Physiol. Res. 55: 285-290, 2006

Insulin Resistance Is Not Related to Plasma Homocysteine Concentration in Healthy Premenopausal Women

F. TANRIKULU-KILIÇ, S. BEKPINAR, Y. ÜNLÜÇERÇI, Y. ORHAN[1]

Department of Biochemistry, and [1]Department of Endocrinology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

Received date December 21, 2004
Accepted date May 16, 2005
On-line available August 5, 2005

Summary

This study was performed to test whether plasma homocysteine concentrations are related to insulin resistance in healthy premenopausal women. For this purpose, the relationship between insulin resistance (as assessed by HOMA index) and fasting plasma homocysteine level was determined in 83 healthy volunteers. The results indicated that homocysteine concentrations did not vary as a function of HOMA index ($r = -0.147$). Plasma homocysteine concentrations also did not vary as a function of other parameters of insulin resistance such as HDL-cholesterol and triglycerides, which they correlated inversely with body mass index (BMI). Furthermore, when individuals were classified according to quartiles of insulin resistance (HOMA index), plasma homocysteine concentrations from the lowest to the highest quartiles were not significantly different. On the other hand, the HOMA index correlated significantly with triglyceride concentrations ($r = 0.377$, $p< 0.001$), HDL-cholesterol ($r = -0.310$, $p< 0.01$) and BMI ($r = 0.468$, $p< 0.001$). These results suggest that plasma homocysteine concentrations are not related to insulin resistance and/or metabolic abnormalities associated with it in premenopausal women.

Key words

Homocysteine • Insulin resistance • Metabolic syndrome

Introduction

An elevated plasma homocysteine is recognized as a risk factor for cardiovascular disease (Eikelboom *et al.* 1999). Hyperhomocysteinemia appears to have manifold actions that would be expected to adversely affect the vasculature. These include endothelial cytotoxicity, lipid peroxidation, increased platelet adhesiveness, enhanced activation of the coagulation system and stimulation of vascular smooth muscle cell proliferation (Fonseca *et al.* 1999).

Hereditary enzymatic abnormalities and nutritional deficiencies of folate, pyridoxine or cobalamin (B_{12}) as well as chronic renal failure are associated with elevated blood homocysteine levels (Kang and Wong 1996, Boushey *et al.* 1995). On the other hand, the insulin resistance syndrome is characterized by glucose intolerance, hyperinsulinemia, dyslipidemia, abnormal obesity, hypertension, and is associated with an increased risk for cardiovascular disease (Laws and Reaven 1993). Recently, two metabolic disturbances, insulin resistance and hyperhomocysteinemia, have been intensively investigated with regard to their possible roles in the pathogenesis of cardiovascular disease. Several

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

observations suggest that there might be links between insulin resistance and hyperhomocysteinemia. Homocysteine levels have been found to be raised in patients with type 2 diabetes, both in the fasting state (Araki *et al.* 1993) and after methionine loading (Munshi *et al.* 1996).

Animal studies also suggested a role for insulin and/or insulin resistance in determining plasma homocysteine levels (Jakobs *et al.* 1998, Fonseca *et al.* 2000). However, there is conflicting evidence whether there is a more general relationship between insulin resistance and homocysteine levels in healthy humans. Giltay *et al.* (1998) measured insulin resistance as glucose utilization during a 2-hour euglycemic hyperinsulinemic clamp in 24 healthy non-obese men and women and found significantly plasma homocysteine levels in the lowest tertile of insulin sensitivity. In contrast, Abbasi *et al.* (1999) found no such relationship when they explored associations between insulin resistance, measured by the insulin suppression test, and plasma homocysteine levels in 55 healthy men and women. Godsland *et al.* (2001) also found that homocysteine concentrations are unrelated to insulin sensitivity, measured by minimal model analysis of glucose and insulin concentrations during an iv. glucose tolerance test. On the other hand, Rosolová *et al.* (2002) reported the negative association of insulin resistance and serum homocysteine.

To make any contribution to this issue, we measured plasma homocysteine levels and vitamins levels related with homocysteine metabolism in 83 premenopausal females, each of whom had undergone measurement of insulin resistance by HOMA index. We also investigated the relation between homocysteine levels and other components of insulin resistance syndrome such as fasting serum triglycerides, HDL-cholesterol, uric acid concentrations and BMI.

Methods

We studied 83 healthy premenopausal women, with a median age 32 years (range 17-43 years). All subjects gave their informed consent to be included in this study, which was performed in accordance with the guidelines stated in the Declaration of Helsinki. All women were spontaneously menstrually active and non-diabetic (basal glucose <6.93 mmol/l) and non-hypertensive (systolic blood pressure <140 mm Hg, diastolic blood pressure <90 mm Hg). In order to avoid the confounding effect of other cardiovascular risk factors, smokers, familial hyperlipidemia, and patients with chronic renal failure were not included in this study. No subjects were taking any medication.

Blood samples were taken and immediately centrifuged at 4 °C and stored at –20 °C. Glucose, total cholesterol, LDL- and HDL-cholesterol, triglyceride levels were determined by an automated analyzer, insulin was measured by means of an enzyme-amplified chemiluminescence assay, with quality control of ISO 9001. Insulin resistance was evaluated by "homeostasis model assessment (HOMA)" formula. This formula was calculated by fasting plasma insulin (μU/ml) x fasting plasma glucose (mmol/l) / 22.5.

Homocysteine concentrations were determined by using an HPLC fluorometric method after the samples had been treated with 7-fluorobenzo-2-oxa-1,3diazol-4 sulfonamide to convert homocysteine to a fluorescent compound (Vester and Rassmusen 1991, Ubbink *et al.* 1991). Vitamin B_{12} and folate were measured by radioimmunoassay (RIA) kit method (Diagnostica Products Corp CA 90045-5597).

Data are expressed as median values and ranges for each of the variables. Pearson correlations were calculated to demonstrate relationships between relevant variables.

Results

Median values and ranges for each of the variables considered here are shown in Table 1. Age ranged from 17.0 to 43.0 years, BMI from 17.80 to 44.00 kg/m^2, insulin resistance from 0.48 to 9.71 and homocysteine levels from 5.50 to 18.30 μmol/l. On univariate analysis, homocysteine negatively correlated with folate ($r = –0.298$, $p<0.02$) and BMI ($r = –0.283$, $p<0.02$).

When individuals were classified according to quartiles of insulin resistance (HOMA), median homocysteine concentrations from the lowest to the highest quartile were 10.60 (range, 7.80-18.30), 11.65 (range, 5.50-17.00), 9.20 (range, 5.5-15.00), 8.60 (range, 6.10-13.80). There were no significant differences among the quartiles. Nevertheless, there were significant univariate correlations between the components of the insulin resistance syndrome themselves (Table 1). Insulin resistance correlated significantly with BMI ($r = 0.468$, $p<0.001$), triglycerides ($r = 0.377$, $p<0.01$), HDL-cholesterol ($r = –0.310$, $p<0.01$), uric acid ($r = 0.366$, $p<0.01$) and age ($r = –0.357$, $p<0.01$) (Table 2).

Table 1. Basal characteristics of the studied group of healthy premenopausal women.

	Medians and ranges	
Homocysteine (μmol/l)	10.00 (5.50-18.30)	(n= 76)
Vitamin B_{12} (pmol/l)	174.17 (44.28-746.12)	(n= 77)
Folate (nmol/l)	14.33 (3.38-45.14)	(n= 79)
Age (years)	32.0 (17.0-43.0)	(n= 83)
BMI (kg/m^2)	27.9 (17.8-44.0)	(n= 83)
Glucose (mmol/l)	4.55 (3.55-5.38)	(n= 83)
Insulin (μU/ml)	8.2 (2.6-40.6)	(n= 82)
HOMA index	1.65 (0.48-9.71)	(n= 82)
Cholesterol (mmol/l)	4.77 (2.99-8.61)	(n= 83)
LDL-cholesterol (mmol/l)	2.79 (1.16-6.82)	(n= 67)
HDL-cholesterol (mmol/l)	1.37 (0.77-2.22)	(n= 82)
Triglycerides (mmol/l)	0.97 (0.48-5.47)	(n= 83)
Uric acid (μmol/l)	220.1 (154.6-422.3)	(n= 70)

Table 2. Pearson correlation coefficients between metabolic variables related to cardiovascular disease

	Hcy	B_{12}	Folate	Age	Glucose	Insulin	HOMA	TG	HDL-chol	Uric acid
Homocysteine										
B_{12}	- 0.204									
Folate	- 0.298 [b]	0.168								
Age	0.091	0.097	- 0.024							
Glucose	- 0.074	- 0.083	0.078	0.037						
Insulin	- 0.147	- 0.090	- 0.094	- 0.389 [d]	0.396 [d]					
HOMA	- 0.147	- 0.090	- 0.081	- 0.357 [c]	0.482 [d]	0.991 [d]				
Triglycerides	- 0.049	- 0.113	- 0.122	- 0.084	0.247 [a]	0.359 [c]	0.377 [d]			
HDL-chol	- 0.037	0.243 [a]	- 0.006	0.258 [b]	- 0.107	- 0.334 [c]	- 0.310 [c]	- 0.367 [c]		
Uric acid	0.136	- 0.247 [a]	0.170	- 0.235	0.169	0.388 [c]	0.366 [c]	0.324 [c]	- 0.471 [d]	
BMI	- 0.283 [b]	- 0.259 [a]	0.000	0.038	0.334 [c]	0.476 [d]	0.468 [d]	0.266 [b]	- 0.393 [d]	0.527 [d]

[a]: p< 0.05, [b]: p< 0.02, [c]: p<0.01, [d]: p< 0.001

Plasma levels of vitamin B_{12} and folate which have a role in homocysteine metabolism were also measured and only a significant relationship between homocysteine and folate levels (r = -0.298, p<0.02) was found.

For the diagnosis of insulin resistance, the fasting plasma insulin values and HOMA index were reported to be >16.7 mU/l and >3.8, respectively (Ascado *et al.* 2001). In our study, the prevalence of high fasting plasma insulin values (>16.7 μU/l) and high HOMA index (>3.8) were about 6 % of healthy subjects. The median of the homocysteine values in subjects with high insulin levels and HOMA index was 9.8 (range 7.8-11.9).

In addition to fasting plasma insulin values and HOMA index, the best clinical and biochemical indicators of insulin resistance were fasting glucose levels (>6.10 mmol/l), BMI (>25 kg/m^2) and plasma triglycerides (>1.69 mmol/l). In our study, no subject had glucose level which exceeded 6.10 mmol/l. Prevalence of subjects with high BMI (>25 kg/m^2) was about 60 %. The 10 % of subjects had elevated triglyceride levels (>1.69 mmol/l). However, the homocysteine levels of subjects with increased levels of the above parameters were not found to be different from those of the rest of the group.

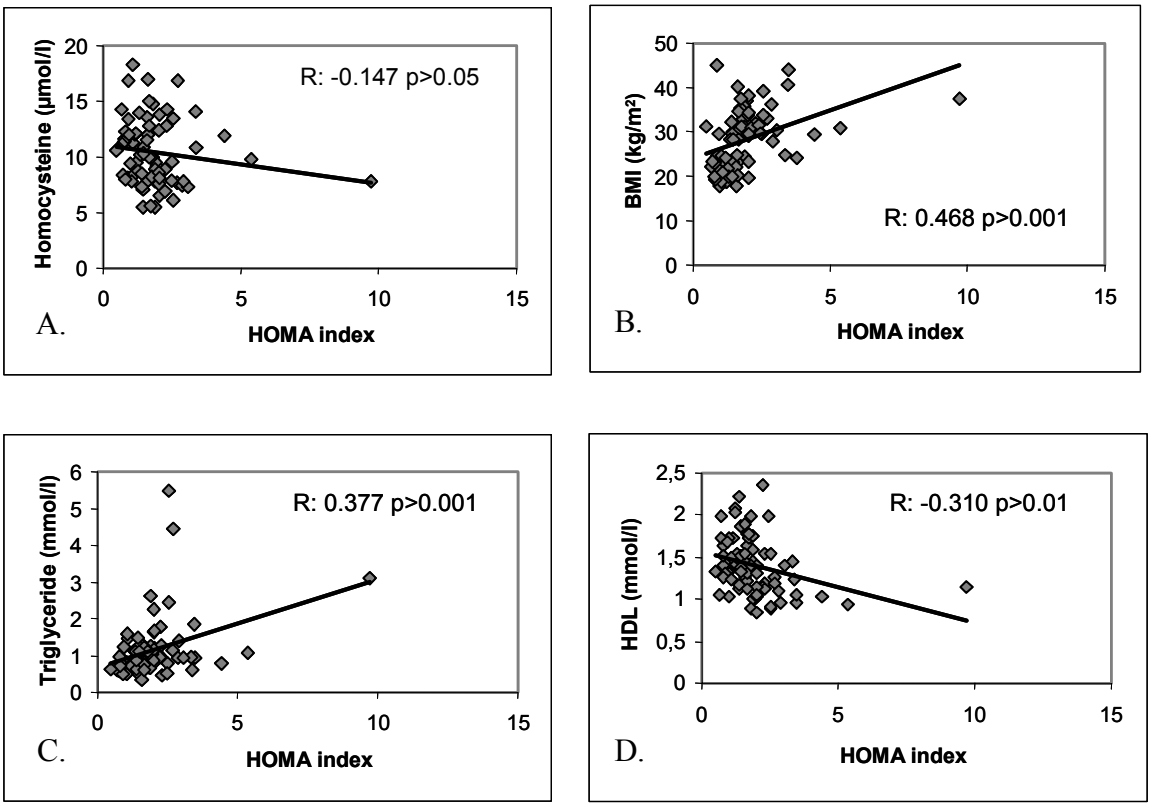

Fig. 1. Relationships of insulin resistance (HOMA index) with homocysteine (**A**), body mass index (**B**), triglycerides (**C**), and HDL-cholesterol (**D**). Pearson's correlation coefficient (r) and p values are indicated.

Discussion

In this study, there was no association between plasma homocysteine and the HOMA index (Fig. 1A) or other putative components of the metabolic syndrome such as elevated triglycerides, HDL-cholesterol and uric acid (Table 2). On the other hand, plasma homocysteine level correlated negatively with BMI, another metabolic syndrome criterion.

The present study indicates that variations in plasma homocysteine concentrations were independent of insulin resistance or metabolic syndrome. This is in accordance with the findings of three studies performed on larger healthy populations (Abbasi *et al.* 1999, Godsland *et al.* 2001, Rosolová *et al.* 2002). Our results did not confirm the positive association found in the study of Giltay *et al.* (1998) performed on a smaller population.

Intake of folate, vitamin B_{12} and vitamin B_6 affect the fasting homocysteine level. In addition, a variety of drugs and hormones play a role in determining plasma homocysteine (Fonseca *et al.* 1999). Unfortunately, Giltay *et al.* (1998) did not provide data on the folate and vitamin status of their subjects.

In our study, folate levels (>7 nmol/l) and vitamin B_{12} levels (>150 pmol/l) were found to be within the normal range in 94 % of the participants. The median of homocysteine values in participants with low vitamin B_{12} and folate levels were found as 12.60 and 12.40, respectively, and these values were not different from those of the rest of group because these vitamin levels were not so extremely low. In our study there was an inverse relationship of plasma homocysteine with folate, demonstrated that plasma homocysteine levels in this population depend on plasma folate levels. Higher folate levels increase remethylation of homocysteine and reduce homocysteine concentrations (Homocysteine Lowering Trialist Group 1998).

Subjects with elevated BMI are characterized by a well-known marker of insulin resistance. As a matter of fact, we found a strong correlation between BMI and insulin resistance ($r = 0.468$, $p<0.001$), (Fig. 1B). On the other hand, plasma homocysteine levels in the population with high BMI was observed to be low. One reason for our results might be an increased rate of renal excretion of homocysteine or its metabolites due to early glomerular hyperfiltration usually present in obese subjects (Hall *et al.* 1999).

Further evidence of our study was observation

that insulin resistance (HOMA index) correlated significantly with fasting plasma insulin, triglycerides, HDL-cholesterol and BMI, i.e. metabolic variables known to be associated with insulin resistance (Laws and Reaven 1993), (Fig.1B,C,D).

Adipose-derived bioactive factors have been described to modulate the physiological function of the other tissues in the body. Our finding of a positive correlation between HOMA index and BMI might be an indicator that the increased levels of adipose-derived factors such as TNF-α and IL-6 that are dependent on obesity, interfere with the insulin action leading the insulin resistance as described previously (Kern *et al.* 2001).

In can be concluded that in healthy premenopausal women, the variations in homocysteine levels are not linked with insulin resistance or with various parameters of the metabolic syndrome.

Acknowledgements

This work was supported by The Research Fund of the University of Istanbul, Project No. T-148/06032003.

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:** 175-178, 1999.

ARAKI A, SAKO Y, ITO H: Plasma homocysteine concentrations in Japanese patients with non-insulin-dependent diabetes mellitus: effect of methyl cobalamin treatment. *Atherosclerosis* **103:** 149-157, 1993.

BOUSHEY CJ, BERESFORD SA, OMENN GS, MOTULSKY AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *J Am Med Assoc* **274:** 1049-1057, 1995.

EIKELBOOM JW, LONN E, GENEST JJ, HEMKEY G, YUSUF S: Homocysteine and cardiovascular disease: a critical review of the epidemiologic evidence. *Ann Intern Med* **131:** 365-375, 1999.

FONSECA V, DICKER-BROWN A, RANGANATHAN S, SONG W, BARNARD RJ, FINK L, KERN PA: Effects of a high-fat-sucrose diet on enzymes in homocysteine metabolism in the rat. *Metabolism* **49:** 736-741, 2000.

FONSECA V, GUBA SC, FINK LM: Hyperhomocysteinemia and the endocrine system: implications for atherosclerosis and thrombosis. *Endocr Rev* **20:** 738-759, 1999.

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

GODSLAND IF, ROSANKIEWICZ JR, PROUDLER AJ AND JOHNSTON DG: Plasma total homocysteine concentrations are unrelated to insulin sensitivity and components of the metabolic syndrome in healthy men. *J Clin Endocrinol Metab* **86:** 719-723, 2001.

HALL JE, BRANDS MW, HENEGAR JR: Mechanism of hypertension and kidney disease in obesity. *Ann N Y Acad Sci* **892:** 91-107, 1999.

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

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

KANG SS, WONG PW: Genetic and non-genetic factors for moderate hyperhomocysteinemia. *Atherosclerosis* **119:** 135-138, 1996.

KERN PA, RANGANATHAN S, LI C, WOOD L, RANGANATHAN G: Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol* **280:** E745-E751, 2001.

LAWS A, REAVEN GM: Insulin resistance and risk factors for coronary heart disease. *Balliere's Clin Endocrinol Metab* **7:** 1063-1078, 1993.

MUNSHI MN, STONE A, FINK L, FONSECA V: Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. *Metabolism* **45:** 133-135, 1996.

ROSOLOVÅ H, ŠIMON J, MAYER O Jr, RACEK J, DIERZE T, JACOBSEN DW: Unexpected inverse relationship between insulin resistance and serum homocysteine in healthy subjects *Physiol Res* **51:** 93-98, 2002.

UBBINK JB, VERMAAK WHJ, BISSBORT S: Rapid high performance liquid chromatographic assay for total homocysteine levels in human serum. *J Chromatogr* **565:** 441-446, 1991.

VESTER B, RASMUSSEN K: High performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and serum. *Eur J Clin Chem Biochem* **29:** 549-554, 1991.

Reprint requests

Selda Bekpinar, Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Çapa 34093-Istanbul, Turkey, Fax: +90 216 418 53 80. E-mail: seldabekpinar@hotmail.com