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PROSPECTS

Mitochondrial Mechanism of Oxidative Stress and Systemic Hypertension in Hyperhomocysteinemia

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Abstract Formation of homocysteine (Hcy) is the constitutive process of gene methylation. Hcy is primarily synthesized by de-methylation of methionine, in which s-adenosyl-methionine (SAM) is converted to s-adenosyl-homocysteine (SAH) by methyltransferase (MT). SAH is then hydrolyzed to Hcy and adenosine by SAH-hydrolase (SAHH). The accumulation of Hcy leads to increased cellular oxidative stress in which mitochondrial thioredoxin, and peroxiredoxin are decreased and NADH oxidase activity is increased. In this process, Ca^{2+}-dependent mitochondrial nitric oxide synthase (mtNOS) and calpain are induced which lead to cytoskeletal de-arrangement and cellular remodeling. This process generates peroxinitrite and nitrotyrosine in contractile proteins which causes vascular dysfunction. Chronic exposure to Hcy instigates endothelial and vascular dysfunction and increases vascular resistance causing systemic hypertension. To compensate, the heart increases its load which creates adverse cardiac remodeling in which the elastin/collagen ratio is reduced, causing cardiac stiffness and diastolic heart failure in hyperhomocysteinemia. J. Cell. Biochem. 96: 665–671, 2005.

Key words: SHR; folic acid; CBS; SAH; SAM; SAHH; collagen; elastin; diastolic function; arterial pressure; 2K1C; aortic stenosis; NO; MMP; nitrotyrosine

Hcy AND ENDOTHELIUM IN HYPERTENSION

Hyperhomocysteinemia has emerged as an independent risk factor for the development of premature arterial fibrosis associated with hypertensive heart disease, coronary occlusion, and myocardial infarction, as well as venous thromboembolism [Boers et al., 1985; McCully, 1996]. Moderately increased Hcy plasma levels were linked with 4%–5% increase in the chance of death by heart disease. Levels of Hcy greater than 20 μM in plasma of patients with coronary heart disease were associated with a 35% increase in mortality [Nygard, 1997].

There are four ways by which homocysteine (Hcy) is accumulated in the plasma and tissues (Figs. 1 and 2): (1) a methionine rich protein diet (de-methylation); (2) a vitamin B_{12}/folate deficiency (re-methylation); (3) a heterozygous/homozygous trait for cystathione β synthase (CBS) activity in humans and B_6 deficiency (transsulphonation); and (4) renovascular stenosis and volume retention. Although Hcy plays a constitutive role in DNA/RNA gene methylation [Tyagi, 1999; Yi et al., 2000], hyperhomocysteinemia leads to endothelial damage [McCully, 1996; Mujumdar et al., 2001], especially, since mammalian endothelial cells lack the CBS enzyme [Finkelstein, 1990, 1998]. Every 3 μmol/L plasma increase in the Hcy level contributes to a 10% increased risk of coronary heart disease and a 20% increased risk of stroke [Hcy Studies Collaboration, 2002]. A common genetic polymorphism, MTHFR C677T, which determines Hcy levels, also has similar effects on heart disease and stroke [Shcherbak et al., 1999; Kaye et al., 2002; Klerk et al., 2002]. The association between this gene polymorphism and heart disease is unlikely to be confounded by other factors, such as smoking or blood pressure, but influences Hcy levels, suggesting a causal association between Hcy and heart

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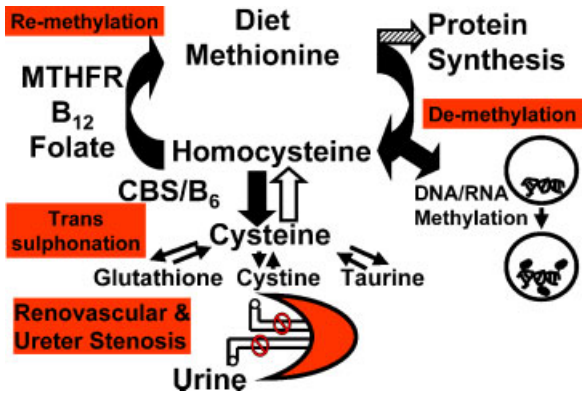

Fig. 1. Metabolic pathways of Hcy clearance. There are four ways by which Hcy is accumulated. 1: By methionine-rich meat diet (de-methylation); (2) b12/folate deficiency and heterozygote or homozygote in MTHFR (re-methylation); (3) hetero or homozygote in CBS activity and b6 deficiency (transsulphonation); (4) renal disease and obstruction in renal ureter clearance. During hyperhomocysteinemia, Hcy is the primary ingredient of thiol-regulated stresses. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

disease and stroke [Davey and Ebrahim, 2003]. A secondary prevention trial [Clarke and Collins, 1998] of folic acid supplementation, which regress the Hcy levels, demonstrated unequivocally that folate and other B-complex vitamins protect against heart disease. In addition, a different study demonstrated a beneficial effect of folate on the rate of revascularization after angioplasty [Schnyder et al., 2002]. Another trial with stroke patients did not

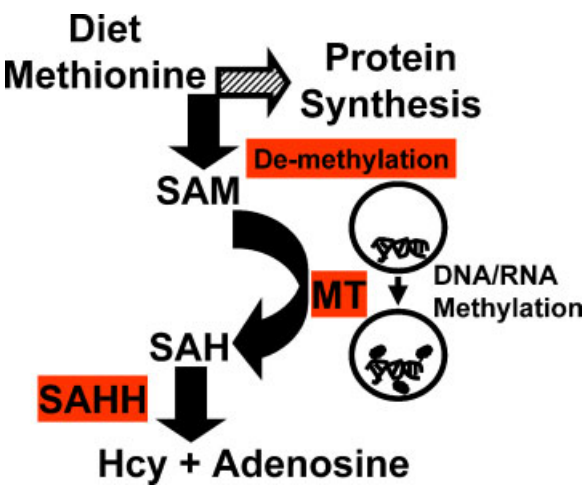

Fig. 2. De-methylation of methionine leads to generation of Hcy. Methionine is converted into s-adenosyl-methionine (SAM). SAM is converted to SAH by methyl-transferase (MT). SAH is hydrolyzed to Hcy and adenosine by SAH-hydrolase (SAHH). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

demonstrate a robust difference in recurrent stroke associated with a reduction of Hcy levels by 2 μmol/L [Toole et al., 2004]. Although the overall risk of heart disease with Hcy is small, there is evidence of synergism between Hcy and other risk factors such as smoking [Graham et al., 1997; Fallon et al., 2003], hypertension [Fallon et al., 2001], diabetes [Audelin and Genest, 2001], and insulin resistance [Fonseca et al., 2003].

Recent studies indicate that a number of forms of hypertension are associated with elevated plasma levels of homocysteine [Malinow and Levenson, 1995; Nygard, 1997]. Elevated circulatory levels of Hcy are associated with structural and functional abnormalities in the vessel wall [Rolland et al., 1995; Matthias et al., 1996] and hypertension [Sutton-Tyrrell et al., 1997]. Although vascular dysfunction (endothelial dysfunction and vascular hypertrophy) is a hallmark of hypertension, several lines of evidence support a role for homocysteine in causing vascular dysfunction [Upchurch et al., 1997a]. The mechanism mediating these abnormalities in vascular dysfunction is unclear. Although Hcy has been shown to affect endothelial and vascular smooth muscle function [Tyagi, 1998, 1999], the importance of Hcy in mediating the vascular dysfunction and hypertension is unclear. Furthermore, the molecular mechanisms whereby hyperhomocysteinemia results in vascular dysfunction are unknown. The purpose of this review is to suggest a role of Hcy in mitochondrial oxidative stress, propagating vascular hypertrophy and vascular resistance, leading to hypertension. In the following sections we discuss: (1) the role of redox-homocysteine in the formation of nitrotyrosine in hypertension; (2) the role of nitrotyrosine in activation of matrix metalloproteinase; and (3) the role of Hcy in vascular stress.

Role of Redox-Homocysteine in the Formation of Nitrotyrosine in Hypertension

Impaired flow mediated vasodilatation has been demonstrated in healthy humans after an acute increase in plasma Hcy [Chambers et al., 1998]. The role of chronic homocysteinemia in nitric oxide-dependent vascular function and its consequences in vascular remodeling is unclear. Nitric oxide, in conjunction with superoxides, forms peroxynitrite in the mitochondria (Fig. 3). In the presence of thiol, the peroxyni-

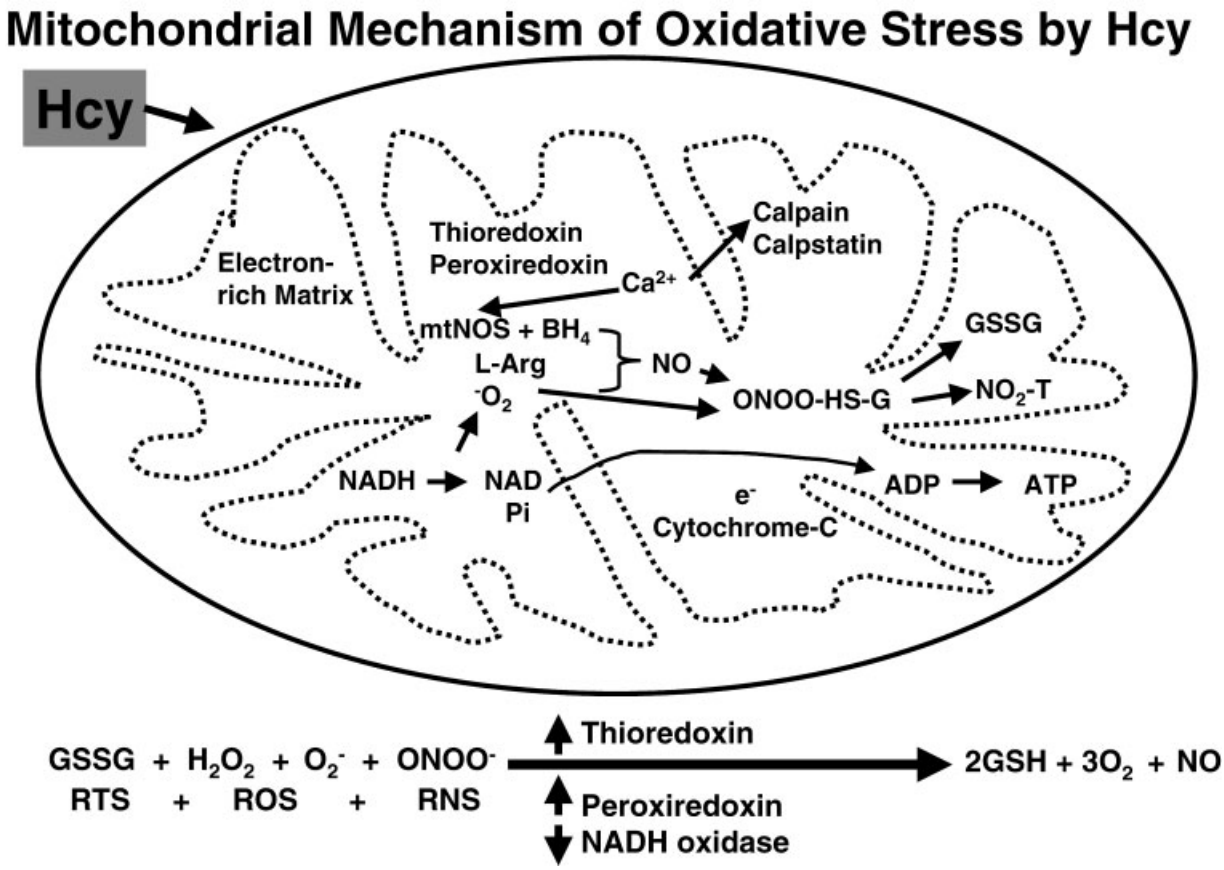

Fig. 3. Primary source of reactive oxygen, nitrogen, and thiol species (ROS, RNS, and RTS, respectively) is the mitochondria. The induction of mitochondrial NOS (mtNOS) in calcium-dependent manner produces NO. Hcy decreases thioredoxin and peroxiredoxin and increases NADH oxidase. This generates peroxinitrite, oxidized thiols, and produces nitrotyrosine (NO2-T). This increases intracellular calpain and decreases its inhibitor (calpstatin), leading disconnect between cytoskeletal and ECM.

trite causes nitration of tyrosine residues in proteins [Beckman et al., 1990; Huie and Padjama, 1993; Simon et al., 1996]. Accumulation of plasma Hcy reduces the levels of cysteine [Wollesen et al., 1999] and glutathione peroxidase activity [Upchurch et al., 1997]. It is possible that other thiol may contribute to peroxynitrite-thiol intermediate prior to nitrotyrosine formation. However, in hyperhomocysteinemia, Hcy may be the primary thiol in regulating redox reaction. Also the levels of Cu^{2+} ions are increased in the conditions of high Hcy [Dudman and Wilcken, 1983]. The Cu^{2+} ion catalyzes the formation of peroxynitrite. These studies, however, did not relate the levels of Hcy to nitrotyrosine formation.

Because BH_4 is a cofactor for eNOS activity [Milstein and Katusic, 1999], treatment with BH_4 may increase the levels of NO and improve vascular function. Additionally, Hcy induces eNOS expression [Upchurch et al., 1997b]. L-NAME, the nitro-derivatives, and other antagonists of substrate, L-arginine, inhibit NO formation [Schmidt et al., 1993]. These studies may suggest that in the presence of high plasma Hcy, NO inhibits its own production and/or the levels of BH_4 are diminished by Hcy and may favor nitration. The nitration may induce conformational changes in proteins and cause inactivation of enzymes and form intermolecular crosslinks. In aortas of rats with pressure overload hypertrophy by aortic coarctation (AC), and two kidney one clip Goldblatt hypertension, increased levels of nitrotyrosine have been observed [Bosse and Bachmann, 1997]. However, the mechanism by which nitrotyrosine is generated in aortas of these rats is unclear. In aortas above the AC, the contractile response was increased and vasorelaxing response was decreased compared to aortas below

the AC, or sham control [Martinez Ayala et al., 1998]. This study suggested a role of increased blood pressure in decreasing levels of endothelial nitric oxide; however, the authors did not measure the effect of nicotinamide and Hcy in aortas above and below the AC. The inhibition of peroxynitrite by nicotinamide prevents cardiovascular dysfunction [Cuzzocrea et al., 1998] and decreases blood pressure [Bushehri et al., 1998]. Decreased NO production leads to increased blood pressure [Johnson and Freeman, 1992]. It is unclear whether decreased NO availability by Hcy is associated with increased nitrotyrosine in hypertension. Our hypothesis is that Hcy reduces bioavailability of endothelial NO by the formation of nitrotyrosine. In an experimental model of hyperhomocysteinemia, administration of Hcy in drinking water increases plasma Hcy within 24 h. However, the blood pressure increased at about 4–5 weeks (Fig. 4). These results suggested that Hcy causes structural changes that lead to an increase in vascular resistance and systemic blood pressure. Similar results were obtained in genetic models of hyperhomocysteinemia in which Hcy is elevated due to heterozygote in cystathionine beta synthase (CBS−/+) enzyme activity. CBS trans-sulphonates Hcy to cysteine prior to clearance (Fig. 5).

Role of Nitrotyrosine in Activation of Metalloproteinase

Elastin turnover is lower compared to collagen. The degraded elastin is replaced by stiffer collagen. Diminution of vascular elastic content is one of the hallmarks of increased vascular stiffness. Studies suggest that high levels of Hcy are associated with atherosclerotic lipid rich lesions as well as arteriosclerotic and thrombotic lesions [Matthias et al., 1996; Hofmaim, 1999]. The reasons for this controversial and dual role of Hcy are not clear. Jourdheuil-Rahmani et al. [1997] have demonstrated that Hcy leads to elastin breakdown by increasing elastase activity in vascular smooth muscle cells. In vitro, Hcy activates latent resident MMPs [Tyagi et al., 1998]. Elastin and collagen peptides induce hypertrophic phenotype in vascular cells and facilitate vascular contractile dysfunction [Faury et al., 1995] (Fig. 6). In culture conditions, inhibition of cytokine-induced NOS reduced both expression and activity of MMPs [Sasaki et al., 1998]. In contrast, cytokine inducible MMPs in immortalized cells were not modified by NOS inhibition [Horton et al., 1998]. The reasons for such diverse effects of NO on MMPs are not clear. However, a differential regulation of MMPs,

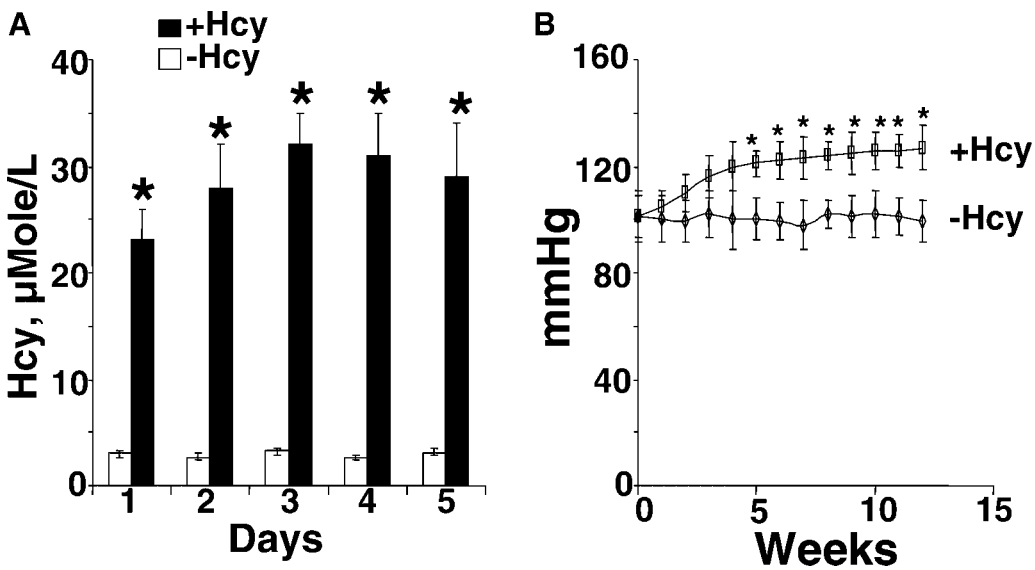

Fig. 4. **A**: The condition of HHCy is created by direct administration of Hcy (0.67 mg/ml) in drinking water of normotensive rats (NWR). Total plasma levels of Hcy were measured in tail vein blood by HPLC and spectrophotometer. Plasma Hcy selectively increased in Hcy-administered rats to 32 ± 1 µmole/L at day 1 compared to 4 ± 1 µmole/L in untreated NWR. **B**: Mean arterial pressure (MAP) in conscious rats with and without Hcy administration, measured by radio-telemetry.

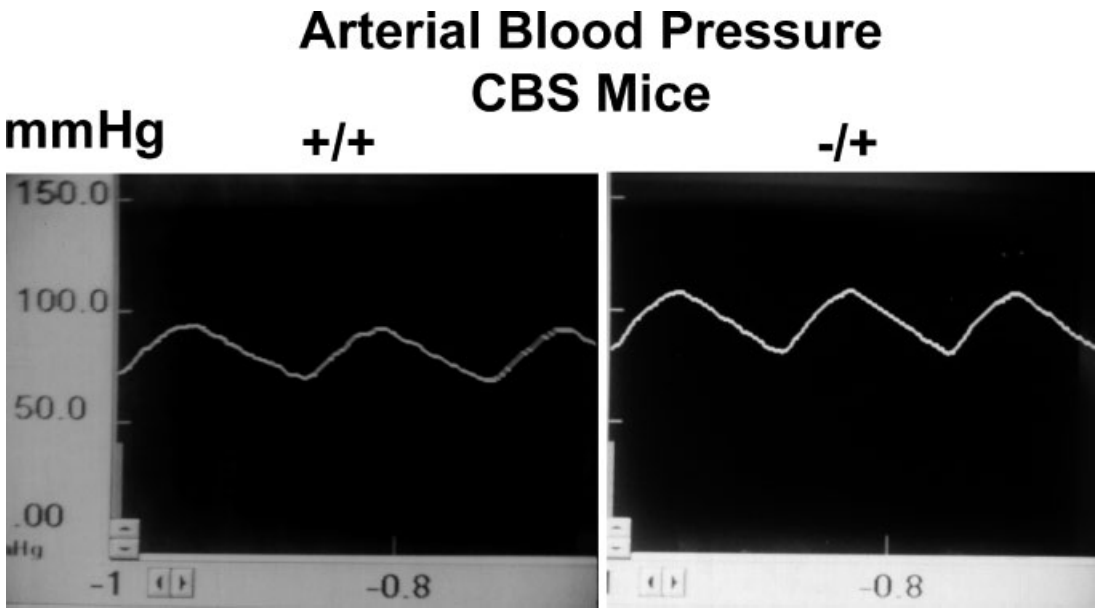

Fig. 5. Carotid artery blood pressure waves of wild type (+/+) and heterozygote cystathione beta synthase (CBS−/+) hyperhomocysteinemic mice.

release and activation in vivo versus in vitro may account for this discrepancy. Oxygen species stimulate MMPs [Rajagopalan et al., 1996] and in vivo inhibition of NO production increases MMP activity [Radomski et al., 1998]. Inactivation of MMP by NO may be multifactorial: (1) redox-sensitive MMP gene activation may be inhibited by NO because NO is an antioxidant; (2) independent of MMP, NO may induce TIMP; (3) NO may block the Zn^{2+} ion active site in MMP. The activity of MMP transforms into collagenolysis-elastinolysis. Hcy reduces bioavailability of endothelial nitric oxide and activates MMPs. The mechanism of NO-mediated MMP activation and ECM disruption is unclear. We hypothesize that reduction in the levels of nitric oxide by Hcy increases MMP activity, decreases TIMP-4, and leads to collagenolysis-elastinolysis.

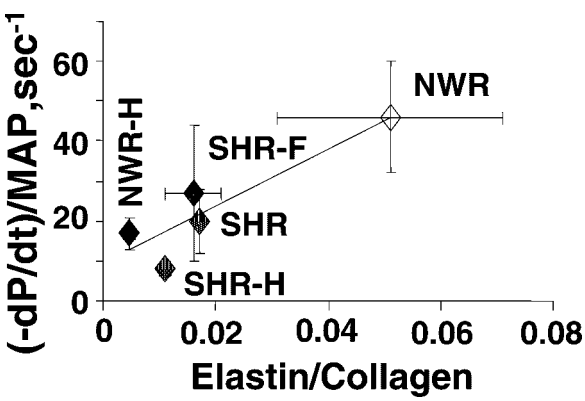

Fig. 6. Relationship between cardiac diastolic relaxation and elastin/collagen ratios in NWR and spontaneously hypertensive rats (SHR) treated with Hcy (NWR-H and SHR-H) for 12 weeks. SHR were also treated with folic acid (SHR-F) in drinking water for 12 weeks.

Role of Hcy in Vascular Stress

In humans, hyperhomocysteinemia is associated with systolic hypertension [Sutton-Tyrrell et al., 1997]. The elevation of Hcy increases blood pressure in pigs [Rolland et al., 1995]. Hcy induces tachycardia, hyperpulsatility, and hyperreactivity of the caudal artery in pigs [Rolland et al., 1995]. Hyperhomocysteinemia causes lowering of reactive mean blood flow following hypoperfusion resulting from a moderately increased systolic blood flow. There is an associated decrease in diastolic blood flow. Hcy lowers intrinsic elasticity of the hyperpulsatile aortic wall, and increases blood pressure, deforming stress, resulting in increased aortic volumetric compliance [Rolland et al., 1995]. This study, however, did not elucidate the role of NO in Hcy-mediated decreased vascular elastic compliance. Hcy proliferates vascular smooth muscle cells [Tsai et al., 1994] and elicits calcium-dependent vascular contraction [Mujumdar et al., 2000]. Nitric oxide inhibits proliferation of vascular muscle cells [Sharma et al., 1999]. We have demonstrated

in culture that aorta with 2-weeks treatment with hornocysteine elicits vascular hypertrophy and inhibition of peroxynitrite by nicotinamide improves vascular function [Mujumdar et al., 2001]. Inhibition of peroxynitrite improves vascular function in vivo [Cuzzocrea et al., 1998]. It is not clear, however, whether this improvement is associated with a decrease in vascular hypertrophy and stress in hyperhomocysteinemia. In conclusion, we hypothesize that Hcy induces vascular muscle hypertrophy and stress by decreasing the availability of NO and promoting nitrotyrosine formation.

SUMMARY

Several studies have implicated hyperhomocysteinemia as a potential risk factor for hypertension. In the Hordaland Homocysteine Study of about 16,000 people from 40 to 67 years old with no history of hypertension, diabetes, or coronary vascular disease, the plasma Hcy levels were positively related to blood pressure [Nygard and Vollset, 1995]. Similarly, Malinow and Levenson [1995] found that hypertensive men with no history of atherosclerotic disease had higher Hcy levels than non-hypertensive men. Sutton-Tyrrell et al. [1997] also found a significant association between Hcy levels and systolic hypertension. This review addresses two novel mechanisms by which Hcy causes hypertension: (1) the reduction in the levels of NO by Hcy leads to increase MMP activity. This instigates collagenolysis, vascular hypertrophy and stress; (2) Hcy alters vascular matrix in a way that decreases the vascular lumen. Therefore, to overcome vascular resistance, the heart increases systolic pressure.

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