Elevated Homocysteine Levels Are Associated With the Metabolic Syndrome and Cardiovascular Events in Hypertensive Patients

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BACKGROUND
Hyperhomocysteinemia and the metabolic syndrome are established cardiovascular risk factors and are frequently associated with hypertension. The relationship of plasma homocysteine (Hcy) with the metabolic syndrome and insulin resistance, however, is debated and studies in hypertensive patients are limited. In this study, we have investigated the association of Hcy with the metabolic syndrome and cerebro-cardiovascular events in hypertension.

METHODS
In 562 essential hypertensive patients who underwent accurate assessment of fasting and postload glucose metabolism, insulin sensitivity, and renal function, we measured plasma levels of Hcy, vitamin B12, folate, and fibrinogen and assessed the prevalence of the metabolic syndrome and of coronary heart and cerebrovascular disease (CVD).

RESULTS
Patients with the metabolic syndrome had significantly higher plasma Hcy levels. After correction for covariates, increasing Hcy levels were associated with an increasing prevalence of the metabolic syndrome, coronary heart disease, and CVD. Plasma Hcy was directly correlated with age, waist circumference, fasting glucose, triglyceride, uric acid, and fibrinogen levels, and homeostatic model assessment index and inversely with creatinine clearance and high-density lipoprotein cholesterol, vitamin B12, and folate levels. Logistic regression analysis showed an independent association of Hcy levels with age, male gender, vitamin B12 and folate levels, and the metabolic syndrome. Logistic regression indicated also an independent association of Hcy with cerebro-cardiovascular disease that was independent of the metabolic syndrome.

CONCLUSIONS
Elevated plasma Hcy is associated with the metabolic syndrome in hypertensive patients. Prevalence of events increases with increasing plasma Hcy levels suggesting a contribution of Hcy to cerebro-cardiovascular diseases in these patients.

Keywords: blood pressure; cerebrovascular disease; coronary heart disease; folate; homocysteine; hypertension; insulin resistance; metabolic syndrome; vitamin B12.

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Population studies conducted 2 decades ago established evidence of a direct and independent relationship of plasma homocysteine (Hcy) levels with atherosclerosis-related morbidity and mortality.[1,2] These initial studies and subsequent clinical observations led to include this thiol-containing intermediate metabolite among the emergent cardiovascular risk factors, although correction of increased levels of Hcy in intervention studies was not associated with evidence of benefit on the cardiovascular outcome.[3] Circulating Hcy is maintained at relatively low levels by ongoing enzymatic conversion to either methionine or cysteine,[4] but some conditions including aging, smoking, decreased folate and vitamin B12 levels, renal failure, and rare genetic abnormalities are associated with increasing levels.[5] Many possible mechanisms linking Hcy with atherogenesis have been suggested including prothrombotic and proinflammatory effects, increased oxidative stress, endothelial dysfunction, and smooth muscle cell proliferation.[5]

The metabolic syndrome is identified by a cluster of abnormalities in which insulin resistance with related hyperinsulinemia and visceral adipose tissue play a key pathogenetic role.[6] Robust evidence indicates that subjects with this syndrome are at increased risk of major cardiovascular events and death.[7,8] Animal studies have shown that insulin might affect the activity of enzymes involved in Hcy turnover[9] and hyperhomocysteinemia has been suggested as a possible additional component of the metabolic syndrome.[10] However, findings of studies that examined the association between plasma Hcy and the metabolic syndrome in the general population[11–13] and in groups of patients with hypertension[14–16] were highly inconsistent. Also, and to a greater importance, it was not clear whether the possible interaction between Hcy and components of the metabolic syndrome might affect the prevalence of cardiovascular events, an issue that might be particularly relevant in high-risk patients such as those with high blood pressure. Therefore,

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the aim of the present study was to investigate the relationship between plasma Hcy levels and the metabolic syndrome in a large group of patients with primary hypertension and its possible relevance for cardiovascular and cerebrovascular disease (CVD).

METHODS

Patients

A total of 562 patients (301 males and 261 females; age: 56 ± 13 years) with mild-to-moderate hypertension, who were consecutively referred to the outpatient service of the Hypertension Clinic at our University, were included in a cross-sectional study. Blood pressure was measured by an automated device (Omron M6, OMRON Healthcare Co., Kyoto, Japan) after each subject had been supine for 15 minutes and the average of 3 readings was recorded.[17] Diagnosis of hypertension was established in all patients according to current guidelines.[18] All patients were White, lived in North-East of Italy, and were representative of the hypertensive population in this area.[19] Patients younger than 18 years and older than 80 years and pregnant women were excluded, together with patients with history of alcohol abuse, glomerular filtration rate of less than 30 ml/min/1.73 m^2, use of fibrates, niacin, or other types of drugs that could interfere with Hcy levels, and acute illness including recent (less than 3 months) acute coronary syndrome, coronary revascularization, stroke, and congestive heart failure. Metabolic syndrome was diagnosed according to the American Heart Association criteria,[20] when 2 or more of the following conditions were associated with hypertension: waist circumference of more than 102 cm in men or 88 cm in women, fasting plasma glucose of 100 mg/dl or more or use of hypoglycemic drugs, triglycerides of 150 mg/dl or more, and high-density lipoprotein cholesterol of less than 40 mg/dl in men or 50 mg/dl in women. In all patients, causes of secondary hypertension were excluded on the basis of extensive clinical and laboratory investigations that included analysis of medical records, physical examination, urine analysis, blood chemistry, duplicate measurements of 24-hour creatinine clearance, plasma active renin and aldosterone, urinary cortisol and catecholamines, and renal ultrasound examination with measurement of renal resistance index. Renal angio magnetic resonance imaging/computed tomography scan and additional functional tests were performed when appropriate.[21]

One-hundred fifty-eight (28%) of 562 patients had never been treated with antihypertensive drugs. The remaining 404 patients (72%) were treated with drugs that were washed-out for a minimum of 2 weeks before the study and all patients were closely monitored during the wash-out period. Patients taking 2 or more antihypertensive agents were admitted to the hospital for the duration of the wash-out and in those with blood pressure persistently higher than 180/110 mm Hg, alpha-blockers and/or calcium channel blockers were given (N = 14). All these patients were already taking calcium channel blockers before inclusion in the study. No patients were taking either folate or vitamin B12 oral supplements. Before the study, patients ate a standard diet for 7 days to keep a sodium intake of 100–150 mmol/day that was checked with measurement of sodium excretion in 24-hour urine collections. Patients were defined as smokers if they had smoked for at least 5 years and up to 1 year before the study and smoking was quantified by the number of cigarettes smoked per day. Alcohol intake was quantified by a questionnaire[22] as grams/day and patients were defined as heavy drinkers when consumption was of 30 g/day or more. The study was approved by the local Institutional Review Board and informed consent was obtained from all patients.

Assessment of cardiovascular and cerebrovascular damage

Prevalence of coronary heart disease (CHD) (i.e., angina, myocardial infarction, coronary revascularization procedure, or coronary angiography showing at least one coronary artery stenosis > 50%) was verified in all patients by analysis of clinical records and laboratory tests that included electrocardiography, echocardiography and, when appropriate, exercise testing, myocardial perfusion scan, and coronary angiography.[23] Prevalence of ischemic CVD (i.e., ischemic stroke, transitory ischemic attack, or carotid arterial disease with ultrasound evidence of a stenosis > 50%) was verified in all patients by analysis of clinical records, physical examination and, when appropriate, cerebral computerized tomography or magnetic resonance, and cerebral angiography.

Laboratory measurements

A sample of venous blood was collected in the morning between 8:00 and 9:00 AM, after an overnight fast with the patients in sitting position. Blood was collected into silicone-treated tubes containing trisodium citrate and plasma was immediately separated and frozen at −80 °C until assaying, usually within 1 month from sampling. Plasma Hcy was determined by a nephelometric method (Dimension Vista System, Siemens Healthcare Diagnostics, Milan, Italy) with inter- and intra-assay coefficients of variation of 8.2% and 7.0%, respectively, and lower limit of detection of 2.0 μmol/l. Total cholesterol and triglycerides were assayed enzymatically by an automated method. High-density lipoprotein cholesterol was assayed enzymatically after magnesium chloride–dextran sulfate precipitation of apolipoprotein B-containing lipoproteins and low-density lipoprotein cholesterol was calculated with the formula of Friedewald. Vitamin B_{12} and folate were measured by standard automated competitive displacement assays. Glomerular filtration rate was measured by duplicate measurement of 24-hour creatinine clearance and normalized for body surface area.[24] Fibrinogen was measured by a functional assay in an automatic coagulometer autoanalyzer.[25] Plasma glucose was assayed using the glucose oxidase method and plasma insulin was measured by radioimmunoassay. The homeostatic model assessment (HOMA) index was calculated as an index of insulin sensitivity from fasting plasma glucose (mmol/l) and insulin (μU/ml) using the formula: ((glucose × insulin)/22.5). Glucose tolerance was evaluated with the use

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Table 1. Clinical characteristics and biochemical variables of the study patients grouped according to the presence or absence of the metabolic syndrome

	All patients (*n* = 562)	Metabolic syndrome (no) (*n* = 356)	Metabolic syndrome (yes) (*n* = 206)	*P*
Clinical characteristics				
Age, year	56 ± 13	55 ± 14	58 ± 12	0.02
Males, *n* (%)	301 (53)	183 (51)	118 (57)	<0.01
Family history of CV disease, *n* (%)	301 (53)	188 (53)	113 (55)	0.66
Body mass index, kg/m^2	28.4 ± 5.2	26.8 ± 4.4	31.2 ± 5.3	<0.01
Waist circumference, cm	97 ± 13	92 ± 12	104 ± 12	<0.01
Heart rate, bpm	72 ± 11	73 ± 11	73 ± 10	0.99
Systolic blood pressure, mm Hg	147 ± 20	146 ± 21	148 ± 18	0.16
Diastolic blood pressure, mm Hg	89 ± 12	89 ± 12	89 ± 12	0.51
Duration of hypertension, year	10.6 ± 9.6	9.8 ± 9.2	12.1 ± 10.1	0.01
Antihypertensive treatment, *n* (%)	404 (72)	239 (67)	165 (80)	<0.01
Alcohol, g/day	13 ± 25	11 ± 18	16 ± 34	0.04
Smokers, *n* (%)	139 (25)	75 (21)	64 (31)	0.01
Diabetes, *n* (%)	91 (16)	28 (8)	63 (30)	<0.01
Coronary heart disease, *n* (%)	54 (10)	28 (8)	26 (13)	0.06
Cerebrovascular disease, *n* (%)	27 (5)	16 (4)	11 (5)	0.65
Biochemical variables				
Creatinine, mg/dl	1.02 ± 0.31	1.00 ± 0.30	1.06 ± 0.34	0.04
Creatinine clearance, ml/min/1.73 m^2	87 ± 27	90 ± 26	84 ± 28	0.03
Urinary protein excretion, mg/day	90 (65–139)	89 (61–131)	97 (68–156)	0.02
Glucose, mg/dl	102 ± 30	94 ± 19	116 ± 39	<0.01
Insulin, μUI/ml	9.1 (6.0–13.0)	7.9 (5.3–10.7)	11.9 (8.5–17.5)	<0.01
HOMA index	2.13 (1.36–3.27)	1.74 (1.20–2.58)	3.11 (2.09–4.94)	<0.01
AUC-G, mg/dl/min	397 ± 107	375 ± 96	442 ± 114	<0.01
AUC-I, μUI/ml/min	177 (118–289)	158 (103–263)	232 (151–354)	<0.01
Triglycerides, mg/dl	105 (78–148)	91 (70–115)	159 (112–210)	<0.01
Total cholesterol, mg/dl	206 ± 42	203 ± 39	211 ± 47	0.05
HDL cholesterol, mg/dl	58 ± 16	62 ± 15	50 ± 15	<0.01
LDL cholesterol, mg/dl	122 ± 37	122 ± 36	124 ± 38	0.59
Uric acid, mg/dl	5.59 ± 1.51	5.28 ± 1.44	6.14 ± 1.47	<0.01
Homocysteine, μmol/l	11.9 (9.3–14.7)	11.4 (9.1–14.3)	12.5 (10.1–15.4)	0.01
Vitamin B12, pg/ml	386 (293–508)	385 (288–495)	387 (299–532)	0.72
Folate, ng/ml	5.20 (3.60–7.20)	5.0 (3.50–7.14)	5.30 (3.87–7.27)	0.30
Fibrinogen, mg/dl	356 (311–409)	345 (303–399)	376 (328–420)	0.01

Values are expressed as mean ± SD. Median and interquartile range given in parenthesis under "Biochemical variables" are shown for variables with skewed distribution. Comparisons between patients with or without the metabolic syndrome were done by the Student's *t*-test after log transformation for variables with skewed distribution. Pearson's chi-square test was used to compare frequency distributions. To convert to international units, multiply creatinine by 88.4 (μmol/l), glucose by 0.05551 (nmol/l), insulin by 7.175 (pmol/l), cholesterol by 0.0259 (mmol/l), triglycerides by 0.0113 (mmol/l), and uric acid by 59.485 (μmol/l).

Abbreviations: AUC-G, area under the curve of plasma glucose after oral glucose tolerance test; AUC-I, area under the curve of plasma insulin after oral glucose tolerance test; CV, cardiovascular; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein.

of a 180-minute oral glucose tolerance test (OGTT) and the area under the curve for plasma glucose (AUC-G) and insulin (AUC-I) concentration during the OGTT was calculated by the trapezoidal rule as described previously.[26]

Statistical analysis

Values are expressed as mean ± SD for normally distributed variables, with median and interquartile ranges used

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Table 2. Clinical characteristics of the study patients grouped according to quartiles of plasma homocysteine levels

	I (*n* = 143)	II (*n* = 139)	III (*n* = 139)	IV (*n* = 141)	*P*
Clinical characteristics					
Age, year	52 ± 13	54 ± 13	57 ± 14	60 ± 13	<0.01
Males, *n* (%)	58 (41)	71 (51)	85 (61)	87 (62)	0.01
Family history of CV disease, *n* (%)	76 (53)	67 (48)	71 (51)	87 (62)	0.23
Body mass index, kg/m^2	28.2 ± 5.5	29.0 ± 5.6	28.6 ± 5.0	27.9 ± 4.6	0.31
Waist circumference, cm	94 ± 14	95 ± 14	98 ± 13	99 ± 11	0.07
Heart rate, bpm	73 ± 11	73 ± 11	73 ± 12	72 ± 9	0.45
Systolic blood pressure, mm Hg	146 ± 17	145 ± 18	148 ± 21	149 ± 19	0.27
Diastolic blood pressure, mm Hg	89 ± 11	90 ± 11	90 ± 12	88 ± 12	0.45
Duration of hypertension, year	9.9 ± 9.3	10.2 ± 9.1	11.1 ± 9.3	11.5 ± 10.5	0.51
Antihypertensive treatment, *n* (%)	89 (62)	104 (75)	107 (77)	104 (74)	0.03
Alcohol, g/day	12 ± 23	14 ± 34	12 ± 23	14 ± 20	0.86
Smokers, *n* (%)	33 (23)	34 (24)	40 (29)	32 (23)	0.63
Metabolic syndrome, *n* (%)	37 (26)	53 (38)	56 (40)	60 (42)	0.02
Coronary heart disease, *n* (%)	11 (8)	6 (4)	10 (7)	27 (19)	<0.01
Cerebrovascular disease, *n* (%)	3 (2.1)	6 (4.3)	5 (3.6)	13 (9.2)	0.03
Biochemical variables					
Creatinine, mg/dl	0.92 ± 0.20	0.95 ± 0.19	1.05 ± 0.34	1.17 ± 0.42	<0.01
Creatinine clearance, ml/min/1.73 m^2	102 ± 33	103 ± 31	96 ± 3 2	79 ± 28	<0.01
Urinary protein excretion, mg/day	78 (54–116)	90 (69–130)	92 (65–132)	108 (70–172)	<0.01
Glucose, mg/dl	97 ± 25	103 ± 33	102 ± 26	104 ± 35	0.20
Insulin, μUI/ml	8.10 (5.35–11.80)	9.50 (5.90–12.80)	9.75 (6.28–14.23)	9.30 (6.40–13.00)	0.12
HOMA index	1.88 (1.18–2.90)	2.21 (1.32–3.25)	2.24 (1.53–3.31)	2.18 (1.41–3.70)	0.10
AUC-G, mg/dl/min	393 ± 110	398 ± 114	393 ± 85	406 ± 121	0.85
AUC-I, μUI/ml/min	176 (124–300)	168 (97–297)	180 (121–275)	195 (128–344)	0.66
Triglycerides, mg/dl	92 (71–125)	107 (80–150)	113 (82–159)	111 (85–160)	<0.01
Total cholesterol, mg/dl	203 ± 37	213 ± 48	205 ± 38	202 ± 44	0.14
HDL cholesterol, mg/dl	59 ± 16	59 ± 18	55 ± 15	56 ± 15	0.08
LDL cholesterol, mg/dl	122 ± 36	125 ± 36	123 ± 36	119 ± 38	0.54
Uric acid, mg/dl	5.25 ± 1.40	5.49 ± 1.43	5.66 ± 1.47	6.00 ± 1.65	<0.01
Homocysteine, μmol/l	8.1 (7.3–8.8)	10.5 (10.0–11.2)	13.4 (12.5–14.0)	17.5 (15.8–20.7)	<0.01
Vitamin B12, pg/ml	427 (318–531)	383 (305–517)	392 (294–511)	356 (256–467)	0.03
Folate, ng/ml	5.90 (4.10–8.25)	5.58 (4.05–7.90)	5.30 (3.50–6.49)	4.20 (3.00–5.40)	<0.01
Fibrinogen, mg/dl	350 (303–402)	360 (305–409)	350 (311–396)	369 (323–432)	0.02

Values are expressed as mean ± SD. Median and interquartile range given in parenthesis under "Biochemical variables" are shown for variables with skewed distribution. Comparisons among homocysteine quartiles were done by analysis of variance with the *post hoc* Bonferroni test used to correct for multiple comparisons. Pearson's chi-square test was used to compare frequency distributions. To convert to international units, multiply creatinine by 88.4 (μmol/l), glucose by 0.05551 (nmol/l), insulin by 7.175 (pmol/l), cholesterol by 0.0259 (mmol/l), triglycerides by 0.0113 (mmol/l), and uric acid by 59.485 (μmol/l).

Abbreviations: AUC-G, area under the curve of plasma glucose after oral glucose tolerance test; AUC-I, area under the curve of plasma insulin after oral glucose tolerance test; CV, cardiovascular; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein.

for variables with skewed distributions. Normality of distribution was assessed with the Kolmogorov–Smirnov test, and variables with skewed distributions were analyzed after logarithmic transformation. The Student's *t*-test was used for comparison between 2 independent groups. Analysis of variance was used for comparisons of more than 2 groups,

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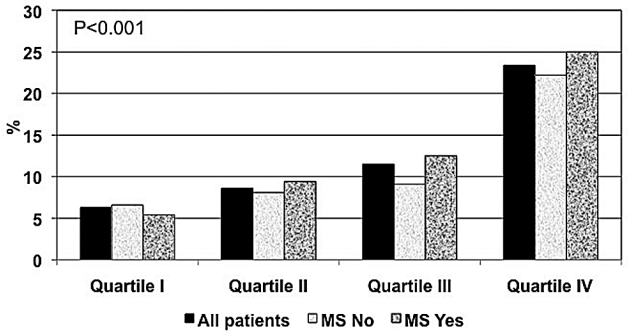

Figure 1. Prevalence of coronary heart and cerebrovascular disease according to quartiles of plasma homocysteine levels in hypertensive patients with or without the metabolic syndrome.

Table 3. Univariate correlation analysis with plasma homocysteine level as the dependent variable

Variables	*r*	*P*
Age, year	0.190	<0.01
Body mass index, kg/m^2	−0.028	0.51
Waist circumference, cm	0.110	0.02
Systolic blood pressure, mm Hg	0.056	0.19
Diastolic blood pressure, mm Hg	−0.010	0.81
Duration of hypertension, year	0.065	0.14
Alcohol intake, g/day	0.017	0.68
Creatinine clearance, ml/min/1.73 m^2	−0.181	<0.01
Glucose, mg/dl	0.096	0.02
Insulin, μUI/ml	0.090	0.05
HOMA index	0.098	0.04
AUC-G, mg/dl/min	0.028	0.61
AUC-I, μUI/ml/min	−0.003	0.96
Triglycerides, mg/dl	0.127	<0.01
Total cholesterol, mg/dl	−0.017	0.69
HDL cholesterol, mg/dl	−0.094	0.03
LDL cholesterol, mg/dl	−0.005	0.73
Uric acid, mg/dl	0.190	<0.01
Vitamin B12, pg/ml	−0.186	<0.01
Folate, ng/ml	−0.240	<0.01
Fibrinogen, mg/dl	0.093	0.03

Abbreviations: AUC-G, area under the curve of plasma glucose after oral glucose tolerance test; AUC-I, area under the curve of plasma insulin after oral glucose tolerance test; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein.

with the *post hoc* Bonferroni test used to correct for multiple comparisons. Pearson's chi-square test was used to compare frequency distributions. Relationship between continuous variables was examined through linear regression analysis, with correlation expressed by Pearson's correlation coefficient.

Multivariate logistic regression analysis was used to assess independence of association of plasma Hcy levels that were treated as the primary independent variable with metabolic syndrome that was the dependent variable. In a separate model, we assessed independence of association of Hcy levels that were the independent variable with cardiovascular events that were the dependent variable, with subsequent inclusion in the model of the metabolic syndrome. Data analyses were done with the Stata 9.2 software system (StataCorp LP, College Station, TX).

RESULTS

The demographic and clinical characteristics of the study patients are summarized in Table 1, where patients are also divided according to the presence or absence of the metabolic syndrome. The prevalence of the metabolic syndrome in the entire hypertensive group was 36.7% and the prevalence of CHD (angina: 41 patients; acute coronary syndrome: 34 patients; coronary revascularization: 28 patients) and CVD (stroke: 16 patients; transient ischemic attack: 18 patients) was 9.6% and 4.8%, respectively. Male sex was more prevalent among patients with the metabolic syndrome who were also older, more often diabetics, smokers, and more frequently treated with antihypertensive agents, had greater body mass index and waist circumference, and had longer duration of hypertension than patients without the metabolic syndrome. No significant differences in prevalence of CHD and CVD were observed between patients with or without the metabolic syndrome. In addition to higher fasting plasma glucose, triglycerides, and insulin, HOMA index, and plasma glucose and insulin responses to OGTT, hypertensive patients with the metabolic syndrome had lower high-density lipoprotein cholesterol, higher Hcy and fibrinogen levels, and worse renal function with greater urinary protein losses than patients without the metabolic syndrome.

Table 2 shows the demographic and clinical characteristics of the study patients who were divided into quartiles of Hcy levels. Age, frequency of male sex, antihypertensive treatment, and prevalence of the metabolic syndrome and both CHD and CVD increased progressively with increasing plasma Hcy. Combined prevalence of CHD and CVD increased progressively across quartiles of plasma Hcy levels with no significant differences between patients with or without the metabolic syndrome (Figure 1). Increasing Hcy levels were also associated with increasing plasma triglycerides and uric acid levels, decreasing vitamin B12 and folate, and progressively worse renal function with greater urinary protein excretion, whereas no differences were observed in other plasma lipids, fasting plasma glucose and insulin, HOMA index, glucose and insulin response to OGTT, and fibrinogen levels (Table 2). The number of patients who required use of calcium channel blockers during the washout period did not differ across quartiles of plasma Hcy.

On univariate regression analysis, Hcy was significantly and directly related with age, waist circumference, fasting glucose, triglycerides, uric acid, and fibrinogen levels, and HOMA index and inversely related with creatinine clearance and high-density lipoprotein cholesterol, vitamin B12,

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Table 4. Multivariate logistic regression analysis was performed to assess independence of association with plasma homocysteine levels

	Model 1			Model 2		
Variables	Odds ratio	CI	*P*	Odds ratio	CI	*P*
Age	1.002	0.96–1.05	0.94	1.056	1.00–1.12	0.05
Male sex	0.070	0.02–0.26	<0.01	2.053	0.48–8.85	0.33
Waist circumference	1.136	1.07–1.20	<0.01	1.027	0.97–1.09	0.36
Duration of hypertension	0.984	0.93–1.04	0.54	1.037	0.98–1.10	0.23
Creatinine clearance	1.010	0.99–1.03	0.36	0.999	0.98–1.02	0.91
Glucose	1.096	1.05–1.14	<0.01	1.005	0.98–1.03	0.74
HOMA index	0.999	0.99–1.00	0.55	0.998	0.99–1.00	0.10
Triglycerides	1.008	1.00–1.01	<0.01	0.999	0.99–1.00	0.43
HDL cholesterol	0.954	0.92–0.99	<0.001	0.983	0.94–1.03	0.45
Homocysteine	1.010	1.00–1.02	0.02	1.011	1.00–1.02	0.01
Vitamin B12	1.002	1.00–1.03	<0.01	1.000	0.99–1.02	0.86
Folate	1.001	1.00–1.02	0.27	1.001	0.99–1.03	0.34
Uric acid	1.021	0.98–1.06	0.31	0.985	0.94–1.03	0.52
Fibrinogen	1.000	0.99–1.00	0.90	1.001	1.00–1.01	0.81

In a first model (Model 1), metabolic syndrome was treated as the dependent variable and plasma homocysteine levels as the primary independent variable, showing that association of metabolic syndrome with homocysteine levels is independent of confounders adjusted in the model. In a second model (Model 2), cerebro-cardiovascular disease was treated as the dependent variable and plasma homocysteine levels as the primary independent variable, showing that association of cerebro-cardiovascular disease with homocysteine levels is independent of confounders adjusted in the model.

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; HOMA, homeostatic model assessment.

and folate levels (Table 3). In a logistic regression analysis that included the metabolic syndrome as the dependent variable and Hcy as the primary independent variable, Hcy was associated with the presence of the metabolic syndrome independent of demographic and anthropometric variables, duration of hypertension, antihypertensive treatment, and additional variables that were related with Hcy on univariate regression analysis (Table 4). In a further logistic regression model that included cardiovascular events (CHD and CVD) as the dependent variable and adjusted for the same confounders, Hcy was associated with the presence of events independent of other confounders (Table 4). Independent relationship of Hcy with cerebro-cardiovascular disease persisted significant after inclusion of metabolic syndrome in the logistic model ($P = 0.01$).

DISCUSSION

The findings of this study demonstrate that elevated plasma Hcy levels are associated with greater prevalence of the metabolic syndrome in hypertensive patients. This association is independent of demographic and anthropometric variables, duration of hypertension, renal function, and plasma levels of vitamin B12, folate, and inflammatory markers. Moreover, increasing Hcy levels are associated with progressively greater prevalence of both CHD and CVD, although no interaction between Hcy and the metabolic syndrome was observed for this association, suggesting that the impact of Hcy on cerebro-cardiovascular disease is independent of the metabolic syndrome in these patients.

Both the metabolic syndrome and elevated plasma Hcy are established independent risk factors for cardiovascular events in the general population[1,2,7,8] and both animal[10] and human[13] studies have suggested that hyperhomocysteinemia might be an integral component of the metabolic syndrome. Findings previously obtained in the general population on the association between Hcy and the metabolic syndrome were highly controversial[11–13,27–30] and also studies conducted in special populations such as adolescents[31] and patients with obesity[32] or CHD[33] did not provide consistent findings. On the other hand, investigations that have looked for an association of Hcy levels with insulin resistance either in the general population[34,35] or in special groups of patients such as women with the polycystic ovary syndrome[36] or psychiatric disorders[37] reported a significant association that was not confirmed in other studies conducted in the general population[38,39] and subjects predisposed to type-2 diabetes.[40] Many factors could account for these substantial discrepancies including age of patients, ethnic differences, methods used to assess insulin sensitivity, and the limited sample size of some of these studies.

Elevated fasting plasma Hcy levels have been reported in hypertensive patients of different age and ethnicity.[14–16] However, only 2 studies were specifically designed to investigate the relationship of plasma Hcy with insulin resistance and the metabolic syndrome in hypertensives, an issue that could be relevant because of the high cardiovascular risk of these patients. Sheu *et al.*[14] reported that plasma levels of Hcy are higher in Chinese hypertensive subjects than matched normotensive controls. They also found a significant

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correlation of plasma Hcy with post-OGTT insulin levels, but not with markers of insulin resistance. Ustundag *et al.*[16] examined 114 Turkish patients with uncomplicated hypertension and plasma Hcy levels higher than those of normotensive controls and reported significantly higher Hcy levels in patients with insulin resistance as defined by a HOMA index of 4 or more. The present study was conducted in a much larger cohort of hypertensive patients and demonstrates that elevated plasma Hcy has a highly significant association with the metabolic syndrome that is independent of factors that may affect Hcy levels such as age, smoking habit, and, most important, renal function and vitamin B12 and folate levels. Furthermore, Hcy levels are associated with higher prevalence of CHD and CVD. Relevant to this point, however, we did not observe a significant interaction between Hcy and the metabolic syndrome, suggesting an independent contribution of Hcy to the cardiovascular outcome of hypertensive patients.

The present study has limitations that should be considered. First, use of a clinical sample might limit the possibility to extend findings to a more general context, because of a possible bias in the referral of patients to the source of care. Second, the cross-sectional design does not permit to draw any conclusion on a causal relationship between elevated Hcy levels and incidence of CHD and CVD. Also, a tight control of cardiovascular risk factors in these patients that were followed in our tertiary center could explain the relatively small proportion of patients who had cerebro-cardiovascular disease, a fact that might have limited the strength of some associations. This might also explain why we did not observe significant differences in prevalence of CHD and CVD between patients with or without the metabolic syndrome. Third, inclusion of a relevant number of hypertensive patients who were not treatment-naive might have introduced, despite a relatively long wash-out period, a possible confounder affecting some of the metabolic variables measured in this study. It must be noticed, however, that no significant differences in these metabolic variables were observed between untreated patients and patients who were treated with different types of antihypertensive drugs. Fourth, although plasma levels of vitamin B12 and folate were measured in all patients and were included together with other possible confounders in the statistical analysis, use of a questionnaire providing information on the dietary intake of food containing these vitamins or metabolic precursors of Hcy and genotyping of patients for methylenetetrahydrofolate reductase would have helped in the interpretation of data. Last, we did not measure the levels of glycated hemoglobin in nondiabetic patients which also might have helped to better define the glicometabolic status of our hypertensive patients.

In conclusion, this study demonstrates in a large group of hypertensive patients that elevated plasma Hcy levels are associated with the presence of the metabolic syndrome independent of all major factors involved in regulation of circulating Hcy. Also, elevated Hcy is associated with greater prevalence of cerebro-cardiovascular disease. These observations could be relevant for the assessment of the cardiovascular risk in hypertensive patients and for their management because detection of high Hcy levels might guide physicians towards better control of additional metabolic risk factors. Also, vitamin supplementation in hypertensive patients with elevated Hcy levels might result beneficial for cardiovascular protection of these patients, but this possibility will have to be tested in future studies.

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DISCLOSURE

The authors declared no conflict of interest.

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