Physiol. Res. 51: 93-98, 2002

Unexpected Inverse Relationship between Insulin Resistance and Serum Homocysteine in Healthy Subjects

H. ROSOLOVÁ, J. ŠIMON, O. MAYER Jr., J. RACEK[1], T. DIERZÉ, D. W. JACOBSEN[2]

Center of Preventive Cardiology, Second Department of Internal Diseases and [1]Institute of Biochemistry of the Medical Faculty Charles University, Plzeň, Czech Republic, [2]Department of Cell Biology, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio, USA

Received January 10, 2001
Accepted March 28, 2001

Summary

Mild hyperhomocysteinemia has been established as a new independent risk factor for atherosclerosis and thrombosis. The metabolic syndrome of insulin resistance is associated with a high risk of coronary heart disease. Our objective was to determine if any relationship exists between the metabolic syndrome of insulin resistance in non-diabetic subjects and total serum homocysteine levels. Sixty-six healthy volunteers (33 males and 33 females) were selected from the population of Pilsen. Insulin resistance was measured by the Insulin Suppression Test using Octreotide. Steady-state plasma glucose concentrations at the end of the test period provided a quantitative measure of insulin resistance. Serum homocysteine level was estimated by high-pressure liquid chromatography. Serum folate and vitamin B_{12} were estimated using commercial kits on an Abbott IMx analyzer. All other laboratory tests were performed by standard methods in a routine biochemical laboratory. Subjects with the highest tertile of steady-state plasma glucose showed a significantly higher body mass index, blood pressure, fasting plasma triglyceride levels, plasminogen activator inhibitor-1 and lower HDL-cholesterol, i.e. an insulin resistance pattern. These subjects had significantly lower serum homocysteine levels compared with non-insulin resistant subjects. The negative association of insulin resistance and serum homocysteine was unexpected. The contribution of plasma folate levels to serum homocysteine levels and serum creatinine was significantly negative and positive, respectively.

Key words
Homocysteine • Insulin resistance • Coronary risk factors

Introduction

Mild hyperhomocysteinemia is an established independent risk factor for atherosclerotic vascular diseases (Boushey *et al.* 1995). If it appears in subjects with standard risk factors such as smoking, hypertension, and/or hypercholesterolemia, the relative risk for atherosclerosis and its complications rises multiplicatively (Graham *et al.* 1977). Homocysteine (Hcy) is a product of methionine metabolism. Serum levels of total Hcy (tHcy) are determined by both genetic and acquired factors. The enzymes involved in the methionine cycle are methylentetrahydrofolate reductase (MTHFR), methionine synthase, and their vitamin

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

substrates (folic acid) and cofactors (vitamin B_{12} and B_2), and the transsulfuration pathway initiated by the vitamin B_6-dependent cystathionine β-synthase (Jacobsen 1997). A relatively common thermolabile variant of MTHFR has been reported among patients with coronary artery disease (Frosst *et al.* 1995). Several studies have shown that supplementation with folic acid, B_{12}, and B_6 decrease the serum tHcy levels; however, a reduction of risk for atherosclerosis after homocysteine lowering is yet to be demonstrated (Homocysteine lowering trialist group 1998).

The metabolic syndrome of insulin resistance (IR) is another well described cluster of risk factors associated with a highly elevated coronary risk and, also, a risk for subsequent type 2 diabetes mellitus (Reaven 1988). Several studies on the relationship between Hcy and IR have appeared over the last three years (Giltay *et al.* 1998, Abbasi *et al.* 1999, Gallistl *et al.* 2000); however, these authors have not furnished consistent results that were obtained in populations different from ours. We studied the relationship between the metabolic syndrome of IR in non-diabetic subjects and serum tHcy levels (two relevant risk factors for coronary heart disease) in a middle-aged population sample of men and women with rather high mean tHcy levels and low folate levels, a feature typical of the Czech population (Mayer *et al.* 2001) in contrast to the above studies.

Methods

Sixty-six healthy subjects (33 men with a mean age of 52 ± 1 years, 33 women with a mean age of 51 ± 1 years) were selected from the PILS Study (Rosolová *et al.* 1994) on the basis of their willingness to participate. We excluded subjects with a medical history of chronic disease, with any pharmacological treatment, and with impaired glucose tolerance and/or newly established diabetes (using standard oral glucose tolerance test). The study protocol was approved by a local ethics committee and informed consent of the subjects was obtained. In each subject, their resistance to insulin-mediated glucose uptake was assessed by a modification of the original insulin suppression test (Pei *et al.* 1994) described previously (Rosolová *et al.* 1997). After an overnight fast, i.v. catheters were placed in a superficial vein of each arm. One arm was used for continuous 180-min infusion of glucose (240 mg/m^2/min), octreotide (a bolus of 25 μg followed by 0.5 μg/min), and insulin (25 mU/m^2/min). Venous blood samples for glucose and insulin determinations were obtained from the contralateral arm every 30 min (up to 150 min) and then every 10 min for the last 30 min of the infusion. The mean of these last four values was used for calculating the steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations. Under these experimental conditions, endogenous insulin secretion is suppressed by octreotide; the SSPI concentration achieved was comparable in all individuals, and the SSPG concentration provided a measure of insulin-mediated glucose disposal; the higher the SSPG, the more insulin resistant the subject. Immunoreactive insulin in plasma was evaluated by radioimmunoassay using Insulin-CT kits (CIS bio international, ORIS Group France), PAI-1 was measured immunochemically using Asseachrom Stago kits. Plasma glucose, cholesterol, and triglycerides were evaluated by standard spectrophotometric enzymatic methods using standard GOD-PAP, CHOD-PAP, and GPO-PAP kits, respectively (Hitachi 917 analyzer). Total serum tHcy was estimated by high-pressure liquid chromatography using SpectraPhysics chromatographic devices (SP8780, San Jose, California, U.S.A) with fluorescence detection (FL3000, Spectra Physics, Abbott). The method was regularly validated by ERNDIM (European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism) reference laboratory. Serum folate and B12 levels were measured immunochemically using Abbott standard kits on an IMX analyser. Creatinine levels were measured, using Jaffe´s method, with Roche standard kits on a Hitachi 917 analyser. Blood pressure (BP) was measured by a mercury sphygmomanometer twice in the sitting position after a 5-min rest. The mean of the two measurements was taken. Statistical evaluation of the data was performed with unpaired Wilcoxon's test, Spearman's correlation, Kruskal-Wallis ANOVA and multiple linear (step-wise) regression on a PC using standard statistical software.

Results

All subjects were divided into tertiles by their SSPG. The mean of SSPG in each tertile and standard error (S.E.M.) are given in Table 1. Subjects in the third tertile of SSPG had a higher body mass index (BMI, in kg/m^2), systolic and diastolic blood pressure (SBP, DBP),

higher triglycerides (TG), plasminogen activator inhibitor 1 (PAI-1) and fasting immunoreactive insulin (IRI). In contrast, HDL-cholesterol and tHcy were significantly lower (Tab. 1). We observed a significant negative simple correlation between SSPG and tHcy in the subjects as a whole: r =-0.31, p<0.05 (Fig. 1, Tab. 3). Subjects with the highest IR (third tertile of SSPG) had higher serum methionine (p<0.039) and lower serum creatinine concentrations (p<0.056) and higher plasma folate (p<0.033) (Tab. 2). No significant relations were found between tertiles of SSPG and vitamin B_{12}. The contribution by plasma folate levels to serum tHcy levels and serum creatinine was significantly negative and positive, respectively. A negative association between SSPG and tHcy persisted even in multiple linear regression after adjustment for age, gender, folate, and creatinine (Tab. 3).

Table 1: Variables (means ± S.E.M.) in 66 healthy subjects in tertiles of SSPG. Differences of variables evaluated by: A/ Kruskal-Wallis test, B/ Wilcoxon's test between TI – T III

Variable	**T – I**	**T – II**	**T – III**	**A – p**	**B – p**
SSPG (mmol/l)	4.5 (0.2)	8.7 (0.2)	13.3 (0.4)	0.001	0.001
SSPI (mU/l)	46.7 (3.9)	47.0 (3.4)	45.9 (3.9)	0.886	0.938
Age (years)	50.3 (1.2)	52.4 (1.4)	50.7 (1.9)	0.547	0.912
BMI (kg/m^2)	24.7 (0.7)	26.3 (0.5)	28.3 (0.8)	0.005	0.003
SBP (mmHg)	136 (4)	143 (4)	152 (4)	0.024	0.011
DBP (mmHg)	84 (2)	89 (2)	92 (3)	0.035	0.021
T-chol (mmol/l)	5.6 (0.2)	5.8 (0.3)	5.9 (0.3)	0.674	0.402
TG (mmol/l)	1.2 (0.1)	1.6 (0.3)	2.0 (0.2)	0.010	0.002
HDL-chol (mmol/l)	1.6 (0.1)	1.5 (0.1)	1.3 (0.1)	0.054	0.019
PAI – 1 (AU/ml)	9.1 (1.5)	15.6 (1.9)	18.6 (1.9)	0.003	0.002
Fasting glu (mmol/l)	4.5 (0.2)	4.7 (0.2)	4.8 (0.3)	0.418	0.278
Fasting IRI (mU/l)	8.7 (1.2)	8.7 (1.3)	18.0 (1.8)	0.001	0.001
tHcy (μmol/l)	12.9 (0.9)	12.6 (1.3)	9.75 (0.5)	0.044	0.029

SSPG: steady-state plasma glucose, SSPI: steady-state plasma insulin, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, T-chol: total cholesterol, TG: triglycerides, HDL-chol: high density lipoprotein cholesterol, PAI-1: plasma activator inhibitor 1, glu: glucose, IRI: immunoreactive insulin, tHcy – total homocysteine, T: tertile

Table 2. Variables (means ± S.E.M.) known to influence serum tHcy level in tertiles of SSPG. Differences of variables evaluated by : A/ Kruskal Wallis test B/ Wilcoxon's test between TI and T III

Variable	**T – I**	**T – II**	**T – III**	**A - p**	**B - p**
S-methionine (μmol/l)	20.2 (1.0)	23.5 (0.9)	23.1 (1.2)	0.039	0.095
S – creatinine (μmol/l)	92.0 (2.8)	89.4 (2.6)	83.3 (2.2)	0.056	0.028
S-folate (ng/ml)	6.2 (0.6)	6.4 (0.7)	8.6 (0.5)	0.0167	0.033
S-vit B_{12} (ng/l)	338 (23)	372 (33)	376 (37)	0.543	0.419

S: serum, T: tertile

Table 3: Multiple linear regression (step wise) between total serum homocysteine and SSPG (insulin resistance) , adjusted for age, gender, plasma folate levels and plasma creatinine. Dependent variable, total serum Hcy μmol/l (continuous).

Independent variables	**Simple correlation**	**p**	**Regression coef.**	**p**
Age (year)	-0.249	NS	4.512	NS
Gender (M/F)	0.254	NS	1.081	NS
Plasma folate (ng/ml)	-0.354	**	-0.004	**
SSPG (mmol/l)	-0.310	*	-0.020	**
Creatinine (μmol/l)	0.219	NS	0.059	*

*Hcy = homocysteine, M = male, F = female, SSPG = steady state of plasma glucose, ** p< 0.001, * p < 0.05, NS = not statistically significant*

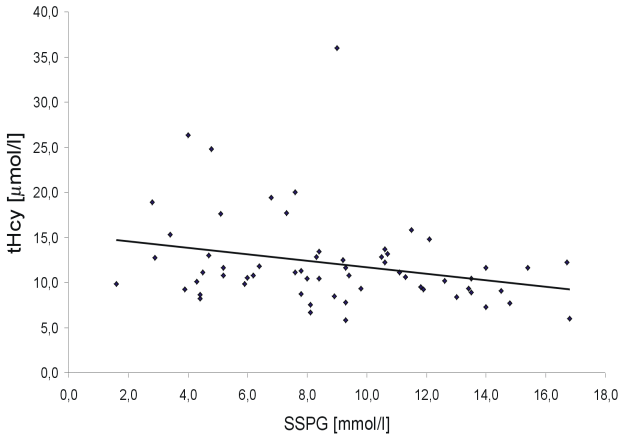

Fig. 1. *Simple correlation of serum total homocysteine (tHcy) and steady state of plasma glucose (SSPG): r = -0.31, p<0.05*

Discussion

The finding of an inverse relationship between two well-established risk factors of atherosclerotic vascular disease, namely the metabolic syndrome of IR and tHcy, was quite unexpected. Three studies have recently been published on the relationship of insulin resistance and plasma tHcy (Giltay *et al.* 1998, Abbasi *et al.* 1999, Gallistl *et al.* 2000). In the study of the Reaven group (Reaven 1988, 1994), 55 healthy subjects were included and no relationship between SSPG and total plasma Hcy was found; however, the tHcy levels reported in both insulin-sensitive and insulin-resistant subjects were lower (8.2 and 8.7 μmol/l respectively) than in our study (12.9 and 9.5 μmol/l, respectively). The plasma folate levels in Reaven's study were not given. On the other hand, Giltay *et al.* (1998) found significantly higher tHcy levels in insulin-resistant healthy subjects; however, the tHcy levels reported for the whole group of studied subjects were again considerably lower than in our sample. The number of subjects was smaller (n=24); moreover, plasma folate was not estimated in this study. Gallistl *et al.* (2000) assessed the relationship of Hcy and IR in obese children and adolescents in Austria. The sample of boys and girls aged about 10 to 12 years were characterized by rather low tHcy levels (6.2-7.1 μmol/l) and comparable folate levels (about 18.9-21.5 nmol/l). These adolescents were obese with a BMI of 28.8 in boys and 29.3 kg/m^2 in girls. After adjustment for age and sex, tHcy correlated significantly with BMI, fat mass, insulin, and inversely with the folate levels. In contrast to our population-based sample, tHcy and folate levels varied within the normal ranges of these estimates.

In our study, subjects with evaluated IR were consistently characterized by well-known markers of IR, i.e. high fasting IRI levels, high TG, low HDL-cholesterol, high BMI, BP and PAI-1, and we considered them to be at a high risk of coronary heart disease (Reaven 1994). The reason why these subjects, who are at a high risk for cardiovascular disease, had decreased tHcy values needs to be explained. One reason for our results might be a difference in folate intake. It has been clearly demonstrated that tHcy levels in the population depend on plasma folate levels, which reflect dietary folate intake. Higher folate levels increase remethylation of Hcy and reduce tHcy concentrations (Homocysteine Lowering Trialist Group 1998). Multiple linear regression showed that plasma folate and SSPG influenced tHcy levels negatively and creatinine levels positively (Tab. 3). We can thus speculate on a metabolic interference of IR and

hyperinsulinemia with Hcy metabolism leading to increased remethylation and/or transsulfuration and, consequently, reduced tHcy concentrations (Fonseca *et al.* 1998). Another reason might be an increased rate of renal excretion of homocysteine or its metabolites due to hyperfiltration usually present in the early stage of diabetic nephropathy. This possibility is supported by our previous study, in which we showed a higher glomerular filtration rate and microalbuminuria already in healthy subjects with insulin resistance, i.e. in subjects with SSPG and fasting insulin in the third tertile (Rosolová 2000). Wollesen showed that hyperfiltration is significantly associated with lower-than-normal mean plasma tHcy and total cysteine concentrations observed in type 1 and type 2 diabetes (Wollesen *et al.* 1998).

It was recently reported that high plasma tHcy is a stronger (1.6 fold) risk factor for cardiovascular disease in patients with type 2 diabetes than in non-diabetic subjects (Hoogeveen *et al.* 1998). The high tHcy levels were attributed to the co-existence of impaired renal function, since these patients also had higher plasma creatinine levels. Patients with both types of diabetes and nephropathy had higher plasma tHcy concentrations than those without nephropathy (Chico *et al.* 1998). These studies suggested that increased tHcy concentrations developed in diabetic subjects secondary to late micro- and macrovascular complications. Thus, hyperhomocysteinemia, a marker of a tissue damage, develops in line with the development of angiopathy.

In our previous study, we observed that higher mean levels of tHcy (13.1±0.32 μmol/l in men, 11.5±0.91 μmol/l in women) and lower plasma folate exist in the Czech population compared with other West European populations (Mayer *et al.* 1998). This study shows that tHcy in healthy insulin-resistant subjects does not contribute considerably to the global cardiovascular risk in insulin-resistant subjects. The lower serum tHcy concentrations in insulin-resistant subjects compared with the mean of the general population seem to be influenced by hyperinsulinemia and, also, probably by glomerular hyperfiltration. Casual tHcy depends on the casual state of renal functions and/or on existing renal angiopathy.

Acknowledgements

This work was partially supported by grants from the Ministry of Health (IGA 3734-3), Charles University Prague, Medical Faculty in Plzeň, MSM 111400001 Czech Republic and from the National Institutes of Health (USA; Grant HL 52234 to DWJ).

Preliminary results of this study were presented at the XIth International Symposium on Atherosclerosis in Paris, France, Oct 5-9, 1997. Atherosclerosis 134:167-8, 1997 (Abstract)

References

ABBASI F, FACCHINI F, HUMPHREYS MH, REAVEN GM: Plasma homocysteine concentrations in healthy volunteers are not related to differences in insulin-mediated glucose disposal. *Atherosclerosis* **146**: 175-8, 1999

BOUSHEY CJ, BERESFORD SAA, OMENN GS, MOTULSKY AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA* **274**: 49-57, 1995.

CHICO A, PEREZ A, CORDOBA A, ARCANS R, CARRERAS SG, DE LEVA A, GOUZABS-SASTRE F, BLANCO-VACA F: Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? *Diabetologia* **41**: 684-693, 1998.

FONSECA VA, MUDALIAR S, SCHMIDT B, FINK LM, KERN PA, HENRY RR: Plasma homocysteine concentrations are regulated by acute hyperinsulinemia in nondiabetic but not type 2 diabetic subjects. *Metabolism* **47**: 686-689, 1998.

FROSST P, BLOM HJ, MILOS R, GOYETTE P, SHEPPARD CA, MATTHEWS RG: A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* **10**: 111-113, 1995.

GALLISTL S, SUDI K, MANGGE H, ERWA W, BORKENSTEIN M: Insulin is an independent correlate of plasma homocysteine levels in obese children and adolescents. *Diabetes Care* **23**: 1348-1352, 2000.

GILTAY EJ, HOOGEVEEN EK, ELBERS JM, GOOREN LJ, ASSCHEMAN H, STEHOUWER CD: Insulin resistance is associated with elevated plasma total homocysteine levels in healthy, non-obese subjects. *Atherosclerosis* **139**: 197-198, 1998.

GRAHAM IM, DALY LE, REFSUM HM, ROBINSON K, BRATTSTROM LE, UELAND PM AND THE RESEARCH GROUP of The European Concerted Action Project: Plasma homocysteine as a risk factor for vascular disease. *JAMA* **277**: 1775-1781, 1977.

HOMOCYSTEINE LOWERING TRIALIST GROUP: Lowering blood homocysteine with folic acid based supplements. Meta-analysis of randomized trials. *Br Med J* **316**: 894-898, 1998.

HOOGEVEEN EK, KOSTENSE PJ, BEKS PJ, MACKAAY AJ, JACOBS C, BOUTER LM, HEINE RJ, STEHOUWER CD: Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. *Arterioscler Thromb Vasc Biol* **8**: 133-138, 1998.

JACOBSEN DW: Homocysteine and vitamins in cardiovascular disease. *Clin Chem* **44**: 1833-1843,1997.

JACOBS RL, HOUSE JD, BROSNAN ME: Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. *Diabetes* **47**: 1967-1970, 1998.

MAYER O, ŠIMON J, ROSOLOVA H: Age and sex differences in homocysteine mediated coronary risk in the Czech population. In: *Proceedings of the XIII World Congress of Cardiology*. Monduzzi Editore, Bologna, 1998, pp 21-24.

MAYER O Jr, ŠIMON J, ROSOLOVÁ H: A population study of the influence of beer consumption on folate and homocysteine concentrations. *Eur J Clin Nutr* **55**: 1-5, 2001

PEI D, JONES CNO, BHARGAVA R, REAVEN GM: Evaluation of octreotide to assess insulin-mediated glucose disposal by the insulin suppression test. *Diabetologia* **37**: 843-845, 1994.

REAVEN GM: Role of insulin resistance in human disease. *Diabetes* **37**: 1595-1607, 1988.

REAVEN GM. Syndrome X 6 years later. *J Intern Med* **236**: 13-22, 1994.

ROSOLOVÁ H, SIMON J. The influence of education on the risk profile in Czech men and women: The Pilsen Longitudinal Study (PILS). *Acta Cardiol* **4**: 369-371, 1994.

ROSOLOVÁ H, MAYER O Jr, REAVEN G: Effect of variations in plasma Magnesium concentration on resistance to insulin-mediated glucose disposal in non-diabetic subjects. *J Clin Endocrinol Metab* **8**: 3783-3785, 1997.

VACCARO O, INGROSSO D, RIVELLESE, GRECO G, RICCARDI G: Moderate hyperhomocysteinemia and retinopathy in insulin dependent diabetes. *Lancet* **349**: 1102-1103, 1997.

WOLLESEN F, BRATTSTROM L, REFSUM H, UELAND PM, BERGLUND L, BERNE C: Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. *Kidney Int* **55**: 1028-1035, 1999.

Reprint requests

H. Rosolová, Center of Preventive Cardiology, Second Department of Internal Medicine, E. Beneše 13, 305 99 Plzeň, Czech Republic, e-mail : rosolova@fnplzen.cz