Homocysteine: Discovery and Metabolism

2

2.1 Introduction

It is estimated that there are more than 50,000 human proteins and that the number of distinct proteins in each cell is 3000–5000. Amino acids are the basic structural units of proteins. Of the several amino acids present in nature, about 22 are required for the synthesis of human proteins. Of these 22 amino acids, 10 amino acids cannot be synthesized in the human body, and, hence, they must be derived from the diet. These amino acids have been coined "essential amino acids." Cysteine, a semi-essential amino acid, is an integral constituent of several human proteins and is derived from the essential amino acid methionine. Methionine serves as a source for available sulphur for the synthesis of cysteine and taurine, and, as *S*-adenosyl methionine, it is the most important methyl group donor in cellular metabolism. Homocysteine is an intermediate product in methionine metabolism.

During his experiments on intermediary metabolism, Vincent de Vigneaud[1] discovered homocysteine as an intermediate product of methionine metabolism (de Vigneaud 1952). Almost four decades later, McCully first associated homocysteine with atherothrombosis, and then several reports described mental retardation and specific vascular lesions (including atherosclerosis, tunica media thickness, tunica intima focal sclerosis, narrowing of arteries of all sizes and thromboembolism) in patients with homocystinuria, leading to its association with these phenomena (Carson and Neill 1962; Gerritsen and Waisman 1964; McCully and Ragsdale 1970).

To understand its pathology, it is imperative to know its physiology and metabolism.

Homocysteine is derived primarily from the breakdown of dietary methionine in the activated methylation cycle. It is not, per se, incorporated into proteins but is converted back to methionine (remethylation cycle) and further broken down by

[1] Vincent du Vigneaud was awarded the Nobel Prize in 1955 for his work on sulphur-containing compounds.

S. Bhargava, *The Clinical Application of Homocysteine*,
https://doi.org/10.1007/978-981-10-7632-9_2

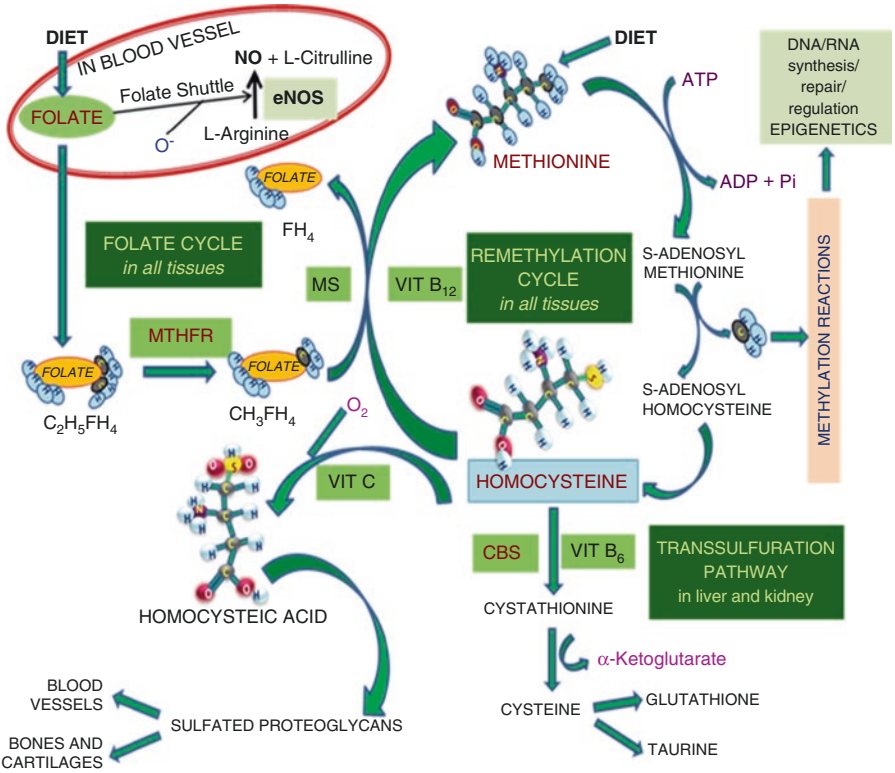

Fig. 2.1 Metabolism of homocysteine. The metabolism of homocysteine and its association with folate cycle, remethylation cycle and the transsulphuration pathway. *NO* Nitric oxide, *eNOS* endothelial nitric oxide synthase, $C_2H_5FH_4$ methylene tetrahydrofolate, CH_3FH_4 methyl tetrahydrofolate, FH_4 tetrahydrofolate, *MS* methionine synthase, O_2 oxygen, *MTHFR* methylene tetrahydrofolate reductase, *CBS* cystathionine beta synthase (Modified from Bhargava S, Srivastava LM. Hyperhomocysteinemia and its clinical implications—A short review. Current Medicine Research and Practice 2014; 4(3):112–118)

enzymes into cysteine and α-ketoglutarate (transsulphuration pathway), all of which are involved in specific protein syntheses.

The remethylation cycle is integrally related to the folate cycle (Fig. 2.1). In the folate cycle, circulating folate is converted to tetrahydrofolate which, on methylation, yields 5,10-methylene tetrahydrofolate (5,10-MTHF) first and then 5-methyltetrahydrofolate (5-MTHF) by the reducing action of the enzyme methylene tetrahydrofolate reductase (MTHFR).

The name "remethylation" cycle comes from the chemical alteration of homocysteine which gets "remethylated" to methionine by acquiring a methyl group from 5-MTHF from the folate cycle through a reaction catalysed by the enzyme methionine synthase (MS) which is cobalamin dependent. Homocysteine actually acquires a methyl group from methyl cobalamine, which is then remethylated by a methyl group from MTHF.

Thus the cobalamin cofactor serves as both donor and acceptor of the methyl group. Hence, 5-MTHF and cobalamin are necessary for the remethylation of homocysteine, and MTHFR is a rate-limiting enzyme for the remethylation cycle. Sometimes in the liver, homocysteine acquires a methyl group from betaine through a reversible reaction. All these reactions are vitamin B_{12} dependent. Approximately every 2000 catalytic cycles, the cobalamin is oxidized and reactivated.

On activation by adenosine triphosphate (ATP), most of the methionine goes into the formation of *S*-adenosyl methionine (SAM). SAM serves primarily as a universal methyl donor to a variety of acceptors involved in protein and nucleotide syntheses. *S*-adenosyl homocysteine (SAH) is the by-product of these methylation reactions, and its formation is governed by the SAM/SAH ratio.

It is subsequently hydrolysed by SAH hydrolase, in a reversible reaction, to yield homocysteine. Homocysteine, thus regenerated, becomes available to start a new cycle of methyl transfer. Here, it would be of utmost importance to note that this hydrolysis is a reversible reaction that favours the synthesis of SAH and that elevated cellular concentrations of this metabolite are likely to precede and accompany all forms of homocysteinemia (Selhub 1999; Gellekink et al. 2005).

The transsulphuration pathway, on the other hand, is not a circular pathway leading to the regeneration of homocysteine. It is a one-way condensation of homocysteine with serine to form cystathionine in an irreversible reaction catalysed by the pyridoxal-5-phosphate (PLP)-containing enzyme, cystathionine-β-synthase (CBS), the rate-limiting enzyme of this pathway. Another PLP-containing enzyme, γ-cystathionase, catalyses cystathionine to form cysteine and α-ketobutyrate. Thus, this pathway performs a dual function—firstly, catabolizing excess homocysteine (which is not required for methyl transfer) and secondly, synthesis of cysteine which gets further degraded to release hydrogen sulphide (H_2S). Vitamin B_6 is the cofactor for these reactions.

In addition, in the presence of vitamin C, homocysteine is also oxidized to homocysteic acid which is required for the synthesis of sulphated proteoglycans of the vessel walls, bones and cartilages.

Thus, homocysteine is located at a critical metabolic crossroad and, both directly and indirectly, impacts all methyl and sulphur group metabolisms occurring in the body. Decreased metabolism of homocysteine leading to homocysteinemia, therefore, causes decreased methylation reactions as well as decreased synthesis of sulphated proteoglycans, which though found in all tissues are highest in concentration in the cartilages, tendons, ligaments, synovial fluid, skin, finger- and toenails, heart valves and the basement membrane of all blood vessels (Bhargava et al. 2012a).

It is pertinent here to note that folate is required for the synthesis of nitric oxide (NO) by the vascular endothelium. In the presence of oxidative stress, the requirement for NO increases, and more folate is shunted towards its synthesis, and, consequently, decreased folate is available for remethylation, thus leading to homocysteinemia. Conversely, when there is homocysteinemia, folate utilization shifts from synthesis of NO to the remethylation cycle, causing a decreased synthesis of NO and, thereby, increased oxidative stress. This would explain why some scientists say that homocysteinemia causes oxidative stress, and yet others hold that

oxidative stress leads to homocysteinemia. This "folate shuttle" model was proposed by Hayden and Tyagi (2004).

2.2 Biological Reference Interval

Reports on biological reference interval (BRI) for concentrations of homocysteine in plasma differ greatly from one laboratory to another. In 1989, Malinow et al. (1989) had recommended keeping homocysteine below 10.0 μmol/L. This was further emphasized, by Boushey et al. (1995), when they elucidated in their meta-analysis that homocysteine does not have a threshold beyond which it is pathogenic; its pathogenicity increases with its concentration even within the biological reference interval which was confirmed to be 5–15 μmol/L. A homocysteine >15 μmol/L was termed homocysteinemia. Malinow et al. (1996) graded homocysteinemia as mild (homocysteine = 16–30 μmol/L), moderate (homocysteine = 31–100 μmol/L) and severe (homocysteine >100 μmol/L).

2.3 Hyperhomocysteinemia (or Homocysteinemia): Causes and Modulation

2.3.1 Causes

The methylation cycle has three limiting factors—the enzyme, methylene tetrahydrofolate reductase (MTHFR) and vitamins folic acid and B_{12}—whereas the transsulphuration reaction is dependent on the enzyme, cystathionine-β-synthase (CBS) and the vitamin, pyridoxine (B_6). Deficiency of any of these vitamins or polymorphisms in the genes of either of these enzymes will reduce the rate of metabolism of homocysteine and cause its accumulation in plasma, i.e. homocysteinemia.

A scrutiny of the metabolism of homocysteine, as detailed above, reveals that homocysteinemia can be a result of either:

A. *Genetic defects in one of the enzymes of homocysteine metabolism*. The MTHFR gene is present on the short arm of chromosome 1 at position 36.3 (Goyette et al. 1998), and the CBS gene is present on the long arm of chromosome 21 at position 22.3 (Münke et al. 1988). The most common defects in either of these enzymes are single-nucleotide polymorphisms (SNPs), which result in either synthesis of a defective enzyme or synthesis of less quantity of enzyme.

   MTHFR is encoded by a 20,328 base pair gene comprised of 11 exons. There are 18 known polymorphisms of this gene, but the most common are the C677T and the A1298C. The former occurs on exon 4, and the resultant thermolabile enzyme shows decreased activity due to dissociation of a dimer into monomers and decrease in its FAD-biding capacity. The latter occurs on exon 7 and apparently does not affect the functioning of the enzyme, with consequently no effect on circulating homocysteine levels (Radha Rama Devi et al. 2004).

The CBS gene is the most common site for mutations resulting in homocystinuria. It is a 23,678 base pair located on chromosome 21q22.3. More than 150 mutations causing homocystinuria have been identified in this gene, but the most common are the T833C, G919A and G1330A.

Interestingly, Alessio et al. (2008), evaluated CBS gene polymorphisms (T833C, G919A and 844ins68) in the samples of 220 children whose samples had already been analysed for MTHFR (C677T, A1298C) and MSR (A66G) polymorphisms. They observed that the insertion of 68 base pairs at position 844 (844ins68) was always associated with T833C (prevalence 19.5%); on the other hand, the G919A polymorphism was not observed, all children exhibiting only GG genotype.

In addition, there are several SNPs that modulate the other enzymes of homocysteine metabolism (e.g. SAH hydrolase, methionine synthase (MS), methionine synthase reductase (MSR)), but they do not culminate in overt metabolic disorders even though homocysteine is mildly raised. Most common among these are the A2756G of MS and the A66G of MSR, as mentioned above.

B. *Nutritional deficiency of one or more of the vitamins that participate in homocysteine metabolism* (Boushey et al.). Three vitamins of the B group—folic acid, vitamin B_{12} and vtamin B_6—play a pivotal role in the metabolism of homocysteine as detailed above. Hence, a deficiency of either one or more of these three leads to accumulation of homocysteine.

Sengupta and his team evaluated the dietary factors and polymorphisms associated with homocysteinemia in Indians, with the basis that due to the prevalent vitamin B_{12} deficiency in our population, the impact of polymorphisms is exaggerated. They observed that homocysteinemia was more prevalent in those on a vegetarian diet ($p = 0.019$) or those with the MTHFR A1298C polymorphism ($p = 0.006$). The minor allele frequency for MTHFR C677T and A1298C is 0.15 and 0.44, respectively, indicating a greater prevalence of the latter polymorphism, unlike in other populations where MTHFR C677T is more prevalent (Kumar et al. 2005).

2.3.2 Modulation

By SNPs or Vitamin Deficiencies By virtue of the different parts of the metabolic cycles affected, several SNPs and vitamin deficiencies affect plasma homocysteine concentrations to different extents. Sometimes these genetic and dietary variants coexist, and the resultant homocysteine concentration varies further (Fig. 2.2).

By Nutrition Utilization of homocysteine molecules by the transsulphuration or remethylation pathways is nutritionally regulated:

a. When a basal *methionine*-containing diet is administered, homocysteine moieties are found to go through the remethylation pathway approximately 1.5–2.0 times before being catabolized through the transsulphuration pathway (Mudd and Poole 1975).

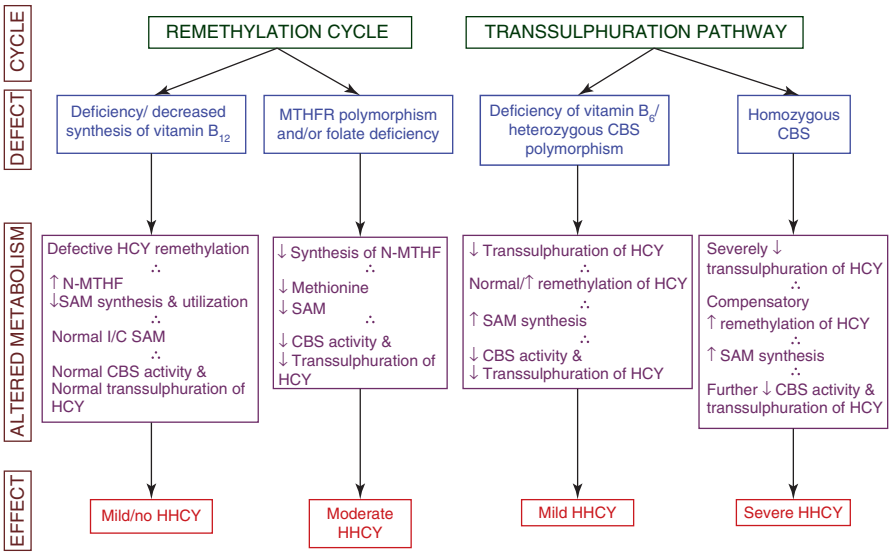

Fig. 2.2 Modulations of plasma homocysteine concentration by SNPs and vitamin deficiencies. *SNP* Single-nucleotide polymorphisms, *MTHFR* methylene tetrahydrofolate reductase, *CBS* cystathionine β synthase, *HCY* homocysteine, *SAM* *S*-adenosyl methionine, *HHCY* hyperhomocysteinemia

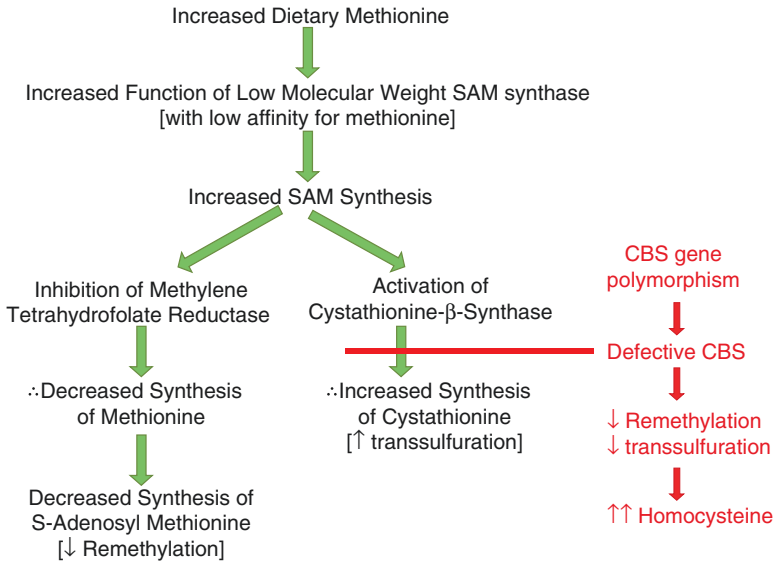

Fig. 2.3 Effects of increased dietary methionine on homocysteine metabolism

b. When dietary methionine is enhanced, this homocysteine cycling falls below basal level.
c. This capacity of the body to discriminate between remethylation and transsulphuration pathways in response to varying amounts of dietary methionine implies the existence of a coordinated regulation between these two pathways (Fig. 2.3).

   Increased dietary methionine leads to a decreased rate of remethylation and consequently an increased homocysteine concentration to be dealt with by the transsulphuration pathway. When there is excess of methionine, despite the feedback mechanism, all the homocysteine is not metabolised by the transsulphuration pathway and, hence, accumulates with resultant homocysteinemia.

   Similarly, in conditions of CBS deficiency, increased dietary methionine leads to severe homocysteinemia. Also as mentioned earlier, decreased SAM synthesis results in an inhibition of methylation reactions as well as a decrease in the formation of sulphated proteoglycans, which impact the integrity of the target organs individually or even in combination. An example of the double-pronged effect is the impairment of the vascular endothelial proteins (due to methylation) as well as impaired formation of collagen of the blood vessels (due to decreased synthesis of sulphated proteoglycans). Both these events initiate and promote the process of atherothrombosis (Gellekink et al. 2005).
d. McCully also established the correlation between the oxidative and reductive properties of *ascorbic acid* and the metabolic and pathologic abnormalities of the connective tissues in scurvy, i.e. vitamin C deficiency (McCully 1971). He demonstrated that lower levels of dihydroascorbic acid led to slower oxidation of homocysteine to homocysteic acid and a consequent accumulation of homocysteine (Fig. 2.4).

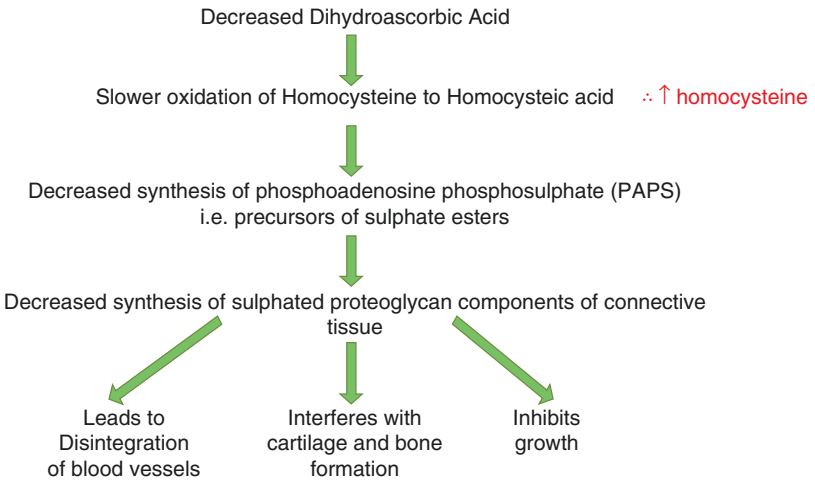

Fig. 2.4 Interplay between vitamin C (ascorbic acid) and homocysteine

Through its effect on homocysteine metabolism, deficiency of ascorbic acid leads to disintegration of walls of the blood vessels, interference in cartilage and bone formation, and consequent inhibition of growth.

To perpetuate its pathological effects on the endothelium, homocysteine has to enter these cells. In 2001, Sengupta et al. (2001) demonstrated experimentally that when in circulation, homocysteine is bound to albumin. This albumin is known to bind to several endothelial cell membrane proteins through sites other than that by which homocysteine binds to it. Thus, albumin enables homocysteine to be transcytosed via the plasmalemmal vesicles (gp 60) or endocytosed via receptor-mediated endocytosis (gp 30, 18). Once it enters these cells, homocysteine is degraded by the lysosomes and released into the cytosol where it modifies several proteins and alters the redox potential resulting in oxidative stress and endothelial cell dysfunction.