

Metabolism of Homocysteine and its Relationship with Cardiovascular Disease

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Abstract. Hyperhomocysteinemia, or the rise of plasmatic homocysteine levels above 15 $\mu\text{g/dL}$, is accepted nowadays as an independent risk factor for cardiovascular disease in men and women. Homocysteine (Hcy) is a non-protein forming aminoacid (aa) derived from the loss of the methyl group, found within methionine. Methionine regenerates by retrieving the methyl radical from 5-methyltetrahydrofolate (5-MTHF) creating tetrahydrofolate (THF) which will then regenerate to 5-MTHF through the action of methyltetrahydrofolate reductase (MTHFR). This process is called remethylation. Alternatively, Hcy can follow the transsulfuration route, where through cystationine- β -syntetase (CBS), it irreversibly converted into cystationine, a precursor of cysteine, glutathione, and other substances that are finally excreted in the urine. Hyperhomocysteinemia results from inhibition of the remethylation route, or inhibition or saturation of the transsulfuration pathway. Main factors causally associated increased plasmatic Hcy are mutations of the enzymes MTHFR and CBS; varying nutritional and health states; demographic factors; and, others. The most accepted hypotheses about Hcy action in cardiovascular disease are direct endothelial and vessel wall damage; oxidative stress generation; and, stimulation of a procoagulant and proinflammatory state of blood components. Since hyperhomocysteinemia can be effectively treated with folic acid, prospective trials are underway to determine if folate therapy is required to lower Hcy levels in plasma. These studies also attempt to address the impact, if any, of folate therapy in the reduction of cardiovascular risk, and to demonstrate if hyperhomocysteinemia is actually an independent risk factor that can be effectively treated.

Key Words. hyperhomocysteinemia, cardiovascular disease, homocysteine metabolism, folic acid

Introduction

Cardiovascular disease remains the main cause of mortality in western countries. The most important known risk factors for developing this disease are dislipoproteinemia [1], hypertension [2], diabetes [3], obesity [4], and smoking [5]. Nevertheless, these factors only explain two thirds of all cardiovascular events [6], and therefore in the last years there has been a great scientific interest in the search of new

markers and risk factors that could be associated and responsible for this pathology.

In 1969, McCully [7] described the vascular pathology in homocysteinuric pediatric patients, which is characterized by smooth muscle proliferation, progressive arterial stenosis, and procoagulant hemostatic changes, revealing the importance of severe hyperhomocysteinemia in the early development of arteriosclerosis and thromboembolism. Afterward, a large number of epidemiological studies followed that related high homocysteine (Hcy) with coronary artery disease (CAD), finding in the majority of cases a strong relationship, suggesting that Hcy could be an independent risk factor for the development of cardiovascular disease.

Metabolism

Hcy is a non-protein forming aminoacid (aa), not codified in DNA, that is characterized by containing sulfur and being an intermediary in the metabolism of methionine and cysteine. Hcy is formed from the essential aa methionine, through the methylation process [8]. The intracellular Hcy is transported to the plasma through an exportation mechanism, where it reaches an average level of 10 $\mu\text{mol/L}$. The exportation mechanism completes the catabolism of Hcy, which is carried out by the transsulfuration mechanism, and contributes to keep intracellular levels low to avoid the potential aa cytotoxicity. When there is hyperhomocysteinemia, the normal Hcy metabolism is damaged at some point, and the exportation mechanism compensates to keep intracellular levels low, thus leaving the vascular tissue exposed to the effects of Hcy excess [9].

Hcy is metabolized through two main routes: methylation and transsulfuration (Fig. 1). Normally, about 50% of Hcy enters the methylation cycle. S-adenosylmethionine (SAM) is an important regulator of Hcy remethylation and transsulfuration. The

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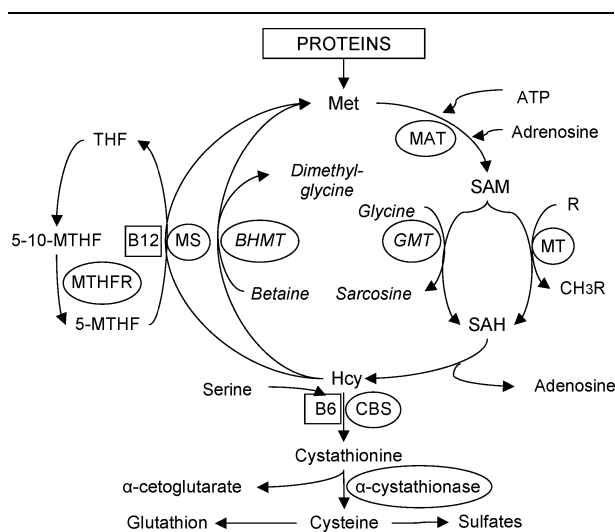


Fig. 1. Hcy Metabolism. ATP: adenosine triphosphate, B₆: vitamin B₆ or pyridoxine, B₁₂: vitamin B₁₂ or cyanocobalamin, BHMT: betaine-homocysteine methyltransferase, CBS: cystathionine β-synthetase, GMT: glycine N-methyltransferase, Hcy: homocysteine, MAT: methionine adenosyl transferase, Met: methionine, MS: methionine synthase, MT: methyltransferase, 5-MTHF: 5-methyltetrahydrofolate, 5-10-MTHF: 5-10-methyltetrahydrofolate, MTHFR: methyltetrahydrofolate reductase, SAH: S-adenosylhomocysteine, SAM: S-adenosylmethionine, THF: tetrahydrofolate.

other 50% catabolizes through the transsulfuration pathway, where Hcy and serine condense in order to form cystathionine, which is transformed to cysteine and alpha-cetoglutarate [8]. It is probable that remethylation is the active state during fasting and that transsulfuration is predominant after a methionine charge, likely to occur after eating a meal rich in protein [10]. Hcy is mainly eliminated through renal catabolism. Only 1% of the Hcy filtered to the glomerulus is normally found in urine. The rest reabsorbs and metabolizes. For this reason it is thought that kidneys are metabolizers rather than excretors of Hcy [8].

Methionine Cycle

Hcy is formed from the essential aa methionine which contains a methyl group that is activated through the activation to SAM. This reaction is mediated by methionine adenosyltransferase (MAT) and adenosine triphosphate (ATP) [8]. The SAM formation is of great importance because it is the main methyl group donor that permits the synthesis of noradrenaline [11], creatine, phosphatidylcholine, DNA, RNA, and a great number of neuromediators [12]. While donating its methyl group through methyltransferase (MT), SAM transforms to S-adenosylhomocysteine and hydrolyzes to Hcy and

adenosine [11]. An alternative to SAM demethylation is the “non productive” methylation of glycine to sarcosine, which is physiologically inert, occurring through glycine N-methyltransferase (GMT) [12].

Hcy remethylates to form methionine in one of two ways. The first, and most frequent, is the remethylation through methionine synthase (MS) in a reaction that requires vitamin B₁₂ as a cofactor (in methylcobalamin form) and that converts N⁵-methyltetrahydrofolate (5-MTHF) into tetrahydrofolate (THF). THF recycles to 5-MTHF in two steps; first THF converts to N⁵-N¹⁰ methyltetrahydrofolate (5-10-MTHF), and later to 5-MTHF. This second step is catalyzed by methyltetrahydrofolate reductase (MTHFR) [13]. The second, and less frequent, creation of remethylation operates independently of vitamin B₁₂ and folate, but uses betaine as a methyl donor and requires betaine-homocysteine methyltransferase (BHMT) for the formation of dimethylglycine and methionine [14]. This route, which takes place mainly in the liver, maintains the necessary methionine tissue levels for synthesis of SAM in situations of folate and/or cobalamin deficiency [12].

Transsulfuration Route

Cysteine is synthesized from methionine through the transsulfuration pathway. The first three steps in this route are the same as the methionine cycle and ends in the formation of Hcy, which is the substrate of the vitamin B₆ (pyridoxal-5'-phosphate)-dependent cystathionine β-synthetase (CBS). This enzyme catalyzes the condensation of Hcy with serine in order to form cystathionine. This is a critical step in the transsulfuration route because it is irreversible under physiological conditions; from here Hcy is obliged to follow this route [15]. After this, cystathionine converts to cysteine (glutathione precursor), and α-cetoglutarate by γ-cystathionase, which is also pyridoxal-5'-phosphate dependent. Cysteine is finally converted into glutathione and sulphates, which are excreted in urine. It is worth mentioning that the preferential distribution of the transsulfuration pathway and the catabolizing enzyme glycine N-methyltransferase occurs in the liver and kidneys. These organs are essential to capturing (through carriers, receptors, or channels) and metabolizing the excess Hcy circulating in the body. The tissues that lack this route require exogenous cysteine in order to produce glutathione [12].

Regulation of Homocysteine Metabolism

SAM plays a central role in the regulation of Hcy metabolism by inhibiting the conservative methionine enzymes with low K_m values and activating those that catabolize methionine with high K_m values. When the SAM tissue level is high enough to keep the vital reactions of transmethylation, SAM

reduces the remethylation rate of Hcy to methionine through MTHF, by inhibiting the MTHFR activity. Furthermore, in order to avoid the Hcy accumulation that can be potentially cytotoxic, SAM promotes Hcy catabolism by stimulating CBS and γ -cystationase [16].

- **Alimentation.** A meal rich in animal protein, or methionine, will cause the SAM tissue level to increase, and 70% of Hcy will be catabolized through the transsulfuration pathway by the CBS. In contrast, in conditions where the protein level is low, the MTHFR-dependent remethylation route will be favored. In this case, just 10% of the Hcy pool enters transsulfuration. However, when sustained over a long period, a diet high in protein can cause the saturation of transsulfuration that associated to remethylation inhibition promotes Hcy intracellular exit, which augments its blood level [17].
- **Folate intracellular level.** The folate intracellular pool is also involved in Hcy regulation by providing MTHF, which maintains optimal levels of SAM when there is a low introduction of methionine from exogenous sources. At the same time, the diminution of MTHF biodisponibility generates a reduction in Hcy remethylation and the acceleration of the alternative transmethylation process. The diminution of SAM concentrations, as a result of folate depletion, induces inhibition of transsulfuration reactions [12]. The combination of a diet low in folic acid coupled with the ingestion of metabolism-interfering drugs will increase the levels of Hcy intracellularly and promote its mobilization, thus raising the plasmatic concentration [17].

Homocysteine forms

Human plasma contains reduced and oxidized Hcy (Fig. 2). Butz and Du Vigneaud [18] established the chemical definitions more than 65 years ago. Sulfhydryl, or the reduced form, is called homocysteine and disulfur, or the oxidized form, is called homocystine. The disulfide forms also exist with cysteine and with proteins that contain cysteine reactive residues (protein bound homocysteine). These oxidized forms are called mixed disulfides. Hcy oxidized forms usually compose 98–99% of the total human plasma Hcy, 80–90% of which is bound to proteins [18]. Nevertheless, researchers do not agree on how to designate the multiple plasma homocysteine forms [19]. Some write “homocyst(e)ine and hyperhomocyst(e)inemia” to designate the plasma multiple forms. This should not contain parentheses. Total homocysteine is the sum of all homocysteine forms that exist in plasma or serum, therefore being hyperhomocysteinemia, the elevation of total homocysteine.

REDUCED:			
Homocysteine	NH_3^+ -OOCCHCH ₂ CH ₂ -SH		1%
OXIDIZED:			
Homocystine	NH_3^+ -OOCCHCH ₂ CH ₂ -S -OOCCHCH ₂ CH ₂ -S NH_3^+		5-10%
Mixed disulfides:			
Protein bound Homocysteine	NH_3^+ -OOCCHCH ₂ CH ₂ -S Protein -S		80-90%
Cysteine - Homocysteine	NH_3^+ -OOCCHCH ₂ CH ₂ -S -OOCCHCH ₂ -S NH_3^+		5-10%

Fig. 2. Total plasma Hcy derived compounds and its composition percentage.

Determinants of Hyperhomocysteinemia and its Measurement

The most important Hcy determinants in plasma, which are complex and diverse, are shown in Table 1.

Physiologic factors

A great number of environmental factors have been found to play a determinant role in Hcy levels [20]. Lussier-Cacan et al. [21] studied a large number of healthy men and women and determined that gender was a determining factor in Hcy. The fasting level in a woman is 21% lower than in a man. This gender difference is noted as early as 10 years of age [22], a fact that could partially be explained by the observation that the remethylation pathway is more efficient in women than in men [23], for whom the transsulfuration route seems to be more efficient [24]. As a person ages, the Hcy level grows. Tonstad et al. [25] determined median concentrations of 5–6 $\mu\text{mol/L}$ in Norwegian children of 8 to 12 years old, while Nygård et al. [26] found, in Norwegian adults of 40 to 42 years old, values of 9.1 to 10.8 $\mu\text{mol/L}$. Race or ethnic origin is also a determining factor. Ubbink et al. [27] demonstrated that black South Africans had lower Hcy levels than white South Africans, although their diets are similar. It is probable that this difference is confined to genetic constitutional differences within both groups.

Genetic factors

- **Alterations in the transsulfuration pathway.** The most common genetic alteration that results in homocysteinuria is the reduced CBS activity, whose homozygous state causes severe hyperhomocysteinemia [28]. Genetic mutations in this enzyme

Table 1. Causes of Hyperhomocysteinemia

Factors	Variables	
Physiological	Age	
	Sex	
	Ethnic origin	
	Menopause	
Genetic	<i>Enzyme abnormalities</i>	
	MTHFR	
	MS	
	CBS	
Acquired	Vitamin B deficiencies (folate, B ₁₂ , B ₆)	
	Smoking	
	Alcohol abuse	
	Coffee abuse	
	Physical inactivity	
	Renal insufficiency and failure	
Pathologic	Cardiac, renal, and other transplants	
	Hypothyroidism	
	Diabetes type 2	
	Diabetes type 1 with nephropathy	
	Psoriasis	
	Cancer	
	Medications	Hormonal contraceptives
		Methotrexate
Sulfasalazine		
Phenytoine and carbamacepine		
Nitrous oxide		
Metformin		
	Cholestipol and niacine	

CBS: cystationine β -synthetase, MS: methionine synthase, MTHFR: methylentetrahydrofolatereductase.

lower the affinity to any of its substrates: phosphate pyridoxal, serine, or Hcy. It has been seen that heterozygotes have variable activity, always inferior to 50% of its capacity [29]. The CBS gene is found in chromosome 21q [30] and its deficiency transmits in an autosomal recessive way, resulting in homozygosity (homocysteinuria) or heterozygosity (homocysteinemia) [31]. Tsai et al. [32] have estimated that the heterozygosity frequency for a CBS point mutation is from 1/20,000 to 1/200,000 and that 30–40% of individuals with premature vascular disease are heterozygotes for the mutation. Patients with deficiency of this enzyme typically present with an alteration in the methionine load test, nevertheless the diagnosis should not be made from this test but from the molecular diagnosis [32].

- *Alterations in the remethylation pathway.* The most common genetic defect associated with mild hyperhomocysteinemia is a transition of a thymine by a cytosine in the 677 position (C₆₇₇T) of the methylentetrahydrofolatereductase gene, causing a substitution of a valine by an alanine in the functional enzyme [33]. This enzyme, the thermolabil variant, with a reduced total activity (30–50%) [34] is an autosomal recessive mutation. The frequency

of this polymorphism is variable among different racial and ethnic groups; Caucasians and Asians have T/T homozygotes levels of 10–13% and C/T heterozygotes levels of 50%, while Afro-Americans have very low incidence rates [35,36]. The MTHFR gene is found in chromosome 1p and has nine other mutations that result in thermostable mutations [37]. Hyperhomocysteinemia does not happen in heterozygote individuals due to the C₆₇₇T polymorphism but it does seem to affect homozygotes who have deficiencies in folate ingestion [38–40].

There are five known mutations that affect the methylcobalamin synthesis, an essential cofactor of methionine synthase. Its incidence is rare and causes moderate hyperhomocysteinemia because it provokes a functional deficiency of MS [41].

Acquired, pathologic and drug factors

Diet deficiencies, or folate, vitamin B₁₂ and vitamin B₆ mal-absorption, have been linked with hyperhomocysteinemia, even in well-nourished populations, as well as inversely correlate with total Hcy [42]. A Hordaland study in Norway found that a rise in Hcy is associated with smoking, high arterial pressure, high cholesterol, and a sedentary lifestyle [26]. Alcoholics have higher total Hcy levels, probably due to malnutrition and mal-absorption [43]. Likewise a high intake of coffee relates to higher Hcy levels [44].

In respect to diseases, it has been found that high Hcy levels exist in the following situations: chronic renal insufficiency where the aa rise has been associated with creatinine elevation [45] and moderate Hcy levels in the terminal stage of the disease [46]; in diabetes I and II [47]; psoriasis [16]; and hypothyroidism [48], where treatment with thyroxine normalizes Hcy levels [49]. Likewise, hyperhomocysteinemia has been found during cardiac transplant procedures [50], a finding that probably could be related with a state of renal insufficiency [51].

In relation to drugs, some of them can cause hyperhomocysteinemia, as is the case of hormonal contraceptives, anticarcinogenic agents (methotrexate), sulfasalazine, anticonvulsives (phenytoin, carbamacepine), nitrous oxide, among others [16,52].

Homocysteine measurement

Although a great part of authors agree that normal plasma Hcy levels for healthy adults is 5–15 μ mol/L [53], some have described that the risk of coronary disease exists at levels of 10 μ mol/L [54]. Thus, it would be convenient to establish the superior limit in this level. Three arbitrary levels of hyperhomocysteinemias have been determined as mild (15–30 μ mol/L), moderate (30–100 μ mol/L) and severe (>100 μ mol/L) [55].

For determining plasma Hcy there exists a controversy among the state of fasting and the anticoagulant to be used. Generally, a 12-hour fast is

recommended, according to O'Broin et al. [56], however other authors such as Rasmussen et al. [57] state that a need does not exist for fasting. In the same way, the anticoagulant of choice for Rasmussen et al. [57] is EDTA, while for Willems et al. [58] it is acidic citrate because it stabilizes blood samples and can stay at room temperature before plasma separation. In the case of EDTA it is necessary to put a sample immediately on ice while preparing to separate plasma. The method to obtain plasma is centrifugation, which does not need to be refrigerated [59]. Once plasma is separated, Hcy is stable for 4–7 days at room temperature, for several weeks at 2°C, and for years at –20°C. Repeated defrosting is not recommended [60]. Several methods to measure Hcy are the total Hcy enzymatic conversion immunoassay, different variants of high-resolution liquid chromatography, and chromatographic gas spectrometry [61].

Methionine load test

The methionine load test (MLT) is used to find anomalies in Hcy metabolism that cannot be detected in a fasting state, the problem being the transsulfuration route [62]. Bostom et al. [63] have emphasized the MLT importance of the hyperhomocysteinemia diagnosis for patients with vascular disease because a great proportion of these patients have normal fasting Hcy levels but have elevated Hcy levels with the MLT. An MLT is done after an all-night fast. Once the first blood sample has been drawn in a fasting state, a methionine dose of 100 mg/kg will be given and two other samples are then taken at hours two and eight. An abnormal MLT is an Hcy level greater than two standard deviations above normal control values [63].

Possible Hyperhomocysteinemia's Physiopathological Mechanisms in Early Vascular Disease

Until this moment there does not exist a unifying hypothesis that explains the hyperhomocysteinemia's atherogenic and thrombotic effects. There probably is a multifactorial effect whose main consequences are endothelial and vascular wall damage and a rise in procoagulant and proinflammatory substances in blood.

- *Hcy and vascular dysfunction.* There exist several *in vivo* studies in humans and animals that support the hypothesis of endothelial cell dysfunction in the presence of hyperhomocysteinemia. Van de Bert et al. [64] studied endothelial dysfunction in young patients with peripheral occlusive arterial disease and mild hyperhomocysteinemia by measuring von Willebrand factor, thrombomodulin, and plasminogen tissue activator. The plasmatic concentrations

of the first two substances were above reference values and diminished after hyperhomocysteinemia treatment with pyridoxin and folic acid.

Using monkeys, Lentz et al. [65] proved the hypothesis that diet-induced mild hyperhomocysteinemia could result in vascular dysfunction. They found that the arterial response to endothelial-dependent vasodilators, such as adenosine diphosphate (ADP) and acetylcholine, was too low in hyperhomocysteinemic animals. Afterwards, this type of research was done in humans. Tawakol et al. [66] described a defective endothelial-dependent vasodilatation in mild hyperhomocysteinemic subjects. The linear regression analysis showed that the Hcy level was the only significant predictor of mediated flow vasodilatation. Woo et al. [67] obtained similar results in moderate hyperhomocysteinemic subjects. It seems that the reduced Hcy is responsible for the suppressed endothelial dependent vasodilatation [68]. Although the exact mechanism of endothelial dysfunction is not known, research studies continuously shows that Hcy exerts this effect thus promoting oxidative damage [69].

- *Hcy and oxidative state.* Hcy rapidly auto-oxidizes when it enters plasma and produces highly reactive oxygen molecules such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals. Harker et al. [70] proposed that the Hcy-induced endothelial damage is mediated by hydrogen peroxide that exposes the vessel matrix and smooth muscle cells, making these cells proliferate and promote platelet and leukocyte activation. Besides this, superoxide formation from hydroxyl radical has shown to be the lipidic peroxidation initiator, an effect that occurs at the level of endothelial membrane and inside of the lipoproteic particles, which presumably creates atheromatose plaque formation [71]. In support to this oxidative damage theory, Chambers et al. [72] showed that with previous antioxidant (vitamin C) treatment, the methionine load did not suppress the endothelial-dependent vasodilatation, effect that is impaired in subjects that did not have this pre-medication.

In the work of Upchurch et al. [73], intracellular endothelial glutathione peroxidase was measured, a key enzyme in the oxidative defense mechanism that has shown to potentiate nitric oxide action. A diminution in glutathione peroxidase activity of 41–81% occurred after incubating endothelial cells for four hours in rising and high concentrations of Hcy (50–250 μ M). An Hcy concentration even higher (1 mM) reduced enzyme activity by 91% in comparison to the control group. These observations indicate that Hcy can cause endothelial damage by promoting peroxide formation as well as impending its activation. Normal endothelial cells detoxify Hcy by liberating nitric oxide,

which take to S-nitrous-homocysteine formation [74]. This compound formation attenuates Hcy pathogenicity by inhibiting oxygen free radicals and sulfhydryl-dependent generation. Nevertheless, this protector effect is eventually bypassed by the endothelial cell chronic Hcy exposure, because it attenuates bioactive nitric oxide production [73].

- *Hcy and procoagulant-proinflammatory state.* The relationship between hyperhomocysteinemia and procoagulant state is deduced because of the following observed effects in the presence of elevated Hcy levels: protein C activation reduction, and therefore inhibition of its anticoagulant effect [75]; more than 75% inhibition of antithrombin III activity [76]; reduction of the anticoagulant sulfate heparan synthesis through an induced alteration in redox potential [77]; thrombomodulin suppression and inactivation of its cofactor activity [78]; blockage of plasminogen tissue activator activity that attaches to endothelial cells [79]; raise in lipoprotein (a) affinity to fibrin inhibiting the conversion of plasminogen to plasmin over fibrin surface [80]; cyclooxygenase activity inhibition in human endothelial cells, diminishing protacyclin production [81]; activity promotion of factors XII [82], V [83], tissue factor [84], and von Willebrand factor [85]. In the same way, some other endothelial production characteristics under Hcy influence have been demonstrated, which make it proinflammatory by promoting interleukin 1 and 6 production [86].

Platelet dysfunction is another abnormality that has been seen in hyperhomocysteinemic patients. It has been demonstrated that in pyridoxine presence platelets have a longer circulatory life, probably because of their proved diminished endothelial adhesion [87]. It has also been studied, that Hcy alters arachidonic acid metabolism, promoting a great production of the procoagulant agent thromboxane A₂ [88]. This procoagulant tendency has also been demonstrated in animal models, because studies made in rats which have been provoked to have mild hyperhomocysteinemia through a folate deficiency diet, have demonstrated that a methionine load causes platelet aggregation, thromboxane biosynthesis, and macrophage derived tissue factor activity [89].

- *Hcy and vascular wall.* In respect to the vascular wall, a study made in laboratory animals, 0.1–0.3 mM of Hcy were injected, and it was seen a thicker intima, smooth muscle cell proliferation, raise in luminal superficial cells desquamation, and high foam cell levels [87]. In fact, Hcy has shown to augment smooth muscle cell DNA synthesis, and at the same time impede endothelial cell regeneration [90]. Besides inducing smooth muscle cells to reinitiate cell cycle and proliferate, it seems that Hcy also interacts in a synergistic

manner with plasma substances as growth factors and cytokines present in atherosclerotic lesions to promote these smooth muscle cell growth during atherogenesis [91].

- *Hcy and chemical alteration.* Another theory that tries to explain Hcy caused damage is hypomethylation [92]. SAM is the main methyl group donator, and methylation, that is involved in phospholipid, nucleic acids, amines, and other neurotransmitters synthesis, and that regulates genetic expression and modifies protein functions, cannot occur without the correct MT function. In adenosine presence, Hcy is converted efficiently to SAH, a potent inhibitor of MT reactions. In fact, SAH is the product of MT, which uses SAM as a substrate. MT bind to SAH with greater affinity that to SAM, and therefore, it is prone to potent negative feedback of its product. Recently it has been described that relevant physiologic Hcy levels (30–100 $\mu\text{mol/L}$) in presence of adenosine inhibit vascular endothelial cells growth, but not smooth muscle's, by a mechanism that involves p21^{ras} carboxylation diminution [93]. Therefore, one of the hyperhomocysteinemia toxic basic biochemical mechanisms is accumulated SAH mediated hypomethylation [92].

Homocysteine and Cardiovascular Disease

The first studies that started to show a relationship among cardiovascular disease with hyperhomocysteinemia in young adults were presented in 1976, when Wilcken and Wilcken [94] described that patients under 50 years old with angiography documented coronary disease had methionine post load Hcy levels higher than controls.

In general, ever since the majority of transversal and retrospective studies have found an association between Hcy plasmatic levels and cardiovascular risk. Clarke et al. [95] documented higher Hcy levels in patients with coronary disease compared with control subjects. Also, in a large multi-centric European study, the COMAC study, hundreds of patients and controls were included, and it was estimated that the cardiovascular risk associated with hyperhomocysteinemia detected in fasting conditions ($>12 \mu\text{mol/L}$) and after methionine load (absolute raise greater than $38 \mu\text{mol/L}$ or net raise of $27 \mu\text{mol/L}$) is similar to that of hyperlipidemia or smoking, although less than that associated to hypertension [96]. Furthermore, in the Boushey et al. [97] meta-analysis, which included 19 case-control, 5 transversal and 3 prospective studies involving 4000 subjects concluded that 10% of coronary arterial disease prevalence was due to hyperhomocysteinemia. They also suggested that a $5 \mu\text{mol/L}$ Hcy plasma rise could augment coronary disease risk to a similar grade as a 20 mg/dl cholesterol plasma raise.

On the other hand, the prospective cohort studies results that evaluated the association between hyperhomocysteinemia and cardiovascular disease risk have been more conflictive. There have been circa 20 statistically representative studies reported that show that an association between these two entities exists [98]. In 1992, the large prospective study "Physicians' Health Study" indicated that high Hcy levels were an independent risk factor for myocardial infarct with a relative risk (RR) of 3.4 in the subjects whose Hcy levels were in the 5% greater compared with the 90% below [99]. Afterwards, another study made in Norway reported a coronary disease RR of 1.4 for each 4 $\mu\text{mol/L}$ rise in plasmatic Hcy levels [100].

More recently, two studies were published that re-examined the relationship between Hcy and cardiovascular disease using new data. In the Cleophas et al. [101] meta-analysis, 33 studies were evaluated. The total risk that was obtained was 1.58 ($P < 0.001$), but the authors suggested that Hcy could be more a marker of unhealthy life styles than a risk factor for CAD. When the prospective (RR 1.49) studies were separated from the retrospective (RR 1.62) ones, the relationship was weaker, suggesting an inherent bias of retrospective studies that have favored the correlation. Besides, five of the eleven prospective studies revised did not show a significant relationship among hyperhomocysteinemia and CAD. In a revision made by Christen et al. [102] that covered 43 prospective studies showed just a weak or inexistent relationship between total Hcy levels and CAD. An even bigger concern arose from the fact that Hcy levels can rise after tissue damage [103], what made suppose that retrospective studies that evaluated patients with CAD could have been showing high Hcy levels as a result, and not as a cause, of coronary events.

What some prospective studies actually do show is that Hcy levels influence in patient's mortality with already established coronary disease. Nygård et al. [104] investigated the relationship between total Hcy levels and mortality in 587 patients with angiographically confirmed CAD. After a 4.6 year follow-up 64 patients died, having only 3.8% of these patients Hcy levels less than 9 $\mu\text{mol/L}$, compared to 24.7% that had values equal to or above 15 $\mu\text{mol/L}$. In the same way, in a similar group of patients, Hcy levels had a mortality predictive value, independently of the traditional risk factor, reactive C-protein, and 5–10 MTHFR genotype [105]. In the Matetzky et al. [106] study, the same observation was confirmed, and demonstrated that in patients with myocardial infarctions diagnosed by electrocardiography and cardiac enzymes, Hcy levels correlated with subsequent cardiac events and death.

When the influence of MTHFR C677T mutation in CAD has been analyzed, the results obtained have been ambiguous. Brattstrom et al. [107] published

a revision of this polymorphism and its effect in Hcy levels and vascular disease. 23 studies were included with almost 6000 patients with all kind of vascular diseases (but predominantly CAD). No difference was found between the vascular diseased patients and controls for the T allele frequency, that is, mutated (34% in both groups) or for genotype TT (12% in both groups). In contrast, a similar analysis made by Wu et al. [108] that included 2000 patients with just CAD, found a positive association between genotype TT (RR 1.3) and CAD. Nevertheless, if data was examined more closely it was observed that the positive correlation for TT genotype came just from three out of the ten studies, and it included Japanese patients only, observation that could suggest certain disparity in the C677T mutation roll among races. A recent study correlated CAD early presentation with Hcy levels and the presence of the same mutation, obtaining as results that hyperhomocysteinemia and T/T genotype have a stronger effect in CAD pathogenesis when they are combined, and that a marked Hcy raise ($>15 \mu\text{mol/L}$) in T/T homocytous patients is a risk factor for the early beginning of CAD, not being the case with homocytotes with normal Hcy levels [109]. In concordance with this last study, a recent meta-analysis published by Klerk et al. [110] that included 11 162 patients with CAD and 12 758 controls, concluded that under good folate ingestion conditions, and therefore low Hcy levels, it does not have any clinical value to look for C677T mutation in order to predict CAD.

Finally, getting away of CAD for a moment, it is worth to mention a recent paper published by Vasan et al. [111] who realized a prospective 8 year study including 2491 patients being 72 year old on average, without myocardium infarcts or failure. Their objective was to investigate the relationship between Hcy and cardiac failure, and it was concluded that Hcy levels above the average correlated positively with future heart failure development. It seems that this analysis is the first research that evaluated prospectively these two aspects, situation that gives more interest to Hcy and its relationship with cardiovascular problems.

Hyperhomocysteinemia Treatment

Hcy elevations in general population are quite common, particularly in elderly people. Vitamin status is the primary determinant in mild and moderate hyperhomocysteinemia, being deficiency in the B complex (folic acid, pyridoxine, cyanocobalamin) the cause in two thirds of the cases [112]. Vitamin supplementation results in near plasma Hcy normalization in the majority of patients, being the exception the subjects with severe MS functional deficiencies or a thermostable MTHFR mutation. In these last two cases, it should be given a supplement with betaine

Table 2. Hyperhomocysteinemia Treatment

Medication	Use
Folates and cobalamine	In all patients with basal hyperhomocysteinemia and methionine post-load;
Folic acid	Folate nutritional deficiency;
400 μg –1 mg per day in acquired deficiencies	MTHFR thermolabil mutation;
5 mg in congenital deficiencies	CBS homozygous deficiency that does not respond to pyridoxine;
	Heterozygous for CBS deficiency.
Folinic acid or 5-MTHF	Instead of folic acid in patients with altered folate metabolism, or MTHFR thermostable mutations.
Cyanocobalamine	
400 μg –2 mg per day	Correction of vitamin B ₁₂ acquired deficiency before folate treatment.
Pyridoxine	In post-load methionine hyperhomocysteinemic patients that do not respond to folate treatment;
3–15 mg per day in acquired deficiencies	Vitamin B ₆ nutritional deficiency;
	Methionine rich animal protein diet excess;
30–100 mg per day in congenital deficiencies	Homozygous and heterozygous CBS deficiency.
Betaine	MS functional deficiency;
	MTHFR thermostable mutations.
Methionine	MS functional deficiency;
	MTHFR thermostable mutations.

CBS: cystationine β -synthetase, MS: methionine synthase, MTHFR: methylentetrahydrofotalereductase.

and/or methione in combination with folinic acid or 5-MTHF (Table 2) [12].

Returning to the majority of hyperhomocysteinemia cases, Brattström et al. [113] showed that administration of 5 mg of folic acid per day for 14 days was sufficient to diminish Hcy levels in healthy people, while 40 mg of pyridoxine of 1 mg of cyanocobalamin had little or no effect when comparing all three-treatment group results. Afterwards the same authors [114] described that administration of 10 mg of folic acid plus 240 mg of pyridoxine per day for four weeks diminished Hcy fasting levels in 53% and methionine post load in 39% in 20 patients with occlusive type cerebral and peripheral premature disease. The same group of investigators in a later paper showed that patients that had suffered a myocardium infarction accomplished to diminish their Hcy levels with the administration of 2.5 mg of folic acid for six weeks [115]. Likewise, Franken et al. [116] treated patients with mild hyperhomocysteinemia with 250 mg of vitamin B₆ per day for six weeks after which Hcy concentration post methionine load diminished in 56% of patients. Ulterior treatment with the addition of folic acid and/or betaine resulted in Hcy levels normalization in 95% of patients. In concordance with these studies, Samman et al. [117] demonstrated that Hcy levels correlated inversely with plasma folic acid level.

In the placebo controlled study made by Ubbink et al. [118] it was investigated the roll of three vitamins as determinants of plasma Hcy concentration. One hundred individuals were enrolled with high Hcy fasting levels and were divided in five differ-

ent treatment groups: (1) placebo; (2) 0.65 mg of folic acid; (3) 10 mg of pyridoxine; (4) 0.4 mg of cyanocobalamin; (5) combination of the three vitamins. Hcy levels were taken after four and six weeks of treatment beginning. Folic acid group diminished 42% its Hcy level, vitamin B₁₂ group 15%, and pyridoxine group did not have substantial effects. The three-vitamin combination group did not vary significantly from the folic acid group.

Following with the same investigation line, many studies have been published that show the beneficial vitamin effects lowering Hcy levels. The obtained information shows that folic acid administration reduces fasting Hcy as well as post methionine load levels, including the patients with partial congenital CBS and MTHFR deficiencies. This information also indicates that pyridoxine supplementation just reduces hyperhomocysteinemia detected after methionine load in patients with vitamin B₆ deficiencies or in heterozygotes for CBS. Cyanocobalamin treatment would just be of benefit in the cases of vitamin B₁₂ deficiency [119].

The main folic acid roll in diminishing Hcy levels could be explained by the fact that, in contrast to 5-MTHF, cobalamin and pyridoxine act just as cofactors and are not consumed during Hcy metabolism [120]. In this way, folic acid supplementation is the only effective treatment to reduce plasma Hcy levels in healthy subjects [119].

The optimal vitamin therapeutic dosage is not clear yet, although the more commonly used is 5 and 10 mg of folic acid per day; nevertheless it has been seen that even dosages of 500 μg can revert

hyperhomocysteinemia efficiently [121]. In Malinow et al. [122] study it was investigated the effect of different folic acid dosages in fortified cereals, being the dosages of 127 μg , 499 μg , and 665 μg , and it was seen a rise in plasma folic acid levels of 30.8%, 64.8%, and 105.7%, and a diminution in Hcy levels of 3.7%, 11.0% and 14.0%, respectively.

Now, as it is clear that there is an efficient way to lower plasma Hcy levels, it results very interesting to see if this treatment can reduce vascular diseases incidence. In this respect, some studies have been published as for example Schnyder et al. [123] who evaluated the efficiency of lowering Hcy levels as a strategy to avoid restenosis after coronary angioplasty. For this purpose, they used a combination of 1 mg of folic acid, 400 μg of vitamin B₁₂, and 10 mg of pyridoxine, for six weeks, and compared against placebo in 205 patients who underwent a successful angioplasty. The results obtained showed that restenosis grade was significantly bigger in the placebo group (37.6%) than in the vitamin treated group (19.6%). The same groups found similar results in another study with a one year follow-up that included 553 patients using the same treatment, having as end points adverse cardiac events (death, non fatal myocardial infarction, need to revascularize), presenting the adverse effect 22.8% of placebo group and 15.4% of the treated one [124]. These results have led some interventionists to adopt this vitamin strategy after coronary interventions, although if analyzed the data deeper, these vitamin treatment seemed to be more effective after balloon angioplasty than after coronary stenting, being the latter now the method of choice for the vast majority of patients undergoing coronary intervention. This is why Lange et al. [125] made a similar study using these vitamin treatment but only after stenting and found that folate therapy had adverse effects on the risk of restenosis in all subgroups except for women, patients with diabetes, and patients with markedly elevated homocysteine levels (15 $\mu\text{mol/L}$) at baseline, results that make the controversy about folate therapy grow.

At this moment there are on their way several multi-centric, randomized, placebo controlled trials addressing the issue of vitamin treatment as a preventive for vascular disease, results that will give many answers in some years. Some of these trials are the Norway Study of Vitamin Intervention (NORVIT) and the West Norway Study of Vitamin B Intervention (WENBIT) that will show the effects of lowering Hcy with vitamins B treatment in patients with CAD. The Trial of Vitamin Intervention for Strokes Prevention (VISIP) in the United States will investigate vitamins B effects in strokes recurrence in patients with cardiovascular disease. Likewise, the Great Britain Study of Efficiency of Additional Reductions of Cholesterol and Homocysteine (SEARCH) will evaluate similar aspects [98].

Conclusion

Mild to moderate hyperhomocysteinemia seems to be an independent and causal risk factor for cardiovascular disease, as well as a death prognostic factor in patients with myocardium infarction or documented coronary disease. Genetic defects, nutritional deficiencies, and demographic factors are some of the causes of high plasma Hcy, elevation that provokes direct and indirect endothelial damage, promoting a procoagulant and proinflammatory status in blood. Until now it has been demonstrated that vitamins B administration, mainly folic acid, diminish in an efficient way plasma Hcy levels, in a dosage proportional effect, fact that in theory should impact in cardiovascular disease prevalence. In order to be able to establish that vitamin B complex administration efficiently diminish morbidity and mortality related to cardiovascular disease, we should wait the results of randomized, double blinded, placebo controlled trials, that will give light and answer to this interrogate. In case these trials actually show beneficial effects of vitamins B supplementation, it will be one of the major advances in prevention and treatment of cardiovascular problems, because through a cheap treatment the survival and life quality of millions of patients will be impacted.

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