In some preparations, L-NAME (NO synthase inhibitor, 100 μ M/20 min) was then added to the perfusate followed by restimulation with ACh and EGCG. Under these conditions, no significant vasorelaxation was observed in response to either ACh or EGCG (Fig. 1A, *right*). In other preparations, after initial treatment with ACh and EGCG, wortmannin (PI 3-kinase inhibitor 100 nM/20 min) was added to the perfusate followed by restimulation with ACh and EGCG. Under these conditions, normal vasorelaxation in response to ACh was observed while the vasodilator response to EGCG was abrogated (Fig. 1B, *right*). Taken together, these results suggest that EGCG, like

insulin, acutely stimulates endothelial production of NO that is dependent on activation of PI 3-kinase signaling pathways and which results in vasodilation.

Effects of chronic EGCG treatment on endothelial dysfunction in SHR. We previously demonstrated that MVB from SHR have endothelial dysfunction with respect to vasodilator actions of insulin (47). Because EGCG has acute vasodilator actions that mimic those of insulin (Fig. 1), we next evaluated whether chronic EGCG treatment may improve endothelial dysfunction in SHR. SHR (9 wk old) were treated daily for 3 wk with vehicle, EGCG (200 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$), or the ACE inhibitor enalapril (30 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$). Vasodilator responses to increasing concentrations of insulin were then evaluated in MVB isolated ex vivo from SHR or vehicle-treated control

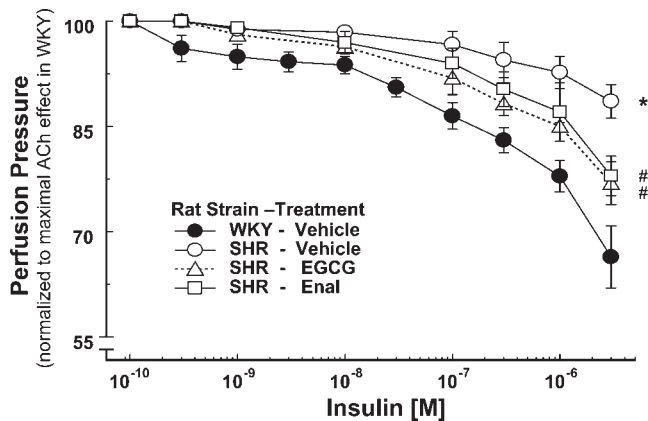


Fig. 2. Therapy (3 wk) of SHR with EGCG or enalapril improves vasodilator actions of insulin in MVB. After daily therapy of SHR with vehicle, EGCG ($200 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), or enalapril (Enal, $30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for 3 wk, insulin-stimulated vasorelaxation in MVB was examined ex vivo (means \pm SE of at least 5 independent experiments for each group). Vasodilator actions of insulin were significantly impaired in vehicle-treated SHR (vs. WKY, $P < 0.000001$). Treatment of SHR for 3 wk with either enalapril or EGCG enhanced the vasodilator response to insulin in MVB (vs. vehicle-treated SHR, $P < 0.001$). Statistical comparisons between dose-response curves were performed using 2-way ANOVA for repeated measures.

WKY rats (Fig. 2). Consistent with our previous study (47, 48), dose-dependent vasorelaxation in response to insulin was significantly impaired in MVB from vehicle-treated SHR when compared with MVB from vehicle-treated WKY rats (Fig. 2). Interestingly, chronic treatment of SHR with either EGCG or enalapril had similar effects to significantly enhance the ability of insulin to acutely mediate vasorelaxation in MVB when compared with MVB from vehicle-treated SHR. However, MVB from EGCG- or enalapril-treated SHR were not as responsive to insulin as MVB from vehicle-treated WKY rats. These results evaluating vasodilator actions of insulin in MVB suggest that chronic therapy with EGCG significantly improves endothelial function in SHR.

In preliminary studies, we did not observe significant differences in phosphorylation status or total content of eNOS when aortas from untreated and EGCG-treated SHR were compared (data not shown). However, these preliminary results may be limited by technical issues related to insufficient number of samples or sensitivity of the assay.

Effects of chronic EGCG treatment on SBP in SHR. Because EGCG has acute vasodilator actions (Fig. 1), and chronic treatment with EGCG improves endothelial function in SHR (Fig. 2), we next evaluated whether 3-wk treatment with EGCG reduces SBP (SBP) in SHR (Fig. 3). As expected, vehicle-treated SHR were hypertensive when compared with age-matched vehicle-treated WKY control rats, and 3-wk treatment with the ACE inhibitor enalapril significantly reduced SBP in SHR when compared with vehicle-treated SHR. A significant reduction in SBP was also observed in SHR after 3-wk treatment with EGCG (although the magnitude of this reduction was not quite as large as with enalapril treatment). Thus EGCG therapy is nearly as effective as enalapril at reducing blood pressure in SHR.

Consistent with the importance of NO in regulating basal blood pressure, SHR receiving L-NAME in drinking water for 3 wk had significantly higher SBP when compared with vehi-

cle-treated SHR drinking regular water (Fig. 4; $P < 0.001$). By contrast with the reduction in SBP observed in EGCG-treated SHR (Fig. 4), treatment with EGCG was unable to reduce the even more elevated SBP observed in SHR given L-NAME in their drinking water (Fig. 4). These findings suggest that the acute actions of EGCG to stimulate and augment production of NO in vascular endothelium, leading to increased vasodilation, are relevant to chronic effects of EGCG therapy to lower blood pressure using an NO-dependent mechanism.

Effects of chronic EGCG treatment on myocardial I/R injury in SHR. Endothelial dysfunction and hypertension both contribute importantly to impaired cardiac function as well as to increased susceptibility to myocardial I/R injury (9). Because EGCG has acute vasodilator actions (Fig. 1) and chronic treatment of SHR with EGCG improves endothelial function and lowers blood pressure (Figs. 2–4), we investigated whether 3-wk therapy with EGCG would also reduce myocardial I/R injury in SHR. Hearts isolated and perfused according to the method of Langendorff were obtained from SHR treated for 3 wk with vehicle or EGCG and then subjected to I/R injury. Isolated hearts from EGCG- and vehicle-treated SHR had similar hemodynamic parameters during aerobic perfusion (Fig. 5, B–D). However, when hearts were exposed to 30 min of ischemia followed by 120-min reperfusion, the myocardial infarct size was significantly reduced by $\sim 30\%$ in hearts from EGCG-treated SHR when compared with hearts from vehicle-treated SHR (Fig. 5A). Consistent with this reduction in infarct size, postischemic recovery of ventricular function was significantly improved in hearts from EGCG-treated SHR when compared with hearts from vehicle-treated SHR (as assessed by coronary flow, LVEDP, and LVDP; Fig. 5, B–D). In addition, heart rate and heart weight were comparable from all groups of animals (data not shown). In hearts from SHR given L-NAME in drinking water, despite a decrease in coronary blood flow, the myocardial infarct size was not significantly different between SHR treated with vehicle or EGCG (Fig. 5A). It is possible that this may be explained by a chronic adaptation in heart perfusion during the 3-wk treatment with L-NAME. When compared with hearts from SHR drinking

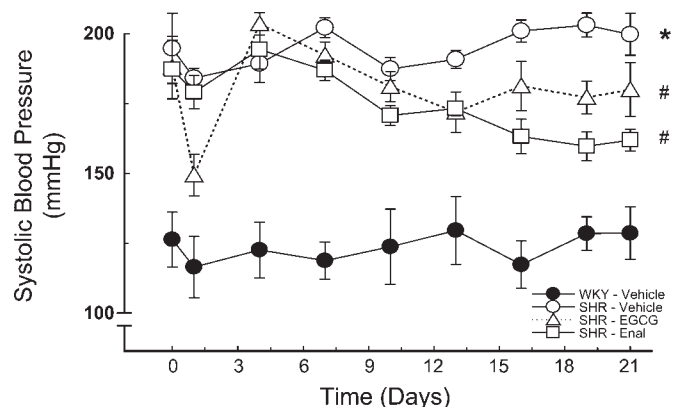


Fig. 3. Treatment (3 wk) with enalapril or EGCG reduces systolic blood pressure (SBP) in SHR. SBP was measured 3 times/wk by tail cuff during daily drug therapy for 3 wk. When compared with vehicle-treated WKY, SBP was significantly higher in vehicle-treated SHR ($P < 0.0001$). Treatment of SHR with either enalapril ($30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or EGCG ($200 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) significantly reduced SBP (vs. vehicle treatment, $P < 0.001$ and 0.03 , respectively). Values shown are means \pm SE of 9 independent experiments for each group of animals.

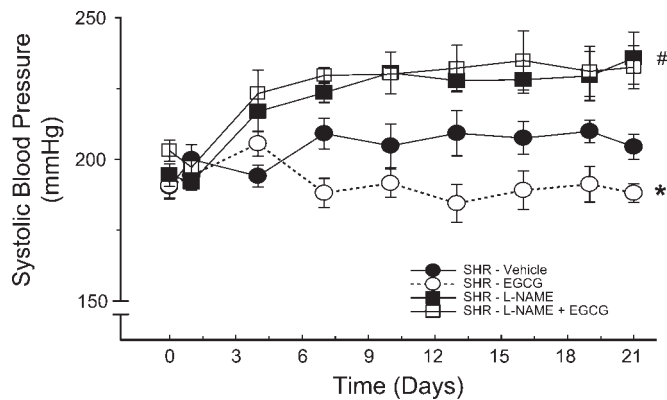


Fig. 4. Increased SBP in SHR treated with L-NAME cannot be improved by concurrent treatment with EGCG. Treatment of SHR with L-NAME significantly increased SBP, and this was not significantly altered by concurrent therapy with EGCG ($200 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$; \square vs. \blacksquare ; $P > 0.9$). Values shown are means \pm SE of 4 independent experiments for each group of animals.

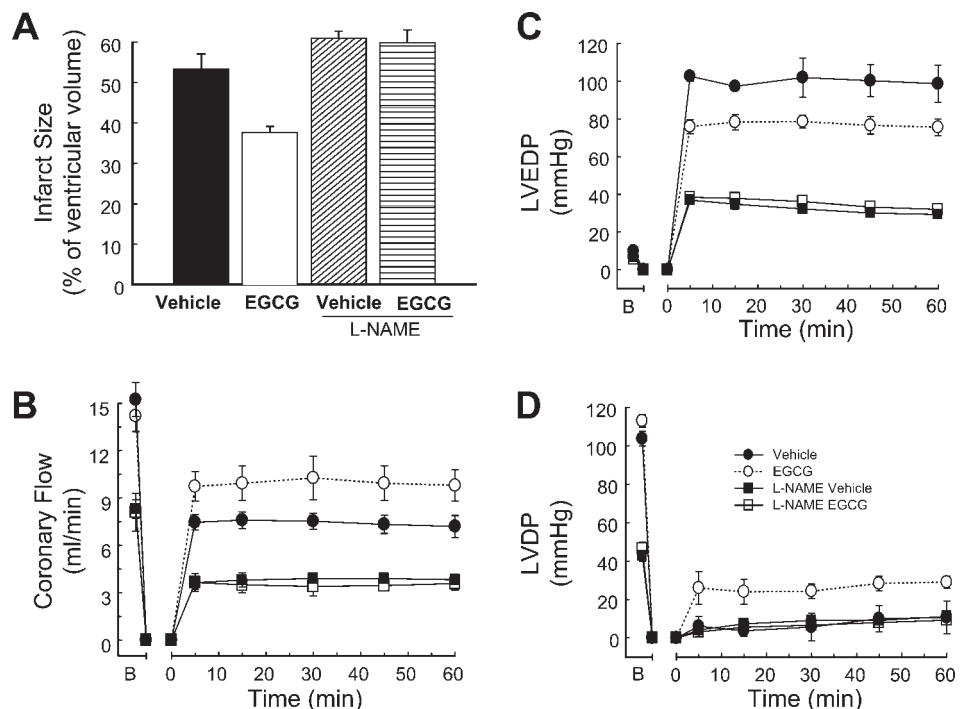
regular water, hearts from SHR with L-NAME in their drinking water exhibited impaired myocardial function in the preischemic period and an impaired ability to recover after 30 min of global ischemia (Fig. 5, B–D). No additional significant differences between EGCG and vehicle treatment were observed in SHR given L-NAME in drinking water with respect to coronary flow, LVDP, or LVDP after I/R injury (Fig. 5, B–D). Thus chronic treatment of SHR with EGCG offers significant protection against myocardial I/R injury that depends on production of NO and that may help to explain the reduced risk of cardiovascular disease associated with consumption of green tea.

Effects of chronic EGCG treatment on insulin resistance and other metabolic parameters in SHR. SHR are typically smaller with a lower body weight when compared with age-matched WKY control rats. However, consistent with results obtained in our previous studies (38, 47), our SHR had significantly higher

body weight than age-matched WKY rats at the age that we studied them. This is probably related to the specific haplotype (SHR/NHsd, haplotype RT1^k) of SHR we investigated. In addition, SHR are not only genetically hypertensive with endothelial dysfunction but are also insulin resistant (47). Because of the reciprocal relationships between endothelial dysfunction and insulin resistance, antihypertensive therapies such as ACE inhibitors and ARBs that improve endothelial dysfunction often also improve insulin resistance (29, 30, 44, 58). Because EGCG therapy improves both endothelial dysfunction and blood pressure in SHR, and EGCG mimics vasodilator actions of insulin, we evaluated the ability of 3-wk EGCG treatment to improve insulin resistance and associated metabolic parameters in SHR. After 3-wk treatment of SHR with vehicle, EGCG, or enalapril, fasting blood samples were obtained for measurement of glucose, insulin, and adiponectin. All groups of animals were normoglycemic (data not shown). In agreement with our previous findings (47, 48), fasting insulin levels were significantly higher in vehicle-treated SHR when compared with vehicle-treated WKY rats (data not shown). Consistent with fasting glucose and insulin values, vehicle-treated SHR were insulin resistant when compared with vehicle-treated WKY (as assessed by the surrogate index QUICKI; Fig. 6A). Therapy (3 wk) of SHR with either enalapril or EGCG resulted in significant improvement in insulin sensitivity when compared with vehicle-treated SHR. Plasma adiponectin levels generally mirrored the findings for insulin sensitivity (Fig. 6B); that is, adiponectin levels in vehicle-treated SHR were significantly lower than in vehicle-treated WKY, and 3-wk therapy of SHR with either enalapril or EGCG significantly increased adiponectin levels when compared with vehicle-treated SHR.

When food intake was evaluated in SHR treated with vehicle, EGCG, or enalapril, we did not observe any differences among the groups for the first 2 wk. However, after the 3rd

Fig. 5. Treatment (3 wk) with EGCG attenuates myocardial ischemia-reperfusion (I/R) injury in SHR only in the absence of concurrent L-NAME treatment. Langendorff-perfused hearts from SHR treated for 3 wk with either vehicle alone or EGCG ($200 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) were made ischemic for 30 min followed by reperfusion for 120 min as described in METHODS. A: infarct volume after I/R assessed by 2,3,5-triphenyltetrazolium chloride (TTC) staining was substantially reduced in hearts isolated from SHR treated with EGCG only in the absence of concurrent L-NAME therapy (vs. vehicle-treated SHR, $P < 0.02$). Coronary flow (B), left ventricular end diastolic pressure (LVDP; C), and left ventricular developed pressure (LVDP; D) were recorded two times before ischemia and after 5, 15, 30, 45, and 60 min of reperfusion. In hearts from SHR treated with EGCG, all 3 of these parameters were significantly improved when compared with hearts from vehicle-treated SHR only in the absence of concurrent L-NAME therapy ($P < 0.05$). Values shown are means \pm SE of 3 independent experiments for each group of animals.



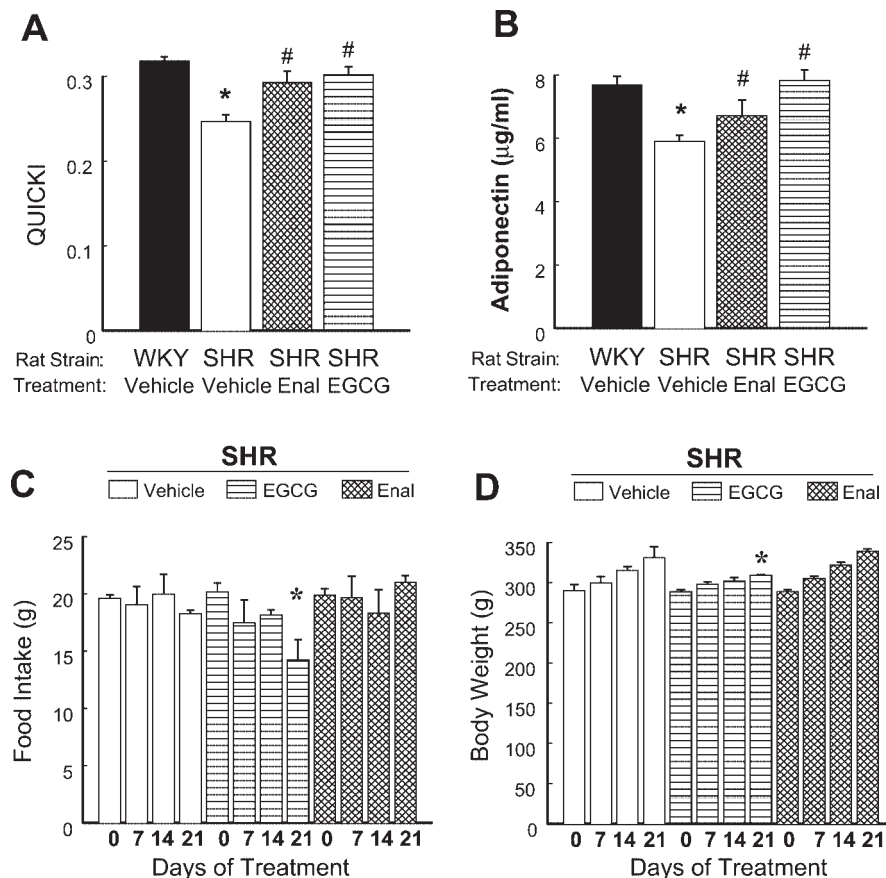


Fig. 6. Treatment of SHR with EGCG increases adiponectin levels and insulin sensitivity. After 3-wk treatment of SHR with vehicle, EGCG (200 mg·kg⁻¹·day⁻¹), or enalapril (30 mg·kg⁻¹·day⁻¹), fasting blood samples were obtained for measurement of glucose, insulin, and adiponectin (means ± SE of 6 independent experiments for each group). Daily food intake and body weight were recorded weekly. *A*: insulin sensitivity in vehicle-treated SHR (assessed by QUICKI) was lower than in WKY ($P < 0.001$). After 3-wk treatment of SHR with enalapril or EGCG, insulin sensitivity increased (vs. vehicle-treated SHR, $P < 0.01$). *B*: plasma adiponectin levels in vehicle-treated SHR were lower than in WKY ($P < 0.001$). After 3-wk treatment of SHR with enalapril or EGCG, adiponectin levels rose substantially (vs. vehicle-treated SHR, $P < 0.05$ and 0.001 , respectively). Daily food intake (*C*) and body weight (*D*) were reduced after 3-wk treatment of SHR with EGCG (vs. vehicle- or enalapril-treated SHR, $P < 0.03$).

week of treatment, SHR treated with EGCG had a moderate decrease in food intake when compared with SHR treated with vehicle or enalapril (Fig. 6C). Moreover, the rate at which SHR gained weight over the 3-wk course of therapy was similar between vehicle- and enalapril-treated animals but slightly slower in EGCG-treated SHR (Fig. 6D). Microscopic examination of gastric mucosa from SHR did not reveal any gross abnormalities in any of the treatment groups (data not shown).

DISCUSSION

A number of so-called functional foods such as green tea, black tea, dark chocolate, and red wine contain bioactive polyphenols, including EGCG, epicatechin gallate, epicatechin, and resveratrol, that may have beneficial effects on cardiovascular health (12, 13, 53). Large epidemiological studies linking consumption of these functional foods with improved cardiovascular outcomes are intriguing (18, 19, 43). Drinking 5–10 cups of green tea daily is sufficient to raise circulating EGCG levels in the micromolar range in humans (57). Although tea polyphenols, including EGCG, are often touted as antioxidants (19), their mechanisms of action with respect to cardiovascular and metabolic physiology are poorly understood. EGCG may actually be a pro-oxidant in intact cells (14), suggesting that beneficial actions of EGCG may involve properties unrelated to its antioxidant capabilities in vitro. Consistent with this view, beneficial effects of black tea consumption on endothelial function may be unrelated to systemic antioxidant effects of tea catechins (55). EGCG acutely activates signaling pathways in liver cells to mimic metabolic

actions of insulin to inhibit gluconeogenesis (54). We previously demonstrated that signaling pathways mediating metabolic actions of insulin are shared in common with signaling pathways mediating vasodilator actions of insulin (39, 40, 59, 60). Therefore, we reasoned that EGCG may have both acute and chronic actions in the vasculature that mimic and/or augment vascular actions of insulin linking hemodynamic and metabolic physiology. If so, these properties of EGCG may be beneficial for simultaneously ameliorating cardiovascular and metabolic pathophysiology in the metabolic syndrome.

Acute and chronic cardiovascular actions of EGCG. To evaluate vascular actions of EGCG, we first assessed endothelial function in MVB isolated ex vivo from 12-wk-old SHR. The behavior of MVB, a minor determinant of SBP with respect to other vascular districts, may nevertheless be a useful proxy for the function of vascular beds that contribute more directly to systemic blood pressure. Moreover, given the large surface area and synthetic capacity of microvascular endothelium, small resistance vessels represent important determinants of plasma levels of endothelium-derived mediators and peripheral vascular resistance. Thus changes in endothelial function in MVB may parallel, or even precede, changes in vascular reactivity, elasticity, and volume of large conductance arteries (41). When compared with arteries isolated from normotensive WKY rats, arteries from SHR are more sensitive and responsive to vasoconstrictor effects of NE (47). Therefore, to achieve a comparable baseline PP of ~120 mmHg, MVB preparations isolated from SHR and WKY were preconstricted with equieffective concentrations of NE. One advantage of this

approach (i.e., normalizing vasorelaxation to results obtained in control animals) is that we can directly compare the differences between pathological and physiological conditions. In the present study, EGCG infusion in MVB acutely induced dose-dependent vasorelaxation mediated by NO that was inhibited by wortmannin. Although wortmannin is not an absolutely specific inhibitor for PI 3-kinase, its ability to completely abrogate vasodilation induced by EGCG (although vasodilator actions of ACh are unaffected) strongly supports the hypothesis that vasodilator effects of EGCG are mediated via a PI 3-kinase-dependent mechanism. Moreover, our results are in agreement with previous reports demonstrating the ability of tea catechins to evoke NO-dependent vasorelaxation by a mechanism involving PI 3-kinase-mediated phosphorylation of Akt and eNOS in vitro (3, 4, 20, 37). Thus EGCG mimics vasodilator actions of insulin in MVB from SHR (47).

We recently reported that MVB from 12-wk-old SHR have impaired insulin-dependent vasodilator responses but intact vasodilator responses to ACh that may contribute to coupling metabolic and hemodynamic pathophysiology (47). Interestingly, the magnitude of vasodilation induced by EGCG in MVB from SHR was comparable to that observed in age-matched control WKY rats (26). This raises the possibility that chronic therapy with EGCG may have beneficial vascular actions to circumvent endothelial insulin resistance and improve endothelial function as well as metabolic and cardiovascular pathophysiology in SHR.

After SHR were given daily oral EGCG therapy for 3 wk, MVB isolated from EGCG-treated SHR had significantly and substantially improved vasodilator responses to insulin when compared with MVB from vehicle-treated SHR. Remarkably, the magnitude of this improvement was comparable to that seen in MVB from SHR treated with the conventional antihypertensive ACE inhibitor enalapril. Consistent with its ability to improve insulin-dependent endothelial function, chronic oral EGCG treatment effectively lowered SBP in SHR nearly as effectively as enalapril. The time course of the progressive lowering in SBP in response to EGCG therapy resembled that typically observed in SHR treated with classical antihypertensive agents (50). Although we did not directly measure plasma levels of EGCG in our study, a bioavailability of $\sim 1 \mu\text{M}$ should result from the dose of EGCG we administered (11). This concentration was able to induce only a weak relaxation in our acute studies on isolated arteries. Nevertheless, the effects we observed in SHR chronically treated with EGCG may be because of small, yet continuous, stimulation of vessels with submaximal doses of EGCG.

We did not exhaustively explore the effects of EGCG in normotensive rats. However, direct administration of EGCG on MVB isolated from WKY rats resulted in a rapid, dose-dependent vasodilation that did not differ significantly from that obtained in MVB from SHR (15). Our results are consistent with modest vasodilator actions of acute intravenous infusion of green tea extract in normotensive rats (36) and a slight decrease in blood pressure in stroke-prone SHR after a 3-wk period of ingesting tea polyphenols (45). Thus our results with purified EGCG suggest that it is a major bioactive component in green tea that may have substantial benefits as a potential therapy for endothelial dysfunction and hypertension associated with insulin resistance.

Interestingly, the effect of chronic EGCG therapy to reduce SBP in SHR was abolished when the rats were concurrently given a drug to inhibit NO synthesis. Given the ability of L-NAME to increase blood pressure per se, a lack of effect for EGCG in SHR treated with L-NAME does not necessarily indicate an involvement of the NO pathway in EGCG-mediated reduction of blood pressure. However, NO appears to be necessary and sufficient to mediate vasorelaxation in response to EGCG in MVB ex vivo. Although we cannot rule out the possibility that EGCG is also acting via additional mechanisms to reduce blood pressure in SHR (e.g., by modulating salt and water homeostasis), it seems reasonable to infer that the acute vascular actions of EGCG to stimulate production of NO and vasodilation may be responsible, at least in part, for the chronic actions of EGCG therapy to lower blood pressure by an NO-dependent mechanism.

Measurement of SBP by the tail-cuff method has some limitations when compared with direct intra-arterial measurements. These include issues related to the stress induced by the procedure and accuracy of tail-cuff determinations of SBP (33). However, because blood pressure measurements in our study detected substantial differences in SBP between experimental and control groups over 3 wk in a large number of animals, we believe that the method we used is satisfactory.

Endothelial dysfunction and hypertension are important contributors to ischemic heart disease and heart failure (34). Green tea consumption has been linked to a reduced risk of myocardial infarction (18). Therefore, in light of our findings that 3-wk therapy with EGCG is effective at improving endothelial function and lowering blood pressure in SHR, we examined the ability of chronic oral EGCG therapy to protect against myocardial I/R injury. In hearts isolated from EGCG-treated SHR that were subjected to I/R injury, the resultant infarct size was substantially reduced by $\sim 30\%$ when compared with hearts isolated from vehicle-treated SHR. Moreover, chronic treatment of SHR with EGCG resulted in hearts that were able to recover coronary flow and cardiac function to a greater extent after I/R injury than hearts from vehicle-treated SHR. Thus beneficial effects of chronic EGCG treatment to improve endothelial function and lower blood pressure also extend to protection against myocardial I/R injury. SHR receiving L-NAME in drinking water for 3 wk had significantly lower coronary blood flow in the preischemic period. Interestingly, this reduction in cardiac perfusion did not further increase the infarct size in SHR exposed to L-NAME. This may be because of a chronic adaptation in heart perfusion during the 3 wk of treatment with L-NAME. However, as with our blood pressure results, beneficial effects of EGCG therapy to protect against I/R injury in SHR were abrogated by treating rats concurrently with L-NAME. This provides further evidence that the beneficial cardiovascular effects of chronic EGCG therapy are NO dependent and likely related to acute actions of EGCG to stimulate production of NO. In normotensive rats, acute intravenous administration of EGCG attenuates myocardial injury after I/R injury by inhibiting the inhibitory factor KK/nuclear factor- κ B/activator protein-1 pathway (2). Moreover, in cell culture, EGCG inhibits STAT1-mediated apoptosis in cardiac myocytes subjected to simulated I/R (52). Finally, insulin-stimulated production of NO from myocardial endothelium may protect against apoptosis induced by I/R injury (15). Thus it is possible that protective cardiac effects of chronic EGCG

therapy we observed in the present study are mediated by both direct actions of EGCG on the myocardium as well as secondary benefits derived from improving vascular endothelial function and lowering blood pressure.

Chronic metabolic actions of EGCG. Insulin resistance in the vascular endothelium may contribute to coupling cardiovascular and metabolic pathophysiology in the metabolic syndrome (27). Importantly, 3 wk of daily oral EGCG treatment of SHR significantly increased insulin sensitivity, lowered fasting plasma insulin levels, and increased fasting plasma adiponectin levels to an extent that was comparable to or greater than that observed with enalapril therapy. Because of reciprocal relationships between insulin resistance and endothelial dysfunction (29, 30, 44, 58), therapeutic interventions aimed at improving endothelial dysfunction may simultaneously increase insulin sensitivity, leading to beneficial effects in metabolic diseases (29, 30, 44, 58). EGCG's ability to ameliorate endothelial function by increasing NO production (via PI 3-kinase) may be one important mechanism by which metabolic actions of insulin are also improved in EGCG-treated SHR. Thus, similar to conventional therapies with ACE inhibitors or ARBs, EGCG therapy has beneficial effects to simultaneously improve both hemodynamic and metabolic homeostasis in a model of the metabolic syndrome that may be due, in part, to improvements in endothelial function and vascular actions of insulin. Our results are consistent with studies demonstrating that 4-wk supplementation with green tea extracts improves lipid and glucose homeostasis and increases adiponectin levels in fructose-fed hamsters (35). Adiponectin, an adipokine secreted exclusively by adipose cells, has anti-inflammatory and antiatherogenic properties in addition to its metabolic and vascular actions that mimic those of insulin (10, 22). Thus effects of EGCG therapy to increase plasma levels of adiponectin may help explain, in part, the reductions we observed in insulin resistance and endothelial dysfunction. In addition, there may be direct effects of EGCG to improve metabolic function since green tea extracts augment insulin-stimulated glucose uptake in isolated rat adipose cells and enhance insulin sensitivity in Sprague-Dawley rats (1, 56).

Interestingly, in SHR treated with EGCG, there was a modest reduction in body weight after the 2nd and 3rd week of treatment when compared with vehicle- or enalapril-treated SHR. With respect to food intake, we observed only a slight decrease after the 3rd week of EGCG treatment when compared with vehicle or enalapril treatment. When we evaluated motor activity, we did not detect any significant increases in activity levels of EGCG-treated animals when compared with vehicle-treated SHR during the 3-wk treatment period. However, decreased weight gain in EGCG-treated animals was coincident with reduced food intake, which may partially account for a decreased growth rate. Taken together, these results suggest that EGCG may have direct or indirect pleiotropic effects to lower body weight that may be beneficial in the context of overweight. Indeed, reduced body weight observed in lean and obese animals has been correlated with the ability of EGCG or green tea extracts to decrease food intake (24), decrease energy absorption and increase fat oxidation (28), inhibit lipolysis of triglycerides (21), and inhibit fatty acid synthase, an important regulator of feeding behavior (51). In our study, the time course of the progressive lowering in SBP in response to EGCG therapy suggests that BP decreases

before significant reduction in body weight occurs. Thus, although it is plausible that a reduction in body weight further improves hemodynamic homeostasis, it seems unlikely that the small reduction in body weight we observed would fully account for the improved insulin sensitivity, endothelial function, blood pressure, and protection against myocardial I/R injury that we documented in EGCG-treated SHR.

Acute NO-dependent vasodilator actions of EGCG that mimic those of insulin may help to explain beneficial effects of chronic oral EGCG therapy to improve endothelial dysfunction and insulin resistance, raise adiponectin levels, lower blood pressure, and protect against myocardial I/R injury in SHR, a genetic model of hypertension with features of the metabolic syndrome. These results may be relevant to understanding the beneficial metabolic and cardiovascular health benefits of green tea consumption in humans.

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