

“THE HOMOCYSTEINE IS A RISK FACTOR FOR CARDIOVASCULAR DISEASE”

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Abstract:

Coronary heart disease (CHD) is the leading cause of death among adults in developed countries. It is noted that with age the fat deposits in the wall of the coronary arteries as well as the other blood vessels supplying the heart. As a result of this deposition, there is a decrease in the blood supply to the heart causing angina and shortness of breath and may also result in a fatal myocardial infarction. There are several modifiable risk factors for CHD and one of them being the increased level of the amino acid i.e homocysteine (HCY) which when treated can reduce the risk of CHD. The positive correlation between hyper homocysteinemia and cardio vascular disease (CVD) has established firmly with the data derived from experimental and epidemiological observations. Clinical data authenticate that HCY is an independent risk factor for CVD. The current article is aiming to evaluate potential role of HCY on CVD risk at molecular level, and deep insights into a pathophysiology of CVD and their associations with CVD.

Key words: Homocysteine, Hyperhomocysteinemia ,Cardiovascular disease.

I. Introduction:

Cardiovascular disease (CVD) is an atypical functioning of the heart and blood vessels of the body.¹ Different types of CVD include stroke, congenital cardiovascular defects, hypertension, congestive heart failure, and hardening or narrowing of the blood vessels (atherosclerosis), including the coronary arteries² The global rise in CVD is the result of an unprecedented transformation in the cause of morbidity and mortality during the twentieth century. Known as the epidemiologic transition, this shift is driven by industrialization, urbanization, and associated

lifestyle changes and is taking place in every part of the world among all races, ethnic groups, and cultures. CVD accounts for nearly 30% of death worldwide, a number that is expected to increase. In 2010, CHD accounted for 13.3% of all deaths globally and the largest portion of global years of life lost (YLLs) and disability –adjusted life-years (DALYs). The second largest cause of death was stroke (11.1% of all deaths), which was also the third largest contributor to global YLLs and DALYs.³

Table 1: Morbidity Related Heart Disease : 2010 -2030.³

Death	2010	2030
CVD deaths : annual number of deaths	15.6 millions	24.2millions
CVD deaths : percentage of all deaths	30.0%	32.5%
CHD deaths: percentage of all male deaths	13.0%	14.9%
CHD deaths : percentage of female deaths	14.0%	13.1%
Stroke deaths : percentage of all male deaths	9.9%	10.4%
Stroke deaths : percentage of all female deaths	13.0%	11.8%

Hence Coronary heart disease (CHD) is the leading cause of death among adults in developed countries. With age, fatty deposits (atherosclerotic plaques) coat the walls of the coronary arteries and the blood vessels that supply the heart with oxygen and nutrients.^[4] The resultant restriction of the heart's blood supply causes shortness of breath, angina (chest pains that are usually relieved by rest), and sometimes fatal heart attacks.^[4] Many established risk factors for CHD, including smoking, physical inactivity, being overweight, and eating a fat-rich diet, can be modified by lifestyle changes. Another possible modifiable risk factor for CHD is a high blood level of the amino acid homocysteine.⁴

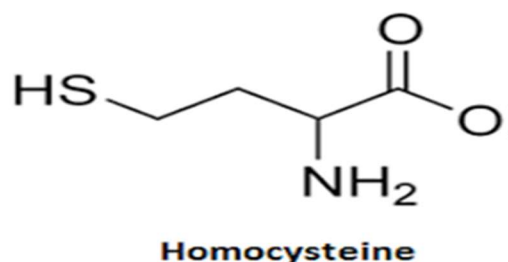
II. History of Homocysteine

The American biochemist Vincent DuVigneaud discovered a new amino acid in 1932 by treating methionine with sulfuric acid. The structure of this amino acid is similar to cysteine, except for one extra carbon atom, hence the name homocysteine. Subsequent investigation established the role of homocysteine as an intermediate in sulfur amino acid metabolism and transmethylation reactions. Little was known, however, about the biomedical significance of homocysteine until 1962, when children with mental retardation, accelerated growth, osteoporosis, dislocated ocular lenses, and frequent thrombosis of arteries and veins were discovered to excrete homocysteine in the urine. Most children with homocystinuria are deficient in the enzyme cystathionine synthase, a pyridoxal phosphate-dependent enzyme that catalyzes the synthesis of cystathionine from homocysteine and serine. Because of this enzyme deficiency, homocysteine and methionine accumulate to high levels in plasma, and homocystine, the disulfide dimer of homocysteine, is excreted in the urine.⁵

III. Properties of Homocysteine

Table 2: Properties of homocysteine have been tabulated below.⁶

<u>Chemical formula</u>	C ₄ H ₉ NO ₂ S
<u>Molar mass</u>	135.18 g/mol
<u>Appearance</u>	White crystalline powder
<u>Melting point</u>	234–235 °C (453–455 °F; 507–508 K) ^[2] (decomposes)
<u>Solubility in water</u>	soluble
<u>log P</u>	-2.56 ^[1]
<u>Acidity (pK_a)</u>	2.25 ^[1]

Figure 1: Structure of Homocysteine.⁷

IV. Discovery of the Homocysteine Theory of Arteriosclerosis

In a 1969 review of an archival case of homocystinuria in an eight-year-old boy, originally published in 1933, it was discovered that the cause of death was a massive stroke resulting from carotid arteriosclerosis and thrombosis. In addition, arteriosclerotic plaques were found to be scattered through arteries to major organs of the body, suggesting a possible connection between homocysteine and atherogenesis.

In a second case of homocystinuria in a two-month-old boy, caused by deficiency of a different enzyme, methionine synthase, widespread, advanced arteriosclerotic plaques were discovered, scattered through the arteries. Because the enzyme deficiency caused elevated blood levels of cystathionine and homocysteine, and decreased levels of methionine, it was concluded that homocysteine causes arteriosclerotic plaques by a direct effect on the cells and tissues of the arteries, since homocysteine elevation was the only metabolic abnormality shared by these two cases. Several years later, investigators in Chicago demonstrated similar arteriosclerotic plaques in a child with methylenetetrahydrofolate reductase deficiency, the third major type of homocystinuria, independently corroborating the conclusion that homocysteine is an atherogenic amino acid.⁵

In the original publication of these cases of homocystinuria, it was suggested that elevation of blood homocysteine is important in the pathogenesis of arteriosclerosis in the general population without these rare inherited disorders of homocysteine metabolism. The individuals with hereditary, dietary, environmental, hormonal, metabolic, or toxic predispositions to arteriosclerosis develop arterial plaques from damage by homocysteine to the lining cells and tissues of arteries.⁵

V. Blood levels of homocysteine and increased risk of cardiovascular disease

Normal homocysteine level in blood is 5 -15 $\mu\text{mol/L}$. In diseases, it may be increased to 50 to 100 times. Moderate increase is seen in aged persons, vitamin B₁₂ or B₆ deficiency, tobacco smokers, alcoholics and in hypothyroidism. Substantial increase is noticed in congenital enzyme deficiencies. Increasing the concentration of homocysteine in blood is called as hyperhomocysteinemia (HHcy).

Classification of hyperhomocysteinemia (according to Selhub, 1999)

Severe hyperhomocysteinemia: High total homocysteine (tHcy) levels at all times ($>100\text{mmol/L}$), caused for example by deficiencies in Cystathionine beta synthase (CBS), methylenetetrahydrofolate reductase (MTHFR), or in enzymes of B12 metabolism

Mild hyperhomocysteinemia: Moderately high tHcy levels (15-30 mmol/L) under fasting conditions; reflects impaired homocysteine methylation (folate, B12 or moderate enzyme defects, e.g. thermolabile MTHFR)

Post-methionine load: Abnormal increase in tHcy ($>15\text{mmol/L}$) after methionine load (100 mg/kg); reflects impaired homocysteine transsulfuration (heterozygous CBS defects, B6 deficiency).

Large amounts of homocysteine are excreted in urine. In plasma, homocysteine (with -SH group) and homocystine (disulfide, -S-S- group) exist. Both of them are absent in normal urine but if present, it will be the homocystine (disulfide) form. If homocysteine level in blood increased, there is increased risk for coronary artery diseases. There is some evidence to associate the increase in homocysteine level in blood and myocardial infarction. An increase of 5 $\mu\text{mol/L}$ of homocysteine in serum elevates the risk of coronary artery disease equivalent to an increase of cholesterol of 20 mg/dl . Homocysteine interacts with lysyl residues of collagen interfering with collagen cross linkage. It forms homocysteine thiolactone, a free radical which thiolates LDL particles. These particles tend to aggregate, are endocytosed by macrophages and increase the tendency for atherogenesis.⁸

Hcy (Homocysteine) is a sulfhydryl-containing amino acid that is formed by the demethylation of methionine. It is normally catalyzed to cystathionine by cystathionine beta-synthase a pyridoxal phosphate-dependent enzyme. Hcy is also remethylated to methionine by 5-methyltetrahydrofolate-Hcy methyltransferase (methionine synthase), a vitamin B12 dependent enzyme and by betaine-Hcy methyltransferase. Nutritional status such as vitamin B12, or vitamin B6, or folate deficiencies and genetic defects such as cystathionine beta-synthase or methylenetetrahydrofolate reductase may contribute to increasing plasma homocysteine levels. The pathogenesis of Hcy-induced vascular damage may be multifactorial, including direct Hcy damage to the endothelium, stimulation of proliferation of smooth muscle cells, enhanced low-density lipoprotein per oxidation, increase of platelet aggregation, and effects on the coagulation system causes the damage of the capillaries leads into Cardiovascular disease.⁹

Another possible modifiable risk factor for CHD is a high blood level of the amino acid homocysteine. Methylenetetrahydrofolate reductase, which is encoded by the *MTHFR* gene, uses folate to break down and remove homocysteine so fortification of cereals with folate can

reduce population homocysteine blood levels. Pooled results from prospective observational studies that have looked for an association between homocysteine levels and later development of CHD suggest that the reduction in homocysteine levels that can be achieved by folate supplementation is associated with an 11% lower CHD risk.⁴

The molecular basis for production of arteriosclerotic plaques is related to the effect of homocysteine on cellular degeneration, damage to arterial intima, cellular growth, connective tissue formation, deposition of lipoproteins in plaques, and enhanced blood coagulation. In each of these critical processes in atherogenesis, homocysteine plays a key role.⁵

Another feature of early plaques is fragmentation and degeneration of elastic fibers. Homocysteine activates the enzyme elastase within arteries, causing fragmentation of the internal elastic membrane. Homocysteine also causes cultured smooth muscle cells to produce excess collagen, explaining the fibrosis that is characteristic of human and experimental plaques. Also, arterial smooth muscle cells proliferate in plaques because homocysteine activates cyclins, signaling proteins that mediate cell division. Homocysteine is involved in skeletal growth by releasing insulin-like growth factor and increasing the sulfation of epiphyseal cartilage of animals, explaining the accelerated skeletal growth in children with homocystinuria and the growth of smooth muscle cells in developing arteriosclerotic plaques.^[5] The first human study of homocysteine in vascular disease in 1976 showed that oral methionine causes increased levels of homocysteine and homocysteine cysteine disulfide in the plasma of patients with coronary heart disease (CHD). Many subsequent studies showed that persons with coronary, cerebral, or peripheral arteriosclerosis have elevated levels of homocysteine in their blood. In patients with early onset of arteriosclerosis, elevation of homocysteine is a more potent risk factor than cholesterol elevation and is similar in strength to the effect of smoking.⁵

VI. Molecular aspects of homocysteine

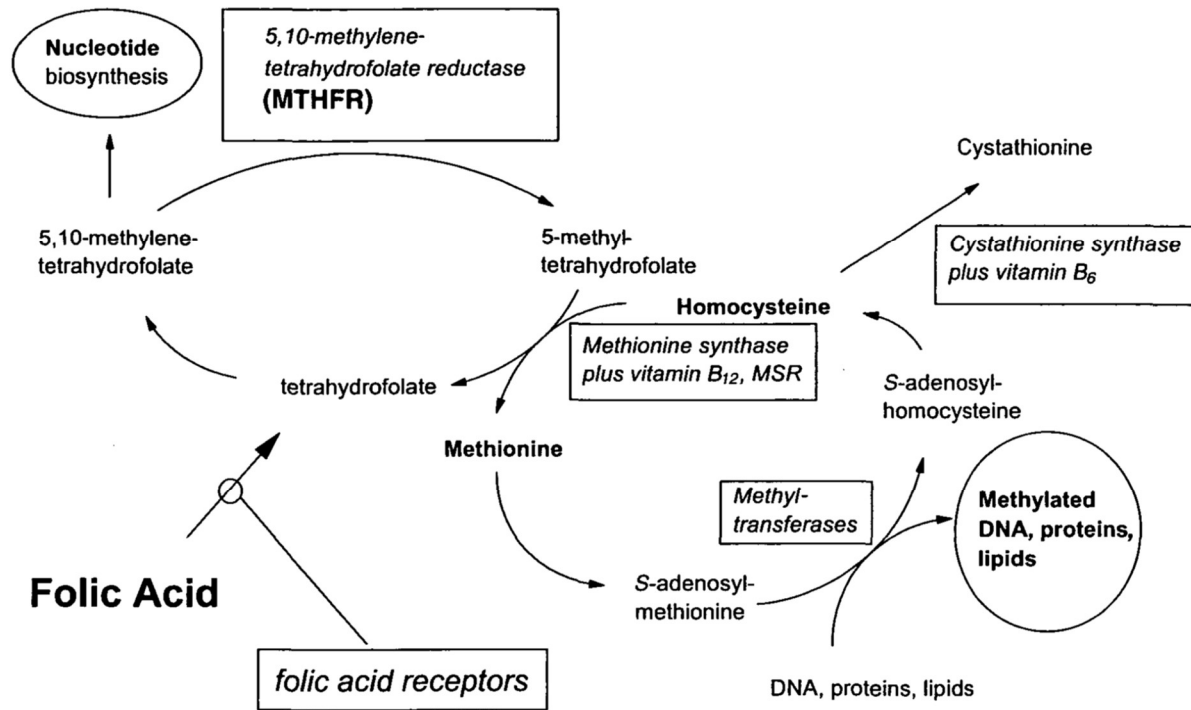
There are 2 genes very importantly controls the homocysteine metabolism

1) MTHFR gene (5,10-methylenetetrahydrofolate reductase) located on chromosome 1 at 1 p36.3 and The cDNA (complementary DNA) sequence is 2.2 kilobases long and consists of 11 exons. The MTHFR gene commonly located on two alleles. The C677T allele and the A1298c allele.

The 5,10-methylenetetrahydrofolate reductase (MTHFR) is a enzyme involved in folate metabolism by conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is the major circulating form of folate. The 5- methyl form, involves in single-carbon transfers as part of the synthesis of nucleotides; the synthesis of S-adenosyl-methionine; the remethylation of homocysteine to methionine; and the methylation of DNA, proteins, neurotransmitters, and phospholipids. usually MTHFR activity may help maintain the pool of circulating folate and methionine and prevents accumulation of homocysteine.¹⁰

Figure 2: Metabolic pathways involving 5,10-methylenetetrahydrofolate reductase

(MTHFR). MSR, methionine synthase reductase.¹⁰



MTHFR gene mutation

C677T allele

Lorenzo D. Botto and Quanhe Yang et al (2000) The C677T allele is characterized by a point mutation at position 677 of the MTHFR gene which converts a cytosine (C) into a thymine (T); this type of point mutation results in an amino acid substitution alanine to valine in the enzyme. The C677T allele is "thermolabile," because the activity of the encoded enzyme is decreased at 37°C or more. Hence, MTHFR activity among C677T homozygotes is 50-60 percent lower at 37°C and 65 percent lower at 46°C than in similarly treated controls. Heterozygotes are in the intermediate range. People who are homozygous for the C677T allele tend to have mildly increased blood homocysteine levels if their folate intake is insufficient but normal blood levels if their folate intake is adequate.¹⁰

A1298C

Lorenzo D. Botto and Quanhe Yang et al (2000) The A1298C allele, a point mutation in exon 7 results in an amino acid substitution (glutamate for alanine) in the enzyme. This allele has also been called the C1289A allele. The activity of the encoded enzyme is decreased, although less than is the case with the C677T allele. People who are homozygous for the A1298C allele do not appear to have higher serum homocysteine levels than controls. However, people who are

compound heterozygous for the A1298C and C677T alleles (i.e., people with the A1298C/C677T genotype) tend to have a biochemical profile similar to that seen among C677T homozygotes, with increased serum homocysteine levels and decreased serum folate levels ¹⁰

2) FTO gene

FTO - Fat mass and obesity-associated protein also called as alpha-ketoglutarate-dependent dioxygenase FTO is an enzyme that in humans is encoded as FTO gene which is located on Fat Mass and Obesity associated gene region (*FTO*) at 16q10 is strongly associated with both increased body weight and also susceptibility to type 2 diabetes (T2D). ¹¹

Genotype effect on homocysteine levels

A study conducted on the possible assessment of association of homocysteine with the FTO gene showed that there was a significant increase in the levels of homocysteine by the presence of FTO rs9939609 AA genotype thereby proving that the presence of this FTO gene can alter the levels of homocysteine significantly. The increased homocysteine levels can be attributed to the significantly increased neuroinflammation (Obeid and Hermann 2006), interference with the response of natural killer cells, adhesion molecules, and both B and T lymphocytes (Kim et al. 1997; Koga et al. 2002), and reduced S-adenosyl methionine (SAM), the important methyl donor in various methylation reactions (Mattson and Shea 2003) results in hypomethylation. ¹² The hypomethylation has been associated with aortic lipid deposition, a predictor of future atherosclerosis.

VII. Comparative analysis of Previous studies showing the relationship between Homocysteine and CVD

Table 3: Cross sectional studies Reports of Homocysteine and Heart disease.¹³

S.No	Source and year	Age, y	Sample Size		Mean Homocysteine, $\mu\text{mol/L}$		
			Cases	Controls	Cases	Controls	P
1.	Murphy-Chutorian et al, 1985	21-65	99 Cases	39 Controls	0.03	0.06	NS
					0.7		0.6 (P)
2.	Kang et al, 1986	<69	241 Cases	202 Controls	5.5	4.3	<.001
3.	Malinow et al, 1990	Mean, 62	99 Cases	259 Controls	13	10.5	<.001
4.	Murphy-Chutorian and Alderman, 1994	17-80	80 Cases	22 Controls	0.76	0.40 (P)	<.001

In above 4 cross-sectional studies of homocysteine and CHD the determination of CHD has been based on angiographic evidence of greater than 50% occlusion of at least one coronary artery. Blood samples were collected around the time of CHD determination. In 2nd to 4th number of studies, mean homocysteine level was higher (approximately 30%-90% higher) in persons with CHD, 10-12 while the 1st number study found no difference in mean homocysteine level in those with and those without CHD. All 4 cross-sectional studies that defined elevated homocysteine level indicated markedly increased odds of CHD in persons with high homocysteine levels.¹³

Table 4: Case control studies Reports of Homocysteine and Heart disease

S.No	Source and year	Age, y	Sample Size		Mean Homocysteine, $\mu\text{mol/L}$		
			Cases	Controls	Cases	Controls	P
1.	Wilcken et al, ¹⁴ 1983	Š50	20 Cases	20 Controls	3.6 13.7	3.7 12.9 (P)	NS NS
2.	Israelsson et al, ¹⁵ 1988	48-58	21 Cases	36 Controls	16.4	13.5	<.05
3.	Genest et al, ¹⁶ 1990	<60	170 Cases	255 Controls	13.7	10.9	<.001
4.	Clarke et al, ¹⁷ 1991	<55	60 Cases	27 Controls	18.7	13.4 (P, G)	<.05
5.	Ubbink et al, ¹⁸ 1991	Mean, 55	163 Cases	195 Controls	16.2	13.4	<.001
6.	Pancharuniti et al, ¹⁹ 1994	30-50	101 Cases	108 Controls	13.5	11.9 (G)	<.001
7.	Von Eckardstein et al, ²⁰ 1994	36-65	199 Cases	156 Controls	8.9	7.8 (G)	<.001
8.	Wu et al, ²¹ 1994	Š65	170 Cases	168 Controls	13.4	10.1	<.001
9.	Dalery et al, ²² 1995	<60	150 Cases	584 Controls	11.7	9.0	NS
10.	Landgren et al, ²³ 1995	28-81	68 Cases	80 Controls	13.9	12.3	<.001
11.	Robinson et al, ²⁴ 1995	Mean, 62	304 Cases	231 Controls	14.4	10.9	..
12.	Gallagher et al, ²⁵ 1996	Mean, 49 (cases), 48(controls)	71 Cases	92 Controls	NG	NG	<.001
13.	Verhoef et al, ²⁶ 1996	<76	130 Cases	118 Controls	10.2	9.1 (G)	.006
14.	Graham et al, ²⁷ 1997	<60	383 Cases	800 Controls	11.2 34.4	9.7 (G) 30.3 (P,	<.001g <.001g

						G)	
15.	Schwartz et al, ²⁸ 1997	18-44	79 Cases	386 Controls	13.4	11.1	<.001
16.	Verhoef et al, ²⁹ 1997	25-65	131 Cases	189 Controls	41.1	12.3i 38.4 (P)i	NS NS

In above 16 case control studies of homocysteine and CHD Fifteen studies examined mean homocysteine levels, and all but 3^{14,23,29} reported significantly higher homocysteine levels (typically 10%- 30% higher), either fasting or after methionine load, in persons with CHD as compared with persons without CHD. Fifteen of 16 studies that compared proportions with elevated homocysteine levels observed an increased risk of CHD for persons with elevated homocysteine levels, and in most studies the CI around the RR estimate excluded the null value of 1.0 indicating that the increase in risk was statistically significant at the P<.05 level.

Table 5 : Studies showing the effect of homocysteine lowering on CHD and stroke (2018).³⁰

Author	Study (type)	Patients (no)	Age (yrs)	Duration (yrs)	Benefit (kind)	HR, OR, RR (95%, CI)
Cui	Prosp	35,611	40–79	14	CHD, W	0.57(0.34-0.96)
					HF, M	0.50 (0.270.94)
Wang	Rev-Meta	223,691	25–79	10.7	CHD	0.88 (0.82-0.94)
Huang	Rev-Meta	47,921	NA	3	CVD	0.98 (0.94-1.03)
	CHD 0.98	(0.92–1.05)	MI 0.97	(0.90–1.15)	Stroke	0.88 (0.82-0.95)
Qin	Rev-Meta	8234	49–72	NA	CVD	0.90 (0.81-1.00)
Huo	RCT	20,702	60	4.5	Stroke	0.79 (0.68-0.93)
		Composite outcome		(CV death, MI, stroke)		0.80 (0.69-0.92)
Li	Rev-Meta	82,334	49–69	0.5–7.0	Overall CVD	0.96 (0.92-0.99)
Park	Rev-Meta	4643	≥35	2–3.4	Stroke	0.71 (0.58-0.88)
Wang	Rev-Meta	7887	35–73	≥1.0	Cerebrovascular Event	0.31 (0.22-0.43)
Tan	Rev-Meta	65,790	50–69	1–7	Stroke	0.90(0.84-0.97)

HR = hazard ratio, OD = odds ratio, RR = relative risk, Prosp = prospective, Rev-Meta = review-meta-analysis, Cross-Sect = cross-sectional, RCT = randomized control trial, CHD =

coronary heart disease, HF = heart failure, CV mort = cardiovascular mortality, CVD = cardiovascular disease, CAD = coronary artery disease, Cerebrovasc = cerebrovascular, NA = not available, M = men, W = women.

In above prospective case control , reviews and meta-analysis, randomized control trials (RCTs) showing that hyperhomocysteinemia is lowering with folic acid \pm B vitamins and reduces the complications of CVD and stroke , hence these studies are demonstrating an association of hyperhomocysteinemia with an increased CVD and stroke incidences

VIII. FUTURE DIRECTIONS

The homocysteine could be potential biomarker for early detection of CVD risk and thereby help in reduction of risk for individual patient and population. The recent findings from prospective studies indicating little predictive ability of plasma homocysteine in CVD underscore the need for a more comprehensive and quantitative overview of all available data. Finally to conclude their exists a positive correlation between elevated homocysteine and risk of CVD. However further in depth research into the epigenetic aspects of homocysteine causing CVD and the homocysteine as an independent risk factor for CVD needs to be investigated

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