

- 1 **HYPERHOMOCYSTEINEMIA AND THROMBOSIS:**
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- 4
- 5 **AN OVERVIEW**

6 **Abstract:**

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9 Context: Homocysteine, a sulfur containing amino acid, absent in natural diets, is a

10 metabolic intermediary in transmethylation and transsulfuration reactions. Such

11 reactions are essential to normal cellular growth, differentiation and function. Excess

12 homocysteine is associated with vascular disease and related disorders. Objective: To

13 review homocysteine metabolism, the pathogenesis and classification of

14 hyperhomocysteinemia and the published literature investigating the association of

15 homocysteine and MTHFR with arterial and venous thromboembolism and related

16 disorders. The role of vitamin supplementation in patients with hyperhomocysteinemia

17 is addressed. Data Sources: Published medical and scientific literature. Data extraction and

18 Synthesis: Articles addressing the objectives were selected and reviewed.

19 Pertinent studies and conclusions summarized, grouped and contrasted. The prepared

20 manuscript was reviewed by both authors. Conclusion: The association of

21 hyperhomocysteinemia and arterial and venous thrombosis is controversial. Severe

22 hyperhomocysteinemia is associated with atherosclerosis. The effect of mild

23 hyperhomocysteinemia is less certain. Coinheritance of MTHFR and Factor V Leiden is likely

24 to increase the risk of venous thromboembolism. The association of MTHFR

25 combined with no additional thrombophilic risk factors with venous thrombosis is less

26 clear. High doses of folic acid to lower homocysteine levels might not be necessary.

27 **Introduction**

28

29

30 Homocysteine is a sulphur containing amino acid absent in naturally occurring dietary sources.

31 It is closely related to the essential amino acid methionine and to cysteine. Butz and du

32 Vigneaud described the formation of homocystine from treating methionine with concentrated

33 acid ⁽¹⁾. Homocysteine is a metabolic intermediary in transmethylation and transsulfuration

34 reactions. S-Adenosylmethionine, an intermediary in the methionine-homocysteine cycle, is an

35 essential methyl donor in over 100 known reactions including methylation of nucleic acids,

36 proteins, phospholipids, myelin, polysaccharides, Choline, and catecholamines. Impaired

37 methylation is associated with abnormal cellular growth, differentiation and function. The

38 synthesis of Gultathione, an important endogenous antioxidant, is dependent on the

39 transsulfuration of homocysteine.

40

41 Aberrant homocysteine metabolism is associated with many disorders. In 1969, McCully first

42 described the association between homocystinemia and premature atherosclerotic vascular

43 disease in homocystinuria ⁽²⁾. Presently, hundreds of publications discuss abnormal plasma

44 homocysteine levels and various diseases. Hyperhomocysteinemia increases the likelihood of

45 developing atherosclerosis. Hyperhomocysteinemia, alone or with other thrombophilic risk

46 factors, may be associated with vascular occlusive pathology underlying varied clinical

47 presentations.

48

49 Coronary vascular disease, stroke, recurrent pregnancy loss, and deep vein

50 thrombosis are some of the presentations. Dementia, depression, retinal artery

51 thrombosis, acquired hypercoagulable states after renal transplant, thrombosis in
52 hemodialysis patients, Parkinson's disease, thrombosis in diabetic patients, acquired
53 thrombophilia in systemic lupus erythematosus are among published disorders
54 associated with hyperhomocysteinemia.

55

56 Collectively, the published studies suggest that elevated plasma homocysteine is
57 injurious to blood vessels leading to vascular occlusive phenomena. Possible
58 pathogenetic mechanisms of the vascular changes have been described. The causes of
59 hyperhomocysteinemia range from aging and vitamin deficiency to genetic defects.

60 Ultimately, the question of disease prevention and management of
61 associated clinical presentations is debated. We review and summarize the
62 published literature to present the current understanding of the relationship of
63 homocysteine and thrombosis and the role of vitamin supplementation in thrombosis
64 prevention.

65 **HOMOCYSTEINE METABOLISM:**

66 Plasma levels of homocysteine are controlled by two distinct metabolic pathways;
67 remethylation of homocysteine to methionine or transsulfuration of homocysteine to cysteine
68 ⁽³⁾. Homocysteine is formed intracellularly from the demethylation of dietary methionine, an
69 essential amino acid, in the methionine cycle (Fig. 1) ⁽⁴⁾. Homocysteine may acquire a methyl
70 group from either N-5-methyl-tetrahydrofolate (MTHF) an intermediary in the folate cycle, or
71 from betaine to reform methionine (Fig. 1). The folate cycle, essential for the MTHF reactions,
72 occurs in all tissues and is Vitamin B12 dependent ⁽³⁾. Betaine is essentially confined to the
73 liver and possibly the kidneys ⁽⁵⁻¹⁰⁾.

74

75 Methylene tetrahydrofolate reductase (MTHFR) reduces 5,10 methylene – tetrahydrofolate, in
76 the folate cycle, to 5-methyltetrahydrofolate. The latter is converted to methionine by
77 methyltransferase (methionine synthase) (Fig. 1) ⁽⁶⁾. Methionine is preferentially activated by
78 ATP to form S-adenosylmethionine (SAM) ⁽⁷⁾. SAM is the universal methyl group donor. S-
79 adenosylhomocysteine (SAH) is formed when SAM donates the methyl group; SAH is
80 hydrolyzed to regenerate homocysteine, propagating the methionine cycle ⁽³⁾.

81

82 Betaine, in the alternative methionine remethylation pathway, helps the folate cycle in
83 sustaining the methionine cycle and the production of SAM. Homocysteine is diverted to the
84 transsulfuration pathway when methionine concentration exceeds the methionine cycle, folate
85 cycle remethylation, capacity or when the synthesis of cysteine is required ⁽⁸⁻⁹⁾ (Fig.1). The
86 initial step in transsulfuration is the union of homocysteine and serine forming cystathionine,
87 catalyzed by cystathionine B-synthase (CBS). Pyridoxal 5' – phosphate (Vitamin B₆) is an
88 essential cofactor for CBS. Cystathionine is hydrolyzed by γ -cystathionase to form cysteine
89 and α -ketobutyrate. Excess cysteine is oxidized to taurine or organic sulfates or is excreted in

90 the urine. Therefore, not only is transsulfuration important for the synthesis of cysteine, but is
91 serves to catabolize homocysteine in excess of the methionine cycle ⁽³⁾. Transsulfuration
92 regulates higher homocysteine concentrations, as in the post prandial state or after methionine
93 loading. Remethylation, the main metabolic pathway of homocysteine, is responsible for the
94 fasting plasma levels ⁽³⁾.

95

96 Factors that can influence plasma homocysteine levels are genetic and acquired. These are
97 listed in Table 1.

98 THE PATHOGENESIS OF HYPERHOMOCYSTEINEMIA:

99 Homocysteine is metabolized intracellularly. A proportion, normally small, of homocysteine is
100 released into the circulation. This release process and the transsulfuration pathway prevent
101 the intracellular accumulation of this cytotoxic sulfur amino acid ⁽¹¹⁻¹²⁾.

102

103 Hyperhomocysteinemia occurs when the kidneys fail to excrete homocysteine or when a
104 metabolic defect results in excess homocysteine entering the blood stream. A genetic defect
105 in one of the enzymes or a nutritional deficiency of cofactors (vitamins) in the remethylation or
106 transsulfuration pathways can be associated with abnormal intracellular homocysteine levels
107 and hyperhomocysteinemia ⁽³⁾. Methionine synthase uses Methyl cobalamin (a Vitamin B₁₂
108 derivative) as a cofactor. MTHFR uses FAD (a riboflavin derivative) as a cofactor. Both CBS
109 and cystathionase use pyridoxal phosphate (a Vitamin B₆ derivative) as a cofactor ⁽¹³⁾. Defects
110 in any of these enzymes or cofactors are known to cause hyperhomocysteinemia. However,
111 the severity of hyperhomocysteinemia appears to correlate with the specific abnormality.
112 Genetic defects of MTHFR leads to impaired synthesis of N-5-methyltetrahydrofolate, the first
113 step in the synthesis of methionine ⁽³⁾. Folate deficiency has a similar consequence. The
114 hyperhomocysteinemia resulting from impaired homocysteine remethylation because of
115 deficiency Vitamin B₁₂ or methionine synthase (methyltransferase) may not be as severe as
116 observed in MTHFR defects, because transsulfuration will be somewhat more active in the
117 catabolism of homocysteine ^(3,14).

118 Abnormalities of the remethylation pathways do not alter the transsulfuration pathway.

119 Abnormalities of the transsulfuration pathway, on the other hand, can affect the remethylation
120 pathway ⁽³⁾. In homozygous CBS defect transsulfuration is severely impaired and homocysteine
121 is diverted toward the remethylation pathway ⁽¹⁵⁾. Methionine synthesis and consequently the
122 intracellular concentration of SAM are increased. Folate remethylation pathway is inhibited

123 when the intracellular concentration of SAM is sufficient for a feedback inhibition of MTHFR.
124 Therefore, severe hyperhomocysteinemia associated with severe impairment of
125 transsulfuration results in the inhibition of the folate remethylation pathway. When
126 homocysteine level is low, as in fasting state, Vitamin B₆ deficiency and heterozygous defect of
127 cystathionine B-synthase lead to a mildly impaired transsulfuration pathway, which together
128 with the remethylation pathway prevent hyperhomocysteinemia. However, when
129 homocysteine burden is high, as in a significant dietary intake of methionine or the oral
130 methionine load test, hyperhomocysteinemia results because homocysteine remethylation is
131 inhibited through the feedback inhibition of MTHFR due to the increase in SAM. Further, in
132 this situation homocysteine generation is accelerated through glycine methylation because
133 glycine N-methyltransferase (GNMT) becomes highly active, as the result of loss of inhibitory
134 action of N-5-methyltetrahydrofolate secondary to MTHFR inhibition ⁽³⁾.

135

136 Disorders possibly associated with hyperhomocysteinemia are listed in Table 2.

137

138 **CLASSIFICATION OF HYPERHOMOCYSTEINEMIA**

139 Hyperhomocysteinemia can be divided into three groups based on severity and pathogenetic
140 mechanisms ⁽³⁾ Table 3.

141

142 Severe hyperhomocysteinemia cases are due to homozygous defects in genes encoding for
143 homocysteine metabolism. Homozygous defects in the gene encoding for Cystathionine beta
144 synthase (CBS) results in congenital homocystinuria ⁽¹⁶⁾. Such patients present usually in
145 childhood, but occasionally as late as the seventh decade. One or more alerting signs might
146 be present; dislocation of ocular lenses, lenticular myopia, marfan-like appearance, thrombosis
147 or thromboembolism, early-onset atherosclerosis and mental retardation.

148

149 Vitamin B₆ supplementation can profoundly influence the clinical picture and the plasma levels
150 of homocysteine and methionine in congenital homocystinuria. Therefore patients should be
151 off vitamin B₆ supplements for at least 1 to 2 weeks before sample collection. The expected
152 fasting total plasma homocysteine values in CBS deficient patients are usually >50 µmol/L
153 (usually in the range of 100-500 µmol/L). Methionine levels are usually elevated, >40 µmol/L,
154 and may reach several hundreds of µmol/L. Rare mutations of MTHFR, methionine synthase
155 and methionine synthase reductase can cause homocystinuria ⁽¹⁵⁻¹⁹⁾.

156

157 Homozygous defects in genes encoding for MTHFR or for any of the enzymes involved in
158 Vitamin B₁₂ metabolism can lead to moderate to severe hyperhomocysteinemia. Severe
159 MTHFR deficiency due to autosomal recessive inheritance is rare and has been described in
160 about 50 patients, ranging in age from birth to adult life. Inborn errors of Vitamin B₁₂
161 metabolism associated with hyperhomocysteinemia include adenosylcobalamin deficiency,
162 combined adenosylcobalamin and Methylcobalamin deficiencies and methylcobalamin

163 deficiency (methionine synthase reductase deficiency and methionine synthase deficiency).
164 The number of patients described with these methionine deficiencies is relatively small ^(3,15).
165
166 The more common causes of hyperhomocysteinemia are polymorphisms of folate and
167 cobalamin metabolism and folate or cobalamin deficiencies. The resulting
168 hyperhomocysteinemia is mild-moderate. Polymorphism refers to the prevalence of a mutation
169 at a frequency of $\geq 1.0\%$ of alleles in a population. Polymorphism of methylenetetrahydrofolate
170 reductase (MTHFR), methionine synthase and methionine synthase reductase, have been
171 described.
172 Five common mutations resulting in sequence changes in MTHFR have been described.
173 Table 4 ⁽²⁰⁻²⁵⁾. The 677C→T substitution (Alanine to Valine) has been studied extensively ^{(20, 26-}
174 ³¹⁾. The 1298A →C(Glutamine to Alanine) has been studied less often ^(24, 29, 32). The 1068T/C
175 (Serine/Serine), 1178+31TC (5' splice site) and the 1317 T/C (phenylalanine/phenylalanine)
176 are not likely to be clinically significant ⁽²¹⁻²⁵⁾. The frequency of homozygous MTHFR 677C→T
177 in North American whites is 10-15% ^(20, 30-31). It is more common in Hispanic Americans with
178 reported frequency of 25% ⁽³³⁾. African Americans have the least frequency 0-1% ⁽³³⁻³⁴⁾. This
179 mutation was identified by Frosst et al, who demonstrated the sensitivity of this variant to heat
180 treatment at 46°C ⁽²⁰⁾. Kang et al, and Engbersen et al, identified this thermolabile MTHFR in
181 coronary artery disease patients by enzymatic assays of lymphocyte extracts ^(26, 35). This
182 mutation decreases specific activity of MTHFR at 37°C. Several studies demonstrated an
183 association of the 677C→T mutation and hyperhomocysteinemia ^(20,27-28,31,36-37). Guttormsen
184 et al, identified 73% homozygosity in Norwegian individuals who were selected to have
185 homocysteine levels greater than 40μM ⁽³⁶⁾. The association between the 677C→T mutation
186 and hyperhomocysteinemia is noted predominantly when the plasma folate level is low ⁽³⁰⁾.

187 Folate supplementation to raise plasma folate levels above the median value can prevent
188 hyperhomocysteinemia. The increase in folate levels might stabilize the mutant enzyme and
189 allow it to function normally or provide exogenous 5-methyltetrahydrofolate for the
190 remethylation pathway ^(36, 38-39). MTHFR is associated with cardiovascular disease. This
191 association is further magnified in the presence of other risk factors such as hypertension and
192 hyperlipidemia ⁽⁴⁰⁻⁴¹⁾. Patients with Factor V Leiden and MTHFR homozygous mutation have a
193 significantly increased risk of thrombosis ⁽⁴²⁻⁴³⁾. Neural tube defects such as spina bifida, pre-
194 eclampsia, recurrent pregnancy loss, and placental abruption have all been described in
195 association with this mutation ^(29, 44-52). Folate supplementation during pregnancy prevents the
196 recurrence of neural tube defects ⁽⁴⁷⁻⁴⁸⁾.

197

198 Homozygous MTHFR 677C→T decreases the risk of colorectal cancer in folate replete
199 individuals by 50%. In folate deficient individuals, no protection is afforded and perhaps the risk
200 is enhanced ⁽⁵³⁻⁵⁵⁾.

201

202 Methionine Synthase 2756A-G mutation homozygosity is found to have frequency less than
203 5%. This polymorphism does not appear to be associated with hyperhomocysteinemia or an
204 increased risk of neural tube defect or vascular disease ⁽⁵⁶⁻⁵⁹⁾.

205

206 Methionine Synthase Reductase 66A-G mutation is extremely common. Wilson et al, reported
207 a homozygosity frequency of 25-30% in the Canadian population ⁽⁶⁰⁾. An increased risk of
208 spina bifida was found in homozygous mutants, but no association with mild
209 hyperhomocysteinemia was observed.

210 **MEASURING HOMOCYSTEINE PLASMA LEVELS AND ASSESSING MTHFR STATUS:**

211 Pathologic homocysteine plasma levels requiring medical intervention is related to the normal
212 plasma reference range, specimen type (fasting, random or post-methionine load test),
213 pretesting specimen handling and test method. The latter two issues shall not be addressed
214 other than to suggest following the manufacturer recommendations.

215 Several non-laboratory related pre-analytical variables affect homocysteine plasma levels.

216 Table 1.

217

218

219 Healthy adults without any of the pre-analytical variables that affect the plasma homocysteine
220 level should be used in setting the reference range. The reference range varies in the
221 literature and should be determined by individual laboratories.

222

223

224 Plasma homocysteine level is affected by the protein content in food intake. Therefore a
225 fasting specimen might be more informative, especially in setting the reference range.

226 However, a different approach might be to order a fasting homocysteine level when a random
227 specimen is abnormal. The evidence in the literature supports that post-methionine load
228 (PML) homocysteine testing identifies a subset of individuals with normal fasting homocysteine
229 levels but abnormal PML tests. Such patients are likely to have a heterozygous genetic defect,
230 MTHFR polymorphisms being the most frequent and probable cause.

231

232

233 PML is impractical and not routinely offered. PML is a global test for homocysteine
234 metabolism. Therefore, PML would likely be abnormal in genetic abnormalities of

235 homocysteine metabolism other than MTHFR polymorphisms. Individuals with MTHFR
236 polymorphisms who are taking vitamin supplements, might have homocysteine plasma levels
237 within the reference range. Further investigation to determine if assessing the MTHFR status
238 is a useful alternative to PML and to diagnose covert hyperhomocysteinemia is needed. While
239 there is lack of agreement in prospective and meta-analysis studies as to the association of
240 hyperhomocysteinemia with arterial thrombosis and venous thromboembolism, retrospective
241 case control studies favor such association. Further, many publications suggest that
242 homocysteine is injurious to the endothelium via a variety of mechanisms. Therefore, it seems
243 prudent, to include measuring plasma homocysteine levels and assessing MTHFR status in
244 initial thrombophilia workup, until such time when solid evidence against this approach is
245 introduced in the literature.

246 **INJURIOUS MECHANISMS OF HYPERHOMOCYSTEINEMIA**

247 Hyperhomocysteinemia is implicated in a wide spectrum of disorders; vascular damage,
248 cognitive impairment, psychiatric and neurological complications, congenital defects,
249 pregnancy complications and neoplastic disorders ⁽⁶¹⁻⁸⁰⁾. There are common underlying
250 pathogenetic mechanisms associated with vascular injury leading to these clinical changes.
251 The proposed pathogenetic mechanisms are, oxidative damage of the endothelium through
252 suppression of the vasodilator nitric oxide ⁽⁸¹⁻⁹²⁾, increasing the levels of dimethylarginine
253 (ADMA), and impaired methylation ^(89,93-98), vascular smooth muscle proliferation ^(88,99-103),
254 promotion of platelet activation and aggregation ^(89,104-109), and disruption of the normal
255 procoagulant-anticoagulant balance favoring thrombosis ^(107,110-117).

256

257 Hyperhomocysteinemia promotes endothelial oxidative damage and dysfunction ^(81-92, 118-119).
258 This might explain one of the benefits of antioxidant therapy ⁽⁸³⁻⁸⁵⁾. Homocysteine inhibits
259 endothelial nitric oxide (NO) synthase and subsequently the bioavailability of NO is markedly
260 decreased resulting in impaired vasodilation ^(106,89, 98).

261

262 NO detoxifies homocysteine by forming S-nitroso-homocysteine (SN) ⁽⁹²⁾. SN is a vasodilator
263 ^(88, 92, 98, 106). Autooxidation of excess homocysteine produces free radicals toxic to endothelial
264 cells ^(86, 91, 106). Normally, glutathione neutralizes free radicals. However, excess
265 homocysteine decreases glutathione peroxidase activity ^(89-90, 118-119). An additional postulated
266 mechanism of endothelial injury is through the diminished catabolism of asymmetric
267 dimethylarginine (ADMA). ADMA is a strong inhibitor of NO synthase ^(89, 93-98).

268 Hyperhomocysteinemia can directly impair DNA methylation resulting in altered gene
269 expression, which may affect both the endothelial and smooth muscle cells of the vascular wall
270 ^(103,120). Several reports suggest that homocysteine induces proliferation of the vascular
271 smooth muscle cells leading to luminal narrowing ⁽¹²⁰⁻¹²¹⁾. Excess homocysteine may be
272 converted to the cyclic thioester homocysteine-thiolactone, (HSL). LDL may form adducts with
273 HSL, which are phagocytized by macrophages and incorporated into foam cells in early
274 atherosclerotic plaques ⁽⁸⁸⁾.

275

276 Platelets have normal life-span and morphology in patients with hyperhomocysteinemia.
277 However, homocysteine might activate platelets, increasing platelet aggregation and adhesion.
278 Platelet thromboxane A₂ biosynthesis is significantly increased in homocystinuria. The
279 enhanced production of thromboxane A₂ may be a major contributor to the risk of thrombosis.

280

281 Homocysteine rapidly auto-oxidizes in plasma. Free oxygen radicals are produced which
282 initiate lipid peroxidation either in endothelial plasma membrane or lipoproteins. Oxidized LDL
283 activate platelets and are atherogenic.

284

285 Several reports show that homocysteine promotes thrombosis by disturbing the
286 procoagulant/anticoagulant balance. Homocysteine either increases or decreases several
287 coagulation factors. Table 5.

288 **HYPERHOMOCYSTEINEMIA AND THROMBOPHILIA**

289 A large number of epidemiological and experimental studies have investigated the association
290 of hyperhomocysteinemia and thrombophilia. Epidemiological studies addressing
291 hyperhomocysteinemia and arterial or venous thrombosis included retrospective case—control
292 and cross-sectional studies and prospective studies. Prospective vitamin therapy clinical trials
293 to address whether hyperhomocysteinemia is a risk factor of atherothrombosis are ongoing.
294 Case-control studies of genetic abnormalities of homocysteine metabolism and
295 atherothrombosis and venous thrombosis have been done.

296

297 **HYPERHOMOCYSTEINEMIA AND ARTERIAL DISEASE**

298 McCully observed premature atherosclerosis in homocysteinemia ^(2,122). Wilcken and Wilcken
299 provided evidence implicating homocysteine in coronary artery disease ⁽¹²³⁾. Several
300 subsequent studies reported an association between mild hyperhomocysteinemia and
301 coronary artery disease, stroke and peripheral arterial disease ⁽¹²⁴⁻¹²⁷⁾. Other studies
302 suggested that hyperhomocysteinemia was independent of established risk factors such as
303 smoking, hyperlipidemia, hypertension and diabetes for vascular occlusive disease ^{(120-121, 128-}
304 ¹³⁰⁾. Boushey et al conducted a meta-analysis of 27 retrospective case-control studies
305 addressing the association of Hyperhomocysteinemia and vascular thrombotic disease ⁽¹²⁴⁾.
306 This analysis demonstrated that a 5µmol/L incremental rise in total plasma homocysteine
307 levels is associated with an increase in the relative risk for coronary artery disease,
308 cerebrovascular disease and peripheral vascular disease of 1.6, 1.5 and 6.8 respectively. The
309 European Concerted Action Project, a multi-center study of 750 patients with vascular disease
310 and 800 controls confirmed that hyperhomocysteinemia is associated with an increased risk of
311 vascular disease ⁽¹²⁵⁾. This risk was independent of, but multiplicative to other risk factors such
312 as smoking, hypertension and additive to hypercholesterolemia. Additional analysis of the
313 same study indicate that red cell folate levels below the 10th percentile and of Vitamin B₆ below
314 the 20th percentile of control subjects were independent risk factors for vascular disorders ⁽¹³¹⁾.
315 Robinson et al, and Folsom et al, showed that low vitamin B₆ (pyridoxal-phosphate) was an
316 independent risk for coronary artery disease. ⁽¹³²⁻¹³³⁾. Both Boers et al, and Malinow et al,
317 showed that hyperhomocysteinemia were associated with peripheral arterial occlusive disease
318 ¹³⁴⁻¹³⁵⁾. Stampfer et al, in a prospective study of plasma homocysteine and risk of myocardial
319 infarction in US physicians, that included 14, 916 subjects, revealed a relative risk for
320 myocardial infarction of 3.1 when homocysteine levels were in the 95th percentile of control
321 values compared to those below the 90% percentile ⁽¹³⁶⁾. Malinow et al, showed, an odds ratio

322 for a thickened carotid intimal wall of 3.15 for patients in the top quintile of plasma
323 homocysteine levels ($>10.5 \mu\text{M}$) compared to those in the lowest quintile ($<5.88 \mu\text{M}$)⁽¹³⁷⁾.
324 Voutilainen et al, reported an increased common carotid artery intimal-media wall thickness in
325 men but not women with plasma homocysteine levels $>11.5 \mu\text{M}$ ⁽¹²³⁾. Konechy et al, revealed
326 an independent correlation between plasma homocysteine levels and aortic atherosclerosis
327⁽¹³⁸⁾. Studies by Wu, Hopkins, Dalery and Verhoef indicate that homocysteine levels are a risk
328 factor for familial and non-familial coronary artery disease⁽¹³⁹⁻¹⁴²⁾. Their work, however,
329 suggests vitamins, especially folate and B₆, rather than homocysteine levels may confer the
330 risk for coronary artery disease. Verhoef et al, in a study of plasma total homocysteine, B
331 Vitamins and risk of coronary atherosclerosis found a graded correlation between occlusive
332 coronary artery disease and both fasting and post methionine load homocysteine levels⁽¹⁴³⁾.
333 Nygard et al, evaluated plasma homocysteine associated mortality in patients with coronary
334 artery disease⁽¹⁴⁴⁾. They found a strong graded relationship between total homocysteine and
335 mortality independent of variables. In a prospective study Wald et al, found higher
336 homocysteine levels in the group that died of ischemic heart disease than in controls⁽¹⁴⁵⁾.
337
338 Other prospective studies shed doubt on the relationship of hyperhomocysteinemia and
339 coronary artery disease. Alftan et al, found no statistical difference in total plasma
340 homocysteine levels in 191 subjects who developed myocardial infarction during the 9-year
341 follow up and the control subjects⁽¹⁴⁶⁾. Additional reports utilizing data from the Physicians'
342 Health Study show that homocysteinemia is associated with a statistically insignificant relative
343 risk to develop coronary artery disease; angina pectoris with subsequent coronary artery
344 bypass surgery and stroke⁽¹⁴³⁾. Evans et al, found no association of plasma homocysteine
345 levels and myocardial infarction⁽¹⁴⁷⁾. Folsom et al, found that total homocysteine levels
346 correlated with the risk of coronary artery disease in women but not in men. While in women,

347 only the level of homocysteine was inversely correlated with the folate levels, that was the
348 case for both men and woman with vitamin B₆ levels ⁽¹⁴⁸⁾. Molgaard et al, and Robinson et al,
349 reported an inverse relationship of plasma homocysteine with folate and with vitamin B₁₂,
350 Vitamin B₆ and folate levels respectively ^(63,149). Robinson showed that low vitamin B₆ was an
351 independent risk factor for coronary artery disease ⁽⁶³⁾. Rimm et al, findings are in accord with
352 Robinson and reported that vitamin B₆ and folate levels were inversely related to the risk of
353 coronary artery disease among women ⁽¹⁵⁰⁾. Selhub et al, report similar findings ⁽¹⁵¹⁾.

354

355 The large number of reports investigating the association of hyperhomocysteinemia and the
356 risk of arterial disease show conflicting results. While hyperhomocysteinemia is likely a risk for
357 arterial disease, that risk appears to be greater and more significant in patients with existing
358 cardiovascular disease or low vitamin B levels. To that end, Donner et al, reported low
359 prevalence of hyperhomocysteinemia in patients with low cardiovascular risk profile ⁽¹⁵²⁾.

360

361 Similarly the correlation of genetic abnormalities of homocysteine metabolism and the risk of
362 cardiovascular disease is uncertain. Kluijtmans et al, and Mudd et al, reported that 677C→T
363 MTHFR was a genetic risk factor for cardiovascular disease ^(15, 41,153). Brattström et al, on the
364 other hand, reported the 677C→T MTHFR mutation is not a causal risk factor for
365 cardiovascular disease ⁽¹⁵⁴⁾.

366

367 Table 6 lists studies showing correlation between hyperhomocysteinemia and arterial occlusive
368 disease, while Table 7 lists studies casting doubt on such correlation.

369

370

371 **HYPERHOMOCYSTEINEMIA AND VENOUS THROMBOSIS**

372 Falcon et al, in 1994, reported a high prevalence of hyperhomocysteinemia in patients with
373 juvenile venous thrombosis ⁽¹⁵⁵⁾. In two subsequent studies by den Heijer et al,
374 hyperhomocysteinemia greater than the 95th percentile of the control range was a risk factor
375 for deep –vein thrombosis ⁽¹⁵⁶⁻¹⁵⁷⁾. This group reported that vitamin supplementation with
376 folate alone, or with folate, B₁₂, and pyridoxine reduced homocysteine levels. den Heijer work
377 showed that several patients with abnormal post-methionine loading total plasma
378 homocysteine levels had normal fasting levels and vice versa. Therefore, the combination of
379 the two tests would identify a larger group of individuals with abnormal homocysteine
380 metabolism than either test alone. A case control study by Simioni et al, identified a
381 statistically significant high prevalence of hyperhomocysteinemia in patients with deep-vein
382 thrombosis ⁽¹⁵⁸⁾. Martinelli et al, found no association of hyperhomocysteinemia and deep-vein
383 thrombosis of the upper extremities ⁽¹⁵⁹⁾. Eichinger et al, found that hyperhomocysteinemia
384 was present in 25% of 264 individuals with a single episode of idiopathic venous
385 thromboembolism. This group identified that the risk of recurrent thromboembolism was 2.7 in
386 the first 24 months after discontinuation of anticoagulation ⁽¹⁶⁰⁾. In a prospective study by
387 Kottke-Marchant et al, high plasma homocysteine levels >13μM was found to be a risk factor
388 for arterial and venous thrombosis in patients with normal coagulation profiles ⁽¹²⁶⁾. An
389 elevated homocysteine level yielded a 7.8 odds ratio for thrombosis. Women had a higher
390 odds ratio than men ⁽¹²⁶⁾. In a quantitative review of hyperhomocysteinemia and venous
391 thrombosis, den Heijer et al, calculated a pooled odds ratio for venous thrombosis of 2.6 ⁽¹⁶¹⁾.

392

393

394 Fermo et al, detected moderate hyperhomocysteinemia in 13.1% of patients with venous and
395 19.2% of patients with arterial occlusive disease ⁽¹⁶²⁾. Other heritable thrombophilic factors
396 were present in same group of patients with venous thrombosis. Fermo et al, calculated the
397 relative risk of venous thrombosis in patients with combined hyperhomocysteinemia and other
398 thrombophilic factors was 1.7 times greater than for patients with hyperhomocysteinemia
399 alone. The age of occurrence of the first thrombotic episode was earlier in the subset of
400 patients with combined risk factors. Ridker et al, reported a tenfold increase in thrombotic risk
401 among patients with both hyperhomocysteinemia and Factor V Leiden ⁽¹⁶³⁾. This group found
402 that hyperhomocysteinemia conferred a relative risk of 3.4 in patients with idiopathic venous
403 thrombosis.

404

405 Legnani et al, found no association between elevated fasting or post-methionine load
406 homocysteine levels and thrombosis in a group of patients with protein C, protein S or
407 antithrombin deficiency or Factor V Leiden ⁽¹⁶⁴⁾. 677C→T MTHFR did not confer additional
408 thrombotic risk to the heritable thrombophilic coagulation effects. Whether 677C→T MTHFR is
409 a risk factor for venous thrombosis is debatable. The published studies show conflicting
410 results. Arruda et al, Salamon et al, and Margaglione et al, show evidence in support of
411 677C→T MTHFR being a risk factor for venous thrombosis ⁽¹⁶⁵⁻¹⁶⁷⁾. De Stefano et al, reviewed
412 nine case-control studies involving 2,225 patients with venous thrombosis and 2,994 healthy
413 controls. There were no significant differences in the cumulative prevalence between
414 homozygous MTHFR genotype in cases with venous thrombosis versus normal controls ⁽¹⁶⁸⁾.
415 Only two studies showed a slightly greater risk for venous thrombosis in the homozygous
416 genotype compared to heterozygous ⁽¹⁶⁵⁻¹⁶⁷⁾. Nevertheless, Trillot et al, and others show that
417 677C→T MTHFR does not modify the risk of venous thrombosis ⁽¹⁶⁹⁾. Further, while Cattaneo
418 et al, indicate that the coexistence of 677C→T MTHFR and Factor V Leiden increased the risk

419

420 of venous thrombosis, Trillot et al, and Kluijtmans et al, suggest that this mutation does not
421 modify the risk for venous thrombosis in patients with heterozygous Factor V Leiden ⁽¹⁶⁹⁻¹⁷²⁾.

422 Kluijtmans et al, suggest the 677C→T MTHFR maybe a risk factor for thrombosis in CBS-
423 deficient patients ⁽¹⁷³⁾. Lalouschek et al, reported an increased risk of transient ischemic

424 attacks or minor strokes in patients with 677C→T MTHFR ⁽¹⁷⁴⁾.

425

426 Table 8 lists studies supporting a correlation between hyperhomocysteinemia and venous
427 thrombosis, while Table 9 lists studies with different conclusions. Table 10 summarizes studies
428 addressing the effect of hyperhomocysteinemia combined with other thrombophilic risk factors.

429

430 **TREATMENT OF HYPERHOMOCYSTEINEMIA**

431 The conventional treatment of hyperhomocysteinemia has been folate supplementation usually
432 with Vitamin B₆ and perhaps vitamin B₁₂. While this approach is successful in lowering total
433 plasma homocysteine levels, its effect on clinical vascular pathology remained untested until
434 recently.

435

436 The Norwegian Vitamin Trial (NORVIT); a randomized trial of homocysteine-lowering with B-
437 vitamins for secondary prevention of cardiovascular disease after acute myocardial infarction,
438 has been completed. This is the largest trial testing the benefit of folate supplementation in
439 reducing the risk of recurrent MI and has reported its findings September, 2005 at the
440 European Society of Cardiology 2005 Congress ⁽¹⁷⁵⁾.

441

442 While a 28% reduction of plasma homocysteine levels was achieved, there was no associated
443 risk reduction for MI or stroke. There was not a significant effect on the risk for cardiovascular
444 disease in patients taking either folic acid alone or vitamin B₆ alone. Interestingly a 21%
445 increased risk of MI was found in patients taking folic acid and vitamin B₆ in combination. An
446 increase in cancer was seen in patients taking either folic acid alone or folic acid and vitamin
447 B₆, but not in those taking vitamin B₆ alone. Tables 11 and 12.

448

449 The NORVIT study suggests that homocysteine is an innocent bystander in patients with
450 cardiovascular disease. It is important to point out that hyperhomocysteinemia was not an
451 inclusion criterion in the NORVIT study. Many questions and possible hypotheses remain
452 unanswered and untested.

453 The results of VITATOPS (Vitamins to Prevent Stroke) and SEARCH (Study of the
454 Effectiveness of Additional Reductions in Cholesterol and Homocysteine) two ongoing trials in
455 large populations should add more insight into the impact of folic acid supplementation in
456 patients with cerebrovascular and ischemic heart disease. Several smaller studies did shed
457 doubt on the usefulness of folic acid supplementation in patients with coronary artery disease.
458

459 In a randomized study of 593 patients with stable coronary artery disease, Liem et al, found
460 that, within the follow-up time of 24 months, folic acid did not seem to reduce clinical end
461 points in patients with stable coronary artery disease, while on statin treatment ⁽¹⁷⁶⁾. The
462 authors conclude that homocysteine might be a modifiable marker of disease and that folic
463 acid supplementation should be treated with reservations. In an outcome trial by Baker et al,
464 1,882 patients with evidence of coronary disease were randomized to folic acid or placebo in
465 addition to the usual drugs for two years. The only predictors of outcome were plasma
466 homocysteine and age. Although homocysteine was reduced from 11.2 ± 6.9 to 9.7 ± 5.3
467 $\mu\text{mol/L}$, there was no difference in composite outcome. There was a twofold difference in non-
468 fatal myocardial infarction (23 vs 12, $P=.05$) but no difference in deaths or revascularization.
469 The authors conclude that routine use of folic acid supplementation in patients with ischemic
470 heart disease and slight elevation of plasma homocysteine is not warranted ⁽¹⁷⁷⁾. Lange et al,
471 tested the effect of a combination of folic acid, vitamin B₆ and vitamin B₁₂ on the risk of
472 angiographic restenosis after coronary-stent placement in a double-blind multi-center trial ⁽¹⁷⁸⁾.
473 A total of 636 patients were enrolled. The authors found at follow-up time, a significantly
474 smaller minimal luminal diameter, greater late luminal loss and higher re-stenosis rate in the
475 folate group compared to the placebo group. Repeated target-vessel revascularization was
476 higher in the folate group. In the VISP (The Vitamin Intervention for Stroke Prevention)
477 randomized controlled trials, Toole et al, tested the effect of lowering homocysteine in patients

478 with ischemic stroke to prevent stroke, myocardial infarction and death ⁽¹⁷⁹⁾. A total of 3600
479 adults with non-disabling cerebral infarction were enrolled. The authors concluded that a
480 moderate reduction of total homocysteine had no effect on vascular outcomes during the 2
481 years of follow-up. Nevertheless, because there was a consistent association of total
482 homocysteine with vascular risk, the authors suggest that further investigations are necessary.
483 In October 2005, Lewis et al, published the largest metaanalysis of the association of MTHFR
484 677C→T polymorphism and coronary heart disease ⁽¹⁸⁰⁾. The authors found no strong
485 evidence to support an association of MTHFR 677C→T and coronary artery disease in
486 Europe, North America or Australia. Geographic variations exist. This study cast doubt on the
487 role of supplemental folic acid in preventing cardiovascular disease, especially in high income
488 countries with folate fortified food. It is important to note that some studies do show a
489 beneficial effect of folic acid supplementations. Williams et al, in a randomized placebo
490 controlled, double blind study of 41 subjects, showed that a 3 week folic acid supplementation,
491 but not placebo resulted in a reduction of brachial artery pulse pressure by 4.7+ 1.6 mm Hg
492 ($P=.05$) without changing mean arterial pressure ⁽¹⁸¹⁾. Systemic arterial compliance increased
493 by 0.15 + 0.03 mL/mmHg ($P=.05$). These results were independent of homocysteine or folate
494 concentration and MTHFR genotype.

495
496 Assanelli et al, in a randomized trial in 30 young subjects with recent acute MI and high plasma
497 homocysteine levels found that a marked reduction in plasma homocysteine concentrations is
498 associated with a significant improvement of endothelial function independent of plasma
499 antioxidant capacity ⁽¹⁸²⁾.

500
501 Finally, the studies by Stott et al, and Nurk et al, published December 2005 are noteworthy ⁽¹⁸³⁻
502 ¹⁸⁴⁾. Stott et al, studied 185 patients, 65 or older with ischemic vascular disease in a

503 randomized, placebo controlled, double-blind study with 3 active treatments: folic acid (2.5
504 mg) plus vitamin B₁₂ (500 mg), vitamin B₆ (25 mg) and riboflavin (25 mg.). Changes in plasma
505 homocysteine, fibrinogen and von Willebrand factor were measured at 3, 6, and 12 months
506 and in cognitive functions at 6 and 12 months. The authors found that while homocysteine
507 levels decreased in the group receiving oral folic acid plus vitamin B₁₂ supplementation, there
508 was no statistically significant beneficial effects on cognition. Nurk et. al, scrutinized the 2,189
509 subjects in the Hordaland homocysteine study population measuring total homocysteine and
510 folate levels and assessing memory performance using the Kendrick Object Learning Test at
511 baseline and 6 years later. The authors conclude that increased plasma total homocysteine is
512 an independent risk factor for memory deficit both cross-sectionally and prospectively. A
513 favorable change in folate and or total homocysteine over time is associated with better
514 cognitive performance.

515

516 Silaste et al, reported that a diet high in fresh berries, citrus fruit and vegetables effectively
517 increases serum and RBC folate and decreases plasma homocysteine ⁽¹⁸⁵⁾. Several studies
518 show that Betaine and Choline supplementation lower plasma homocysteine in healthy men
519 and women ⁽¹⁸⁶⁻¹⁸⁸⁾. N-acetylcysteine therapy is another possible option ⁽¹⁸⁹⁾. Alternative
520 methods to reduce plasma homocysteine might be worth pursuing.

521

522 In conclusion, the answer to the question: Is hyperhomocysteinemia a risk factor for
523 vascular occlusive disease; is a qualified affirmation. The authors suggest that there are several
524 possible hypotheses relating hyperhomocysteinemia and thrombosis, Table 13.

525 Additional studies are needed to determine which of the six hypotheses is true.

526 Hyperhomocysteinemia is related to atherosclerosis and disorders resulting from arterial
527 vascular disease in a graded manner. This association is modulated by pre-existing vascular
528 disease, if any, vitamin levels and other risk factors for cardiovascular disease.

529

530 The association of hyperhomocysteinemia and venous thrombosis is controversial. The
531 interplay of aberrant homocysteine metabolism, vitamin levels and other inherited coagulation
532 defects are likely important factors contributing to the risk of thrombosis.

533

534 Should patients at risk for atherothrombosis or venous thrombosis receive folate
535 supplementation? Perhaps, the most reasonable approach, given the current state of
536 knowledge, is to treat hyperhomocysteinemia patients who have additional risk factors for
537 atherothrombosis or venous thrombosis, including those with MTHFR homozygous 677C→T.

538 Dietary treatment should be first attempted followed by either folate or folate alternatives
539 (Betaine, Choline, N-acetylcysteine) supplementation. Folate alternative therapy should be
540 considered in patients with higher risk for breast or prostate cancer.

541

542 To this end, it is reasonable to assume that the final verdict on folate supplementation has not
543 been reached yet. More studies are needed to investigate various hypotheses and clinical
544 situations. Meanwhile, a conservative approach to normalize plasma homocysteine levels
545 might be best accomplished by a healthy diet of fresh fruit and vegetables and moderate
546 exercise.

547 **FACTORS THAT INFLUENCE PLASMA HOMOCYSTEINE LEVELS**

548 Table 1 (190)

549 Acquired

550 1. Folate Deficiency:

551 a. Dietary inadequacy

552 b. Malabsorption

553 c. Metabolic disorders, including alcohol and drugs

554 d. Increased requirements and increased losses.

555 2. Cobalamin Deficiency:

556 a. Dietary inadequacy

557 b. Gastrointestinal Disorders

558 c. Metabolic and Transport Disorders

559 3. Vitamin B₆ Deficiency

560 a. Inadequate supply

561 b. Vitamin B₆ Antagonists: Natural Antagonists and Drug- B₆ Interactions

562 4. Disease Associated with Hyperhomocysteinemia

563 a. Kidney Dysfunction

564 b. Proliferative disorders: Cancer, Psoriasis

565 c. Rheumatoid Arthritis and Systemic Lupus

566 d. Hypothyroidism

567 5. Drugs

568 a. Hormones: Sex hormones, insulin

569 b. Antiepileptic Drugs

570 c. Nitrous Oxide

571 d. Lipid – Lowering Drugs

572 **FACTORS THAT INFLUENCE PLASMA HOMOCYSTEINE LEVELS**

573 Table 1 (Cont'd)

- 574 e. Metformin
- 575 f. Disulfide exchangers (D-penicillamine)
- 576 g. Gastric Proton Pump Inhibition
- 577 h. Vitamin B₆ Antagonists
- 578 i. Methyl Group Acceptors (L-Dopa, 6-Mercaptopurine)
- 579 j. Other Drugs: (Sulfasalazine, Mega doses of Vitamin C)
- 580 6. Miscellaneous
 - 581 a. Increasing age
 - 582 b. Male sex
 - 583 c. Gastroplasty
 - 584 d. Down syndrome
 - 585 e. Increased Muscle mass
 - 586 f. Carbon monoxide, cyanide
- 587 7. Life Style Factors
 - 588 a. Exercise
 - 589 b. Smoking
 - 590 c. alcohol consumption
 - 591 d. Coffee intake
 - 592 e. Vitamin intake
 - 593 f. Protein intake

594 **FACTORS THAT INFLUENCE PLASMA HOMOCYSTEINE LEVELS**

595 Table 1 (Cont'd)

596 Genetic

- 597 1. Cystathionine – B-synthase deficiency
- 598 2. Inborn errors of Folate metabolism
- 599 a. Hereditary folate malabsorption
- 600 b. Methylenetetrahydrofolate Reductase Deficiency (MTHFR).
- 601 c. Glutamate formiminotransferase deficiency
- 602 3. Inborn errors of Cobalamin Absorption & Transport
- 603 a. Transcobalamin I (Haptocorrin, R Binder) Deficiency
- 604 d. Intrinsic Factor Deficiency
- 605 c. Defective Cobalamin Transport by Enterocytes (Imerslund-Gräsbeck Syndrome)
- 606 4. Inborn Errors of Cobalamin Metabolism
- 607 a. Adenosylcobalamin Deficiency
- 608 b. Combined Adenosylcobalamin and Methylcobalamin Deficiencies
- 609 c. Methylcobalamin Deficiency – Methionine synthase Reductase Deficiency and
- 610 Methionine Synthase Deficiency
- 611 5. Polymorphism of Folate and Cobalamin Metabolism:
- 612 a. Methylenetetrahydrofolate Reductase
- 613 b. Methionine Synthase
- 614 c. Methionine Synthase Reductase

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DISORDERS ASSOCIATED WITH HYPERHOMOCYSTEINEMIA

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Table 2

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1. Atherosclerosis (carotid artery intima-media thickening)

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2. Coronary Artery Disease.

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3. Cerebral Vascular Disease

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4. Peripheral Arterial Disease

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5. Venous thromboembolic Disease

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6. Disordered Hemostasis:

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a. Platelet dysfunction: Increased thromboxane A₂ synthesis

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b. Procoagulant activity: Increased FVIIIc, vWF, thrombin-antithrombin complexes

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and prothrombin F1&2, and Decreased FVII

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c. Decreased Natural Anticoagulant Activity:

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1. Deficiency of antithrombin

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2. Deficiency of Protein C

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CLASSIFICATION OF HYPERHOMOCYSTEINEMIA

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Table 3

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633 Severe – Moderate Hyperhomocysteinemia

634 High Total homocysteine levels at all times; deficiencies in CBS, MTHFR or in enzymes of B₁₂
635 metabolism.

636 Mild – Moderate Hyperhomocysteinemia

637 Moderately high total homocysteine levels under fasting conditions; reflects impaired
638 homocysteine methylation (folate, B₁₂ or moderate enzyme defects, e.g., thermolabile
639 MTHFR).

640 Post-methionine load

641 Abnormal increase in total homocysteine after methionine load. Abnormal net increase reflects
642 impaired homocysteine transsulfuration (heterozygous CBS deficiency, B₆ deficiency).

Polymorphic Mutations in 5, 10 - Methylene tetrahydrofolate Reductase

Table 4

Mutation	Change in Amino Acid or Splice Site	Exon or Intron	Reference
677 CT	Alaine/Valine	Exon 4	20
1068 TC	Serine/Serine	Exon 6	21
1178 + 31 T/C	5' Splice Site	Intron 6	22
1317 T/C	Phenylalanine/Phenylalanine	Exon 7	25
1298 A/C	Glutamate/alanine	Exon 7	23, 24, 25

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HOMOCYSTEINE EFFECT ON VARIOUS COAGULATION FACTORS

Table 5

Factor/Process	Effect	Evidence in Literature
Tissue factor expression	Increase	Suggestive (191)
FVII activity	Increase	Inconsistent (192-194)
Thrombin generation	Increase	Suggestive (194-196)
FV activation	Increase	Suggestive (197, 198)
Fibrinogen modification	Present	Suggestive (199, 200)
Thrombomodulin expression	Decrease	Inconsistent (197, 201, 202)
Inactivation of FVa	Decrease	Inconsistent (203-205)
TFPI activity	Increase	Inconsistent (206, 207)
tPA binding	Decrease	Suggestive (208, 209)
Plasmin generation	Decrease	Suggestive (199, 209, 210)

676 **HYPERHOMOCYSTEINEMIA AND ARTERIAL OCCLUSIVE DISEASE;**
677 **STUDIES SHOWING CORRELATION**

680 **Table 6**

Study	Findings
685 Boushey et al. (120)	685 5 µmol/L rise in total plasma HC 686 increases relative risk of CAD, CVD, PVD
688 European Concerted Action Project (121)	688 HHC associated with increased risk of 689 vascular disease multiplicative to other 690 risk factors.
692 Stampfer et al. (136)	692 Relative risk of MI of 3.1 when HC levels 693 were in the 95th percentile of control 694 values.
696 Malinow et al. (137) & Voutilainen et al. (127)	696 Increase plasma HC levels are associated 697 with thickened carotid wall
699 Nygaard et al. (144)	699 Strong graded relationship between total 700 HC and mortality
702 Kluijtmans et al. & Mudd et al. (14-15, 153)	702 677C→T MTHFR is a genetic risk for 703 CAD

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720 **CAD = Coronary Artery Disease**
721 **CVD = Cerebrovascular Disease**
722 **PVD = Peripheral Vascular Disease**
723 **HHC = Hyperhomocysteinemia**
724 **HC = Homocysteine**

725 **HYPERHOMOCYSTEINEMIA AND ARTERIAL OCCLUSIVE DISEASE;**
726 **STUDIES SHOWING NO CORRELATION**

727
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729 **Table 7**

Study	Findings
731 Alfthan et al. (146)	732 No statistical difference between 733 individuals who developed MI and those 734 who did not.
735 Verhoef et al. (143)	736 No statistically significant relative risk to 737 develop CAD, angina and stroke.
738 Evans et al. (147)	739 No association between plasma HC 740 levels and MI.
741 Folsom et al. (148)	742 Total HC levels correlate with CAD in 743 women but not men.
744 Brattström et al. (154)	745 677C→T MTHFR is not a causal risk for 746 CAD.

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766 **MI = Myocardial Infarction**
767 **CAD = Coronary Artery Disease**
768 **HC = Homocysteine**
769
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**HYPERHOMOCYSTEINEMIA AND VENOUS THROMBOSIS;
STUDIES SHOWING CORRELATION**

Table 8

Study	Findings
Falcon et al. (155)	High prevalence of HHC in juvenile VT
den Heijer et al. (156-157)	HHC > 95 th percentile of control range is a risk factor for DVT
Simioni et al. (158)	Significant high prevalence of HHC in patients with DVT of upper extremities
Eichinger et al. (160)	<ul style="list-style-type: none"> • HHC in 25% of patients with a single episode of idiopathic VT • 2.7 risk of recurrent TE in the first 24 months after discontinuation of anticoagulation
Kottke-Marchant (126)	Plasma HC > 13 μM is a risk factor for arterial and venous thrombosis in patients with normal coagulation profiles
Fermo et al. (162)	Moderate HHC in 13.1% of patients with VT and 19.2% of patients with AOD.
den Heijer et al. (161)	HHC associated with a calculated pooled odds ratio of 2.6 for VTE
Arruda et al, Salomon et al, & Margaglione et al. (165-167)	Evidence in support of 677→T MTHFR being a risk factor for VT (slightly greater risk for VT in homozygous vs. heterozygous genotype)
Kluijtmans et al. (173)	677C→T MTHFR may be a risk factor for thrombosis in CBS deficient patients.
Lalouschek et al. (174)	677C→T MTHFR increased risk of TIA or minor strokes
<p>HHC = Hyperhomocysteinemia DVT = Deep Venous Thrombosis HC = Homocysteine AOD = Arterial Occlusive Disease VTE = Venous Thromboembolism VT = Venous Thrombosis TE = Thromboembolism</p>	

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821 **HYPERHOMOCYSTEINEMIA AND VENOUS THROMBOSIS;**
822 **STUDIES SHOWING NO CORRELATION**

824
825 **Table 9**

Study	Findings
829 Martinelli et al. (159)	826 No association of HHC and DVT of upper 827 extremities
832 Trillot et al & Kluijtmans et al. (169, 172)	831 677C→T MTHFR does not modify risk of VT
834 De Stefano et al. (168)	835 • nine case –control studies involving 2225 836 patients with VT and 2994 healthy controls 837 • No significant differences in cumulative 838 prevalence between homozygous MTHFR in 839 cases with VT vs normal controls

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865 **HHC = Hyperhomocysteinemia**

866 **VT = Venous thrombosis**

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868 **NORVIT; EVENT RATES (PER 1000 PERSON-YEAFFECT OF**
869 **HYPERHOMOCYSTEINEMIA COMBINED WITH OTHER THROMOBOPHILIC RISK**
870 **FACTORS**

871
872 **Table 10**

Study	Findings
874 Fermo et al. (162) 875 876 877 878 879 880 881 882	The relative risk of VT in patients with HHC combined with other thrombophilic factors was 1.6 times greater than for patients with HHC alone and patients developed first thrombotic episode at a younger age.
883 Ridker et al. (163) 884 885	10 fold increase in thrombotic risk among patients with HHC and FVL.
886 Legnani et al. (164) 887 888 889 890 891 892	• No association between HHC and thrombosis in patients with ptn C, ptn S, AT def or FVL. • 677C→T MTHFR did not confer additional thrombotic risk factor to the heritable thrombophilic coagulation defects
893 Cattaneo et al. (170-171) 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909	Coexistence of 677C→T MTHFR and FVL increased risk of VT

910 **VT = Venous Thrombosis**
911 **HHC = Hyperhomocysteinemia**
912 **FVL = Factor V Leiden**
913 **ptn C = Protein C**
914 **ptn S = Protein S**
915 **AT def= Antithrombin deficiency**
916

NORVIT; EVENT RATES (PER 1000 PERSON-YEARS)

Table 11

	Folic Acid + Vitamin B6	Folic Acid	Vitamin B6	Placebo
Primary endpoint	81.6	66.9	70.1	67.2
MI	73.0	57.5	64.0	59.2
Death from any cause	37.5	28.7	33.4	31.7
Cancer	12.0	11.9	8.0	9.0

MI = Myocardial infarction

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NORVIT; RATE RATIOS

Table 12

	Folic acid vs control			Vitamin B-6 vs control			Folic Acid + Vitamin B-6 vs control		
	RR	95% CI	<i>p</i>	RR	95% CI	<i>p</i>	RR	95% CI	<i>p</i>
MI and stroke	1.1	(0.9 – 1.3)	.3	1.1	(1.0 – 1.3)	.09	1.2	(1.0 – 1.4)	.03
MI	1.1	(0.9 – 1.2)	.5	1.1	(1.0 – 1.4)	.04	1.2	(1.0 – 1.4)	.03
Death	1.1	(0.9 – 1.3)	.8	1.1	(1.0 – 1.5)	.11	1.2	(1.0 – 1.5)	.10
Cancer	1.1	(0.9 – 2.0)	.08	1.1	(1.0 – 1.4)	.30	1.3	(0.8 – 1.9)	.30

MI = Myocardial infarction

RR = Relative Risk

CI = Confidence interval

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HOMOCYSTEINEMIA AND THROMBOSIS

978

Table 13

979

1. Hyperhomocysteinemia is a cause of atherosclerosis and venous thrombosis.

980

981

2. Hyperhomocysteinemia is associated with either atherosclerosis or venous thrombosis, but not both.

982

983

3. Hyperhomocysteinemia is not a cause but a marker of vascular disease, an innocent bystander.

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4. Hyperhomocysteinemia is a risk factor for vascular disease only in very high concentrations.

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5. Hyperhomocysteinemia is associated with vascular disease in patients with co-existent risk factors.

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6. Hyperhomocysteinemia is a surrogate for low Vitamin-B levels, which is the true risk for vascular disease.

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996 **Fig.1: Schematic representation of homocysteine metabolism**
997 **THF:tetrahydrofolate, MTHFR: methylenetetrahydrofolate reductase; MS:**
998 **methionine synthase; SAM: S-adenosylmethionine, SAH: S-**
999 **adenoylhomocysteine; BHMT: betaine homocysteine methyltransferase: CBS:**
1000 **Cystathionine-B-synthase; B₁₂;B₆: vitamin B₆**

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Fig. 1

