

PRE-CLINICAL RESEARCH

## Effects of (–)-Epicatechin on Myocardial Infarct Size and Left Ventricular Remodeling After Permanent Coronary Occlusion

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- Objectives** We examined the effects of the flavanol (–)-epicatechin on short- and long-term infarct size and left ventricular (LV) structure and function after permanent coronary occlusion (PCO) and the potential involvement of the protective protein kinase B (AKT)/extracellular signal-related kinase (ERK) signaling pathways.
- Background** (–)-epicatechin reduces blood pressure in hypertensive patients and limits infarct size in animal models of myocardial ischemia–reperfusion injury. However, nothing is known about its effects on infarction after PCO.
- Methods** (–)-epicatechin (1 mg/kg daily) treatment was administered via oral gavage to 250 g male rats for 10 days before PCO and was continued afterward. The PCO controls received water. Sham animals underwent thoracotomy and treatment in the absence of PCO. Immunoblots assessed AKT/ERK involvement 2 h after PCO. The LV morphometric features and function were measured 48 h and 3 weeks after PCO.
- Results** In the 48-h group, treatment reduced infarct size by 52%. There were no differences in hemodynamics among the different groups (heart rate and aortic and LV pressures). Western blots revealed no differences in AKT or ERK phosphorylation levels. At 3 weeks, PCO control animals demonstrated significant increases in LV end-diastolic pressure, heart and body weight, and LV chamber diameter versus sham. The PCO plus (–)-epicatechin group values were comparable with those of the sham plus (–)-epicatechin group. Treatment resulted in a 33% decrease in myocardial infarction size. The LV pressure-volume curves demonstrated a right shift in control PCO animals, whereas the (–)-epicatechin curves were comparable with those of the sham group. The LV scar area strains were significantly improved with (–)-epicatechin.
- Conclusions** These results demonstrate the unique capacity of (–)-epicatechin to confer cardioprotection in the setting of a severe form of myocardial ischemic injury. Protection is sustained over time and preserves LV structure and function. The cardioprotective mechanism(s) of (–)-epicatechin seem to be unrelated to AKT or ERK activation. (–)-epicatechin warrants further investigation as a cardioprotectant. (J Am Coll Cardiol 2010;55:2869–76)  
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Diet is one of the most important lifestyle factors that influence the incidence of cardiovascular disease (CVD). Many plants and natural products contain significant amounts of flavonoids (1). Evidence indicates a negative correlation between consumption of flavonoids and incidence of CVD (2–5). Cacao beans contain very high

amounts of flavonoids, in particular, the flavanol subtype (6). Main cacao flavanols are catechin and (–)-epicatechin, present in monomeric and multimeric forms (3,6). Kuna Indians living on islands near Panama consume large amounts of a local cacao beverage and have a low incidence of CVD, in particular, hypertension. Studies relate their low incidence of CVD to the substantial consumption of cacao and not to other factors such as eating habits, physical activity, or genetic background (7,8). Interestingly, data from a population-based cohort study of 1,169 patients link chocolate consumption with decreased mortality after myocardial infarction (MI) (9).

There are clues as to mechanisms that may explain cacao flavanol effects. Consumption of cacao flavanols leads to nitric oxide synthesis-dependent vasodilation (10,11). Flavonoids can also act as antioxidants and can inhibit platelet

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### Abbreviations and Acronyms

<b>AKT</b>	= protein kinase B
<b>CVD</b>	= cardiovascular disease
<b>ERK</b>	= extracellular signal-related kinase
<b>IR</b>	= ischemia–reperfusion
<b>LV</b>	= left ventricle/ventricular
<b>MI</b>	= myocardial infarction
<b>PCO</b>	= permanent coronary occlusion
<b>RISK</b>	= reperfusion injury salvage kinase

adhesion, low-density lipoprotein oxidation, inflammation, reactive oxygen species generation, and eicosanoid synthesis and can improve insulin resistance (for reviews, see [12]). In humans, the ingestion of (-)-epicatechin causes vasodilatation and reproduces the antioxidant and insulin-sensitizing effects of cocoa (13). We previously reported on the cardioprotective effects of (-)-epicatechin before treatment using a rodent model of ischemia–reperfusion (IR) injury (14). Our results showed a significant reduction in infarct size that was sustained up to 3 weeks

after injury. Reductions in infarct size were accompanied by preserved myocardial inflammation and decreases in matrix metalloproteinase activity and tissue oxidative stress. The activation of the reperfusion injury salvage kinase (RISK) pathway (a term given to a group of prosurvival protein kinases that include protein kinase B [AKT] and extracellular signal-related kinase [ERK] 1/2), has been demonstrated to confer cardioprotection (15). Pre-clinical studies have demonstrated that agents such as insulin, erythropoietin, adenosine, volatile anesthetics, and statins can reduce MI size through RISK pathway activation (15). Because cardioprotection was observed with (-)-epicatechin in the setting of IR injury, we speculated that AKT activation, ERK activation, or both may be involved in mediating these effects.

Permanent coronary occlusion (PCO) models of MI also have been used to examine the cardioprotective potential of candidate agents and to determine the long-term effects that the compounds may exert on LV structure and function. To our knowledge, no compounds have demonstrated a sustained, long-term reduction in MI size in the setting of a PCO. The effects that cocoa flavanols have on these end points are unknown. Given the cardioprotective effects of (-)-epicatechin noted in the setting of myocardial IR injury and the reported pleiotropic actions of flavanols, we hypothesized that pre-treatment with the compound may yield sustained reductions in infarct size and, as a consequence, may improve long-term LV structure and function. We also wished to examine the effects of (-)-epicatechin on AKT activation, ERK activation, or both in the setting of a PCO to identify possible cardioprotective mechanisms.

## Methods

**Surgical procedures.** All procedures were approved by the Institutional Animal Care and Use Committee and conform to published National Institutes of Health guidelines for animal research. Adult male Sprague Dawley rats (Harlan, Indianapolis, Indiana) weighing 250 to 300 g were anesthetized by intraperitoneal injection of ketamine (100 mg/kg)

and xylazine (10 mg/kg), intubated, and ventilated with room air. The heart was exposed via a left thoracotomy, the pericardium was opened, and the left anterior descending coronary artery was occluded. The chest was closed and the animals were allowed to recover. Sham were subjected to the surgical procedure described above and the suture was placed without occluding the coronary artery.

**Animal groups and (-)-epicatechin treatment.** (-)-epicatechin (1 mg/kg daily; Sigma-Aldrich, St. Louis, Missouri) or vehicle (water) was administered by oral gavage once daily beginning 10 days before thoracotomy and continuing until the time of the terminal study (2 h, 48 h, or 3 weeks). Two-hour study groups included PCO (n = 6) and PCO plus (-)-epicatechin (n = 5). The 2-h time point was selected as optimal for the evaluation of the RISK pathway activation, as per published reports (16). For 48-h studies, groups included sham (n = 5), sham plus (-)-epicatechin (n = 5), PCO (n = 8), and PCO plus (-)-epicatechin (n = 11). The 48-h time point was selected to distinguish clearly regions of necrotic tissue from viable myocardium. For 3-week studies, groups included sham (n = 11), sham plus (-)-epicatechin (n = 8), PCO (n = 12), and PCO plus (-)-epicatechin (n = 14). Six animals from each group were used to measure passive mechanics of the LV.

**Hemodynamics.** In vivo hemodynamic measurements were obtained from all animals used in the study just before the terminal studies. For hemodynamic measurements (heart rate and aortic and LV pressures), animals were anesthetized with 5% isoflurane and were maintained with 1% to 2% isoflurane. The LV and aortic pressures were measured with a micromanometer (2-F, 140 cm; Millar Instruments, Inc., Houston, Texas) introduced via the right carotid artery. Pressures were recorded digitally for subsequent analysis using WINDAQ software version 2.15 (DATAQ Instruments, Inc., Akron, Ohio).

**LV sampling and morphologic features.** Hearts were exposed via medial sternotomy and were arrested with an apical injection of ice-cold arrest solution (NaCl, 4 g/l; KCl, 4.48 g/l; NaHCO<sub>3</sub>, 1 g/l; glucose, 2 g/l; 2,3-butanedione monoximine, 3 g/l; heparin, 2,000 U/l) into the LV. The 3-week animal hearts were used for LV pressure-volume and pressure-strain studies (described in the following text). After arrest, hearts were excised and weighed and the right ventricle was removed. A 2-mm ring section was taken from the middle of the LV and stained for approximately 10 min using triphenyltetrazolium chloride. Computer-assisted image analysis was performed by a blinded operator. Results were expressed as percent infarct area of the LV. The images of stained rings also were used to measure internal and external LV chamber diameters and anterior and septal wall thicknesses.

**LV pressure volume and pressure strains.** Ex vivo passive mechanics of the LV and mature scar were analyzed in 3-week subjects as described previously (17). Briefly, a deflated balloon attached to a cannula and pressure trans-

**Table 1** Hemodynamic Data Obtained From Either Sham or PCO Groups 48 h After PCO

	Sham	Sham Plus (–)-Epicatechin	PCO	PCO Plus (–)-Epicatechin
HR (beats/min)	319 ± 12	334 ± 10	333 ± 17	342 ± 8
LVPSP (mm Hg)	100 ± 2	102 ± 2	98.4 ± 4	107 ± 4
LVEDP (mm Hg)	6.8 ± 0.9	4.3 ± 1.2	8.2 ± 4.2	5.8 ± 1.2
MAP (mm Hg)	83 ± 3	83 ± 2	76 ± 6	91 ± 5

Values are mean ± SEM.

HR = heart rate; LVEDP = left ventricular end diastolic pressure; LVPSP = left ventricular peak systolic pressure; MAP = mean arterial pressure; PCO = permanent coronary occlusion.

ducer was inserted into the LV. A triangle of white titanium dots (approximately 3 to 4 mm of separation between dots) was painted on the surface of the LV within the scar to measure 2-dimensional epicardial scar strains with changing LV pressure. A video camera was set up to record the position of the dots during balloon inflation. Water was infused into the balloon to reach 0 pressure. Internal balloon pressure and infused volume were measured simultaneously as the balloon was inflated slowly at a rate of approximately 1 ml/min to a peak pressure of 25 mm Hg. Data were acquired after 2 to 3 cycles of inflation and deflation of the balloon. For pressure-strain analyses, video frames at defined pressures were digitized using Scion Image (Scion Corp., Fredrick, Maryland). Two-dimensional epicardial scar strains were calculated with respect to the cardiac coordinate system yielding circumferential and longitudinal strains as described previously (17) by a blinded operator.

**Western blotting.** Western blots were used to determine increases in phosphorylated AKT, phosphorylated ERK relative to total AKT, and total ERK protein levels. Briefly, equal amounts of total protein were separated by 10% SDS-PAGE gels under reducing conditions and transferred onto a polyvinylidene difluoride membrane. Membranes were blocked in 5% bovine serum albumin in tris-buffered saline that contained 0.1% Tween 20 and were exposed to the proper primary antibody. Membranes were incubated

for 1 h at room temperature with the respective secondary horseradish peroxidase-labeled antibody and then were developed using an ECL Plus detection system (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). All antibodies used were obtained from Cell Signaling Technology (Danvers, Massachusetts).

**Data analysis.** All data are reported as mean ± SEM. Statistical analyses were performed using a Student unpaired *t* test, 1- or 2-way analysis of variance, and Bonferroni post hoc test, as appropriate. Results were considered statistically significant at  $p \leq 0.05$ .

## Results

**Hemodynamics.** Hemodynamic parameters were measured to determine LV contractile function. No significant changes were observed in heart rate, LV end-diastolic or systolic pressure, or mean aortic pressure between groups 48 h after PCO (Table 1). In 3-week studies (Table 2), there was a significant increase in LV end-diastolic pressures in the PCO group compared with both sham groups ( $p < 0.05$ ). A significant, albeit small, decrease in peak systolic pressure was noted in the PCO plus (–)-epicatechin group versus sham. No significant changes were observed in the other parameters measured.

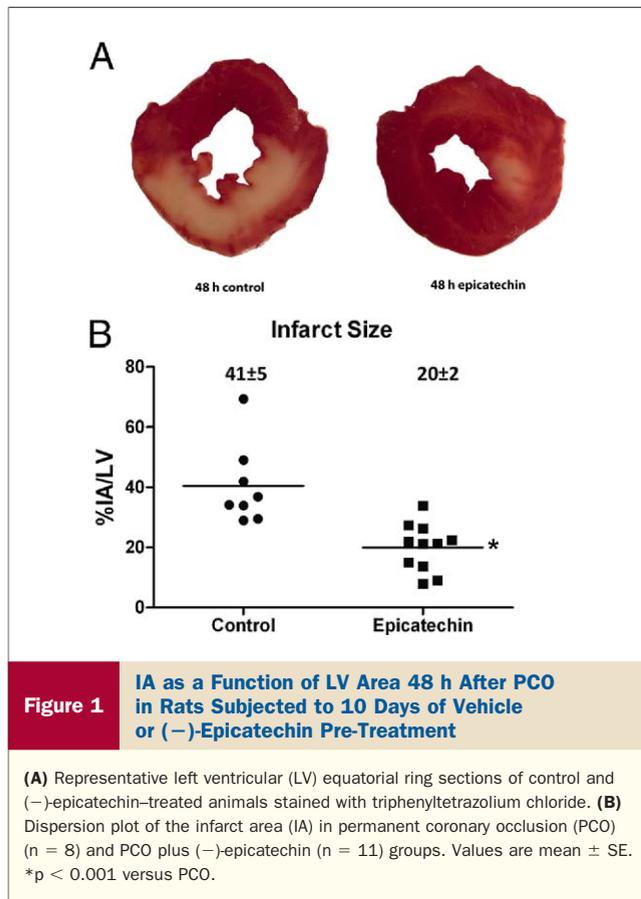
**Infarct size and morphometric analysis.** No differences in area at risk were identified between PCO groups at any of the time points studied. At 48 h after infarction (Fig. 1), the PCO and PCO plus (–)-epicatechin groups had infarct areas  $41 \pm 5\%$  and  $20 \pm 2\%$ , respectively ( $p < 0.001$ ). Figure 2 summarizes the results from the 3-week studies. The infarct area of PCO animals was  $43 \pm 3\%$  versus  $29 \pm 5\%$  ( $p < 0.02$ ). As shown in Table 2, the heart weight-to-body weight ratio and inner LV diameter were significantly higher in the PCO group ( $p < 0.01$ ) versus both sham groups, whereas the PCO plus (–)-epicatechin group demonstrated no differences. Anterior wall versus septal wall

**Table 2** Hemodynamic and Morphometric Data Obtained From Either Sham or 3-Week PCO Groups

	Sham	Sham Plus (–)-Epicatechin	PCO	PCO Plus (–)-Epicatechin
<b>In vivo</b>				
HR (beats/min)	294 ± 11	303 ± 10	273 ± 9	268 ± 10
LVPSP (mm Hg)	112 ± 6	118 ± 4	104 ± 2	102 ± 3*
LVEDP (mm Hg)	7.4 ± 0.4	6.4 ± 0.9	14 ± 2*†	11 ± 1
MAP (mm Hg)	85 ± 6	92 ± 4	83 ± 4	82 ± 2
<b>Ex vivo morphometry</b>				
HW/BW	3.7 ± 0.2	3.8 ± 0.3	5.0 ± 1.2*†	4.4 ± 0.7
Outer LV diameter (mm)	1.47 ± 0.06	1.47 ± 0.08	1.5 ± 0.03	1.495 ± 0.03
Inner LV diameter (mm)	0.51 ± 0.02	0.49 ± 0.05	0.65 ± 0.03†	0.55 ± 0.05
AW thickness (mm)	0.61 ± 0.03	0.58 ± 0.05	0.32 ± 0.03‡	0.45 ± 0.04
SW thickness (mm)	0.38 ± 0.04	0.38 ± 0.04	0.49 ± 0.01	0.48 ± 0.02

Values are mean ± SE. \* $p < 0.01$  versus sham. † $p < 0.01$  versus sham plus (–)-epicatechin. ‡ $p < 0.01$  versus PCO SW thickness (1-way analysis of variance, Bonferroni test, 4 comparisons).

AW = anterior wall; HW/BW = heart weight-to-body weight ratio; LV = left ventricle; SW = septal wall; other abbreviations as in Table 1.



thicknesses yielded a statistical difference only in the vehicle-treated PCO group ( $p < 0.01$ ).

**LV pressure-volume studies.** Figure 3 shows mean LV pressure-volume curves obtained from treated and untreated PCO groups compared with those obtained from shams. Untreated PCO yielded a significant right-shifted pressure-volume curve when compared with sham groups ( $p < 0.02$ ). (–)-epicatechin-treated animals produced a pressure-volume curve comparable with that of both sham groups. No significant interaction between treatment and pressure was detected.

**LV pressure strains.** Figure 4 shows mean LV pressure scar strains obtained in untreated and treated PCO groups. Regardless of treatment, scar tissue is more compliant in the circumferential direction. Untreated PCO had a significantly more compliant scar when compared with both sham groups and the PCO plus (–)-epicatechin groups ( $p < 0.01$ ) in the circumferential direction. Notable differences in longitudinal strain also were detected between untreated PCO and both sham groups ( $p < 0.05$ ). A 2-way analysis of variance revealed no interaction between treatment and pressure.

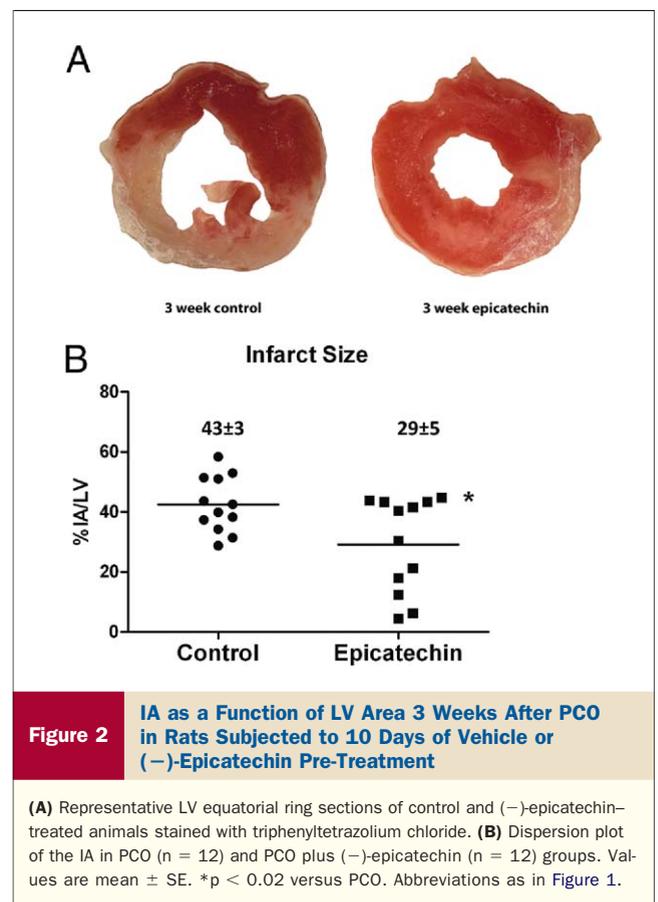
**Western blotting.** The phosphorylation of AKT and ERK was measured 2 h after PCO with or without (–)-epicatechin. Densitometry analysis indicated that 10 days of (–)-epicatechin pre-treatment led to no significant differ-

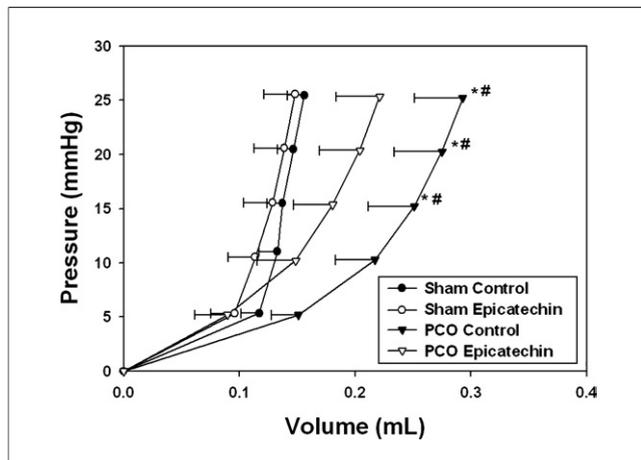
ences in AKT or ERK phosphorylation (Fig. 5). Total AKT and ERK levels were unaltered.

## Discussion

Unique findings derived from this study indicate that pre-treatment of animals with the flavanol (–)-epicatechin can substantially reduce infarct size 48 h after a PCO. The effect is sustained at 3 weeks and is accompanied with left-shifted LV pressure-volume curves, reduction in epicardial scar strains, and improvements in LV post-infarction morphometry. To our knowledge, this is the first time a compound has demonstrated significant and sustained long-term beneficial outcomes in a model of MI secondary to a PCO. A potential mechanism by which (–)-epicatechin may decrease MI size is a reduction in workload. As with our previous findings, (–)-epicatechin treatment in sham or PCO animals did not reduce blood pressure or heart rate, and thus, changes in hemodynamics fail to explain the observed cardioprotective effects. The protective kinases—AKT, ERK, or both—at the assessed time point, seemed to be unaltered.

Previous studies have attempted to identify compounds capable of reducing MI size in the setting of a PCO. Kingma and Yellon (18) reported on the capacity of verapamil given 5 min after embolization to reduce 48-h infarct size in a closed-chest canine model. Smith et al. (19)



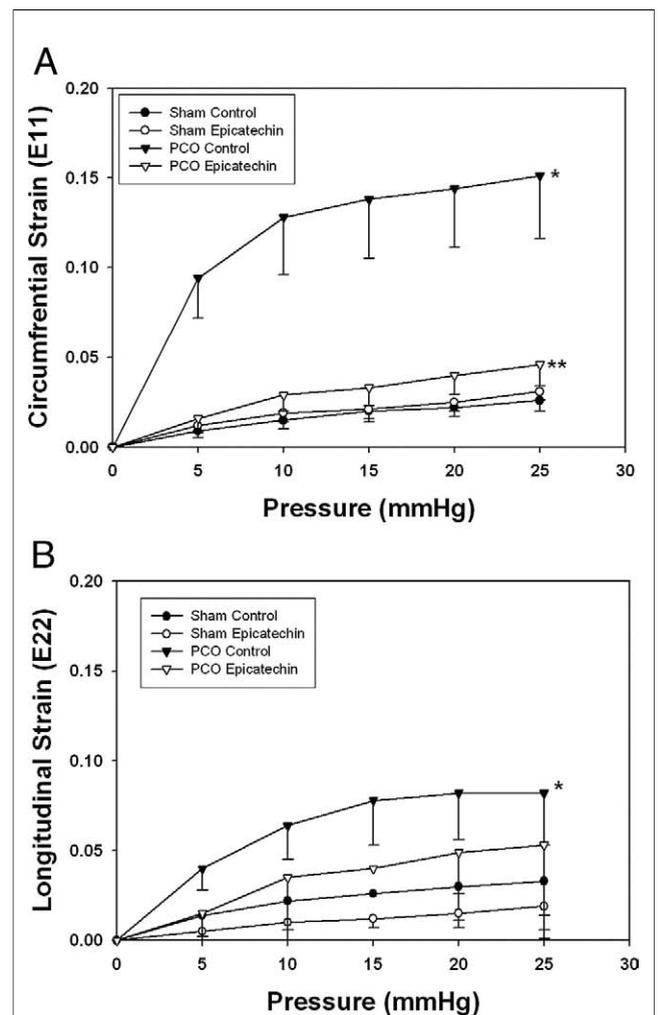


**Figure 3** Average Passive LV Pressure Volume Relations for Sham, Sham plus (-)-Epicatechin, PCO, and PCO Plus (-)-Epicatechin After 3 Weeks

Solid circles = sham (n = 6). Open circles = sham plus (-)-epicatechin (n = 6). Solid triangles = PCO (n = 6). Open triangles = PCO plus (-)-epicatechin (n = 6). Two-way analysis of variance followed by Bonferroni's test (30 comparisons) revealed significant differences between PCO and shams ( $p < 0.02$ ). No interactive effect between treatment and pressure was detected. \* $p < 0.02$  versus sham control. # $p < 0.02$  versus sham (-)-epicatechin. Abbreviations as in Figure 1.

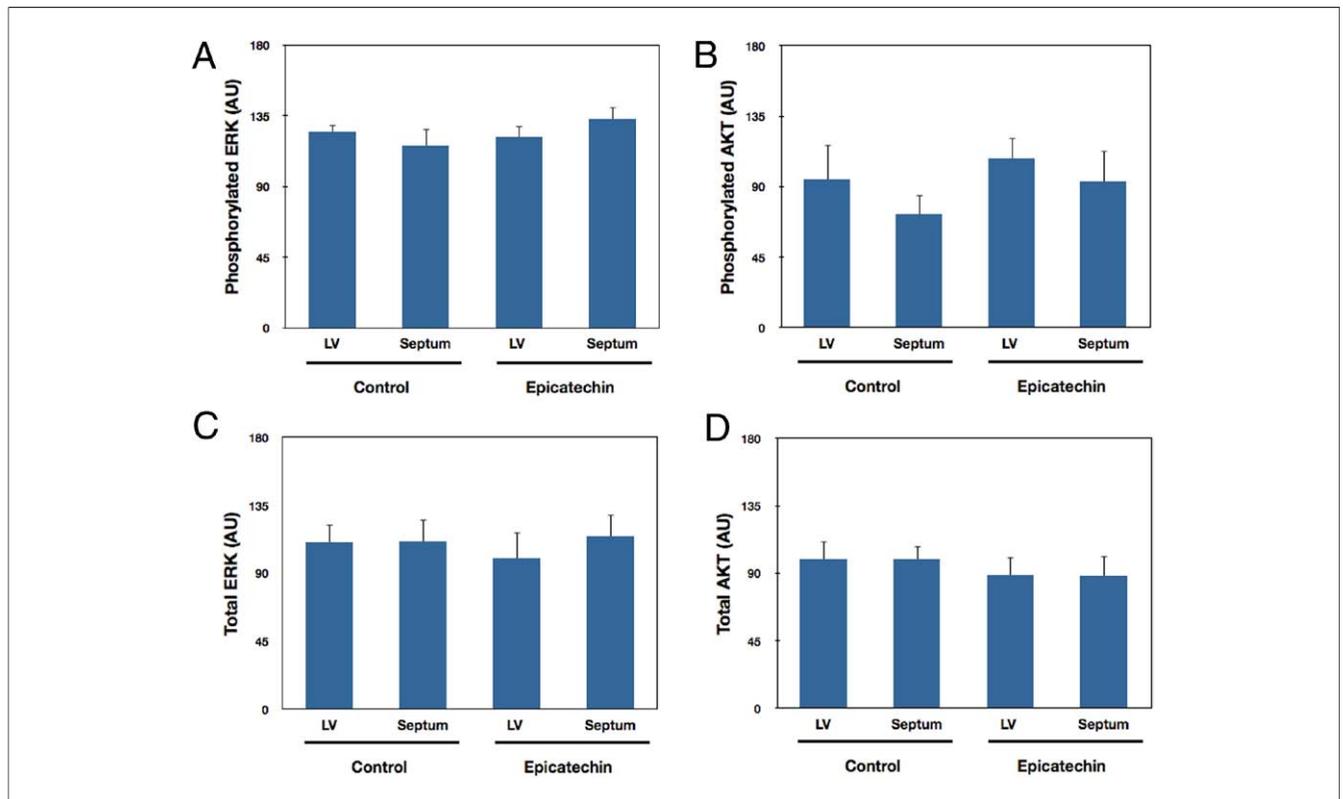
reported the capacity of propranolol given 1 min before PCO to reduce tissue creatinine kinase levels 48 h after infarction. Kaga et al. (20) demonstrated that the flavanoid resveratrol is capable of reducing MI size in a rat 24 h after PCO. In this study, the authors suggested that resveratrol effects may be secondary to an early angiogenesis process, but relevant end points were examined only 4 days after MI. Other compounds such as metformin, erythropoietin, rosuvastatin, and amlodipine have been examined for their potential for reducing MI size in the setting of PCO, but did not improve this end point (21–24). We have examined the capacity of doxycycline and minocycline to yield sustained reductions in MI size both in the setting of IR and of PCO. Our results indicate that although the compounds yield short-term (24 to 48 h) reductions in MI size, they fail to sustain the effect in the long term (25–27). It is worth noting that neither verapamil, propranolol, nor resveratrol are reported as capable of reducing MI size in the magnitude achieved by 1 mg/kg daily (-)-epicatechin at 48 h (approximately 50%). Because (-)-epicatechin can have vasodilatory effects, it was important to measure blood pressure, which was not reduced by treatment in our animals. Interestingly, in a recent report, special formulation high flavanol cocoa given to spontaneously hypertensive rats was able to reduce diastolic and systolic blood pressure noticeably (28). However, in control Wistar rats, blood pressure remained stable, suggesting that (-)-epicatechin has the property of exerting antihypertensive effects in a pathologic state with no hypotensive effects in normal subjects.

The purpose behind 3-week studies was to determine the extent to which there was a sustained reduction in MI with treatment and an improvement in passive mechanics and LV morphometric features. Results yield a reduction in scar (infarct) size of approximately 33%. This result is indeed unique, in that no published studies have demonstrated the capacity of candidate cardioprotective agents to yield such sustained (long-term) effects in the setting of a PCO (29,30). The assessment of the effects of (-)-epicatechin on post-MI chamber structure and function is critical, because little is known about the pleiotropic effects of flavanols on post-MI wound healing and remodeling. As is well noted in



**Figure 4** Average 2-Dimensional Circumferential and Longitudinal Strains in the Scar Area as Functions of Inflation Pressure After 3 Weeks

(A) Circumferential ( $E_{11}$ ) and (B) longitudinal ( $E_{22}$ ) strains. Solid circles = sham (n = 6). Open circles = sham plus (-)-epicatechin (n = 6). Solid triangles = PCO (n = 6). Open triangles = PCO plus (-)-epicatechin (n = 6). Two-way analysis of variance followed by Bonferroni's test (30 comparisons) indicates that PCO plus (-)-epicatechin yields a stiffer scar in the  $E_{11}$  direction relative to PCO. No interactive effect between treatment and pressure was detected. \* $p < 0.05$  versus sham. \*\* $p < 0.05$  versus PCO control. Abbreviations as in Figure 1.



**Figure 5** ERK and AKT Levels in Animals Subjected to 10 Days of Vehicle or (–)-Epicatechin Pre-Treatment and 2 h of PCO

Densitometric analysis of Western blots for (A) phosphorylated extracellular signal-related kinase (ERK), (B) phosphorylated protein kinase B (AKT), (C) total ERK, and (D) total AKT in PCO (n = 6) and PCO plus (–)-epicatechin (n = 5) animals. Abbreviations as in Figure 1.

the literature, the use of anti-inflammatory agents such as steroids in the setting of PCO leads to serious adverse effects on heart structure and function. We recently compared the effects of prednisone administration to those of doxycycline in an animal model of PCO-induced MI (17). Doxycycline treatment yielded improvements in post-MI LV structure and function, including heart and body weight, wall thickness, chamber size, and LV pressure-volume and pressure-strain curves. In contrast, treatment with steroids yielded a worsening of these end points versus untreated or doxycycline-treated MI animals. Results from (–)-epicatechin treatment of animals subjected to PCO indicate improvements in all these end points 3 weeks after MI. Hearts were smaller, LV pressure-volume and pressure-strain curves were comparable with those of shams, and there was a preservation of infarct wall thickness. Thus, the use of (–)-epicatechin does not seem to compromise the ability of the myocardium to heal and function properly. Regarding the mechanisms responsible for the improved long-term outcome, it follows that a smaller infarct would improve 3-week scar size and LV structure and function. However, we cannot exclude other effects, because the use of other flavanoids (epicatechin gallate) has been reported to improve tissue healing and scarring in animals (31).

The activation of proteins belonging to the RISK pathway in the setting of myocardial IR injury has been

associated with protective outcomes. Members of this group of kinases include AKT and ERK. In our previous IR study, we demonstrated that (–)-epicatechin treatment decreases infarct by 50% at 48 h and by 32% by 3 weeks (14). In this study, the magnitudes of these decreases are the same at 48 h and 3 weeks when injury was induced via PCO. These results suggest a potential common mechanism(s) that may be responsible for cardioprotection. Recent reports have documented the capacity of (–)-epicatechin to activate signaling pathways known to be associated with cardioprotection (ERK and AKT) in neuronal cultures (32). Thus, we wished to explore the potential involvement of these kinases in mediating cardioprotective responses in the setting of PCO. Results fail to demonstrate changes in ERK and AKT phosphorylation levels at 2 h after PCO. Thus, (–)-epicatechin likely exerts its cardioprotective actions through other mechanisms. Interestingly, there is controversy as to the cardioprotective roles exerted by AKT and ERK, because they have been claimed to be involved in ischemic post-conditioning with ambiguous results (33). There are other members of the RISK pathway that also need to be examined further, such as p70 ribosomal S6 protein kinase and glycogen synthase kinase 3b (33).

Other possible scenarios by which (–)-epicatechin may exert cardioprotective actions include the induction of a hibernating myocardium-like gene program. Hibernating

myocardium is characterized by the up-regulation of proteins involved in apoptosis proteins, growth (vascular endothelial growth factor, H11 kinase), and cytoprotection (heat shock protein 70, hypoxia induced factor 1 $\alpha$ , glucose transporter 1) (34). The up-regulation of these proteins by (-)-epicatechin may allow the cells to sustain ischemia better when present. Subsequently, ischemic-hibernating myocardium may recover on the reestablishment of blood flow via vessel recruitment, angiogenesis, or both. As noted above, flavanoids such as resveratrol have demonstrated the capacity to induce angiogenesis in ischemic myocardium (35); however, less is known about cocoa flavanols. There is indirect evidence to suggest that (-)-epicatechin actions indeed may occur through changes in gene expression. In studies performed in patients with hypertension, sustained reductions in blood pressure were observed when treatment was given with high flavanol cocoa for at least 7 days, indicating that the response requires a time-dependent effect (36). In our previous study, we used 2 pre-treatment schemes to examine the effects of (-)-epicatechin on hearts subjected to IR injury. Pre-treatment was provided for either 2 or 10 days. Short-term treatment with 1 mg/kg daily (-)-epicatechin did not confer cardioprotection, whereas 10 days yielded reduced infarct size. These data suggest that changes in myocardial gene expression, protein levels, or both may need to develop with longer treatment times (6,37). Alternatively, the accumulation of (-)-epicatechin or sustained changes in blood levels of (-)-epicatechin metabolites may be required to generate cardioprotection (6). It is also well established that flavonoids are pleiotropic, because they possess antioxidant (38,39), anti-inflammatory (40–42), and antithrombotic properties (43). As noted, flavonoids also can induce nitric oxide-mediated vasodilation via endothelial nitric oxide synthase activation (11,44). In a recent report by Gundewar *et al.* (21), metformin was demonstrated to reduce infarct size in a mouse model of myocardial IR injury. The effects were associated with increases in 5' adenosine monophosphate protein kinase and endothelial nitric oxide synthase phosphorylation. Indeed, it is widely accepted that endothelial nitric oxide synthase activation is recognized as being cardioprotective (45). Further investigation is warranted to determine if any of the mechanisms of action discussed above has relevance to (-)-epicatechin's actions on limiting infarct size in the setting of a PCO.

As in our first study, experiments focused on the use of a pre-treatment scheme to induce cardioprotection. These results imply that the regular and sustained consumption of products high in flavanols may induce a prophylactic organ protective phenotype. Just as the regular consumption of moderate amounts of wine has been suggested to yield a beneficial impact on CVD, a similar argument eventually may be validated for the consumption of products enriched for flavanols. There is a need to develop flavanol-enhanced products that are low in calories, because regular commercial products are high in fat and sugar. Many commercial

companies (e.g., Natraceuticals, Inc., Valencia, Spain) have begun to generate such products and to promote the apparent beneficial effects of cocoa consumption.

On the basis of the results presented, further studies need to be undertaken to validate these results and to identify key underlying mechanisms of action of (-)-epicatechin's effects, which are likely complex. The reproducibility of these observations by independent groups is warranted. In addition, further MI pre-clinical studies need to be performed using small and large animal models of human disease, such as those with diabetes, and, importantly, to verify the variability of the effects on the basis of age and sex. Nonetheless, the results presented provide support toward the consideration of (-)-epicatechin as a possible therapeutic agent intended to prevent or limit the development of ischemic heart disease. Recent reports on the association of chocolate consumption to decreases in post-infarct mortality (9), on the reversal of vascular dysfunction in diabetic patients (46), and on the beneficial effects on vascular function in humans (44) suggest that cocoa indeed may have protective effects on the ischemic heart. However, caution must be exercised, because the regular consumption of most commercially available chocolate or cocoa-related products provide a high caloric content and likely vary as to their overall composition (as cocoa has many bioactive molecules) and in the amount of (-)-epicatechin present.

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**Key Words:** cardioprotection ■ catechins ■ flavanols ■ infarction ■ ischemia.