Essential Role of Bone Marrow for Microvascular Endothelial and Metabolic Functions in Mice

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Rationale: **We have previously demonstrated that the importance of endothelium-derived hyperpolarizing factor (EDHF) increases as the vessel size decreases and that endothelium-derived hydrogen peroxide (H_2O_2) is an EDHF in animals and humans, for which endothelial nitric oxide synthase (eNOS) is the major source. Recent studies have suggested the important role of the bone marrow (BM) in modulating cardiovascular and metabolic functions.**

Objective: **We aimed to examine whether BM plays a role in modulating microvascular endothelial and metabolic functions in mice, and if so, to elucidate the mechanisms involved.**

Methods and Results: **Male eNOS$^{-/-}$ mice were transplanted with BM cells from wild-type (WT) or eNOS$^{-/-}$ mice and were maintained for 6 weeks. Endothelium-dependent relaxations and hyperpolarizations of mesenteric arteries to acetylcholine were reduced in eNOS$^{-/-}$ mice and were markedly improved when transplanted with WT-BM but not with eNOS$^{-/-}$-BM. The enhanced component of endothelium-dependent relaxations was abolished by catalase, indicating that the improved responses were mediated by H_2O_2. In contrast, no such beneficial effect was noted in the aorta. Reduced plasma adiponectin levels and impaired glucose tolerance in eNOS$^{-/-}$ mice were also improved by WT-BM transplantation. Neuronal nitric oxide synthase (nNOS) in mesenteric arteries of eNOS$^{-/-}$ mice was significantly upregulated only when transplanted with WT-BM. Importantly, the beneficial effects of WT-BM transplantation were absent in eNOS$^{-/-}$/adiponectin$^{-/-}$ or eNOS$^{-/-}$/nNOS$^{-/-}$ mice.**

Conclusions: **These results provide the first evidence that BM plays an important role in modulating microvascular endothelial and metabolic functions, for which adiponectin and nNOS may be involved. (*Circ Res*. 2012;111:87-96.)**

Key Words: nitric oxide synthases ■ endothelium-derived hyperpolarizing factor ■ bone marrow ■ adiponectin

The endothelium plays an important role in maintaining vascular homeostasis by synthesizing and releasing several vasodilators, including prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF).[1–3] It is widely accepted that EDHF plays an important role in modulating vascular tone, especially in microvessels.[4,5] In addition, endothelial function is impaired by hypertension, glucose intolerance, insulin resistance, dyslipidemia, obesity, and metabolic syndrome.[6–9]

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We have previously demonstrated that endothelium-derived hydrogen peroxide (H_2O_2) is an EDHF in mouse[10] and human[11] mesenteric arteries and porcine coronary microvessels.[12] Other investigators confirmed the importance of H_2O_2 as an EDHF[13] in human[14] and canine[15,16] coronary microvessels and porcine pial arteries.[17] We also have demonstrated that endothelial nitric oxide synthase (eNOS) is a major source of EDHF/H_2O_2,[10] where copper, zinc-superoxide dismutase plays an important role to dismutate eNOS-derived superoxide anions to EDHF/H_2O_2[18,19] and that endothelial NOSs system plays different roles depending on the vessel size, serving as the NO-generating system in large arteries and as the EDHF/H_2O_2-generating system in microvessels in mice.[20] Furthermore, we have recently demonstrated that when the all 3 NOSs [eNOS, neuronal NOS (nNOS), and inducible NOS (iNOS)] are genetically deleted in mice, acute myocardial infarction develops spontaneously associated with unique phenotypes that resemble metabolic syndrome in humans, including glucose intolerance, hypertension, dyslipidemia, and visceral obesity.[21] These results indicate the close coupling between microvascular endothelial functions and metabolic disorders, although the detailed mechanisms remain to be elucidated.

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Non-standard Abbreviations and Acronyms	
ACh	acetylcholine
APN	adiponectin
EDHF	endothelium-derived hyperpolarizing factor
eNOS	endothelial nitric oxide synthase
EPC	endothelial progenitor cell
H_2O_2	hydrogen peroxide
iNOS	inducible nitric oxide synthase
K_{Ca} channels	calcium-activated potassium channels
L-NNA	N^{ω}-nitro-L-arginine
MCP-1	monocyte chemoattractant protein-1
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
PG	prostaglandin
SNP	sodium nitroprusside
WT	wild-type

It has been recently demonstrated that some blood cells [eg, neutrophils, macrophages, and endothelial progenitor cells (EPCs)[22]] are involved in the development of cardiovascular diseases, including atherosclerosis,[23] cardiovascular events,[24] vasospastic angina,[25] and angiogenesis.[26] However, it also has been demonstrated that bone marrow (BM)-derived cells do not directly differentiate into vascular endothelial or smooth muscle cells in mouse models of atherosclerosis[27–29] and of chronic endothelial dysfunction.[30]

We hypothesized that the BM plays an important role in maintaining microvascular endothelial and metabolic functions. To test this hypothesis, we examined whether BM transplantation improves microvascular endothelial and metabolic functions in $eNOS^{-/-}$ mice, an established animal model of atherosclerosis and metabolic disorders, and if so, what mechanisms are involved.

Methods

Animals

An expanded Methods section is available in the online Data Supplement.

The present study was reviewed and approved by the Committee on Ethics of Animal Experiments of Tohoku University. Male mice aged 8 weeks were used. The $eNOS^{-/-}$ mice were originally provided by P. Huang (Harvard Medical School, Boston, MA). The $eNOS^{-/-}$ mice were backcrossed to C57BL/6 mice over 10 generations. Thus, C57BL/6 mice were used as a wild-type (WT) control. We generated $n/eNOS^{-/-}$ mice by crossing $nNOS^{-/-}$ and $eNOS^{-/-}$ mice, as previously reported.[31] $eNOS^{-/-}$/adiponectin $(APN)^{-/-}$ mice were generated by crossing $eNOS^{-/-}$ and $APN^{-/-}$ mice (Jackson Laboratory, Bar Harbor, ME). Systolic blood pressure was measured by tail-cuff method.

BM Transplantation

BM transplantation was performed as described previously.[32] After transplantation, the mice were placed on a regular chow diet for 6 weeks. We generated green fluorescent protein (GFP)-positive WT and GFP-positive $eNOS^{-/-}$ mice by crossing with transgenic mice ubiquitously expressing GFP.[33]

Organ Chamber Experiments

Isometric tension was recorded using isolated small mesenteric arteries (≈200 μm) and the aorta, as previously described.[9,10,18,20] Rings were precontracted with prostaglandin $F_{2\alpha}$. The contributions of vasodilator prostaglandins (PGs), NO and EDHF to acetylcholine (ACh)-induced endothelium-dependent relaxation were determined by the inhibitory effect of indomethacin, N^{ω}-nitro-l-arginine (L-NNA) in the presence of indomethacin, and a combination of charybdotoxin and apamin, respectively.[9,10,18,20] To examine the involvement of endothelium-derived H_2O_2 in the EDHF-mediated responses, the inhibitory effect of catalase, a specific scavenger of H_2O_2, was examined.[9,10,18,20]

Electrophysiological Experiments

The rings of small mesenteric arteries were placed in experimental chambers perfused with Krebs solution containing indomethacin and L-NNA. A fine glass capillary microelectrode was impaled into the smooth muscle from the adventitial side of mesenteric arteries, and changes in membrane potentials produced by ACh were continuously recorded.[9,10,18,20]

Histological Analysis

Adipose tissue was fixed in zinc fixative and embedded in paraffin. After blocking of endogenous biotin and avidin binding sites, sections were subjected to immunohistochemical staining overnight at 4°C with an anti-F4/80 monoclonal antibody. Immune complexes were detected with biotinylated secondary antibodies, horseradish peroxidase–conjugated streptavidin and the peroxidase substrate diaminobenzidine.

Glucose Tolerance Tests

For the glucose tolerance test, mice were fasted overnight. Glucose (1 g/kg body weight) was injected intraperitoneally and blood was collected from the tail vein at different time points.[34]

Statistical Analysis

Statistical analysis was performed with JMP 9.0 (SAS Institute Inc, Cary, NC). Results are shown as mean±SEM. Dose-response curves were analyzed by 2-way ANOVA followed by Tukey HSD test for multiple comparisons. Other variables were analyzed by Student *t* test or 1-way ANOVA followed by Tukey HSD test for multiple comparisons. $P<0.05$ was considered to be statistically significant.

Results

Chimerism in Transplanted Mice

Chimerism in transplanted $eNOS^{-/-}$ mice was analyzed by the percentage of $EGFP^{+}$ nucleated blood cells found in peripheral blood at 5 weeks after BM transplantation. All animals showed >85% chimerism (WT-BM, 90.2±0.6%; $eNOS^{-/-}$-BM, 87.3±0.3%, n=5–7).

Body Weight, Systolic Blood Pressure, and Blood Cell Counts

In the present study, we examined 4 groups of mice, including sham-transplanted (control) WT and $eNOS^{-/-}$ mice and $eNOS^{-/-}$ mice transplanted with WT-BM or $eNOS^{-/-}$-BM (Online Table I). At baseline, body weight was greater in the 3 $eNOS^{-/-}$ groups than in WT. At 6 weeks after BM or vehicle injection, there was no significant difference in body weight among the 4 groups. Systolic blood pressure was significantly higher in the 3 $eNOS^{-/-}$ groups compared with WT throughout the experimental period (Online Table I). At 5 weeks after BM or vehicle injection, control $eNOS^{-/-}$ mice had higher white and red blood cell counts, increased hemoglobin levels, and decreased platelet count compared with

control WT. The increased white and red cell counts and hemoglobin levels were normalized by transplantation with WT-BM or eNOS$^{-/-}$-BM, whereas platelet count was unchanged (Online Table I).

Endothelium-Dependent Relaxations and Hyperpolarizations

In isolated mesenteric arteries of control WT mice, ACh elicited concentration-dependent relaxations, which were resistant to indomethacin (vasodilator PGs component) or indomethacin plus L-NNA (NO component), but was highly sensitive to the combination of apamin and charybdotoxin in the presence of indomethacin and L-NNA (EDHF component) (Figure 1A). In mesenteric arteries of control eNOS$^{-/-}$ mice, endothelium-dependent, EDHF-mediated relaxations to ACh were markedly attenuated compared with those of WT mice (Figure 1B), a consistent finding with our previous studies.[10,20] Transplantation with WT-BM but not that with eNOS$^{-/-}$-BM markedly improved the EDHF-mediated relaxations (Figure 1C and 1D). The enhanced component of EDHF-mediated relaxations was abolished in endothelium-denuded mesenteric artery (Online Figure I, A). In addition, this enhanced component was markedly inhibited by catalase, indicating that the improved responses were mediated by endothelium-derived H_2O_2 (Figure 1E). Similarly, EDHF-mediated relaxations in WT mice mesenteric arteries were markedly inhibited by catalase, whereas NO-mediated relaxations in WT mice aorta were not affected by catalase (Online Figure II, A and B). Absolute values of contractions of mesenteric artery to prostaglandin $F_{2\alpha}$ were comparable among all the groups (Online Table II).

Electrophysiological recordings of membrane potentials with the microelectrode technique in mesenteric arteries demonstrated that endothelium-dependent hyperpolarizations to ACh (10^{-5} mol/L) were significantly reduced in eNOS$^{-/-}$ mice compared with WT mice and were significantly improved with WT-BM transplantation but not with eNOS$^{-/-}$-BM transplantation (Figure 1F).

In contrast to mesenteric arteries, endothelium-dependent relaxations of the aorta to ACh were mainly mediated by NO in WT mice as L-NNA abolished the responses (Figure 2A) and were totally absent in eNOS$^{-/-}$ mice (Figure 2B). No beneficial effect of BM transplantation with WT-BM or eNOS$^{-/-}$-BM was noted in the aorta of eNOS$^{-/-}$ mice (Figure 2C and 2D).

Endothelium-Independent Relaxations

There was no significant difference in endothelium-independent relaxations to NS1619 [an opener of calcium-activated potassium (K_{Ca}) channels] or sodium nitroprusside (SNP) in mesenteric arteries and the aorta between WT-BM and eNOS$^{-/-}$-BM mice (Online Figure III, A through D). In addition, there was no significant difference in endothelium-independent relaxations of mesenteric artery to SNP regardless of the presence or absence of the endothelium in WT-BM eNOS$^{-/-}$ mice (Online Figure I, B). Furthermore, the relaxations to NS1619 were unaltered by catalase in mesenteric arteries when transplanted with WT-BM (Online Figure III, A), and the relaxations to SNP were unaltered by catalase in WT mice mesenteric arteries and aorta (Online Figure II, C and D).

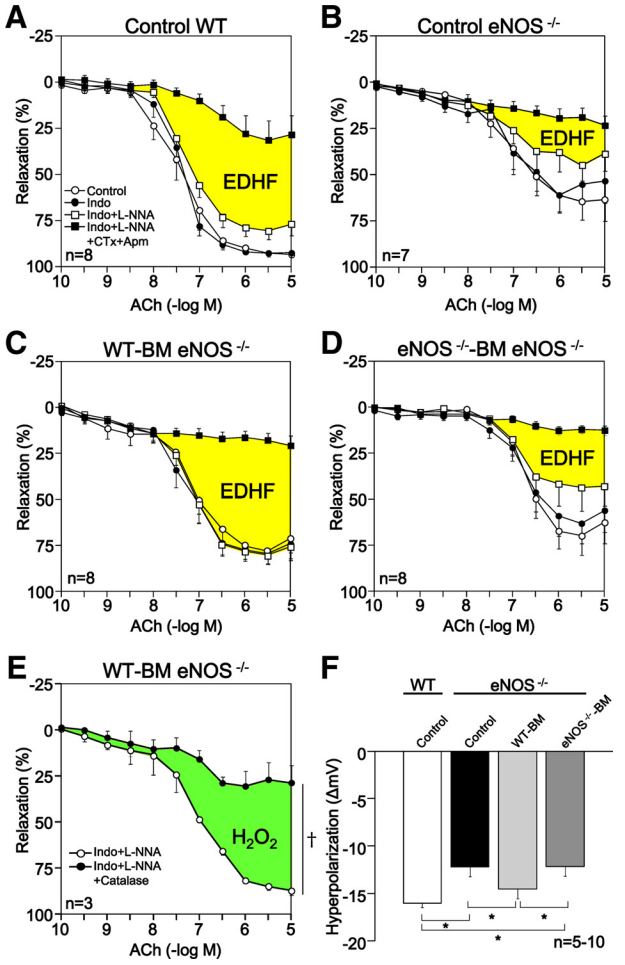

Figure 1. WT-BM transplantation markedly improves EDHF-mediated relaxations and hyperpolarizations of mesenteric arteries from eNOS$^{-/-}$ mice. Endothelium-dependent relaxations to ACh of mesenteric arteries from control WT mice (**A**), control eNOS$^{-/-}$ mice (**B**), eNOS$^{-/-}$ mice transplanted with WT-BM (**C**), and those with eNOS$^{-/-}$-BM (**D**). EDHF-mediated relaxations to ACh (in the presence of indomethacin and L-NNA as shown in yellow) were reduced in eNOS$^{-/-}$ mice (**B**) and were markedly improved with WT-BM transplantation (**C**) but not with eNOS$^{-/-}$-BM transplantation (**D**). **E**, Catalase markedly inhibited the enhanced EDHF-mediated relaxation in eNOS$^{-/-}$ mice transplanted with WT-BM. Indo indicates indomethacin; CTx, charybdotoxin; and Apm, apamin. **F**, Endothelium-dependent hyperpolarizations to ACh (10^{-5} mol/L) of mesenteric arteries in eNOS$^{-/-}$ mice were also improved with WT-BM transplantation but not with eNOS$^{-/-}$-BM transplantation. Results are expressed as mean±SEM. *$P<0.05$, †$P<0.01$.

Immunostaining of Mesenteric Artery and Aorta in eNOS$^{-/-}$ Mice

Immunofluorescence staining was performed in mesenteric arteries and the aorta of GFP^{+}WT-BM eNOS$^{-/-}$ mice and GFP^{+} eNOS$^{-/-}$-BM eNOS$^{-/-}$. We observed a small number of GFP-positive BM-derived cells in the adventitia of mesenteric artery (WT-BM, 0.6±0.6; eNOS$^{-/-}$-BM, 0.8±0.2, n=5 each, not statistically significant) but not in the intima or media of mesenteric artery or in the aorta (Online Figure IV, A). Immunohistochemical staining was performed in mesen-

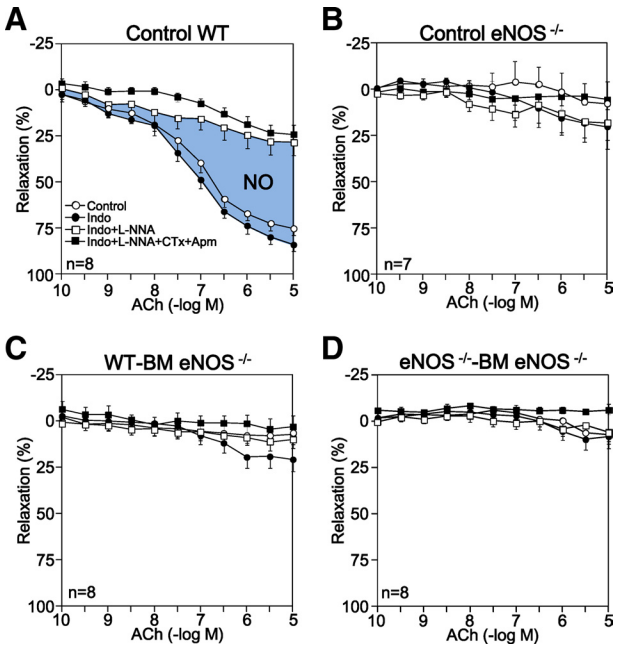

Figure 2. No effect of BM transplantation on endothelium-dependent relaxations of the aorta. Endothelium-dependent relaxations of the aorta from control WT mice (**A**), control eNOS$^{-/-}$ mice (**B**), eNOS$^{-/-}$ mice transplanted with WT-BM (**C**), and those with eNOS$^{-/-}$-BM (**D**). In the aorta of untreated WT mice, endothelium-dependent relaxations to ACh were resistant to indomethacin but were markedly inhibited by L-NNA. In contrast, in control eNOS$^{-/-}$ mice, endothelium-dependent relaxations to ACh were absent. Unlike the case of mesenteric arteries, WT-BM transplantation had no enhancing effect on endothelium-dependent relaxations of the aorta. Results are expressed as mean±SEM.

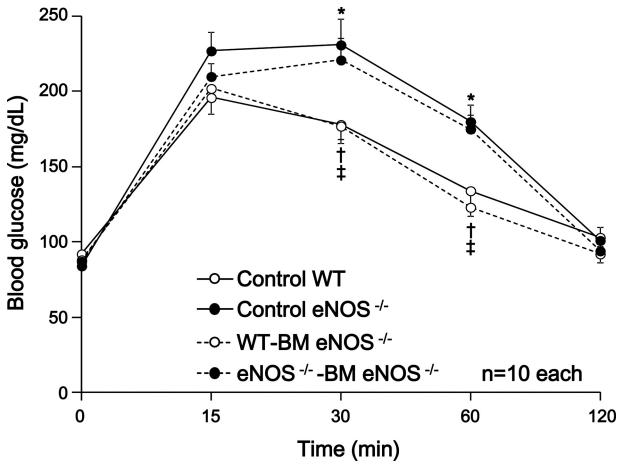

Figure 3. WT-BM transplantation normalizes glucose tolerance in eNOS$^{-/-}$ mice. Glucose tolerance test was performed at 5 weeks after BM or vehicle injection. Although plasma glucose levels were comparable at baseline and 15 minutes after glucose injection in all groups, the levels were significantly higher at the 30 and 60 minutes in control eNOS$^{-/-}$ mice compared with control WT mice. The impaired glucose tolerance in eNOS$^{-/-}$ mice was markedly improved with WT-BM transplantation but not with eNOS$^{-/-}$-BM transplantation. Results are expressed as mean±SEM. *P<0.05 versus control WT, †P<0.05 versus control eNOS$^{-/-}$, ‡P<0.05 versus eNOS$^{-/-}$-BM eNOS$^{-/-}$.

teric artery of WT-BM eNOS$^{-/-}$ mice and eNOS$^{-/-}$-BM eNOS$^{-/-}$ to identify the blood cells in the adventitia. In the adventitia, only F4/80-positive cells were noted but not CD3- or Ly6G-positive cells (Online Figure IV, B). eNOS was not detected at all in eNOS$^{-/-}$ mice, CD31 was stained in the endothelium alone (Online Figure V, A), and nNOS was stained in all layers of the blood vessel (Online Figure V, B).

Flow Cytometry Analysis to Assess EPC

Flow cytometry analysis was performed to examine the number of circulating EPC defined as Sca-1$^+$/Flk-1$^+$ mononuclear cells (Online Figure VI, A and B). There was no significant difference in the EPC number among WT, WT-BM, and eNOS$^{-/-}$-BM eNOS$^{-/-}$ mice (Online Figure VI, C).

Glucose Tolerance Tests and Plasma Levels of Lipids

Glucose tolerance test was performed in all 4 groups at 5 weeks after BM or vehicle injection. Although plasma glucose levels were comparable at baseline, the levels were significantly higher in control eNOS$^{-/-}$ mice at 30 and 60 minutes compared with WT mice (Figure 3). WT-BM transplantation normalized glucose tolerance in eNOS$^{-/-}$ mice to the level in WT mice, whereas eNOS$^{-/-}$-BM transplantation was without the effects (Figure 3). The plasma levels of total cholesterol, LDL cholesterol and triglycerides were also significantly higher in control eNOS$^{-/-}$ mice compared with WT mice, and the levels of total cholesterol and LDL cholesterol were significantly reduced with WT-BM transplantation but not with eNOS$^{-/-}$-BM transplantation (Online Table I). In contrast, the plasma levels of HDL cholesterol were significantly higher in control eNOS$^{-/-}$ mice than in control WT mice and were unchanged with the BM transplantation (Online Table I).

Visceral Fat and Plasma Levels of Adiponectin

The size of epididymal white adipose cells, one of the representative visceral fats, was significantly larger in control eNOS$^{-/-}$ mice than in control WT mice and was diminished when transplanted with WT-BM but not with eNOS$^{-/-}$-BM (Figure 4A and 4B). We also performed immunostaining for the F4/80 antigen, a specific marker for mature macrophages, and calculated the percentage of F4/80-expressing cells in the epididymal white adipose tissue. The percentage was significantly higher in eNOS$^{-/-}$ mice than in WT mice, and aggregates of F4/80-expressing cells were noted in control eNOS$^{-/-}$ but not in control WT mice (Figure 4A through 4C). The percentage was significantly reduced with WT-BM transplantation but not with eNOS$^{-/-}$-BM transplantation (Figure 4A and 4C). Plasma adiponectin level was significantly reduced in eNOS$^{-/-}$ mice compared with WT mice and was significantly improved with WT-BM transplantation but not with eNOS$^{-/-}$-BM transplantation (Figure 4D).

Experiments With eNOS$^{-/-}$/Adiponectin$^{-/-}$ Mice

We also performed the experiments with eNOS$^{-/-}$/APN$^{-/-}$ mice with or without WT-BM transplantation. Unlike eNOS$^{-/-}$ mice, WT-BM transplantation had no beneficial effects on endothelium-dependent relaxations of mesenteric arteries of eNOS$^{-/-}$/APN$^{-/-}$ mice (Figure 5A and 5B). Interestingly, WT-BM transplantation to eNOS$^{-/-}$/APN$^{-/-}$

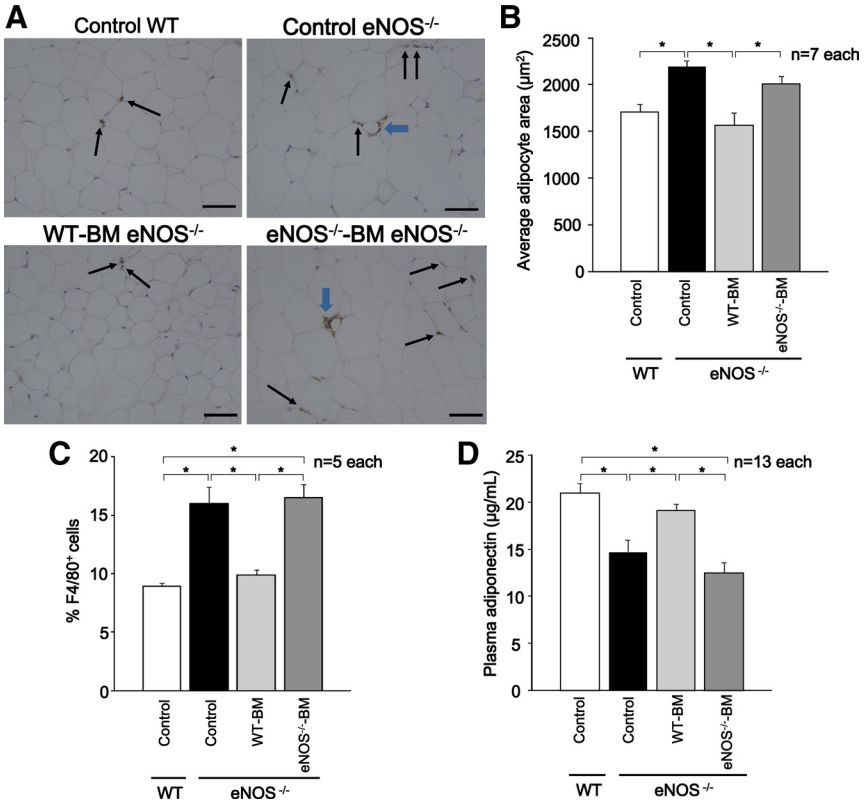

Figure 4. Effects of BM transplantation on the visceral fat and plasma levels of adiponectin in eNOS$^{-/-}$ mice. A, Immunostaining for F4/80, a specific marker for mature macrophages, in the epididymal adipose tissue from the 4 groups. While the adipose tissue contained F4/80-expressing cells in all groups (**black arrows**), aggregates of F4/80-expressing cells (**blue arrows**) were noted in control eNOS$^{-/-}$ but not in control WT mice. Bar, 50 μm. **B**, The size of epididymal white adipose cells was significantly larger in control eNOS$^{-/-}$ mice than in control WT mice. The cell size in eNOS$^{-/-}$ mice was diminished when transplanted with WT-BM but not with eNOS$^{-/-}$-BM. **C**, The percentage of F4/80-expressing cells in the epididymal white adipose tissue was higher in control eNOS$^{-/-}$ mice than in control WT mice. The percentage was significantly reduced with WT-BM transplantation but not with eNOS$^{-/-}$-BM transplantation. **D**, Plasma adiponectin levels were significantly reduced in control eNOS$^{-/-}$ mice compared with control WT mice and were significantly improved with WT-BM transplantation but not with eNOS$^{-/-}$-BM transplantation. Results are expressed as mean±SEM. *P<0.05.

mice significantly enhanced indomethacin-sensitive endothelium-dependent relaxations, suggesting an upregulation of vasodilator prostaglandins (Figure 5 A and 5B). Endothelium-independent relaxations of mesenteric arteries to NS1619 or SNP were unaltered with WT-BM transplantation in eNOS$^{-/-}$/APN$^{-/-}$ mice (Online Figure VII). The impaired glucose tolerance in eNOS$^{-/-}$/APN$^{-/-}$ mice was also unaltered with WT-BM transplantation (Figure 5C). Plasma adiponectin was not detected in control eNOS$^{-/-}$/APN$^{-/-}$ or WT-BM eNOS$^{-/-}$/APN$^{-/-}$ (Figure 5D).

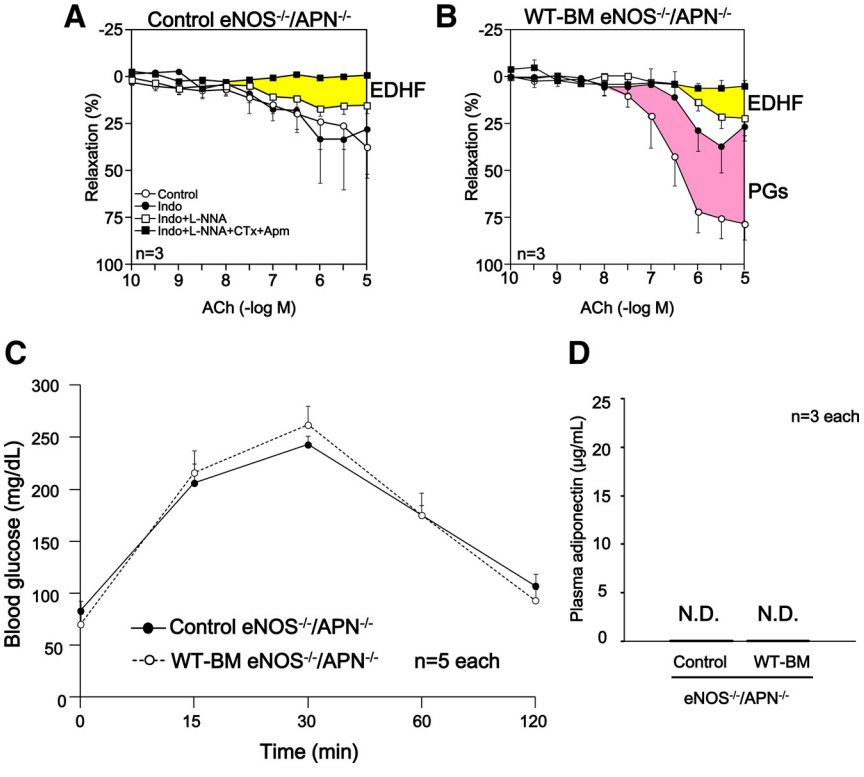

Figure 5. Effect of WT-BM transplantation on endothelium-dependent relaxations and glucose tolerance in eNOS$^{-/-}$/adiponectin$^{-/-}$ mice. A, EDHF-mediated relaxations of mesenteric arteries were reduced in untreated eNOS$^{-/-}$/adiponectin$^{-/-}$ mice. **B**, WT-BM transplantation had no effects on the EDHF-mediated relaxations but significantly enhanced indomethacin-sensitive endothelium-dependent relaxations in eNOS$^{-/-}$/adiponectin$^{-/-}$ mice, suggesting an upregulation of vasodilator prostaglandins. **C**, WT-BM transplantation had no effects on glucose tolerance in eNOS$^{-/-}$/adiponectin$^{-/-}$ mice. **D**, Plasma adiponectin was not detected in control eNOS$^{-/-}$/adiponectin$^{-/-}$ or WT-BM eNOS$^{-/-}$/adiponectin$^{-/-}$ mice. N.D. indicates not detected. Results are expressed as mean±SEM.

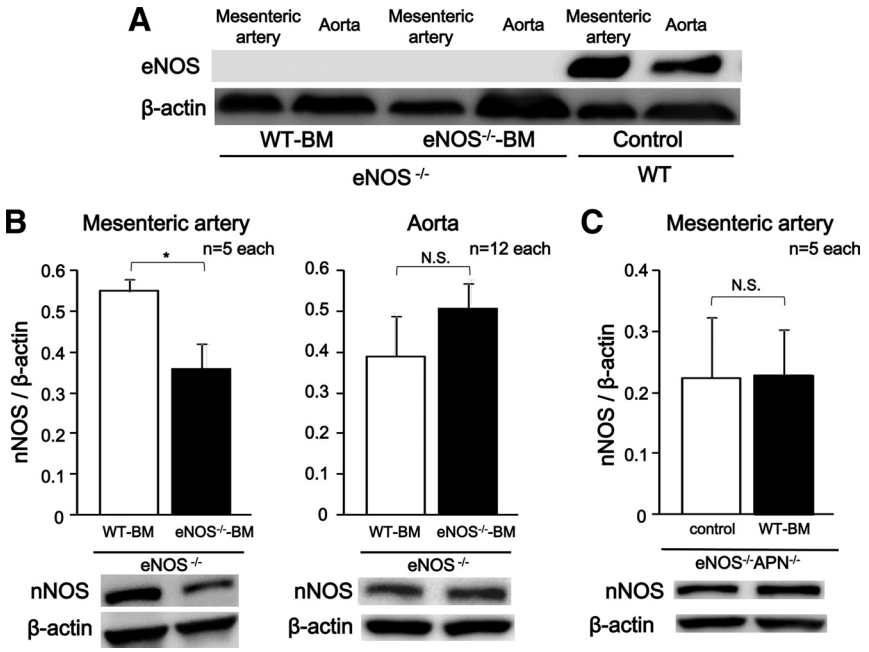

Figure 6. WT-BM transplantation enhances nNOS protein expression in eNOS$^{-/-}$ mice but not in eNOS$^{-/-}$/APN$^{-/-}$ mice. A, eNOS protein expression was noted in WT mice but was absent in eNOS$^{-/-}$ mice transplanted with either WT-BM or eNOS$^{-/-}$-BM. **B**, WT-BM transplantation significantly enhanced nNOS protein expression in mesenteric arteries but not in the aorta. **C**, WT-BM transplantation did not enhance nNOS protein expression in eNOS$^{-/-}$/APN$^{-/-}$ mice. Results are expressed as mean±SEM. *$P<0.05$.

Protein Expression of eNOS and nNOS

As expected, eNOS protein expression of mesenteric arteries was absent in eNOS$^{-/-}$ mice transplanted with WT-BM or eNOS$^{-/-}$-BM (Figure 6A). In contrast, nNOS protein expression of mesenteric arteries was significantly greater in eNOS$^{-/-}$ mice transplanted with WT-BM compared with those with eNOS$^{-/-}$-BM, whereas it was comparable in the aorta (Figure 6B). In eNOS$^{-/-}$/APN$^{-/-}$ mice, WT-BM transplantation did not alter nNOS protein expression of mesenteric arteries (Figure 6C).

Experiments With n/eNOS$^{-/-}$ Mice

We also performed the experiments with n/eNOS$^{-/-}$ mice with or without WT-BM transplantation. Unlike eNOS$^{-/-}$ mice, WT-BM transplantations caused no beneficial effects on endothelium-dependent relaxations of mesenteric arteries (Figure 7A and 7B). In contrast, WT-BM transplantation to n/eNOS$^{-/-}$ mice tended to enhance indomethacin-sensitive endothelium-dependent relaxations, suggesting an upregulation of vasodilator prostaglandins (Figure 7A and 7B). Endothelium-independent relaxations of mesenteric arteries to NS1619 or SNP were unaltered with WT-BM transplantation in n/eNOS$^{-/-}$ mice (Online Figure VIII, A and B).

Effect of Adiponectin Treatment to eNOS$^{-/-}$ Mice

We intraperitoneally injected recombinant mouse adiponectin (500 μg/kg BW) or PBS into male eNOS$^{-/-}$ mice at the age of 13 weeks once daily for 7 days. Treatment with adiponectin significantly improved EDHF-mediated relaxations in mesenteric arteries compared with PBS-treated mice (Figure 8A and 8B). In contrast, the adiponectin treatment had no significant effects in the aorta (Figure 8C and 8D). Endothelium-independent relaxations of mesenteric arteries to NS1619 or SNP were comparable between adiponectin-treated and PBS-treated groups (Online Figure IX).

Discussion

The major findings of the present study were as follows: (1) eNOS$^{-/-}$ mice were characterized by absence of NO-mediated relaxations and reduced EDHF-mediated relaxations associated with impaired glucose tolerance and dyslipidemia, (2) WT-BM transplantation to eNOS$^{-/-}$ mice almost normalized EDHF-mediated endothelium-dependent relaxations of mesenteric arteries and glucose tolerance, (3) the enhancing effects of WT-BM transplantation on the EDHF-mediated responses were mediated primarily by endothelium-derived H_2O_2, and (4) the beneficial effects of WT-BM

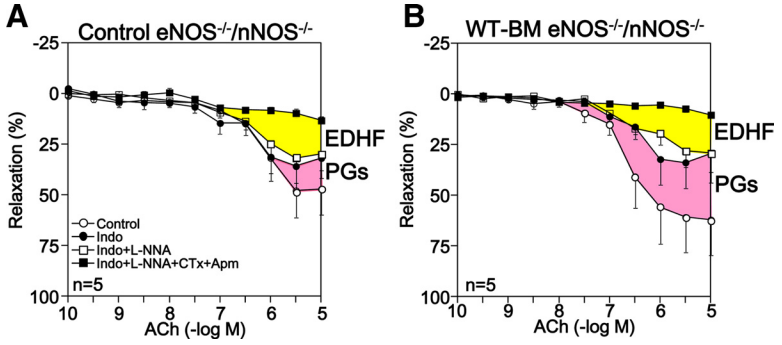

Figure 7. No effect of WT-BM transplantation in eNOS$^{-/-}$/nNOS$^{-/-}$ mice. A, EDHF-mediated relaxations were reduced in untreated eNOS$^{-/-}$/nNOS$^{-/-}$ mice. **B**, WT-BM transplantation had no effect on EDHF-mediated relaxations to ACh of mesenteric arteries from eNOS$^{-/-}$/nNOS$^{-/-}$ mice. Results are expressed as mean±SEM.

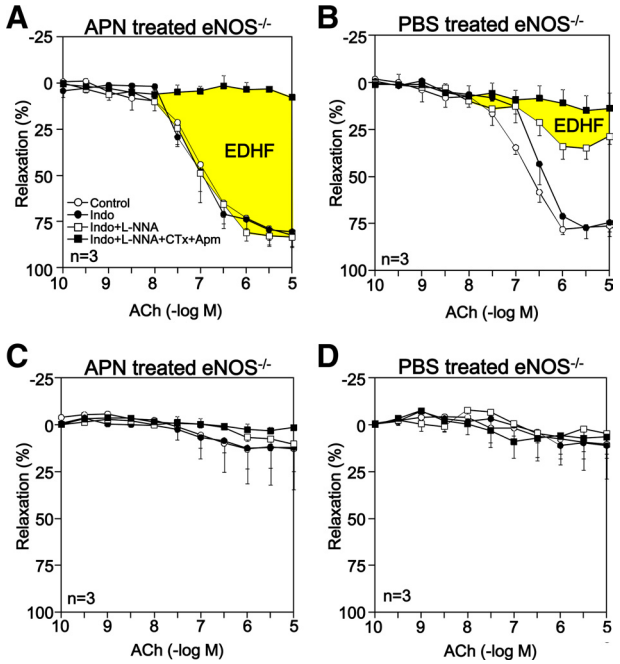

Figure 8. Effect of adiponectin treatment on endothelium-dependent relaxations in eNOS$^{-/-}$ mice. Intraperitoneal adiponectin treatment improved EDHF-mediated relaxations of mesenteric artery in eNOS$^{-/-}$ mice compared with PBS treatment groups (**A** and **B**). On the other hand, it had no effect to aorta (**C** and **D**).

transplantation on EDHF-mediated responses in eNOS$^{-/-}$ mice were mediated by adiponectin and nNOS. To the best of our knowledge, this is the first study that demonstrates that BM plays an essential role for microvascular endothelial and metabolic functions, for which adiponectin and nNOS are substantially involved.

Effects of WT-BM Transplantation

In eNOS$^{-/-}$ mice, WT-BM transplantation markedly improved and almost normalized EDHF-mediated and endothelium-dependent relaxations, whereas eNOS$^{-/-}$-BM transplantation was without the effects. We have previously demonstrated that EDHF-mediated relaxations and hyperpolarizations of mesenteric arteries are markedly reduced (but not absent) in eNOS$^{-/-}$ mice, where the remaining 2 NOSs (nNOS and iNOS) play a compensatory role.[10,20] It is known that eNOS generates superoxide anions under normal conditions from reductase domain, and only when pathologically uncoupled (eg, depletion of BH4 and/or L-arginine) it generates superoxide anions from oxidase domain and that L-arginine analogues inhibits the latter process alone.[35] We have previously reported that H_2O_2 as an EDHF is generated from reductase domain of eNOS, which is not affected by L-arginine analogues.[20] Several studies have previously addressed the effects of BM transplantation; eNOS$^{-/-}$-BM transplantation to WT mice was found to aggravate vascular lesions in a mouse model of carotid artery ligation,[36] BM-derived EPCs from eNOS$^{-/-}$ mice showed reduced angiogenic effects compared with EPCs from WT mice,[37] and WT-BM transplantation to eNOS$^{-/-}$ mice ameliorated cardiac fibrosis and capillary density in pressure overload.[38] Taken together with the present results, it is highly possible that BM plays an important role in modulating microvascular endothelial functions.

The mechanisms of differentiation of BM-derived EPCs have been extensively investigated[39,40]; however, it has been recently demonstrated that BM-derived cells do not differentiate into vascular endothelial or smooth muscle cells in mice.[27–30] In the present study, we also were unable to observe the presence of BM-derived cells in the vascular wall of eNOS$^{-/-}$ mice transplanted with GFP-positive BM or eNOS protein expression in mesenteric arteries of eNOS$^{-/-}$ mice transplanted with WT-BM. Furthermore, WT-BM transplantation did not improve endothelial-dependent relaxations of the aorta from eNOS$^{-/-}$ mice, although it markedly improved the responses of mesenteric arteries. Thus, we consider that the improvement of endothelium-dependent relaxations of mesenteric artery from eNOS$^{-/-}$ mice by WT-BM transplantation was mediated by humoral factor(s) but not by differentiation of EPCs or other BM-derived progenitor cells to endothelial cells.

Roles of Adiponectin

Adiponectin is an abundant plasma protein specifically secreted by adipocytes,[41] playing an important role in the regulation of glucose and lipid metabolism.[42] The serum levels of adiponectin are inversely correlated with the extent of visceral fat.[43] Moreover, hypoadiponectinemia is associated with reduced endothelium-dependent dilatation in both diabetic and nondiabetic human subjects.[44] We and others showed that eNOS$^{-/-}$ mice had low serum levels of adiponectin and hypertrophic visceral adipose cells.[21,45] Interestingly, in the present study, WT-BM transplantation to eNOS$^{-/-}$ mice decreased the size of visceral adipose cells and increased plasma levels of adiponectin, whereas WT-BM transplantation had no effect on the adiponectin level in control eNOS$^{-/-}$/APN$^{-/-}$ or WT-BM eNOS$^{-/-}$/APN$^{-/-}$ mice. Furthermore, the F4/80 antigen, a marker for mature mouse macrophages, was highly expressed in epididymal white adipose tissue of eNOS$^{-/-}$ mice compared with WT mice and WT-BM transplantation to eNOS$^{-/-}$ mice decreased these expressions. Obesity and metabolic syndrome are associated with macrophage infiltration in adipose tissue.[46,47] Macrophage-specific expression of adiponectin suppresses the development of obesity and macrophage infiltration to adipose tissue and improved insulin sensitivity in mice.[48] Transgenic mice expressing monocyte chemoattractant protein-1 (MCP-1) in the adipose tissue exhibit enhanced macrophage infiltration and insulin resistance, whereas in MCP-1–deficient mice, macrophage accumulation in the adipose tissue, insulin resistance, and hepatic steatosis are improved associated with increased serum levels of adiponectin.[34] These results suggest the metabolic syndrome–like phenotypes of eNOS$^{-/-}$ mice is caused by reduced adiponectin levels and enhanced macrophage infiltration, both of which are related to reduced eNOS functions.

Roles of nNOS

Adiponectin promotes phosphorylation of AMP-activated protein kinase, protein kinase Akt/protein kinase B, and eNOS in HUVEC in vitro.[49] The eNOS expression in the aorta from adiponectin$^{-/-}$ mice is decreased in vivo, whereas

adenovirus-mediated delivery of adiponectin increases eNOS expression in the aorta from KK-Ay mice (a mouse model for type 2 diabetes) and adiponectin$^{-/-}$ mice.[50] These results indicate that adiponectin enhances eNOS expression and activity. In the present study, nNOS expression in mesenteric arteries was enhanced in eNOS$^{-/-}$ mice when transplanted with WT-BM but not with eNOS$^{-/-}$-BM. nNOS is usually expressed in nerve tissues but is also constitutively expressed in endothelial cells as is eNOS.[51] Especially when expression and activity of eNOS are reduced, nNOS (and iNOS) exerts compensatory effects.[52,53] Indeed, EDHF-mediated and endothelium-dependent relaxations were totally absent only when all 3 NOSs were deleted in mice.[20]

To confirm our hypothesis on the roles of adiponectin and nNOS, we also performed experiments with eNOS$^{-/-}$/APN$^{-/-}$ mice and eNOS$^{-/-}$/nNOS$^{-/-}$ mice. In both mice, endothelium-dependent and EDHF-mediated relaxations of mesenteric arteries were reduced compared with control eNOS$^{-/-}$ mice. Importantly, unlike eNOS$^{-/-}$ mice, WT-BM transplantation failed to improve EDHF-mediated relaxations in those mice. Furthermore, the treatment with recombinant adiponectin improved EDHF-mediated relaxations in eNOS$^{-/-}$ mice as did WT-BM transplantation. In addition, in eNOS$^{-/-}$/APN$^{-/-}$ mice, WT-BM transplantation enhanced relaxations mediated by vasodilator PGs. These results suggest that both adiponectin-dependent and adiponectin-independent mechanisms are involved in the effects of WT-BM transplantation in eNOS$^{-/-}$ mice.

Although it remains to be examined whether adiponectin also enhances the expression and activity of nNOS as in the case of eNOS, it is conceivable that increased adiponectin levels by WT-BM transplantation may upregulate nNOS in the endothelium of mesenteric arteries from eNOS$^{-/-}$ mice, enhancing endothelium-dependent and EDHF-mediated relaxations. Although the molecular mechanism of nNOS upregulation by adiponectin remains to be elucidated, it is conceivable that anti-inflammatory effects of adiponectin and those of eNOS-derived H_2O_2/EDHF may be involved.

Study Limitations

Several limitations should be mentioned for the present study. First, although we were able to elucidate the important roles of adiponectin and nNOS in the beneficial effects of WT-BM transplantation in eNOS$^{-/-}$ mice, the detailed molecular mechanisms remain to be elucidated. Second, it remains to be determined why WT-BM transplantation had no beneficial effects on endothelial function of the aorta. Third, although WT-BM transplantation improved EDHF-mediated relaxations in eNOS$^{-/-}$ mice, blood pressure was not improved. Several possibilities should be considered to explain this observation, including the limitation of tail-cuff method to measure blood pressure and the partial improvement of the responses as compared with genetic modulation.[13] Fourth, in the present study, WT-BM transplantation improved (almost normalized) microvascular endothelial functions, glucose tolerance, lipid profiles, adiponectin levels, and macrophage infiltration in the visceral adipose tissue, the cause-result relationships among those changes remain to be established. In addition, the background underlying these changes (eg, renin-angiotensin system and oxidative stress) remains to be examined.

Clinical Implications

The present study has important clinical implications. eNOS$^{-/-}$ mice are characterized by the phenotypes resembling metabolic syndrome in humans[21,54,55] and thus could be regarded as an animal model of atherosclerosis and metabolic disorders. Since WT-BM transplantation almost normalized microvascular endothelial and metabolic functions, the present results suggest that BM function could be a new therapeutic target of cardiovascular and metabolic disorders. It remains to be examined in future studies what therapeutic options could improve BM functions in atherosclerotic animals models as well as patients with athero-metabolic disorders.

Conclusions

The present study provides the first evidence that BM plays an important role in modulating microvascular endothelial and metabolic functions, for which adiponectin and nNOS may be involved.

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Disclosures

None.

References

1. Shimokawa H. Primary endothelial dysfunction: atherosclerosis. *J Mol Cell Cardiol*. 1999;31:23–37.
2. Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH. EDHF: bringing the concepts together. *Trends Pharmacol Sci*. 2002;23:374–380.
3. Feletou M, Vanhoutte PM. Endothelium-derived hyperpolarizing factor: where are we now? *Arterioscler Thromb Vasc Biol*. 2006;26:1215–1225.
4. Shimokawa H, Yasutake H, Fujii K, Owada MK, Nakaike R, Fukumoto Y, Takayanagi T, Nagao T, Egashira K, Fujishima M. The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. *J Cardiovasc Pharmacol*. 1996;28:703–711.
5. Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, Takeshita A. Importance of endothelium-derived hyperpolarizing factor in human arteries. *J Clin Invest*. 1997;100:2793–2799.
6. Vitale C, Mercuro G, Cornoldi A, Fini M, Volterrani M, Rosano G. Metformin improves endothelial function in patients with metabolic syndrome. *J Intern Med*. 2005;258:250–256.
7. De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. *Br J Pharmacol*. 2000;130:963–974.
8. d'Uscio LV, Smith LA, Katusic ZS. Hypercholesterolemia impairs endothelium-dependent relaxations in common carotid arteries of apolipoprotein E-deficient mice. *Stroke*. 2001;32:2658–2664.
9. Hosoya M, Ohashi J, Sawada A, Takaki A, Shimokawa H. Combination therapy with olmesartan and azelnidipine improves EDHF-mediated responses in diabetic apolipoprotein e-deficient mice. *Circ J*. 2010;74:798–806.

10. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J Clin Invest*. 2000;106: 1521–1530.
11. Matoba T, Shimokawa H, Kubota H, Morikawa K, Fujiki T, Kunihiro I, Mukai Y, Hirakawa Y, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. *Biochem Biophys Res Commun*. 2002;290:909–913.
12. Matoba T, Shimokawa H, Morikawa K, Kubota H, Kunihiro I, Urakami-Harasawa L, Mukai Y, Hirakawa Y, Akaike T, Takeshita A. Electron spin resonance detection of hydrogen peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary microvessels. *Arterioscler Thromb Vasc Biol*. 2003;23:1224–1230.
13. Prysyazhna O, Rudyk O, Eaton P. Single atom substitution in mouse protein kinase G eliminates oxidant sensing to cause hypertension. *Nat Med*. 2012;18:286–290.
14. Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, Gutterman DD. Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. *Circ Res*. 2003;92:e31–e40.
15. Yada T, Shimokawa H, Hiramatsu O, Kajita T, Shigeto F, Goto M, Ogasawara Y, Kajiya F. Hydrogen peroxide, an endogenous endothelium-derived hyperpolarizing factor, plays an important role in coronary autoregulation in vivo. *Circulation*. 2003;107:1040–1045.
16. Yada T, Shimokawa H, Hiramatsu O, Haruna Y, Morita Y, Kashihara N, Shinozaki Y, Mori H, Goto M, Ogasawara Y. Cardioprotective role of endogenous hydrogen peroxide during ischemia-reperfusion injury in canine coronary microcirculation in vivo. *Am J Physiol Heart Circ Physiol*. 2006;291:H1138–H1146.
17. Lacza Z, Puskar M, Kis B, Perciaccante JV, Miller AW, Busija DW. Hydrogen peroxide acts as an EDHF in the piglet pial vasculature in response to bradykinin. *Am J Physiol Heart Circ Physiol*. 2002;283: H406–H411.
18. Morikawa K, Shimokawa H, Matoba T, Kubota H, Akaike T, Talukder MA, Hatanaka M, Fujiki T, Maeda H, Takahashi S, Takeshita A. Pivotal role of Cu,Zn-superoxide dismutase in endothelium-dependent hyperpolarization. *J Clin Invest*. 2003;112:1871–1879.
19. Morikawa K, Fujiki T, Matoba T, Kubota H, Hatanaka M, Takahashi S, Shimokawa H. Important role of superoxide dismutase in EDHF-mediated responses of human mesenteric arteries. *J Cardiovasc Pharmacol*. 2004;44:552–556.
20. Takaki A, Morikawa K, Tsutsui M, Murayama Y, Tekes E, Yamagishi H, Ohashi J, Yada T, Yanagihara N, Shimokawa H. Crucial role of nitric oxide synthases system in endothelium-dependent hyperpolarization in mice. *J Exp Med*. 2008;205:2053–2063.
21. Nakata S, Tsutsui M, Shimokawa H, Suda O, Morishita T, Shibata K, Yatera Y, Sabanai K, Tanimoto A, Nagasaki M, Tasaki H, Sasaguri Y, Nakashima Y, Otsuji Y, Yanagihara N. Spontaneous myocardial infarction in mice lacking all nitric oxide synthase isoforms. *Circulation*. 2008;117:2211–2223.
22. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275:964–967.
23. Björkerud S, Björkerud B. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and t cells), and may contribute to the accumulation of gruel and plaque instability. *Am J Pathol*. 1996;149:367–380.
24. Schmidt-Lucke C, Rossig L, Fichtlscherer S, Vasa M, Britten M, Kamper U, Dimmeler S, Zeiher AM. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation*. 2005;111:2981–2987.
25. Kikuchi Y, Yasuda S, Aizawa K, Tsuburaya R, Ito Y, Takeda M, Nakayama M, Ito K, Takahashi J, Shimokawa H. Enhanced rho-kinase activity in circulating neutrophils of patients with vasospastic angina: a possible biomarker for diagnosis and disease activity assessment. *J Am Coll Cardiol*. 2011;58:1231–1237.
26. Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, Silver M, Ma H, Kearney M, Isner JM. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation*. 2001;103:634–637.
27. Bentzon JF, Weile C, Sondergaard CS, Hindkjaer J, Kassem M, Falk E. Smooth muscle cells in atherosclerosis originate from the local vessel wall and not circulating progenitor cells in apoE knockout mice. *Arterioscler Thromb Vasc Biol*. 2006;26:2696–2702.
28. Bentzon JF, Sondergaard CS, Kassem M, Falk E. Smooth muscle cells healing atherosclerotic plaque disruptions are of local, not blood, origin in apolipoprotein e knockout mice. *Circulation*. 2007;116: 2053–2061.
29. Hagensen MK, Shim J, Thim T, Falk E, Bentzon JF. Circulating endothelial progenitor cells do not contribute to plaque endothelium in murine atherosclerosis. *Circulation*. 2010;121:898–905.
30. Perry TE, Song M, Despres DJ, Kim SM, San H, Yu ZX, Raghavachari N, Schnermann J, Cannon RO III, Orlic D. Bone marrow-derived cells do not repair endothelium in a mouse model of chronic endothelial cell dysfunction. *Cardiovasc Res*. 2009;84:317–325.
31. Morishita T, Tsutsui M, Shimokawa H, Sabanai K, Tasaki H, Suda O, Nakata S, Tanimoto A, Wang K, Ueta Y. Nephrogenic diabetes insipidus in mice lacking all nitric oxide synthase isoforms. *Proc Natl Acad Sci U S A*. 2005;102:10616–10621.
32. Nakano M, Fukumoto Y, Satoh K, Ito Y, Kagaya Y, Ishii N, Sugamura K, Shimokawa H. Ox40 ligand plays an important role in the development of atherosclerosis through vasa vasorum neovascularization. *Cardiovasc Res*. 2010;88:539–546.
33. Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y. Green mice as a source of ubiquitous green cells. *FEBS Lett*. 1997;407:313–319.
34. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. Mcp-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest*. 2006;116:1494–1505.
35. Stuehr D, Pou S, Rosen GM. Oxygen reduction by nitric-oxide synthases. *J Biol Chem*. 2001;276:14533–14536.
36. Furuno Y, Morishita T, Toyohira Y, et al. Crucial vasculoprotective role of the whole nitric oxide synthase system in vascular lesion formation in mice: involvement of bone marrow-derived cells. *Nitric Oxide*. 2011;25: 350–359.
37. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med*. 2003;9:1370–1376.
38. Kazakov A, Muller P, Jagoda P, Semenov A, Bohm M, Laufs U. Endothelial nitric oxide synthase of the bone marrow regulates myocardial hypertrophy, fibrosis, and angiogenesis. *Cardiovasc Res*. 2012;93: 397–405.
39. Wassmann S, Werner N, Czech T, Nickenig G. Improvement of endothelial function by systemic transfusion of vascular progenitor cells. *Circ Res*. 2006;99:e74–e83.
40. Chen J, Li H, Addabbo F, Zhang F, Pelger E, Patschan D, Park HC, Kuo MC, Ni J, Gobe G. Adoptive transfer of syngeneic bone marrow-derived cells in mice with obesity-induced diabetes: selenoorganic antioxidant ebselen restores stem cell competence. *Am J Pathol*. 2009;174:701–711.
41. Hu E, Liang P, Spiegelman BM. Adipoq is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem*. 1996;271:10697–10703.
42. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein acrp30 enhances hepatic insulin action. *Nat Med*. 2001; 7:947–953.
43. Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT. Adiponectin multimeric complexes and the metabolic syndrome trait cluster. *Diabetes*. 2006;55:249–259.
44. Ouchi N, Ohishi M, Kihara S, et al. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension*. 2003;42:231–234.
45. Koh EH, Kim M, Ranjan KC, Kim HS, Park HS, Oh KS, Park IS, Lee WJ, Kim MS, Park JY, Youn JH, Lee KU. ENOS plays a major role in adiponectin synthesis in adipocytes. *Am J Physiol Endocrinol Metab*. 2010;298:E846–E853.
46. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112:1796–1808.
47. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest*. 2007;117: 175–184.
48. Luo N, Liu J, Chung BH, Yang Q, Klein RL, Garvey WT, Fu Y. Macrophage adiponectin expression improves insulin sensitivity and protects against inflammation and atherosclerosis. *Diabetes*. 2010;59: 791–799.
49. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, Funahashi T, Walsh K. Adiponectin stimulates angiogenesis by promoting cross-talk

between amp-activated protein kinase and akt signaling in endothelial cells. *J Biol Chem*. 2004;279:1304–1309.
50. Ohashi K, Kihara S, Ouchi N, Kumada M, Fujita K, Hiuge A, Hibuse T, Ryo M, Nishizawa H, Maeda N. Adiponectin replenishment ameliorates obesity-related hypertension. *Hypertension*. 2006;47:1108–1116.
51. Bachetti T, Comini L, Curello S, Bastianon D, Palmieri M, Bresciani G, Callea F, Ferrari R. Co-expression and modulation of neuronal and endothelial nitric oxide synthase in human endothelial cells. *J Mol Cell Cardiol*. 2004;37:939–945.
52. Huang A, Sun D, Shesely EG, Levee EM, Koller A, Kaley G. Neuronal NOS-dependent dilation to flow in coronary arteries of male ENOS-KO mice. *Am J Physiol Heart Circ Physiol*. 2002;282:H429–H436.
53. Chlopicki S, Kozlovski VI, Lorkowska B, Drelicharz L, Gebska A. Compensation of endothelium-dependent responses in coronary circulation of ENOS-deficient mice. *J Cardiovasc Pharmacol*. 2005;46:115–123.
54. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, Vollenweider P, Pedrazzini T, Nicod P, Thorens B, Scherrer U. Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation*. 2001;104:342–345.
55. Cook S, Hugli O, Egli M, Vollenweider P, Burcelin R, Nicod P, Thorens B, Scherrer U. Clustering of cardiovascular risk factors mimicking the human metabolic syndrome X in ENOS null mice. *Swiss Med Wkly*. 2003;133:360–363.

Novelty and Significance

What Is Known?

- The endothelium plays an important role in vascular homeostasis by synthesizing and releasing vasodilator prostaglandins, nitric oxide, and endothelium-derived hyperpolarizing factor (EDHF).
- Endothelial nitric oxide synthases (eNOS)-derived hydrogen peroxide (H_2O_2) is a major source of EDHF in microvessels of animals and humans.
- Several leukocyte populations contribute to the pathogenesis of cardiovascular diseases.

What New Information Dose This Article Contribute?

- Transplantation of wild-type bone marrow to eNOS-deficient mice improves endothelial vasodilator functions in small mesenteric arteries that are mediated by EDHF/H_2O_2 derived from neuronal NO synthase (nNOS).
- Wild-type bone marrow transplantation in eNOS-deficient mice improves glucose intolerance and dyslipidemia.
- These beneficial effects of wild-type bone marrow transplantation are mediated by adiponectin and nNOS as they are abolished in eNOS/adiponectin-deficient or eNOS/nNOS-deficient mice.

The endothelium plays an important role in maintaining vascular homeostasis by synthesizing and releasing endothelium-derived relaxing factors, including vasodilator prostaglandins, NO, and EDHF. The contribution of NO increases as the vessel size becomes larger, and, conversely, that of EDHF increases as the vessel size becomes smaller. Microvascular endothelial functions are closely linked to cardiovascular and metabolic diseases. In this study, we demonstrated that transplantation of wild-type bone marrow equals enhanced EDHF-mediated endothelial functions in eNOS-deficient mice through EDHF/H_2O_2 derived from nNOS. Moreover, transplantation of bone marrow from wild-type mice also improved glucose intolerance and dyslipidemia in eNOS-deficient mice. Importantly, the beneficial effects of wild-type bone marrow transplantation were abolished in eNOS/adiponectin- or eNOS/nNOS-deficient mice, indicating important roles of adiponectin and nNOS. Thus, the present findings provide new evidence that bone marrow plays an important role in modulating microvascular endothelial and metabolic functions in the organ network involving bone marrow, fat tissue, and blood vessels. We suggest that bone marrow functions could be a new therapeutic target in management of cardiovascular and metabolic disorders.