Introduction

Evidences from in vitro and in vivo studies established that hyperhomocysteinemia, an elevated homocysteine level, is associated with vascular remodeling [1-3] and dysfunction [4-5]. Although the exact mechanism of homocysteine-induced vascular dysfunction is not clear and may be complex, one of the well documented mechanisms is oxidative stress [6]. Among the sources of oxidative radicals, NADPH oxidase plays a major role in hyperhomocysteinemia and causes changes in vascular phenotype including endothelial dysfunction [7], smooth muscle proliferation [8], and accumulation of collagen in the extracellular matrix (ECM) [9] leading to remodeling of the vessels [10].

Remodeling is a process of degradation and/or accumulation of matrix protein including collagen and elastin [11]. This is an active mechanism by which vessels are formed, maintain their integrity and repair in the event of injury [12]. While this mechanism is a part of normal physiological process in the healthy vessels, dysregulated remodeling causes vascular diseases, such as atherosclerosis and stroke [13].

Remodeling of the vessels is largely regulated by a family of calcium-dependent and zinc containing endopeptidases known as matrix metalloproteinases (MMPs) [14]. The MMPs are secreted in an inactive (latent) form called a zymogen or a pro-MMP [15]. These pro-MMPs require an activation step before they are able to cleave extracellular matrix component [14]. Reactive oxygen species (ROS) are a group of oxidative radicals which activates MMPs and initiates degradation of matrix protein in various pathological conditions including arthritis, cancer and vascular diseases [16-18]. Of particular interests MMP-2 and -9 are activated by ROS.

Original Article

Remodeling in vein expresses arterial phenotype in hyperhomocysteinemia

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Received October 6, 2011; accepted October 26, 2011; Epub November 15, 2011; Published December 15, 2011

Abstract: Accumulating evidences suggest that homocysteine, a non-protein amino acid, is involved in vessel remodeling and blood flow at elevated level, although the exact mechanism is unclear. Here we hypothesized that homocysteine affects vein in such a way that vein develops arterial phenotype. We tested our hypothesis employing wild type (WT, C57BL/6J) and CBS+/- (cystathionine β-synthase heterozygote, a genetic model of hyperhomocysteinemia) supplemented with or without folic acid (FA, a homocysteine lowering agent). Vena cava blood flow was measured by ultrasound transonic flow probe. Tissue collagen and elastin were detected by histochemistry. Super oxide was detected by dihydroethidium (DHE) staining. Expressions of MMP-2, -9, -12, TIMP-2, -4, were measured by Western blot. MMP-13, TIMP-1, -3, and vein and aortic markers, EphB4 and EphrinB2, respectively were measured by RT-PCR. The results indicated relatively low blood flow and significant increase of collagen/elastin ratio in the CBS+/- mice compared to WT. Although FA treatment did not alter blood flow in CBS+/- mice, the collagen/elastin ratio was normalized. A relatively increased content of super oxide and gelatinase activity was observed in CBS+/- vena cava vs WT and normalized by FA treatment. Western blot analyses showed significant increase in MMP-9, -12 and decrease in TIMP-2, -4 expressions. Expressions of MMP-13, TIMP-1 and -3, Ephrin B2 were increased, whereas EphB4 was decreased with reverse change in FA treatment, with no change in MMP-13 and TIMP-1. We conclude that chronic HHcy causes vascular remodeling that expresses arterial phenotype in vein.

Keywords: Homocysteine, vascular remodeling, matrix, metalloproteinase, collagen, elastin
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and reported to become involved in atherosclerotic vascular diseases associated with homocysteine-mediated generation of oxidative stress [9, 19].

Endogenously, these MMPs are primarily regulated by a class of inhibitory enzymes named tissue inhibitor of matrix metalloproteinase (TIMPs) [20]. TIMPs may be secreted by the cells or they may be associated with membrane bound MMPs [17]. The integrity of vessels is largely dependent on the balance between MMPs and TIMPs [15]. Dysregulated MMP/TIMP balance leads to degradation of matrix protein and is a characteristic of diverse pathological conditions including vascular hypertrophy and dysfunction [21-22].

Although MMPs are involved in tissue remodeling and cell migration, other intrinsic factors, such as Ephrin B2 and EphB4 are well known for their role in remodeling of vessels and proper vascular development [23-30]. While the roles of homocysteine in regulation of MMPs, particularly MMP-2 and -9 in vascular remodeling are well documented, the role of homocysteine in regulation of Ephrin B2 and Eph4 in mature vessels is not studied. We have previously reported that blood flow interplays with elastin/collagen and MMP/TIMP ratios to maintain normal vascular structure and function [31]. In this study we report that in addition to altered blood flow and MMP/TIMP imbalance, homocysteine induces arterial phenotype in vena cava as evidenced by EphB4 upregulation during remodeling process.

Materials and methods

Animal model

C57BL/6J (wild type, WT) and CBS+/- mice of C57 background of ages 14-18 weeks were used for this study. Mice were obtained from Jackson Laboratories Inc, Maine, USA, and housed in the animal care facility of the University of Louisville. CBS+/- and WT mice were divided into four groups and supplemented with or without folic acid (FA, 0.03 g / L) in the water for a period of 45 days. The groups were: 1) WT, 2) WT +FA, 3) CBS+/-, 4) CBS+/- + FA. The mice were fed with regular rodent chow and water supplied ad libitum. At the end of the experiments, animals were deeply anesthetized with Tribromoethanol (TBE, 240 mg/Kg body weight) and sacrificed to harvest the inferior vena cava (VC). All animal procedures were in accordance with the National Institutes of Health guidelines for animal research and were approved by the Institutional Animal Care and Use Committee (IACUC # 09083) of the University of Louisville.

Blood flow measurement

The blood flow measurement was taken in anesthetized condition (TBE, 240 mg/kg b.w.). Abdomen was surgically opened, and a small window was made below the kidney, to isolate the vena cava. A transit time perivascular flow meter (Transonic System Inc, Ithaca, NY), equipped with transonic flow probe 0.5PSB630 was used to measure blood flow in the vena cava. The equipment was calibrated using a standard flow meter in ml/min. The waveform of the blood flow was recorded using DMSI-100 software.

Tissue sectioning and staining

The vena cava was isolated from each experimental groups of animal at the end of experiments. The tissues were cleaned off with phosphate-buffered saline (PBS) containing 20 unit/ml heparin. Heparin was used to remove blood clot. The tissues were then cut into approximately 2 mm pieces, placed vertically in tissue freezing media (Triangle Biomedical Sciences, Inc., Durham, NC) and were frozen in liquid nitrogen. Frozen blocks with the molds were placed in a −70°C freezer until serial sections were made. Cryosections (Leica CM1850) of 7 μm thickness were put on glass slides and stained with one of the following staining kits according to the supplied protocol: Masson Trichrome, van Gieson’s or Hematoxylin and Eosin (Richard Allen Scientific, Kalamazoo, MI).

Measurement of elastin and collagen

Cryosections of vena cava were stained with Masson Trichrome, van Gieson or Hematoxylin Eosin and mounted. Slides were observed under light microscope and images were taken with an attached digital camera. To measure intensity of collagen and elastin staining in the vena cava sections, Un-ScanIt software was used to scan the images and densitometric data were collected. From the scanned densitometric data, elastin : collagen ratio was calculated. Medial thickness was measured in the hema-
toxylin eosin-stained vessels.

Detection of ROS

The oxidative fluorescent dye, dihydroethidium (DHE; Invitrogen, Carlsbad, CA) was used in frozen, 7 µm vena cava sections (40 µmol, 30 mins), and the fluorescence intensity was measured by the laser scanning confocal microscopy (Fluo View 1000, Olympus). Negative control sections were incubated for 25 min with 250 U/ml polyethylene glycol-superoxide dismutase (PEG-SOD; Sigma-Aldrich), before incubation with DHE. Fluorescent images were analyzed with ImagePro software (Media Cybernetics, Bethesda, MD).

Antibodies and reagents

MMP-2 antibody was obtained from Novas Biologicals, Littleton, CO. MMP-9 anti-body was purchased from Abcam Antibodies, Cambridge, MA, and TIMP-2 and TIMP-4 was obtained from Chemicon International, Tamecula, CA. MMP-12 and HRP conjugated secondary antibodies were bought from Santa Cruz Biotechnology, Santa Cruz, CA. All other reagents were used from commercially available highest grade.

Western blotting

Protein from isolated and finely capped vena cava was extracted using RIPA lysis buffer (Thermo Scientific Inc., Rockford, IL), containing protease inhibitors and PMSF. Protein content in the extracted samples was estimated by BCA assay, and 30 µg of total protein was loaded in each well of SDS-PAGE gels. Protein separated by electrophoresis, transferred to PVDF membrane and incubated with primary antibody followed by secondary HRP conjugated antibody. An ECL plus Western blotting detection reagent (GE Health Care, Little Chalfont, Buckinghamshire) was used to detect the protein of interests. For loading control, the membranes were stripped with membrane-stripping buffer (Boston BioProducts, Worcester, MA) and reprobed with GAPDH (Chemicon International). Intensity of bands was detected by Gel –Doc software, and was normalized with their corresponding GAPDH control.

In situ MMP activity

Seven µm frozen sections were incubated with 40 µmol DQ gelatin (Invitrogen) for 2 hours and the fluorescence was measured by the laser scanning confocal microscopy without washing. Fluorescent images were analyzed with ImagePro software.

Isolation of RNA and RT-PCR

Total RNA was isolated from the tissues by using Trizol reagent (Invitrogen) following manufacturer's instructions. Complementary total DNA was made by incubation of RNA with oligo dT at 70°C for 6 min. The RT cycle was 25°C for 2 min, 42°C for 50 min, 75°C for 5 min and 4°C forever as described previously [32]. All the primers were bought from Invitrogen and the primer sequences are shown in Table 1.

Table 1. Sequences of primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sense Sequence</th>
<th>Antisense Sequence</th>
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<tbody>
<tr>
<td>TIMP-1</td>
<td>5'-TCATCGGCGCAGCGGATCT</td>
<td>5'-TAGGCCTCACTGACCTTC</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>5'-GCGCTCCGATGATGATC</td>
<td>5'-TCATCGGCGCAGCGGATCT</td>
</tr>
<tr>
<td>MMP-13</td>
<td>Forward: 5'-AGCCTCTCAGAAAGCTTTC</td>
<td>Reverse: 5'-CCCCCATGTTTTGTCAG</td>
</tr>
<tr>
<td>EphrinB2</td>
<td>5'-TGGAGGAGGAGGTTCGTTG</td>
<td>5'-GAAGAGTGTGAGATGCTCAG</td>
</tr>
<tr>
<td>EphB4</td>
<td>5'-ATGGAGGAGAGTGTGAGATG</td>
<td>5'-CTTTCACATAGCATGTCAG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-GAGGGATCCCGTAAAGTCG</td>
<td>5'-TTCAGGGATCCCGTAAAGTCG</td>
</tr>
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Vascular reactivity study

The vena cava was isolated from the animal and placed in ice cold physiological salt solution (PSS). The recipe of PSS solution was (in mM), NaCl 118, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 12.5 and glucose 11.1, pH 7.4). The vessels were cut into 2-5 mm rings, fat and connective tissues were removed, and mounted with two tungsten wires. Wires were attached to the myobath and placed in a 25 ml organ bath filled with PSS at 37°C. The PSS in the myobath was constantly aerated with 20% O₂, 5% CO₂, and 75% N₂. Rings were stretched gradually to obtain an optimal resting tension of 0.1 g, and equilibrated for an hour. After equilibration dose (10⁻⁶ to 10⁻⁵ M) dependent response of phenylephrine (Phe) was detected. Acetylcholine (Ach) was added to the organ bath in the same manner as Phe to detect endothelial dependent response. Similarly, after washing with PSS, tissues were treated with sodium nitroprusside (SNP) after a brief treatment of Phe (10⁻⁵ M) to measure endothelial-independent...
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Blood flow and medial thickness

Blood flow (ml/min) in the vena cava of WT and WT + FA was similar (Figure 1). In the WT it was 0.22 ± 0.02 ml/min and WT + FA was 0.23 ± 0.03 ml/min. Although in CBS+/- vena cava blood flow showed a tendency to decrease (0.19 ± 0.02 ml/min), the difference was not significant compared to WT. FA very slightly decreased the flow in CBS+/- (0.18 ± 0.02 ml/min) mice compared to CBS+/- alone (Figure 1). Again, no significant deference was observed.

Medial thickness of vena cava in WT was 2.1 X 10^2 ± 0.02 µm and in CBS +/- was 2 X 10^2 ± 0.02 µm. However, the difference was not significant. Similarly, although FA treatment slightly increased medial thickness in CBS+/- vena cava (2.1 X 10^2 ± 0.05 µm) compared to CBS+/-, the difference was not statistically significant (Figure 2).

Statistical analysis

Each experiment was carried out using 4–6 specimens in each group. Paired T-Test was used to determine significance between groups; p < 0.05 was considered significant.

Values were taken as means ± standard deviation of measurement (SD).

Results

Blood flow and medial thickness

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Figure 1. Blood flow in the vena cava. Blood flow was measured by Transonic perivascular flow meter. Data represent mean ± SD, n = 7.

Figure 2. H&E staining of the vena cava. (A) The H&E staining is showing gross morphological structure. The images were taken in 6.4X magnification and the inset images were taken in 32X magnification to show the details of the structures. (B) The bar graph shows medial thickness. Data represent mean ± SD, n = 5.
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Folic acid mitigated increased collagen/elastin ratio in CBS+/- vena cava

The elastin content of WT mice vena cava was 88.7±8 A.U. (Figure 3). In CBS+/- mice treated without or with FA elastin content were 84.2±4 A.U. and 86.3±3 A.U., respectively. No significant changes of the elastin content were observed between the groups (Figure 3). Collagen content in the vena cava of WT was 72 ± 5 A.U.), and it was slightly increased in CBS+/- (80 ± 8 A.U.) group (Figure 4). This increase was mitigated in CBS+/- mice treated with FA (70 ± 5 A.U.). Collagen/elastin ratio in the vena cava of WT was 0.81 ± 0.2 A.U. This ratio was significantly increased in CBS +/- mice (0.95 ± 0.4 A.U.). Interestingly, FA treatment attenuated the increased collagen/elastin ratio in CBS+/- mice (0.81±0.3 A.U.) (Figure 5).

Super oxide production

The intensity of dihydroethidium staining as an indicator of ROS, particularly super oxide in CBS+/- vena cava was slightly increased, although it was not significant compared to WT (Figure 6). The ROS intensity in the vena cava of three groups were 0.8 ± 0.1 A.U. in WT, 0.9 ± 0.16 A.U. in CBS +/- and 0.89 ± 0.6 A.U. in FA treated CBS+/- mice (Figure 6). FA mitigated this increase of superoxide production in CBS+/- vena cava (Figure 6).

Expression of MMP/TIMP and vena cava remodeling in CBS+/- mice

The expression of MMP-2 in the vena cava did not change significantly among the groups (Figure 7). However, MMP-9 and -12 significantly increased in CBS+/- vena cava (Figure 7). Although FA treatment attenuated MMP-12 expression in CBS+/- vena cava, the increased expression of MMP-9 remained unchanged in CBS+/- vena cava after FA treatment (Figure 7). Both TIMP-2 and -4 expressions showed significant decrease in CBS+/- vena cava, compared
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Vascular MMP activity

The MMP activity level as measured by \textit{in situ} zymography, showed increased tendency in CBS+/- vena cava compared to WT (Figure 8); however, it was not significant. FA treatment normalized the activity of MMP (Figure 8).

MMP/TIMP mRNA and aorta marker in CBS+/- vena cava

TIMP-3 and mMMP-13 RNA expression showed significant increase in the CBS+/- mice compared to WT vena cava (Figure 9), whereas TIMP-1 expression did not change significantly (Figures 9). Treatment with FA mitigated TIMP-3 expressions in CBS+/- mice, whereas MMP-13 remained unchanged in vena cava (Figure 9).

The expression of aorta marker EphrinB2 signifi-

Figure 4. Masson-trichrome staining of the vena cava. (A) Masson-trichrome staining of the vena cava is showing collagen content in the tissue. The images were taken in 6.4X magnification and the inset images were taken in 32X magnification to show details of the structures. (B) The bar graph represents relative collagen content among the groups. Data represent mean ± SD, n = 6.

Figure 5. Collagen/elastin ratio in the vena cava. Bar graphs indicate collagen/elastin ratio among the groups. Data represent mean ± SD, n = 5; *indicates $p < 0.05$ vs WT and † indicates $p<0.05$ vs CBS+/-.
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Figure 6: DHE staining of the vena cava. (A) The ROS content in the vena cava tissue were detected by dihydroethidium (DHE) staining. (B) The bar graph is showing relative intensity of fluorescence activities among the groups. Data represent mean ± SD, n = 5.

Discussion

Remodeling of vascular bed in pathophysiological hyperhomocysteinemia (HHcy) conditions have been well documented by various independent laboratories, including our own, in human as well as in vivo animal and in vitro cell culture models [6-7, 33-36]. To our knowledge none of these studies were done to investigate whether there is any shift of arterial phenotype in vena cava or vice versa during HHcy. We have previously reported that HHcy causes expression of venous phenotype in artery. Whether or not a reverse expression i.e. expression of arterial phenotype in vein during chronic HHcy occurs is unknown. Here we report that vena cava develops arterial phenotype in HHcy. This is a part of adaptive mechanism where vena cava accommodates with changing physiochemical environment in HHcy.
Homocysteine (Hcy) is formed during the metabolism of methionine and can be disposed out of the body by remethylation or transsulfuration pathway [37]. In transsulfuration pathway the CBS enzyme breaks down the Hcy to cysteine [37]. Individuals deficient of homozygous CBS (CBS-/-) develop severe HHcy and experience major clinical manifestations of premature vascular death, if untreated; whereas, CBS+/- individuals apparently live normal with cardiovascular abnormalities [38-39]. To mimic human disease processes we used CBS+/- mice, a well established model of HHcy, for our study because these animals live normal with vascular dysfunction due to moderate HHcy. Folic acid (FA) treatment was given to these mice to minimize the effect of Hcy, as the FA is an essential factor in remethylation pathway. Previously we reported that FA lowered Hcy level in CBS+/- animals (21 µmole/L in CBS+/- vs 12 µmole/L in CBS+/- + FA) [40]. Independent study have also documented that FA reduced Hcy level in patients of HHcy [41-43]. In accordance with these previous studies, in the present study we have also recorded similar results with FA treatment (data not shown).

In our present study we observed an active remodeling process, such as alteration of collagen and collagen/elastin ratio, increased ROS production and altered expression of MMPs and TIMPs in the vena cava of CBS+/- mice. Among matrix protein, collagen is stiffer than elastin and deposition of collagen in the basement membrane of vascular smooth muscle cells and in the extracellular matrix makes the vessel rigid. Although we have not observed changes in the elastin protein in CBS+/- vena cava, the content of collagen was increased, and therefore collagen/elastin ratio was higher (Figure 5). We also observed increased expression (Figure 7) and activities (Figure 8) of MMP-2 and -9 in...
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Endogenously, MMPs are regulated by TIMPs. Although, by definition, TIMPs family inhibits MMPs activity, selective inhibition of MMPs has been reported [17, 47]. A classic example is TIMP-2, which in addition to inhibiting MMPs activities, interacts with membrane type-1(MT1)-MMP to facilitate activation of pro-MMP-2 [17, 47-48]. TIMP-1 is a prototypic inhibitor of MMPs and abundant in fibrotic tissues [49-50], whereas TIMP-3 primarily inhibits ADAM [51] and is apoptotic [52]. Compared to other TIMPs, relatively few studies are focused on TIMP-4 [51]. We previously reported that Hcy mitigates TIMP-4 in microvascular endothelial cells [53]. We have also shown that CBS+/- mice are deficient of TIMP-4 expression, both in mRNA and protein level, in neurovascular tissue [54]. Surprisingly, to our knowledge, none of the studies are focused to asses any involvement of TIMP-2 in vascular remodeling in HHcy. Since TIMP-2 and -4 are general MMP inhibitors, our present findings of increased MMP-2,-9 and -12 suggest that in HHcy TIMP-2 and -4 were actively participated in vena cava remodeling as evidenced with their mitigated expressions (Figure 7). Folic

our present study. Additionally, we measured upregulated mRNA expression of MMP-13 (Figure 9). Since one of the substrates of MMP-2, -9 and -13 are collagen [15], with increase expression and activities of these MMPs, collagen should have been digested and expressed in lower amount in HHcy vessels compared to WT control. Instead, we have detected paradoxical increase (Figure 5). The possible explanation is that due to increased oxidative radicals, especially superoxide in CBS+/- animal (Figure 6), matrix collagen oxidatively modified. When it is oxidized, MMPs can not cleave, and therefore accumulates in ECM [44-45]. Additionally, MMP-12 is a macrophage metalloelastage and increased expression of this MMP is related to elastin degradation [46]. Our present report in agreement with this previous finding suggest that increased MMP-12 is associated with lower, although not significant, elastin content in CBS+/- vena cava (Figure 3). FA treatment normalized MMP-12 expression with slightly increased elastin content in CBS+/- vena cava, suggesting a possible regulation of MMP-12 by Hcy.

Figure 8: In situ zymography. (A) In situ zymography was performed using DQ gelatin as substrate and details were described in the methods. (B) Bar diagram indicates relative intensities of MMP activities among the groups.
acid treatment partially ameliorated this mitigation by Hcy. This result suggests that Hcy played a crucial role in vena cava remodeling. Additionally, we have detected relatively higher mRNA expressions of TIMP-1 and -3 in CBS+/- vena cava; however, FA did not significantly alter expression of these TIMPs. From this result it appeared that reduction of Hcy level by FA did not have effect on TIMP-1 and -3 regulations, although high Hcy induced these two TIMPs. Whether the statistical power was enough to exclude the possibility or there may be transitional protein expressions of these TIMPs, which required FA treatment for a longer period of time needs to be investigated in future.

In addition to matrix protein, metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), a group of cell surface signaling molecules, known as ephrin family, actively participate in vascular cell assembly and proliferation of vascular smooth muscle cells (VSMC); thus, contribute to the vessel remodeling. EphrinB2 and EphB4 belong to this family of cell surface signaling molecules, and known as receptor tyrosine kinase (RTK); play an important role in regulating vascular boundaries and integrity [55]. EphrinB2 is normally expressed on arterial wall, whereas its cognate receptor EphB4 dominantly expressed on venous endothelial cells [56]. Dysregulated EphrinB2 expression and signaling can result disrupted recruitment of VSMC in the vessel resulting in pathophysiological weak vessels [57-58]. Homocysteine is the only known molecule that induces apoptosis of endothelial cells and at the same time proliferate VSMC at higher levels [59-61]. In our study we have measured higher mRNA expression of EphrinB2 in CBS+/- compared to WT. In addition, venous EC marker EphB4 was low in CBS+/- vena cava (Figure 9). Although FA partially mitigated EphrinB2 in CBS+/- mice, the

Figure 9. Expressions of mRNA in the vena cava. (A) Total RNA was isolated from vena cava as indicated in the methods and mRNA expressions were measured by RT-PCR. (B) The bar diagram indicated relative expression of mRNA among the samples. Data represents mean ± SD, n = 5. The * represents p<0.01 vs WT and † represents p<0.05 vs CBS+/-.
expression of EphB4 did not augment to normal level (Figure 9). This result may suggest that while down regulating the venus endothelial marker EphB4, Hcy is mediating EphrinB2 expression and promoting VSMC in vena cava, which causing vascular phenotypic shift. Expression of protein levels of these two markers and detection of VSMC proliferation, if any, would have further strengthened and reinforced our hypothesis. Extracted protein from the vena cava of our animal model did not result detectable range of these two ephrin protein molecules in Western blot. Using greater amount of pooled vessels or larger animal may give further insights of our findings.

Results of vascular reactivity partially supported the hypothesis that Hcy mediates VSMC proliferation (Figure 10). In our study, CBS+/- vena cava relaxed at greater extent in endothelial-independent way (sodium nitroprusside response), which suggesting possible presence of more VSMC in CBS+/- compared to other two groups. This is a striking result, because, although FA treatment did not normalized im-paired endothelial function, neither it did changed endothelial-independent vascular relaxation in CBS+/-, but increased relaxation was observed in CBS+/- mice with out FA treatment.

In conclusion, one important aspect of our study is that the expression of arterial endothelial markers, Ephrin B2 and venus endothelial marker EphB4 expressions in the vena cava is altered in the HHcy condition, which we correlate with a shift towards arterial phenotype. This is an interesting and novel finding, where HHcy contributes to vascular remodeling by phenotypic change, in addition to its well established role on imbalance of collagen/elastin and MMP/TIMP ratio.
Acknowledgements

This study was supported, in part, by NIH grants HL-104103 (to US) and HL-71010 and HL-88012 (to SCT).

Conflict of interest

None declared.

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