DIABETES, LIPIDS AND METABOLISM

# Levels of homocysteine are increased in metabolic syndrome patients but are not associated with an increased cardiovascular risk, in contrast to patients without the metabolic syndrome

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**Aim:** The metabolic syndrome is associated with increased cardiovascular risk. Elevated plasma homocysteine may cause or result from insulin resistance, and may indicate vascular risk or be actively involved in atherogenesis. The aim of the study was to investigate the relationship between homocysteine, the metabolic syndrome and the incidence of cardiovascular events in patients with manifest vascular disease.
**Methods:** A cohort of 2169 patients with manifest vascular disease was followed for a mean period of 2.8 years. Plasma homocysteine was measured at baseline. Metabolic syndrome was defined by NCEP criteria.
**Results:** Homocysteine levels were higher in metabolic syndrome patients compared to patients without the metabolic syndrome (14.9±0.2 *v* 14.1±0.2 μmol/l; p=0.002) and increased with the presence of its components (from 0 to 5) (12.7 to 15.9 μmol/l; p<0.001). During follow-up, 52 strokes, 67 myocardial infarctions, 5 fatal ruptures of aortic aneurysms and 53 vascular deaths occurred. Patients without the metabolic syndrome and homocysteine levels in the highest tertile had increased risk for events (HR 1.9; 95% CI 1.0 to 3.5) compared to patients without the metabolic syndrome and homocysteine levels in the lowest tertile. The presence of the metabolic syndrome increased the risk (HR 2.2; 95% CI 1.2 to 4.2), but elevated homocysteine levels further increased the risk only marginally (2.5; 95% CI 1.4 to 4.6).
**Conclusions:** Metabolic syndrome patients have elevated homocysteine levels, but these higher levels are not associated with an increased risk for new cardiovascular events. In contrast, elevated homocysteine levels confer increased risk in patients without the metabolic syndrome.

The clustering of risk factors associated with central obesity (elevated glucose, dyslipidaemia and elevated blood pressure), often referred to as the metabolic syndrome, is associated with a two- to fivefold increased risk for the development of type 2 diabetes and a three- to fourfold increased incidence of cardiovascular diseases.[1–5] The prevalence of the metabolic syndrome varies from 9% to 27% depending on geographical location and age of the study population.[4 6] In patients with manifest vascular disease, the prevalence of the metabolic syndrome is even higher (46%) and associated with advanced vascular damage.[7 8] In patients who have experienced a myocardial infarction, the presence of the metabolic syndrome worsens the prognosis of survival and increases the incidence of future cardiovascular events.[9]

Insulin resistance is considered to be the major underlying pathophysiological feature of the metabolic syndrome, as it interferes in many metabolic pathways.[10] It is not yet known whether the increased cardiovascular risk associated with the metabolic syndrome can be explained by the individual components only, or whether other risk factors associated with both atherosclerosis and insulin resistance are involved.

Homocysteine, a thiol-containing amino acid which is produced during the metabolism of methionine, is considered to be a risk factor or an indicator of risk for the development of cardiovascular disease.[11–13] In an insulin resistant state, elevated homocysteine plasma levels may be the result of hyperinsulinaemia, as observed in animal models.[14 15] At the same time, homocysteine may lead to insulin resistance through inhibition of insulin-receptor kinase activity in vitro.[16 17] Therefore, homocysteine may be a cause and/or a consequence of insulin resistance.

Studies investigating the association between the metabolic syndrome and homocysteine levels have shown conflicting results.[18–25] The aim of the present study is to determine whether the presence of the metabolic syndrome is associated with elevated levels of homocysteine and to determine the relationship between homocysteine and the incidence of new cardiovascular events in patients with manifest vascular disease with and without the metabolic syndrome.

## METHODS

### Study design and patients

Data from patients enrolled in the SMART study (Second Manifestations of ARTerial disease), an ongoing single centre prospective cohort study carried out at the University Medical Center Utrecht, were used. Patients newly referred to our institution with clinically manifest atherosclerotic vascular disease (coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm) or with cardiovascular risk factors (hyperlipidaemia, diabetes or hypertension) are included. The aims of the SMART study are to determine (i) the risk factors for atherosclerosis, (ii) the prevalence of additional vascular disease and (iii) the incidence

**Abbreviations:** 95% CI, 95% confidence intervals; HR, hazard ratio; SD, standard deviation

of future cardiovascular events. The Medical Ethics Committee has approved the study and all patients gave written informed consent. Patients were asked to complete a health questionnaire covering medical history, risk factors, smoking habits and medical treatment. A standardised diagnostic protocol was followed consisting of physical examination and laboratory testing in a fasting state. A more detailed description of the design of the study has been published previously.[26]

The present study was based on the data of 2169 consecutive patients included in the SMART study between September 1996 and November 2004. Only data from patients with manifest vascular disease were used for analyses with the reservation that participants did not use folate therapy; 72 patients (3%) on folic acid therapy were therefore excluded from analyses.

### Definitions

The metabolic syndrome was defined according to the Adult Treatment Panel III (ATPIII) criteria of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.[27] Subjects were diagnosed with the metabolic syndrome if three or more of the following abnormalities were present:

- Abdominal obesity: waist circumference >102 cm in men or >88 cm in women.
- High blood pressure: ⩾130 mm Hg systolic or ⩾85 mm Hg diastolic or use of blood pressure lowering agents.
- Hypertriglyceridaemia: serum triglycerides ⩾1.70 mmol/l (150 mg/dl).
- Low HDL-cholesterol: serum HDL-cholesterol <1.04 mmol/l (40 mg/dl) in men or <1.29 mmol/l (50 mg/dl) in women.
- High fasting glucose: fasting serum glucose ⩾6.1 mmol/l (110 mg/dl) or use of glucose lowering agents.

Diabetes mellitus was defined as the use of glucose lowering agents and/or a fasting serum glucose concentration ⩾7.0 mmol/l.[28] Smoking and alcohol consumption were defined as smoking or use of alcohol within the last 12 months. Creatinine clearance (ml/min) was estimated by the Cockroft-Gault formula.

### Homocysteine measurement and methionine loading test

High performance liquid chromatography with fluorescence detection was used to determine plasma homocysteine levels. From September 1996 until May 1998, as part of a sub-study within the SMART cohort aiming at identifying risk factors in young patients with a recent clinical manifestation of atherosclerosis, methionine loading tests were performed in all consecutive patients <50 years of age. Plasma homocysteine was measured when fasting and 6 h after standardised methionine loading (100 mg/kg bodyweight). From May 1998 on, only fasting homocysteine levels were measured in all patients. As there are no international reference values for abnormal methionine loading tests, hyperhomocysteinaemia was defined as levels of homocysteine >55 μmol/l or an increase of 30 μmol/l above fasting levels as recommended by the Netherlands Heart Foundation.[29]

### Vascular endpoints

During follow-up, patients were asked twice a year to fill in a questionnaire on hospitalisations and outpatient clinic visits. When a possible event was reported by a participant, correspondence and relevant data were collected. Vascular death was reported by relatives of the participant, the general practitioner or by the vascular specialist to members of the SMART study group. Based on the information from the questionnaire and/or the family, all events were audited by three members of the SMART study Endpoint Committee, comprising physicians from different departments. In cases of disagreement, the opinion of other members of the Endpoint Committee was sought and final adjudication was based on the majority of the classifications obtained. The endpoints of interest for the present study were vascular death, ischemic stroke, coronary ischemic disease and these vascular events combined. If a patient had multiple events, the first recorded event was used in the analysis. In the present study of 2169 patients, 105 patients were excluded from follow-up analysis because of limited follow-up time (<6 months), and four subjects left the study directly after inclusion.

### Statistical analyses

Data are presented as percentages with number of patient in parenthesis for categorical variables, as mean (standard deviation, SD) for normally distributed variables and as median with the interquartile range in parenthesis for non-normally distributed variables.

The mean levels of homocysteine and 95% confidence intervals (95% CI) were calculated firstly for patients with and without the metabolic syndrome and secondly according to the number of metabolic syndrome components. Adjustment for age, gender and creatinine clearance was carried out with the general linear model (SPSS) since these factors were considered to be confounders. p Values <0.05 were considered significant. Binary logistic regression was used to analyse the association between the methionine loading test and the metabolic syndrome. Cox proportional hazard analysis was performed to estimate adjusted hazard ratios with 95% CI for the association between tertiles of homocysteine levels and the occurrence of cardiovascular events. To investigate whether relationships between homocysteine and vascular events were modified by the presence of the metabolic syndrome, we compared the model with the interaction term metabolic syndrome and homocysteine tertiles with a model without that interaction term and compared the −2log likelihood of the two models. Patients without the metabolic syndrome and levels of homocysteine in the lowest tertile were the reference group. All statistical analyses were performed with the Statistical Package for Social Sciences version 12.0 (SPSS) for Windows.

## RESULTS

### Study population

Patient characteristics of the 2169 study subjects with manifest vascular disease are presented according to tertiles of homocysteine plasma levels in table 1. In the study population 1021 patients were recently diagnosed with coronary heart disease, 506 with cerebrovascular disease, 462 with peripheral arterial disease and 180 with an abdominal aortic aneurysm.

In the study population, 78% of patients were male (from 69% within the lowest homocysteine tertile to 86% in the highest) and the mean age increased from tertile 1 (57±10 years) to tertile 3 (63±10 years). The prevalence of the metabolic syndrome within the total study population was 43%. Patients within the highest homocysteine tertile had lower creatinine clearance compared to patients within the lowest tertile (67 *v* 86 ml/min). Of the entire study population, 55% had a creatinine clearance between 60 and 90 ml/min and 19% had a creatinine clearance <60 ml/min. Diabetes mellitus was almost equally distributed between the tertiles, although the use of glucose lowering agents was more prevalent in the lowest tertile (13% *v* 10%). No difference was observed in the proportion of patients using blood pressure lowering agents in all tertiles.

**Table 1** Patient characteristics according to homocysteine tertiles at baseline

| | T1 (n = 724) | T2 (n = 734) | T3 (n = 711) |
|---|---|---|---|
| Homocysteine, μmol/l* | 9.8 (SD 1.3) | 13.3 (SD 1.0) | 20.4 (SD 8.5) |
| Male gender, % (n) | 69 (503) | 78 (576) | 86 (610) |
| Age, years* | 56.8 (SD 9.8) | 59.3 (SD 9.8) | 62.8 (SD 10.4) |
| Body mass index, kg/m$^2$* | 26.7 (SD 3.8) | 26.8 (SD 3.8) | 26.4 (SD 3.6) |
| Smoking†, % (n) | 30.9 (224) | 32.3 (237) | 34.0 (242) |
| Alcohol use†, % (n) | 73.1 (529) | 65.7 (482) | 68.5 (487) |
| Total cholesterol, mmol/l* | 5.3 (SD 1.1) | 5.3 (SD 1.2) | 5.3 (SD 1.0) |
| Diabetes mellitus‡, % (n) | 22 (161) | 19 (139) | 20 (139) |
| Creatinine clearance, ml/min (Cockroft) | 86.0 (SD 17.8) | 79.3 (SD 18.4) | 67.2 (SD 20.8) |
| Glucose-lowering agents, % (n) | 13 (96) | 10 (74) | 10 (70) |
| Blood pressure-lowering agents, % (n) | 27 (193) | 31 (229) | 41 (290) |
| Lipid-lowering agents, % (n) | 15 (110) | 15 (111) | 17 (118) |
| Metabolic syndrome, % (n) | 43.5 (315) | 40.5 (289) | 44.8 (328) |
| Metabolic syndrome components | | | |
| Waist circumference, cm* | 94.7 (SD 10.7) | 97.0 (SD 10.5) | 96.9 (SD 10.6) |
| Blood pressure systolic, mm Hg* | 138 (SD 22) | 139 (SD 20) | 144 (SD 23) |
| Blood pressure diastolic, mm Hg* | 80 (SD 11) | 80 (SD 11) | 81 (SD 12) |
| HDL-cholesterol, mmol/l# | 1.16 (0.96–1.40) | 1.14 (0.94–1.39) | 1.16 (0.93–1.38) |
| Triglycerides, mmol/l# | 1.6 (1.1–2.2) | 1.6 (1.1–2.2) | 1.6 (1.2–2.4) |
| Fasting glucose, mmol/l* | 6.4 (SD 2.3) | 6.3 (SD 1.8) | 6.2 (SD 1.6) |

*Mean (standard deviation, SD) or #median with interquartile range; †still smoking or drinking or recently stopped smoking or drinking; ‡fasting serum glucose ≥7.0 mmol/l or self-reported diabetes.

### Homocysteine concentrations and the metabolic syndrome

In table 2 we present the homocysteine plasma concentrations (mean (SD)) in patients with and without the metabolic syndrome and by the number of metabolic syndrome components. Patients with the metabolic syndrome had significantly increased homocysteine fasting plasma levels compared to subjects without the metabolic syndrome after adjustment for age, creatinine clearance and gender (14.9 μmol/l; 95% CI: 14.5 to 15.3 μmol/l *v* 14.1 μmol/l; 95% CI: 13.8 to 14.5 μmol/l; p = 0.002). The last part of table 2 shows a gradual and significant increase in plasma homocysteine concentrations with increase in the number of metabolic syndrome components after adjustment for age, gender and creatinine clearance (from 12.7 μmol/l; 95% CI: 11.7 to 13.7 μmol/l to 15.9 μmol/l; 95% CI: 14.8 to 17.0 μmol/l; p<0.001).

### Methionine loading test

Methionine loading tests were performed in a group of 114 patients (mean age 44 (SD 5) years, 62% male). At the time of inclusion, 27 of these patients were diagnosed with cerebrovascular disease, 42 with peripheral arterial disease and 45 with coronary arterial disease. Forty four patients (38%) fulfilled the criteria of the metabolic syndrome. The proportion of patients with abnormal levels of plasma homocysteine after the methionine loading test was similar in patients with (22.7%) and without the metabolic syndrome (22.8%, odds ratio 1.1, 95% CI 0.4 to 2.8). The same analysis was carried out with a different cut-off level (50 μmol/l) for the methionine loading test. The odds ratio remained unchanged.[30]

**Table 2** Homocysteine plasma concentration in patients with and without the metabolic syndrome, and by the number of metabolic syndrome components

| Metabolic syndrome | Homocysteine, μmol/l (mean (95% CI)) | p Value |
|---|---|---|
| Crude | | |
| No | 14.4 (14.0 to 14.8) | |
| Yes | 14.5 (14.1 to 14.9) | 0.6 |
| Adjusted for age, gender and creatinine clearance | | |
| No | 14.1 (13.8 to 14.5) | |
| Yes | 14.9 (14.5 to 15.3) | 0.002 |
| Adjusted for age, gender and creatinine clearance | | |
| 0 | 12.7 (11.7 to 13.7) | |
| 1 | 13.6 (13.1 to 14.2) | |
| 2 | 14.8 (14.3 to 15.3) | |
| 3 | 14.6 (14.0 to 15.1) | |
| 4 | 15.2 (14.5 to 15.9) | |
| 5 | 15.9 (14.8 to 17.0) | <0.001 |

### Homocysteine and vascular events

In the 2060 patients who were followed up, 177 vascular events occurred in a mean period of 2.8 years (range 0.1–7.5 years). During follow-up, 52 strokes, 67 myocardial infarctions, 5 fatal ruptures of abdominal aorta aneurysms, 29 acute vascular deaths and 24 other vascular deaths occurred. For the study population in total, patients with homocysteine levels in the highest tertile had a 40% increased risk for new cardiovascular events compared to patients with homocysteine levels in the lowest tertile (hazards ratio (HR) 1.4, 95% CI 0.9 to 2.2). For patients in the middle tertile, this risk was increased by 10% (HR 1.1, 95% CI 0.7 to 1.7).

The adjusted hazard ratios for new vascular events in patients with and without the metabolic syndrome for different homocysteine levels are shown in table 3. In patients without the metabolic syndrome, increasing levels of homocysteine were associated with an increased risk of future cardiovascular events. Patients within the highest homocysteine tertile had an increased risk, HR 1.9 (95% CI 1.0 to 3.5), of experiencing a new vascular event compared to the reference group, who were patients without the metabolic syndrome and levels of homocysteine in the lowest tertile. However, in patients with the metabolic syndrome there was no relationship between homocysteine level and future cardiovascular events (p value for interaction = 0.049). The HR in the highest homocysteine tertile for developing cardiovascular events was 2.5 (95% CI 1.4 to 4.6), while patients with the metabolic syndrome and low levels of homocysteine (tertile 1) had a 2.2-fold (95% CI 1.2 to 4.2) increased risk compared to patients without the metabolic syndrome and within the lowest homocysteine tertile. Figure 1 displays a Cox proportional hazard survival curve according to the homocysteine tertiles and the presence of the metabolic syndrome and adjusted for age, gender and creatinine clearance.

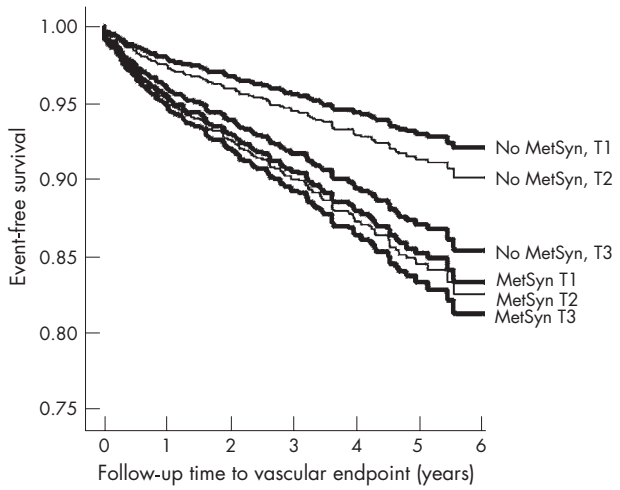


**Figure 1** Survival curve according to the presence of the metabolic syndrome and tertiles of homocysteine levels. No MetSyn/MetSyn: patients without and with the metabolic syndrome. T1–T3: tertiles of plasma homocysteine concentrations, from low to high levels. Adjusted for age, gender and creatinine clearance.

**Table 3** Cardiovascular risk in patients with and without the metabolic syndrome according to homocysteine plasma concentrations

| Metabolic syndrome | Tertile | Number of patients | Number of CV events | Hazard ratio* (95% CI) |
|---|---|---|---|---|
| No | 1 | 391 | 37 | Reference |
| | 2 | 399 | 48 | 1.3 (0.7 to 2.4) |
| | 3 | 384 | 87 | 1.9 (1.0 to 3.5) |
| Yes | 1 | 302 | 49 | 2.2 (1.2 to 4.2) |
| | 2 | 275 | 61 | 2.3 (1.2 to 4.4) |
| | 3 | 309 | 85 | 2.5 (1.4 to 4.6) |

*Adjusted for age, gender and creatinine clearance. CV, cardiovascular.

## DISCUSSION

In the present study we show that clustering of risk factors related to central obesity, often referred to as the metabolic syndrome, in patients with manifest vascular disease is associated with elevated fasting plasma levels of homocysteine compared to patients without the metabolic syndrome and show that homocysteine increases with the number of metabolic syndrome components. Elevated levels of plasma homocysteine are associated with an increased incidence of new cardiovascular events in patients without the metabolic syndrome but not in patients with the metabolic syndrome.

The relationship between insulin resistance and levels of homocysteine has been investigated previously in different populations but with conflicting results.[18–25] Studies in non-diabetic healthy men and women showed that plasma homocysteine concentrations are not influenced by insulin resistance,[18 22] while in similar populations, others found an inverse relationship between homocysteine and insulin resistance.[19 24] In yet other study populations (healthy non-obese, obese non-diabetic subjects and patients with the metabolic syndrome), a positive relationship was found between homocysteine and insulin resistance.[20–23 25]

Several mechanisms may explain the association between elevated homocysteine levels and insulin resistance or the metabolic syndrome. Increased homocysteine plasma concentrations may be the cause and/or the consequence of insulin resistance.

Decreased insulin receptor activity was observed in rat hepatoma cells overexpressing the human insulin receptor after incubation with homocysteine thiolactone,[16 17] a metabolite of homocysteine which is present in human vascular endothelial cells.[31] This resulted in reduction of glycogen synthesis and reduced insulin-stimulated DNA and protein synthesis. Folate therapy given to patients with the metabolic syndrome (NCEP criteria) resulted not only in lower homocysteine concentrations but, more interestingly, also in reduced levels of insulin and improved insulin sensitivity.[32] Conversely, induction of insulin resistance in rats resulted in elevated plasma homocysteine levels.[14 15]

Cystathionine-β-synthase, the key enzyme of the transsulfuration pathway in homocysteine metabolism, is downregulated in an insulin resistant state.[14] During methionine loading tests this pathway is more challenged than the remethylation pathway,[33] and so these loading tests may therefore identify more precisely disturbances in homocysteine metabolism. The results of the present study do not indicate any differences in homocysteine metabolism after methionine loading in patients with or without the metabolic syndrome.

Different studies have investigated the impact of elevated levels of homocysteine on the development of cardiovascular events but lack indisputable proof of causality.[34 35] Elevated plasma homocysteine levels may be a cause of insulin resistance and be actively involved in atherogenesis, and therefore may be a risk factor. On the other hand, elevated levels of homocysteine could be considered an indicator of vascular risk and only be used for risk estimation. In the present study elevated homocysteine levels were associated with an increased risk of future cardiovascular events in patients with manifest vascular disease but without the metabolic syndrome. This risk was similar to that observed in patients with a MTHFR gene mutation, a mutation which leads to increased homocysteine levels.[13] In contrast, elevated levels of homocysteine did not substantially increase the already increased cardiovascular risk in patients with the metabolic syndrome. Apparently, in metabolic syndrome patients the cardiovascular risk is mainly determined by the individual metabolic syndrome components and other vascular risk factors associated with insulin resistance (for example, inflammation, hyperinsulinaemia, hypoadiponectinaemia). Another argument suggesting that elevated homocysteine levels are an indicator of risk instead of a risk factor is suggested by the results of two recent homocysteine lowering trials showing no positive effect on the cardiovascular risk in patients with manifest vascular disease.[36 37] These results are supported by previous findings,[38 39] although other studies show conflicting results.[40 41] In the present study the relatively high homocysteine plasma concentrations in patients with and without the metabolic syndrome may be explained by the high prevalence of decreased renal function.[24 42]

We acknowledge study limitations. In the present study we used the Adult Treatment Panel III definition for the metabolic syndrome.[27] Although this definition is most often used, other definitions for the metabolic syndrome do exist.[43] Secondly, the cut-off values for hyperhomocysteinaemia after methionine loading are arbitrary because no stringent normal ranges have been defined. However, levels above 55 μmol/l or an increase of 30 μmol/l after methionine loading are considered as elevated according to the Netherlands Heart Foundation.[29] It is likely that cardiovascular risk increases gradually and there is no particular threshold. Finally, it could be argued that plasma concentrations of vitamins B6, B12 and folate may provide additional information on the relationship between the metabolic syndrome and cardiovascular disease. Information on these vitamins were not available in the present study.

However, it is not likely that vitamins are confounders or effect modifiers in the analyses reported.

In conclusion, patients with the metabolic syndrome have elevated plasma homocysteine levels, but these elevated homocysteine levels are not associated with an increased risk for new cardiovascular events. These data indicate that elevated plasma homocysteine levels are not a risk factor for cardiovascular events in metabolic syndrome patients in contrast to patients without the metabolic syndrome.

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