

## The Impact of Homocysteine on Coronary Heart Disease: a look at the Most Recent Studies and Trials

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### ABSTRACT

Coronary Heart Disease (CHD) are leading cause of death in Bulgaria and other countries from East and Central Europe. Ecological studies show that traditional risk factors could not entirely explain the variations in population trends, suggesting need of search for new determinants of coronary risk, including homocysteine (Hcy). The results from many studies strongly suggest that plasma or serum concentration of total Hcy (tHcy) is an important marker in coronary risk assessment. Although, many data are supportive to the deteriorating role of Hcy, significant discrepancies exist yet in this information. The aim of paper is to review existing data on relationship between Hcy and coronary risk, published after 1995, based on the controversial results from different types of epidemiological studies and high number of new studies conducted in the last ten years. Although many data are supportive to the adverse effect of Hcy on CHD and the relationship is consistent, strong, dose related and biologically plausible the causal role of elevated Hcy and atherosclerosis remain inconclusive. The reviewed cross-sectional and retrospective case-control studies support the association between high plasma tHcy concentration and risk of CHD. The data from prospective studies, however, suggest controversial results. The only one finished till now trial of Hcy-vitamin lowering therapy did not find a treatment effect on CHD endpoints during the two years of follow-up. The reasons for the difference in results between the prospective studies still remain unclear. Absence of population studies on Hcy in Bulgaria is an emerging circumstance for research in that field.

**KEYWORDS:** *homocysteine, coronary risk, cardiovascular disease*

Coronary Heart Disease (CHD) are leading cause of death in Bulgaria and other countries from East and Central Europe. Ecological studies show that traditional risk factors could not entirely explain the variations in population trends, suggesting need of search for new determinants of coronary risk, including homocysteine (Hcy). A causal role of elevated total homocysteine (tHcy) in development of CHD was proposed approximately 30 y ago [1]. Since then, the results from clinical, early cross-sectional and case-control studies strongly suggest that plasma or serum concentration of tHcy is an important marker in coronary risk assessment [2, 3].

The literature concerning role of Hcy in relation to coronary risk has expanded enormously in recent years. A lot of new studies on relationship between Hcy and CHD have been published after the publication of an overview of 17 studies, 14 of which with a positive association between Hcy and CHD (Boushey et al, 1995) [4]. Although, many data are supportive to the deteriorating role of Hcy, significant discrepancies exist yet in this information. Eleven of the reviewed by Boushey et al (1995) studies were retrospective. Prospective studies were only three and the results of those studies were not consistent with one another. There is a lack of sufficient data supportive to the effect of Hcy, coming as from earlier as well as from recent prospective studies [5, 6] and almost absence of the data from large randomized trials of Hcy-lowering therapy [7].

The aim of this paper is to review existing data on relationship between Hcy and coronary risk, published after 1995, based both on the controversial results from different types of epidemiological studies and the high number of new studies conducted in the last ten years.

### METHODS

We performed a literature search of the electronic database Medline between 1995 and 2004, using the exploded terms homocysteine and cardiovascular

disease. We limited our search to observational studies and clinical trials on both fatal and non-fatal CHD, excluding those investigating cerebrovascular disease only, or reporting them not separately. As studies investigating the association between tHcy levels and anatomic extend of coronary atherosclerosis have as an endpoint only a surrogate measure of coronary event, they have not been included in this review. The reviewed studies have been divided into two main sections by the type of study (observational and clinical trial) arranged into each section by the year of presentation.

### RELATIONSHIP BETWEEN PLASMA HOMOCYSTEINE AND CHD

#### Mechanisms of action

Independent role of Hcy has been suggested by experimental evidence of mechanisms, by which Hcy might cause vascular disease. Elevated tHcy induces thrombogenicity and procoagulant state both related to subsequent higher risk of thromboembolic events, including myocardial infarction. Hcy contains a sulfhydryl group that can react with plasma constituents and thus may promote oxidative damage and oxidation of low-density lipoprotein (Blom et al 1992) [8]. Hcy could also cause a direct cytotoxic effect on endothelial cells through formation of reactive oxygen species, impaired production of nitric oxide and subsequent endothelial dysfunction and stimulation of smooth cell proliferation (Blundell et al 1996) [9]. Plasma Hcy concentrations are increased after tissue damage. Elevated plasma Hcy levels could further cause additionally endothelial damage (Knekt et al, 2001) [10].

### OBSERVATIONAL STUDIES

#### Cross-sectional and retrospective case-control studies

Alfthan et al in a cross-sectional (ecological study) conducted on 1990 healthy men aged 40-49 from Europe found elevated tHcy levels in countries with higher cardiovascular mortality. The correlation coefficient of

tHcy levels with cardiovascular death was statistically significant in order of 0.71 [11].

Recent meta-analysis on 26 case-control studies, involving approximately 3315 cases and 4001 controls reported that hyperhomocysteinemia (Hcy levels over 90 or 95th percentile, for a 5  $\mu\text{mol/l}$  increase in Hcy concentration) was associated with an increased risk of both fatal and non-fatal CHD (OR 1.7, 95%CI 1.5-1.9) (Ford et al 2002) [12]. The Odds ratio was 1.63 (95% CI 1.44-1.85) for men and 2.11 (95%CI 1.30 - 3.42) for women. The recent large European Concerted Action Project conducted on 750 patients and 800 controls and not included in the meta-analysis by Ford et al (2002) confirmed the independent role of elevated Hcy level on coronary risk (OR 2.2; 95%CI 1.6-2.9) (Graham et al 1997) [13].

#### Prospective (nested case-control and cohort) studies

There were only two cohort studies both conducted on men (Stewhower et al 1998 and Ubbink et al 1998) investigating the relationship between CHD and Hcy. The rest of the prospective studies were designed as nested case-control studies. Data from prospective studies suggest controversial results (Tab.1). Seven prospective studies have found higher coronary risk associated with higher tHcy levels (Arnesen et al 1995, Nygard et al 1997, Ubbink et al 1998, Wald et al 1998, Stewhower et al 1998, Bots et al 1999, Wincup et al 1999) [14-20]. Strong, consistent and graded associations between Hcy and CHD were found in Tromso, Rotterdam and British United Provident Association (BUPA) studies. The rest of the reviewed studies failed to show an association between plasma Hcy and CHD (Chasan-Taber et al 1996, Jing Ma et al 1996, Evans et al 1997, Verhoef et al 1997, Folsom et al 1998, Knekt et al 2001) [21-26]. Moreover, two of the reviewed studies, the Multiple Risk Factor Intervention Trial (Evans et al 1997) and Finnish Mobile Clinic Health Examination Survey (Knekt et al 2001) reported values of OR for CHD below 1.0 (Tab.1). The reasons for negative results of the two studies and for the difference in results between the prospective studies remain unclear. Differences in nutritional status and genetic factors may modify the association between tHcy and CHD in different populations. Increased intake of vitamin supplements among the US population, for instance, during the follow up of MRFIT might play role in attenuating the relationship between AMI and tHcy concentration. Difference in the length of follow up seems to be unlikely explanation as studies of similar design and follow up period (Finnish Mobile Clinic Health Examination Survey and British Regional Heart study) appear to present with different results. In confirmation, it has been suggested attenuation of risk during in the second decade of follow-up (Chasan-Taber et al, 1996) [27]. Randomized controlled trials of homocystein-lowering vitamin therapy

Meta analysis of 12 randomized controlled trials shows that a daily supplementation with 500 mg of vitamin B12 decreases plasma Hcy concentration by 25-30% (Homocysteine Lowering Trialist Collaboration 1998). Vitamin B supplementation could lead to improvement of clinical outcome of the disease: Schnyder et al (2002) in the Swiss Heart Study observed lower rate of restenosis after coronary angioplasty in supplemented with folic acid, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> [28].

There have been published results from only one trial evaluating the effect of decrease in Hcy levels on major cardiovascular events (recurrent stroke, AMI and death). The Vitamin Intervention for Stroke Prevention Trial (VISP) investigating the effect of effect of 25 mg pyridoxine, 0.4 mg cobalamin and 2.5 mg of folic acid vs 200  $\mu\text{g}$  pyridoxine, 6  $\mu\text{g}$  cobalamin and 20  $\mu\text{g}$  of folic acid on 3680 adults with non disabling cerebral infarction from centers in USA, Canada and Scotland did not find the treatment effect on any endpoint during the two years of follow-up. The unadjusted risk ratio for CHD event was 1.0 (95%CI 0.8-1.1). It has been, however, reported, a persistent and graded association between baseline Hcy and outcomes. A 3  $\mu\text{mol/l}$  lower total Hcy, for instance, was associated with a 26% lower risk of CHD ( $p < 0.001$ ) in the low-dose group. Moderate reduction of total Hcy after cerebral infarction had no effect on vascular outcomes. The received results suggest the need of longer trials in populations with elevated total Hcy concentrations [29]. Relationship between Hcy and major CAD is still object of many ongoing experimental studies of Hcy-lowering vitamin therapy (Doshi SN et al, 2002).

#### Differences in results between studies

Many factors could account for the differences in results between different studies. Methodological considerations (quality control, timing and storage of blood sample collection, measuring method, after the methionine loading or at baseline, patient characteristics in terms of fasting state and posture) should be taken in mind. It is known that tHcy decreases during and increases after the acute coronary event, so as the elevated Hcy concentrations found in cases could be a consequence rather than a cause of the disease making interpretation of the results form retrospective case-control studies cautious.

Although many data are supportive to the adverse effect of Hcy on CHD and the relationship is consistent, strong, dose related and biologically plausible the causal role of elevated Hcy and atherosclerosis remain inconclusive:

1. Atherosclerotic patients may have higher level of Hcy because they have more advanced inflammatory disease, not because Hcy caused their vascular disease. Low levels of folic acid, proposed by retrospective studies as an independent risk factor for fatal CHD may reflect the repairing process in those patients, rather than the higher Hcy itself;
2. The strength of association of Hcy with CHD is weaker in prospective studies. Prospective studies are generally considered superior to retrospective in terms of better elucidation of the time-dependent relationship between the investigated exposure and effect, and thus on causality. Observational studies (both retro- and prospective), however, are prone to uncontrolled confounding and bias. Therefore, the causal relationship is not enough to make the recommendations on screening and treatment for prevention of CHD. It could be, however, considered screening and treatment patients at high risk, if tHcy levels are above 10  $\mu\text{mol/l}$  [30].
3. The results from the only one finished till now trial (VISP) are not encouraging to confirming the causal role of Hcy on major CV endpoints.

Study	Study population	Cases, events/controls	Age/Sex	Follow up	Outcome	Plasma tHcyases/Controls (µmol/l)	Adjusted Relative risks, 95%CI
Tromso Study (Arneson et al 1995)	21826	122/478	12-61/M, F	3.5	CHD fatal, non fatal	12.7/11.3	1.4 <sup>a</sup> (1.1-1.7)
Physicians' Health Study (Chasan-Taber et al 1996)	14916	333/333	40-84/M	7.5	MI	not reported	1.7 <sup>a</sup> (0.9-3.3)
Physicians' Health Study (Jing Ma et al 1996)	22071	293/290	35-64/M	8	MTHFR polymorphism, MI	11.3/10.6	1.1 <sup>f</sup> (0.8-1.5) 0.8 <sup>k</sup> (0.5-1.4)
Multiple Risk Factor Intervention Trial (Evans et al 1997)	12866	93/86 147/286	35-57/M	10	MI, CHD (fatal)	12.6/13.1 12.8/12.7	0.8 <sup>b</sup> (0.5-1.5)
Nygard et al 1997	587	64	46/2M, F	4.6	CHD* (fatal)	>20/<9	4.5 <sup>e</sup> (1.2-16.6)
Physicians Health Study (Verhoef et al 1997)	14916	109/427	40-84/M	9	New angina, CABG	11.4/10.6	1.0 <sup>f</sup> (0.4-2.4)
Zutphen, Netherlands (Stehouwer et al 1998)	878	162	64-84/M	10	MI	-	1.8 <sup>b</sup> (1.0-3.0)
Caerphilly study (Ubbink et al 1998)	2290	154/21361	50-64/M	5.0	CHD	12.4/11.7	1.07 <sup>h</sup> (0.9-1.2)
British United Provident Association Study (BUPA) (Wald et al 1998)	21250	229/1126	35-64/M	8.7	CHD (fatal)	13.1/11.8	2.9 <sup>b</sup> (2.0-4.1)
Atherosclerosis Risk in Communities (Folsom et al 1998)	15792	232/527	45-64/M, F	3.3	CHD	8.9/8.5	1.3 <sup>c</sup> (0.5-3.2)
Caerphilly study (Ubbink et al 1998)	2290	154/21361	50-64/M	5.0	CHD	12.4/11.7	1.07 <sup>h</sup> (0.91.2)
The Rotterdam study, (Bots et al 1999)	7983	224/533	> 55/M, F	3.4	MI	17.3/15.2	2.4 <sup>c</sup> (1.15.3)
British Regional Heart study (Wincup et al 1999)	5661	386/454	40-59/M	12.8	MI	14.2/13.5	1.7 <sup>f</sup> (1.2-2.4)
Finnish Mobile Clinic Health Examination Survey (Knekt et al 2001)	3471	272/524	45-64/M	13	MI, CHD (fatal)	10.8/11.2	0.9 <sup>c</sup> (0.5-1.6)

CABG – coronary artery bypass graft, MI myocardial infarction, \*angiographically demonstrated, †median ‡) per 4 µmol/l increase in tHcy; §) highest compared with the lowest quartile of tHcy; ¶) highest compared with the lowest quartile of tHcy; d) to 5% compared with lowest 10% of tHcy levels; e) tHcy ≥20 µmol/l vs those with less than 9 µmol/l; f) ≥95 percentile compared with <75 percentile of tHcy levels; g) highest compared with the lowest third of tHcy levels; h) per 5 µmol/l increase in tHcy; i) top fifth (≥ 16.5 µmol/l) vs compared to the lower levels; j) (+/-) genotype for MTHFR as compared to (-/-) genotype; k) (+) genotype

**Tab.1** Prospective studies on relationship between plasma homocysteine concentration and cardiovascular disease endpoint.

**In Conclusion**, cross-sectional and retrospective case-control studies support the association between high plasma Hcy concentration and risk of CHD. The data from prospective studies suggest controversial results. The reasons for the difference in results between the prospective studies still remain unclear. The only one, finished till now trial of Hcy-vitamin lowering therapy, with a relatively short time of follow up, did not find a

treatment effect on CHD endpoints. Based on the current state of knowledge for the role of Hcy in CHD risk, the American Heart Association and International Task Force on the Prevention of Coronary Artery Disease, state that any change in public health policy must wait for results from the ongoing large clinical trials [31,32]. The absence of the population studies on Hcy in Bulgaria is an emerging circumstance for research in that field.

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## Воздействие гомоцистеина на ишемическую болезнь сердца: обзор новейших исследований и

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### Р Е З Ю М Е

Ишемическая болезнь сердца (ИБС) является главной причиной смерти в Болгарии и других странах Восточной и Центральной Европы. Экологические исследования показывают, что традиционные факторы риска не могут полностью объяснить изменения в популяциях, что свидетельствует о необходимости поиска новых детерминантов коронарного риска, в частности, гомоцистеина (Hcy). Данные многих исследований свидетельствуют, что концентрация общей Hcy в плазме или сыворотке (tHcy-total Hcy) - важный маркер в оценке коронарного риска. Однако по этому вопросу данные противоречивы. Цель работы состояла в анализе существующих данных о взаимоотношениях между Hcy и коронарным риском. Существуют данные о побочных эффектах Hcy на ИБС; между ними отчетливо прослеживаются, дозозависимые и биологические эффекты. Тем не менее важнейшая роль повышения Hcy и атеросклероза остается невыясненной. Кросс-секционные и ретроспективные контрольные исследования указывают на ассоциацию между высокой плазменной концентрацией tHcy и риском ИБС, хотя данные спорны. Единственный законченный в настоящее время труд по оценке эффекта Hcy-витаминпонижающей терапии не выявил лечебного эффекта при ИБС в течение двух лет наблюдений. Причины подобных различий результатов исследования все еще остаются неясными. Отсутствие популяционных исследований Hcy в Болгарии является наиболее актуальным для проведения исследований в этой области.

**Ключевые слова:** гомоцистеин, коронарный риск, ишемическая болезнь сердца