Review Article

Homocysteine: A Risk Factor for Coronary Artery Disease?

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Coronary artery disease (CAD) is one of the main health problems concerning health care services in western societies. In recent years, attempts to combat this disease have extended beyond treatment and have centred mainly on prevention. The recognition of a variety of risk factors for CAD and their effective management have represented an important step in this direction.

Despite the established significance of the classical risk factors, there continues to be a large number of CAD patients who have no relation to any of them. It has recently been discovered that elevated plasma levels of the amino acid homocysteine are associated with a greater risk and increased mortality from CAD in the general population.[1-4]

However, before homocysteine can be definitely established as a risk factor for the disease many questions remain to be answered, such as whether elevated homocysteine levels to lead to CAD, or vice versa. We also do not know whether reducing homocysteine levels with suitable treatment can modify the relative risk of CAD.

Biochemistry and molecular genetics

Homocysteine is produced from methionine as a product of a large number of transmethylation reactions dependent on S-adenosylo-methionine.[5] Three enzymes contribute to homocysteine metabolism, as shown in figure 1.[5,6] When there is an excess of methionine, homocysteine follows the transulphydryliosis pathway, through which homocysteine is converted automatically to cysteine. The first reaction in this pathway is catalysed by an enzyme dependent on vitamin B6, cystathionine β-synthase (CBS).[5] Under conditions with a negative methionine balance homocysteine follows two pathways. In the liver a significant quantity of homocysteine is remethylated by betaine-homocysteine-methylo-transferase (BHMT), which uses betaine as methyl group donor. In most tissues, though, the remethylation of homocysteine is catalysed by methionine synthase (MS), which uses B12 as coenzyme and methylene-tetrahydrofolate (MTHF) as substrate. The formation of MTHF is catalysed by methylene-tetrahydrofolate reductase (MTHFR), an enzyme that has an indirect but powerful effect on the remethylation of homocysteine and whose action depends on vitamin B12.[7]

Very often, one or more of the homocysteine metabolism pathways are inhibited by enzyme deficiencies or because of vitamin deficiencies, and the result is an accumulation of homocysteine and an increase of its levels in the blood.[2] This is the metabolic basis for using total homocysteine as a functional index of B12 and folate levels.[8] The enzyme deficiencies

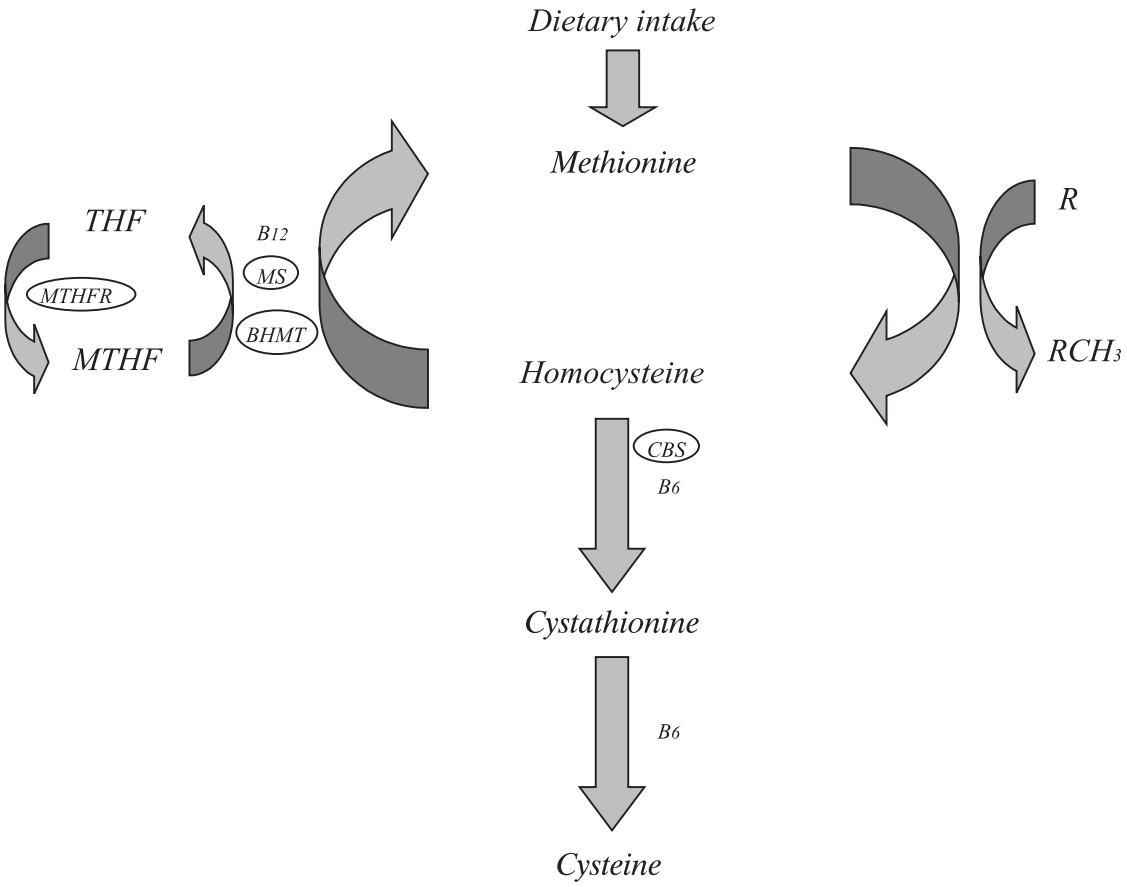

Figure 1. Homocysteine metabolism. Dietary methionine intake leads to the generation of homocysteine donating a methylene-group. Homocysteine may further be transformed to cystathionine and after that to cysteine, a reaction catalysed by the enzyme CBS, which uses B6 as co-factor. Furthermore, homocysteine may be re-converted to methionine by receiving a methylene group from MTHF, under the effect of enzyme MS, which uses B12 as co-factor. MTHF is regenerated after MHF methylation which is catalysed by MTHFR. Homocysteine methylation may also occur through the action of BHMT enzyme, which uses betaine as a methylene-donating substance.

B6: pyridoxal phosphate; B12: vitamin B12 (methylocobalamin); BHMT: betaine-homocysteine methyl-transferase; CBS: cystathionine β-synthase; MS: methionine synthase; MTHFR: methylenetetrahydrofolate reductase; THF: tetrahydrofolate.

along homocysteine's metabolic route constitute a variety of metabolic diseases that are together described as hyperhomocysteinaemia.

In recent years the genes that code for the enzymes CBS, MTHFR, MS and BHMT have been identified.[4] Mutations that result in the disease of hyperhomocysteinaemia have been identified on the genes for CBS and MS.[8,9]

Determination of total homocysteine levels

The sulphydril group of homocysteine gives it the property of oxidising at normal pH to form disulphides. However, the term homocysteine refers to both the oxidised and the reduced forms. In the plasma traces of homocysteine are encountered as reduced homocysteine (1%), in combination with albumin (70%), and the remaining 29% as low molecular weight disulphides, mainly cysteine. The total of all kinds of homocysteine is described by the term "total homocysteine."[4]

Since the 1980s, new methods have been developed to determine total homocysteine levels and to overcome the problems associated with the multiple and unstable types of homocysteine.[4] Briefly, the principle on which the new methods is based is that the oxidised form of serum homocysteine is converted by the action of a reductase to reduced homocysteine, which can then be measured quantitatively.[4] Most measurement methods are based on chromatographic techniques (high output liquid chromatography, or gas chromatography with mass spectrometry). Both

the collection and the handling of the blood samples are important. A measurement procedure that does not ensure absolute precision in the collection and storage of samples may generate false elevated total homocysteine levels.

One fact that should be taken into account in the assessment of total homocysteine is that the consumption of a light meal (such as breakfast) only has a slight effect on the measurements, slightly raising homocysteine levels, but a meal rich in protein can cause an increase of 15-20%.[4]

In addition, it should be borne in mind that in the presence of blood cells there is a temperature- and time-dependent increase in homocysteine levels that approaches 5-15% per hour at room temperature.[4] Consequently, immediate centrifugation of the blood is essential; otherwise, the samples must be kept on ice. Centrifugation causes the cells to settle out so that the total homocysteine is stable in the serum or plasma for days at room temperature, for weeks at 0-2°C and for years at -20°C.[4]

In clinical practice two methods are used for the diagnosis of hyperhomocysteinaemia. The first and simpler method is used for screening the general population for hyperhomocysteinaemia and measures the fasting or baseline levels of plasma homocysteine. The normal levels range from 5 to 15 μmol/L with a mean of 10 μmol/L. In hyperhomocysteinaemia homocysteine levels are above 15 μmol/L. Hyperhomocysteinaemia is classified as mild (15-30 μmol/L), moderate (30-100 μmol/L) or severe (>100 μmol/ L).[4] The normal variations in homocysteine levels are given in table 1.[10]

The second method of diagnosing hyperhomocysteinaemia in the general population is based on the measurement of total homocysteine levels after methionine loading. Methionine loading means the ingestion of large doses of methionine (0.1 g/kg body weight) and total homocysteine is measured 2, 4 and 6 hours after the methionine is given.[2,11] The reference values for normal values after methionine loading have not yet been determined precisely. In relation to fasting levels, total homocysteine levels at the fourth and sixth hour after loading are usually two or three times higher. Elevated fasting levels or elevated homocysteine levels after methionine loading have been linked with a high risk of cardiovascular diseases and especially with early atherosclerosis, as well as with thromboembolic episodes in the cerebral, peripheral and coronary vessels.[4,12]

Recently, immunoassay methods have been developed for the sensitive measurement of plasma homocysteine levels, such as fluorescence polarisation FPIA (Abbott Laboratories IMx System, Abbott Park, Ill) and microplaque FPIA (Bio-Rad, Hercules, CA).

Table 1. Normal reference ranges of plasma homocysteine levels.

	Age 12-19	Age ≥60	Threshold for elevated values
Men	4.3-9.9	5.9-15.3	11.4
Women	3.3-7.2	4.9-11.6	10.4

Values expressed in μmol/L

Determination of plasma total homocysteine levels. Relation with genetic and acquired risk factors

The determination of total homocysteine levels depends on genetic and acquired factors, which are listed in table 2.[4]

Table 2. Factors affecting homocysteine levels.[4]

Causes		Effects
Genetic factors		
1.	Homozygous defect for CBS	+++
2.	Homozygous defect for MTHFR	+++
3.	Cobalamin mutations	+++
4.	Down's syndrome	-
5.	Heterozygous defect for CBS	+
6.	Heterozygous defect for MTHFR	+
Drugs		
1.	Folate antagonists (methotrexate)	+
2.	Vitamin B6 antagonists	+
3.	Vitamin B12 antagonists	++
4.	Antiepileptic drugs	+
5.	Contraceptives	-
6.	Aminothiols (penicillamine, acetylcysteine)	-
7.	Others (niacine, cholestyramine, L-dopa)	+
Clinical conditions		
1.	Folate deficiency	++
2.	Vitamin B12 deficiency	+++
3.	Vitamin B6 deficiency	+
4.	Renal failure	++
5.	Hypothyroidism	+
6.	Neoplasms	+
Lifestyle		
1.	Vitamin intake	-
2.	Smoking	(+)
3.	Coffee	(+)
4.	Alcohol	±
5.	Exercise	-
Other factors		
1.	Increasing age	(+)
2.	Male sex	(+)
3.	Increased muscle mass	(+)

Age, sex and renal function

Women have lower total homocysteine than men. Additionally, an increase in total homocysteine has been noted with increasing age.[4] These observations could be attributable to differences in vitamin levels and to the effect of sex hormones.[13] Homocysteine levels typically increase after menopause.[4,13,14]

The difference between the sexes could involve the formation of homocysteine in relation with creatine/creatine synthase, which is proportional to the difference in muscle mass and is consequently higher in men than in women.[4]

Renal function may be used as an index of total homocysteine.[4] This is probably related with the fact that homocysteine clearance takes place in the kidneys. The physiological reduction in kidney function with increasing age probably explains the rise in homocysteine levels.[4]

Lifestyle

Consumption of vitamins B6, B12 and folate is inversely related with plasma total homocysteine levels. Smoking and caffeine cause an increase in homocysteine, while physical activity causes a reduction. It should be stressed that these factors affect women more than men. Chronic alcohol consumption is associated with elevated homocysteine levels, probably because of the effect of alcohol on vitamin levels. In contrast, moderate alcohol consumption appears to be associated with low homocysteine levels.[4]

Heredity

Homocystinuria is related with severe hyperhomocysteinaemia and is a genetically determined disease. Homozygous defect for CBS is the most common cause of homocystinuria, with an incidence of 1/30,000 births and a varied geographic distribution. Rare types of homocystinuria involve a defect for MTHFR or, even more rarely, cobalamin metabolism. Heterozygotes for CBS defect make up <1% of the general population. Those individuals usually have low fasting homocysteine levels, but those levels increase with methionine loading.[15,16] Recent genetic studies show that heterozygotes for CBS defect are rare; their genetic defect is thus not a frequent cause of hyperhomocysteinaemia in the general population.[4]

The common point mutation C677 in the MTHFR gene shows differences from race to race, with a high incidence approaching 40% in Caucasians, while being virtually nonexistent in Africans and Americans.[4] The mutation in question causes a reduction in the potency and the stability of the enzyme and predisposes to moderate hyperhomocysteinaemia under conditions of reduced folate levels.[4,9] Low daily doses of folate are likely to help in maintaining normal homocysteine levels.[4]

Clinical condition and drugs

Folate or cobalamin deficiency is a frequent cause of hyperhomocysteinaemia in the general population.[4] Elevated homocysteine levels are seen in renal failure and in a variety of other clinical conditions (Table 2).[4] Hyperhomocysteinaemia can be caused by a variety of drugs, mainly those that affect vitamin levels and are related with homocysteine metabolism. Aminosulphates such as penicillamine and acetylcysteine reduce plasma homocysteine levels.[4]

Homocysteine and CAD

The clinical studies that have been carried out so far concerning the relation between homocysteine and CAD have produced conflicting results. In spite of that, following the completion of large epidemiological studies in recent years many now maintain that homocysteine is a new risk factor for CAD. The first useful meta-analysis, by Boushey et al[17] in 1995, showed that an increase in homocysteine levels of 5 μmol/L raises the relative risk for CAD by the same amount as does an increase in total cholesterol levels of 20 mg/dL. This meta-analysis included 17 studies (5 cross sectional case control, 3 population-based, 2 nested case-control, and 7 of other kinds), but so far no large prospective study has been completed.

The data from a newer, large meta-analysis published by Christen et al[18] in 2000 has only added to the confusion. This meta-analysis included 31 studies (5 cross sectional, 18 case-control, and 8 prospective studies). The data concerning the relation between homocysteine and CAD supported the connection in most of the case-control studies, but the results from the prospective studies were rather disappointing. Thus, the authors concluded that while plasma homocysteine is elevated in patients with CAD, elevated homocysteine probably does not itself cause the disease. However, the data from the above meta-analysis cover the period up to 1998. From then until today the results from a series of prospective studies tend to contradict the prior hypothesis, since they found that elevated plasma

homocysteine can be used at least as an index of a predisposition for the development of CAD (Table 3).

If the relationship between homocysteine and CAD is indeed a causal one, then what are the actual mechanisms of this relationship? Homocysteine seems to cause endothelial dysfunction, induces LDL oxidation and thus leads to the formation of vascular foam cells, while being related with disturbances of blood coagulability.[4,12]

Homocysteine and classical risk factors for CAD

A correlation between total homocysteine levels and HDL and LDL has been found in many studies. Recent studies show that homocysteine is an independent prognostic index for the development of atherosclerosis in dyslipidaemic patients.[2,4,12]

Total homocysteine levels are related with increased blood pressure in the general population,[41,42] in diabetics,[43] and probably in patients with multi-vessel disease.[44]

Tobacco use is associated with a lower nutritional intake and thus with reduced blood levels of folate, vitamin B12 and pyridoxal phosphate.[45-48] From another study it appears that total homocysteine levels increase in proportion with the number of cigarettes smoked per day, which means that smoking plays an important role in determining these levels.[4] It should be stressed that total homocysteine levels are elevated in patients who have vascular disease and who smoke.[4] In spite of this, even in those who stop smoking the relation between homocysteine and cardiovascular disease does not appear to change.[4]

Patients with kidney failure also show increased homocysteine levels.[4] In a prospective study it was found that this increase may be related with the high incidence of fatal and non-cardiovascular episodes that occur during the final stage of this disease.[49] In diabetic patients with intact renal function homocysteine levels are normal or below normal, because of the increased renal glomerular infiltration in those patients.[50] In contrast, in diabetics with proteinuria homocysteine is elevated, a fact that may be related with the increased tendency these patients show for atherogenesis.[48,51]

In the "European Concerted Action Project" the correlation between homocysteine and manifestations of cardiovascular diseases was studied in 750 vascular patients (coronary, cerebral and peripheral arteries) and 800 controls.[52] This study monitored the interaction between homocysteine and the three most significant risk factors: cholesterol, smoking and arterial hypertension. Homocysteine as a risk factor was equally significant and was independent of the other factors. On the other hand, raised levels of total homocysteine interacted powerfully with smoking and hypertension. The combined effect was somewhat greater than multiplicative in both sexes, but was more evident in women.[52]

To conclude, there appears to be a positive correlation between homocysteine and the other known risk factors for CAD, especially smoking and hypertension.[4]

Relation between high homocysteine levels and endothelial dysfunction

As we have seen, homocysteine has already been recognised as an independent risk factor for CAD and the risk is related to endothelial function.[53-56] Endothelial function can be estimated in forearm circulation by gauge-strain plethysmography. Endothelium-dependent vasodilation is expressed as the percentage change of forearm blood flow from baseline to the maximum flow during reactive hyperaemia, following ischaemia of the distal forearm and wrist lasting five minutes. Indications that homocysteine is connected with vascular damage come from studies showing a strong and reversible relation between plasma homocysteine and endothelial dysfunction. Specifically, an acute increase in homocysteine concentrations (methionine loading) is associated with a reduced endothelium-related vasodilation. However, these studies were unable to determine whether the endothelial damage was related directly with the elevated homocysteine or whether it was an epiphenomenon having to do with atherosclerosis.[57-60]

The mechanism through which homocysteine affects the endothelium is unclear, although mechanisms of free radical production play a major role. In vitro cultures of endothelial cells with homocysteine show that the latter decreases nitric oxide bioavailability. On the other hand, however, homocysteine causes an increase in the production of free radicals and an increase in lipid peroxidation. The production of peroxide anions and hydrogen peroxide, however, leads to reduced production and increased deactivation of NO. An increase in homocysteine is thus related with acute endothelial dysfunction, and oxidative stress is implicated in this process.[57-59] Administration of vitamin C, a powerful antioxidative factor, may prevent this damaging effect.[59] The same holds true for administration of folic acid and vitamins of

Table 3. Clinical studies.

Cases/controls/sexes	tHcy type	Study type	Mean tHcy μmol/l		
			Cases	Controls	P
241/262/both	Fasting	Cross sectional[39]	Men: 5.4 Women: 5.7	4.37 4.2	<0.001 <0.001
178/178/both	Fasting	Cross sectional[20]	23.83	19.69	<0.001
170/255/both	Fasting	Case control[21]	13.7	10.9	<0.001
163/195/men	Fasting	Case control[22]	16.2	13.4	<0.001
199/156/men	Basal	Case control[23]	8.9	7.8	<0.001
150/584/both	Fasting	Case control[24]	Men: 11.7 Women: 12.0	9.7 7.6	<0.001 <0.01
304/231/both	Fasting	Case control[25]	14.4	10.9	<0.001
383/800/both	Fasting 6h PML	Case control[26]	Fasting: 11.2 PML: 30.3	9.7 30.3	<0.001 <0.001
551/1025/both	Fasting 6h PML	Case control[27]	Fasting: 11.1-12.0 PML: 33.8-36.1	10.2-10.8 32.9-34.3	0.002 0.07
131/189/both	Fasting 6h PML	Case control[28]	Fasting: 13.5 PML: 41.1	12.3 38.4	NS NS
191/269/both	Basal	Prospective[29] Follow up: 9 years 7,424 (Finnish study)	9.6	9.6	NS
333/333/men	Basal	Prospective[30] Follow up: 7.5 years 14,916 (Physicians Health Study)	Not given	Not given	
232/395/both	Fasting	Prospective[31] Follow up: 3.3 years 15,792 (ARIC Study)	8.9	8.5	NS
154/-/men	Unspecified	Prospective[32] Follow up: 5 years 2,290 (Caerphilly cohort)	12.4	11.7	NS
123/492/both	Basal	Retrospective[33] Follow up: 4 years 21,826 (Tromso Study)	12.7	11.3	0.002
229/1126/men	Fasting	Retrospective[34] Follow up: 8.7 years 21,520 (BUPA Study)	13.1	11.8	<0.001
153 men, 347 women	Unspecified	Prospective[35] Follow up: 31 ± 9 months 500 elderly subjects	16.8	12.8	<0.001
386/454/men	Unspecified	Retrospective[36] Follow up: 12.8 years 7,735 (British Regional Heart Study)	14.2	13.5	0.06
122/244/women	Basal	Prospective[37] Follow up: 3 years 28,263 postmenopausal women (Women's Health Study)	14.1	12.4	0.02
271/271/men	Unspecified	Prospective[38] Follow up: 5 years 14,916 (US Physicians study)	11.1	10.5	0.03
405/-/both	Unspecified	Prospective[39] Follow up: 9-11 years 1,788 (Jerusalem Study)	Not given	Not given	
120/533/both	Unspecified	Prospective[40] Follow up: 2.7 years 7,983 (Rotterdam Study)	17.3	15.2	<0.05

tHCY: total homocysteine. PML: post methionine loading

the B complex, via a reduction in homocysteine levels.[56]

Homocysteine inhibits the action of thrombomodulin, a powerful antithrombotic of the endothelial surface that contributes to the activation of protein C by thrombin. Through the inhibition of thrombomodulin and protein C, homocysteine contributes to causing thrombosis in patients with CBS deficiency.[60]

Treatment for reducing plasma homocysteine levels

Total homocysteine levels may be reduced by the administration of vitamins B12, B6 and folate, regardless of the levels prior to treatment.[4] The treatment may prevent the vascular complications of homocystinuria and the use of vitamins is likely to reduce the risk of cardiovascular disease in the general population.[4,58] An increased dietary intake of fruit and vegetables, which are rich sources of folate, can contribute to this goal. Some authors suggest that B6 administration can delay the development of CAD,[61] although this has not been proved by prospective studies. The administration of B12 and folate has also been found to improve endothelium-dependent vasodilation in the brachial artery of CAD patients, and this is probably due to the reduction in homocysteine achieved by this combined therapy.

Folic acid

Various studies have shown that the administration of 0.65-10 mg/day folic acid, by itself or in combination with B12 and/or B6 can reduce plasma homocysteine levels in general and after methionine loading, in healthy individuals and in patients with elevated homocysteine levels or with vascular disease. Folic acid is non-toxic, well-tolerated for long-term use, but it can conceal the symptoms of B12 deficiency if this occurs during its administration.[4,55-59]

Vitamin B12

If patients suffering from B12 deficiency are excluded, B12 administration can cause a reduction in homocysteine levels by 10-15% and a significant improvement in endothelial function. An increase in NO concentrations has also been seen after vitamin B12 administration in individuals with hyperhomocysteinaemia.[4,55-57,62] The recommended dose of B12 is 1 mg/day.

Pyridoxine (vitamin B6)

Oral pyridoxine in a dosage of more than 300 mg/day does not appear to reduce plasma homocysteine levels in either healthy individuals or vascular patients. However, the administration of 10-250 mg/day pyridoxine in combination with folate leads to a greater reduction in homocysteine levels in patients undergoing methionine loading than does monotherapy with folate. Chronic B6 administration may cause peripheral neuropathy, although in a dosage of 100 mg or less it does not appear to have side effects.[4,61]

Conclusions

Many people claim that up to 10% of cardiovascular events could be prevented by a reduction in total homocysteine in patients with hyperhomocysteinaemia. A high dietary intake of folate, low coffee consumption, cessation of smoking and an increase in physical exercise can all contribute to a reduction in homocysteine in the general population. As far as therapy is concerned, folic acid is used widely and safely, its combination with B12 is becoming more accepted, whereas the usefulness and safety of B6 need further investigation.

On the initial diagnosis of hyperhomocysteinaemia the aetiology must first be investigated based on table 2 and the cause must be treated if it is apparent. Methionine loading may also be of help in the diagnosis of hyperhomocysteinaemia in patients with borderline values (around 15 μmol/L).

Treatment with B12/folate/B6 may be given initially until plasma levels have normalised, from which point B12/folate treatment may be continued. Even though the value of this treatment in the prevention of CAD has not been fully determined, there is no reason not to give it (unless there is some other contraindication) since it causes no side effects and has a relatively low cost.

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