Amino Acids (2000) 18: 289–298

Amino
Acids

Impaired glucose tolerance (IGT) is not associated with disturbed homocysteine metabolism

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Accepted September 16, 1999

Summary. Elevated plasma total homocysteine (tHcy) has been suggested to be an additional risk factor for cardiovascular disease in subjects with impaired glucose tolerance (IGT) and Type 2 diabetes (T2D). In order to investigate whether an insulin resistant/chronic hyperinsulinemic situation in male diabetic and prediabetic subjects directly influences the tHcy metabolism, fasting tHcy and post-methionine load tHcy plasma levels (PML-tHcy) were determined in 15 men with IGT, 13 men with newly diagnosed T2D, and 16 normoglycemic controls (NGT). Fasting tHcy (IGT, 13.1 $\pm$ 4.6; T2D, 12.8 $\pm$ 4.0; NGT, 10.7 $\pm$ 4.4 μmol/L) and PML-tHcy (IGT, 46.5 $\pm$ 17.39; T2D, 41.1 $\pm$ 6.8; NGT, 38.0 $\pm$ 9.7 μmol/L) showed no differences between the groups. Fasting tHcy and PML-tHcy correlated with fasting proinsulin ($r = 0.395$, $p < 0.05$; $r = 0.386$, $p < 0.05$) and creatinine ($r = 0.489$, $p < 0.01$; $r = 0.339$, $p < 0.05$), resp. Multiple regression analysis showed only a relationship between fasting tHcy and creatinine. No relationships have been found between fasting tHcy and PML-tHcy, resp., and indicators of an insulin resistant state, e.g., insulin and proinsulin, as well as serum cobalamin and folate concentrations. In conclusion, our data suggest that the degree of glucose intolerance has no direct impact on the metabolism of homocysteine. However, tHcy levels tend to be elevated with the development of nephropathy, indicating an association between tHcy and renal function in these subjects.

Keywords: Amino acids – Impaired glucose tolerance – Type 2 diabetes – Homocysteine – Renal function

Introduction

Homocysteine, a thiol-containing non-protein amino acid, is an intermediate in the metabolism of methionine (Duell and Malinow, 1997). In numerous case control studies and several prospective epidemiological studies elevated plasma total homocysteine (tHcy) levels have been recognized as an independent risk factor for coronary artery disease (Chasan-Taber et al., 1996; Graham et al., 1997; Nygard et al., 1997), cerebrovascular disease (Selhub et al., 1995; Giles et al., 1998; Yoo et al., 1998), peripheral arterial occlusive disease (van den Berg et al., 1996; Taylor et al., 1999), and venous thromboembolic disease (Falcon et al., 1994; den Heijer et al., 1996). Several factors are known to affect plasma homocysteine levels, among them certain vitamin deficiencies (Ubbink, 1997; Robinson et al., 1998). Both folate and cobalamin (vitamin B_{12}) levels have been shown to correlate negatively with plasma tHcy concentrations. The influence of vitamin B_6 deficient states on tHcy levels has not been clearly demonstrated (Ubbink, 1997). Increased homocysteine concentrations are also present in genetic disorders such as cystathionine β-synthase deficiency, in which homocysteine cannot be converted to cystathionine (Mudd et al., 1995).

Subjects with impaired glucose tolerance (IGT), a strong predictor of Type 2 diabetes (T2D) (Edelstein et al., 1997), and subjects with overt T2D are frequently affected by premature cardiovascular disease (CVD). Both conditions are known to be associated with several adverse arteriosclerotic risk factors, including insulin resistance, chronic compensatory hyperinsulinemia, hyperglycemia, dyslipidemia, hypertension, and obesity (Haffner, 1997). However, the relationship between plasma tHcy and the excess of atherosclerotic risk in these subjects has not been clearly demonstrated (Guba et al., 1996; Munshi et al., 1996; Hoogeven et al., 1998). In spite of a higher prevalence of CVD compared with persons with normal glucose tolerance (NGT), the mean plasma tHcy concentrations have been found in the lower normal range in patients with manifested T2D (Araki et al., 1993; Munshi et al., 1996). The plasma concentration of tHcy is determined by intracellular metabolism of homocysteine within the activated methyl cycle (Dudman et al., 1996), by *trans*-sulfuration to cysteine (Selhub and Miller, 1992), and by renal clearance (Arnadottir et al., 1996). Lower levels of plasma tHcy might be a result of abnormal homocysteine metabolism or renal clearance of homocysteine under diabetic conditions. However, recent studies showed tHcy concentrations to be regulated by acute hyperinsulinemia in normal subjects but not in subjects with T2D (Fonseca et al., 1998; Nagai et al., 1999). The aim of the present study was to investigate whether an insulin resistant/chronic hyperinsulinemic situation in male subjects with IGT and newly diagnosed T2D directly influences the homocysteine metabolism. To challenge the tHcy metabolizing pathways an oral methionine loading test was performed. Furthermore the relationships between tHcy and indicators of an insulin resistant/hyperinsulinemic state, e.g., plasma glucose, insulin, proinsulin, and indicators of the vitamin state, e.g., cobalamin and folate, were explored.

Materials and methods

Fasting tHcy and post-methionine load (PML-tHcy) homocysteine plasma levels as well as vitamins involved in tHcy metabolism were determined in 15 subjects with IGT, 13 subjects with newly diagnosed T2D, and 16 normoglycemic controls (NGT). Only men were studied because of the difficulty in interpretation of data in perimenopausal women (Lussier-Cacan et al., 1996). The diagnosis of IGT and T2D was confirmed by two consecutive 75 g oral glucose tolerance tests (OGTT) according to the WHO criteria and guidelines (120 min plasma glucose values between 7.8–11.1 mmol/L for IGT, and >11.1 mmol/L for T2D). Men with clinical signs and/or history of macrovascular disease (myocardial infarction, undergone coronary bypass surgery or percutaneous transluminal coronary angioplasty, ischemic ECG changes, cerebral infarction, transient ischemic attack, intermittent claudication, foot ulcers), renal insufficiency (serum creatinine > 125 μmol/L), hepatic dysfunction (ALAT > 0.80 μmol/L $\times$ s^{-1}; ASAT > 0.80 μmol/L $\times$ s^{-1}; γGT > 1.00 μmol/L $\times$ s^{-1}), severe dyslipidemia (triglycerides > 5 mmol/L; total cholesterol > 7.5 mmol/L), and men receiving a supplementation of folic acid, cobalamin, and vitamin B_6 have been excluded.

The diagnosis of hypertension and dyslipidemia was established when subjects were treated with antihypertensive and lipid lowering drugs. After an overnight fast, venous blood samples were obtained into EDTA vacutainers before and 1, 2, 4 and 6 hours after an oral load of L-methionine (0.1 g/kg body weight). During the test participants were given water and tea. Food intake was not allowed. Blood samples were immediately centrifuged within 30 minutes at 4°C (4,000 rpm, 10 min.). EDTA plasma was separated from cells immediately after centrifugation and stored at −80°C until analyzed. tHcy concentrations were determined by a homocysteine enzyme immunoassay based on enzymatic conversion of Hcy to S-adenosyl-L-homocysteine (SAH), followed by quantification of SAH in a competitive immunoassay with the use of monoclonal enzyme-labelled anti-SAH antibodies, intra(inter)assay CV: <6.2% (<8.0%) (BioRad Diagnostics, Germany) (Pietzsch and Pixa, 1998). Cobalamin and folate levels in serum were analyzed by specific EIA (Ciba Corning Diagnostics, Germany). Plasma glucose was measured by the hexokinase method (Boehringer, Germany) and HbA_{1C} by HPLC on a Diamat analyzer (BioRad Diagnostics, Germany). HDL-cholesterol was determined after precipitation with dextran sulfate. Triglycerides and total cholesterol were measured by commercially available test kits (Boehringer, Germany). LDL-cholesterol was calculated using the Friedewald formula. Insulin and proinsulin were measured by a specific EIA (BioSource Europe, Belgium; DRG Diagnostics, Germany; within-day precision < 3.8%, between-day precision < 6.5%; no cross reaction with human proinsulin and insulin, resp.). Serum creatinine was determined by enzyme colorimetric assay and albumin concentrations in fresh morning urine samples by nephelometry (Nephelometer BN II, Germany). The latter has been shown to give a fairly strong correlation to albumin excretion rate (AER) in urine samples collected over 24-hours (Marshall, 1991).

Descriptive data were expressed as means ± standard deviation (SD). Differences between the groups were tested with Mann-Withney test. Spearman's rank correlation coefficient was used to assess relationships between study variables and fasting tHcy/PML-tHcy concentrations. Multiple regression analyses were performed with log tHcy as dependent variable. Statistical analyses were performed using the SPSS software package.

Results

Table 1 summarizes the clinical and biochemical characteristics of the groups. There were no differences with respect to age, total cholesterol, LDL-cholesterol, and parameters of renal function between the groups. Subjects with IGT and newly diagnosed T2D exhibited significantly higher levels of

Table 1. Clinical and biochemical characteristics of IGT, T2D, and controls

	NGT	IGT	T2D
n	16	15	13
Age [y]	55.8 ± 5.3	56.7 ± 5.3	57.6 ± 4.2
Body mass index [kg/m^2]	25.2 ± 2.3	27.4 ± 2.3	28.6 ± 3.1$^{\$\$}$
Waist to hip ratio	0.94 ± 0.05	0.98 ± 0.05$^{\bullet}$	1.00 ± 0.05$^{\$}$
Fasting plasma glucose [mmol/L]	5.7 ± 0.3	6.4 ± 0.3$^{\bullet\bullet}$	7.9 ± 0.9$^{\$\$,\#\#}$
HbA_{1c} [%]	5.6 ± 0.4	5.7 ± 0.4	6.5 ± 0.6$^{\$\$,\#\#}$
Fasting plasma insulin [pmol/L]	52.7 ± 23.8	103.5 ± 23.8$^{\bullet}$	113.2 ± 82.9$^{\$}$
Fasting plasma proinsulin [pmol/L]	1.80 ± 1.10	3.30 ± 1.10$^{\bullet}$	5.43 ± 4.01$^{\$}$
Triglycerides [mmol/L]	1.11 ± 0.56	2.07 ± 0.56$^{\bullet}$	2.58 ± 0.98$^{\$\$}$
Total cholesterol [mmol/L]	5.15 ± 0.91	5.03 ± 0.91	5.00 ± 0.62
HDL-cholesterol [mmol/L]	1.32 ± 0.3	1.07 ± 0.3	0.96 ± 0.22$^{\$\$}$
LDL-cholesterol [mmol/L]	2.78 ± 0.68	2.89 ± 0.68	2.59 ± 0.76
Serum creatinine [μmol/L]	96.1 ± 8.2	97.9 ± 8.2	97.3 ± 15.3
Albumin in urine [mg/L]	5.7 ± 7.1	15.4 ± 7.1	29.3 ± 55.5
Hypertension [%]	25	47	62
Dyslipidemia [%]	44	67	77
Familial history of T2D [%]	38	60	62

Values are expressed as mean ± SD. Differences between the groups: (•) NGT vs. IGT; (#) IGT vs. T2D; ($) NGT vs. T2D; $^{\bullet,\#,\$}p < 0.05$; $^{\bullet\bullet,\#,\$\$}p < 0.01$ (Mann-Whitney).

fasting plasma glucose, insulin, proinsulin, triglycerides, and a higher waist to hip ratio (WHR), whereas HDL-cholesterol levels tended to be decreased when compared with NGT subjects. The prevalence of hypertension, dyslipidemia, and familial history of T2D was higher in IGT and T2D subjects than in controls. Table 2 presents the results of fasting tHcy, 6-hour PML-tHcy, and delta-tHcy (6-hour PML-tHcy minus fasting tHcy) determinations as well as the cobalamin and folate concentrations. Fasting tHcy, PML-tHcy, and delta tHcy showed no differences between the groups. To estimate the area under the concentration curve (AUC) the sum of tHcy concentration (Σ_{tHcy}) for the 6-hour period (fasting value and 1, 2, 4, 6 hours after methionine load) were calculated for each subject. No significant differences for Σ_{tHcy} were found between the groups. All study participants showed a rapid raise in tHcy within 1 hour after methionine load followed by a peak of plasma tHcy at 6 hours. No differences were found between the groups at any given time point after the methionine load (Fig. 1). An elevated fasting tHcy and PML-tHcy, respectively, was defined as one above the 95th percentile in controls (fasting tHcy $> 15.15\,\mu$mol/L; PML-tHcy $> 49.3\,\mu$mol/L) (Pietzsch et al., 1997). Table 2 also shows the prevalences of fasting hyperhomocysteinemia and post-methionine load hyperhomocysteinemia somewhat higher in IGT subjects than in T2D or controls. Univariate correlation analysis showed a direct relationship between fasting tHcy and PML-tHcy ($r = 0.636$; $p < 0.01$). The individuals with abnormal methionine loads and normal fasting tHcy represented only 6.8% of all subjects investigated. In addition, fasting tHcy and PML-tHcy in the total population correlated directly with creatinine (Fig. 2; $r = 0.489$; $p < 0.01$; $r = 0.339$; $p < 0.05$) and fasting proinsulin ($r = 0.395$;

Table 2. Homocysteine metabolism in IGT, T2D, and controls

	NGT	IGT	T2D
Fasting tHcy [μmol/L]	10.7 ± 4.4	13.1 ± 4.6	12.8 ± 4.0
PML-tHcy [μmol/L]	38.0 ± 9.7	46.5 ± 17.3	41.1 ± 6.8
Delta-tHcy [μmol/L]	27.3 ± 7.2	33.4 ± 13.9	28.4 ± 5.2
serum cobalamin [pg/mL]	471 ± 150	416 ± 164	396 ± 242[$]
serum folate [ng/mL]	10.4 ± 6.7	9.1 ± 5.2	9.3 ± 6.5
prevalence of fasting hyperhomocysteinemia [%]	12.5	33.3	30.1
prevalence of PML-hyperhomocysteinemia [%]	6.3	33.3	7.7

Data are expressed as means ± SD; Differences between the groups: ($) NGT vs. T2D, $p < 0.05$ (Mann-Whitney); Delta-tHcy (6-hour PML-tHcy minus fasting tHcy); fasting hyperhomocysteinemia: tHcy > 15.15μmol/L; PML-hyperhomocysteinemia: tHcy > 49.30μmol/L

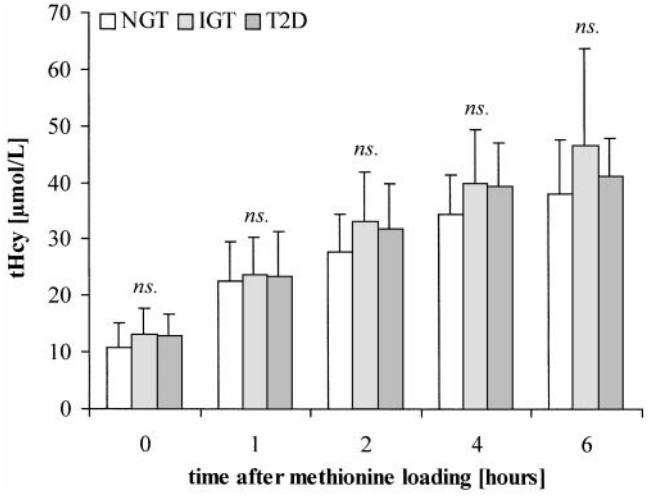

Fig. 1. tHcy concentrations before and during an oral methionine load in NGT (n = 16), IGT (n = 15), and T2D (n = 13). Means and SD are demonstrated

$p < 0.05$; $r = 0.386$; $p < 0.05$; data not shown in detail), respectively. Multiple regression analysis in the total population with homocysteine as dependent variable and insulin, proinsulin, and creatinine as independent variables showed only a direct relationship between fasting tHcy and creatinine ($p < 0.01$). There were no relationships between fasting tHcy and PML-tHcy, respectively, and indicators of an insulin resistant/hyperinsulinemic state, e.g., insulin, proinsulin or other variables of metabolic decompensation in IGT and T2D, e.g. fasting plasma glucose, HbA_{1C}, albumin (urine), triglycerides, total cholesterol, LDL- and HDL-cholesterol. Furthermore in the subjects studied fasting tHcy and PML-tHcy were not inversely related with vitamin concentrations involved in homocysteine metabolism as cofactors. However,

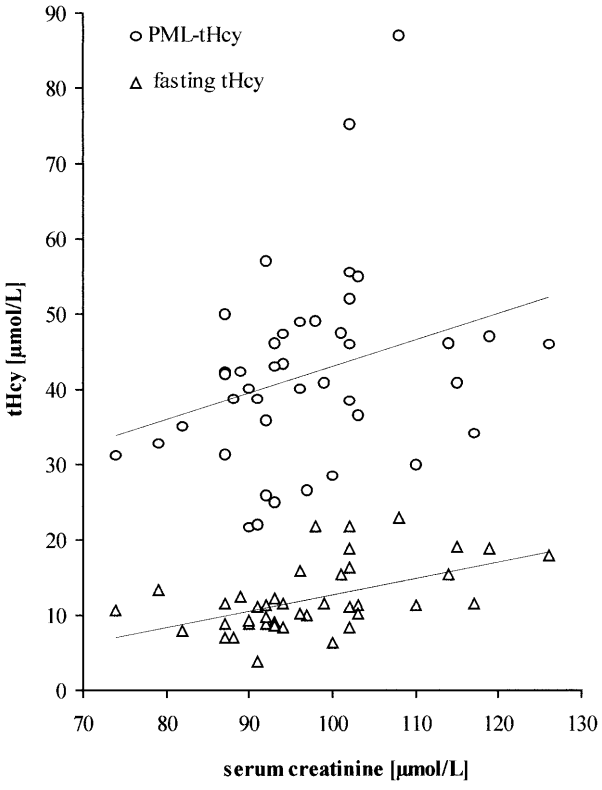

Fig. 2. Relationship between fasting tHcy and serum creatinine (triangles; $r = 0.489$; $p < 0.01$) and 6-hour PML-tHcy and serum creatinine (circles, $r = 0.339$; $p < 0.05$)

serum cobalamin levels were significantly lower in T2D compared with controls. In contrast, serum folate levels did not differ between the groups.

Discussion

The present study was designed to investigate whether the degree of glucose intolerance is a potential factor influencing homocysteine metabolism. Our data show that a dysregulation of glucose homeostasis *per se* seems not to be a risk factor for disturbed homocysteine metabolism. The degree of glucose intolerance had no influence on plasma homocysteine levels. Only serum creatinine concentration appeared to be a strong independent predictor for elevated fasting tHcy concentrations, whereas indicators of an insulin resistant/hyperinsulinemic state as well as vitamins involved in homocysteine metabolism did not. Furthermore, with respect to the strong direct correlation between fasting tHcy and PML-tHcy, and additionally the low prevalence of subjects with isolated post-methionine load hyperhomocysteinemia, determination of fasting tHcy should be adequate enough to identify prediabetic subjects at a higher risk for vascular disease due to this risk factor.

In previously published studies the impact of diabetes on plasma tHcy was controversially discussed. Hoogeveen et al. found no important differences in fasting serum tHcy levels between subjects with T2D and nondiabetic subjects (Hoogeven et al., 1998). In contrast, Araki et al. (Araki et al., 1993) and Munshi et al. (Munshi et al., 1996) measured significantly higher fasting and PML-tHcy values in subjects with T2D who also had macrovascular disease when compared with diabetics without macrovascular disease and controls, respectively. Lanfredini et al. (Lanfredini et al., 1998) found no differences between subjects with T2D and controls for fasting and PML-tHcy, whereas T2D subjects with cardiovascular complications had significantly higher fasting tHcy levels than those without. Chico et al. (Chico et al., 1998) measured significantly elevated fasting tHcy levels in T2D with high prevalence of macrovascular disease and nephropathy. Summarizing these data, diabetic subjects with late complications, i.e. macrovascular disease and/or nephropathy seem to have higher tHcy levels when compared with T2D subjects without these complications.

In contrast to other studies all of our investigated diabetic subjects were newly diagnosed and characterized by the absence of clinical signs of cardiovascular disease. Our data demonstrate that in early prediabetic and diabetic states without complications neither fasting tHcy nor PML-tHcy concentrations are significantly different to normoglycemic control subjects. However, if tHcy is elevated, estimated odd ratios (OR) for cardiovascular disease were 1.55 in IGT and 2.33 in T2D subjects per 5 μmol/L increment in fasting serum tHcy, as reported by Hoogeeven et al. (1998).

Hyperhomocysteinemia is very frequent in patients with renal failure which suggests the importance of the kidney for homocysteine metabolism. In spite of the fact that all investigated men had normal serum creatinine levels, multivariate correlation analyses showed only serum creatinine to be independently associated with fasting tHcy in the whole population. These findings are in agreement with other reports who found strong correlations between tHcy and creatinine or other markers of glomerular function in diabetics (Hultberg et al., 1991; Araki et al., 1993; Agardh et al., 1994) and non-diabetics (Arnadottir, 1996; Lussier-Cacan et al., 1996). The increment in tHcy seems to be more closely related to glomerular filtration rate (GFR) than to the increase in serum creatinine (Arnadottir et al., 1996; Wollesen et al., 1999). On the other hand, glomerular hyperfiltration is a characteristic feature of subjects with newly diagnosed T2D and subjects with IGT as reported by Nelson et al. (Nelson et al., 1999). This phenomenon represents an initial stage in the development of renal dysfunction in (pre)diabetic subjects and may explain the normal tHcy and PML-tHcy values in our subjects. In addition, Wollesen et al. found tHcy levels lower than normal in diabetics with renal hyperfiltration (Wollesen et al., 1999). Usually years after clinical manifestation of diabetes patients develop mild diabetic nephropathy with microalbuminuria and a decline of GFR (Mogensen, 1999). In our subjects we could not find any relationships between tHcy plasma concentrations and urinary albumin excretion.

In hyperinsulinemic insulin resistant patients with T2D no change in tHcy during a hyperinsulinemic-euglycemic clamp study was observed, whereas in normal subjects tHcy levels significantly decreased (Fonseca et al., 1998). No correlation has been found between the change in plasma tHcy and the glucose disposal rate or insulin in this study. The authors suggested that resistance to the effects of insulin on glucose disposal rate may be associated with resistance to the suppressive effect on tHcy levels in subjects with T2D. In animal studies, Jacobs et al. found that insulin is involved in the regulation of plasma homocysteine levels by affecting the hepatic transsulfuration pathway of homocysteine in streptozotocin-induced diabetic rats (Jacobs et al., 1998). In our study IGT and T2D subjects were characterized to be hyperinsulinemic and displayed discrete features of an insulin resistant situation. Nevertheless, we did not find any relationships between indicators of an insulin resistant/hyperinsulinemic state and fasting tHcy or PML-tHcy concentrations in multiple regression analyses.

In conclusion, an impairment of tHcy metabolism could be an important risk factor worsening the prognosis in diabetic subjects, especially in individuals with nephropathy. Prediabetic and diabetic subjects could be particularly prone to hyperhomocyst(e)inemia, probably due to renal dysfunction with a reduction in the removal of tHcy. However, in early stages of diabetes or glucose intolerance with pathophysiological features of insulin resistance and/or hyperinsulinemia homocysteine metabolism is not essentially impaired suggesting that tHcy concentrations tend to be elevated even with the progression of diabetic nephropathy.

Acknowledgements

The authors wish to thank Mrs Sigrid Nitzsche, Mrs Uta Buro, and Mrs Katja Fücker for their expert technical assistance.

References

Agardh CD, Agardh E, Andersson A, Hultberg B (1994) Lack of association between plasma homocysteine levels and microangiopathy in type 1 diabetes mellitus. Scand J Clin Lab Invest 54: 637–641

Araki A, Sako Y, Ito H (1993) Plasma homocysteine concentrations in Japanese patients with non-insulin-dependent diabetes mellitus: effect of parenteral methylcobalamin treatment. Atherosclerosis 103: 149–157

Arnadottir M, Hultberg B, Nilsson-Ehle P, Thysell H (1996) The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. Scand J Clin Lab Invest 56: 41–46

Chasan-Taber KL, Selhub J, Rosenberg IH, Malinow MR, Terry P, Tishler PV, Willett WD, Hennekens CH, Stampfer MJ (1996) A prospective study of folate and vitamin B_6 and risk of myocardial infarction in US physicans. J Am Coll Nutr 15: 136–143

Chico A, Perez A, Cordoba A, Arcelus R, Carreras G, de Leiva A, Gonzalez-Sastre F, Blanco-Vaca F (1998) 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

den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, Vandenbroucke JP, Rosendaal FR (1996) Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med 334: 759–762

Dudman NP, Guo XW, Gordon RB, Dawson PA, Wilcken DE (1996) Human homocysteine catabolism: three major pathways and their relevance to development of arterial occlusive disease. J Nutr 126: 1295S–3000S

Duell PB, Malinow MR (1997) Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease. Curr Opin Lipidol 8: 28–34

Edelstein SL, Knowler WC, Bain RP, Andres R, Barrett-Connor EL, Dowse GK, Haffner SM, Pettitt DJ, Sorkin JD, Muller DC, Collins VR, Hamman RF (1997) Predictors of progression from impaired glucose tolerance to NIDDM: an analysis of six prospective studies. Diabetes 46: 701–710

Falcon CR, Cattaneo M, Panzeri D, Martinelli I, Mannucci PM (1994) High prevalence of hyperhomocysteinemia with juvenile venous thrombosis. Arterioscler Thromb Vasc Biol 14: 1080–1083

Fonseca VA, Mudaliar S, Schmidt B, Fink LM, Kern PA, Henry RR (1998) Plasma homocysteine concentrations are regulated by acute hyperinsulinemia in nondiabetic but not type 2 diabetic subjects. Metabolism 47: 686–689

Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ (1998) Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: results from the third national health and nutrition examination survey, 1988–1994. Stroke 29: 2473–2477

Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, Medrano JS, Condito M, Evans AE, Andria G (1997) Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 277: 1775–1781

Haffner SM (1997) Impaired glucose tolerance – is it relevant for cardiovascular disease? Diabetologia 40: S138–S140

Hoogeveen EK, Kostense PJ, Beks PJ, Mackaay AJ, Jakobs C, Bouter LM, Heine RJ, Stehouwer CD (1998) 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 18: 133–138

Hultberg B, Agardh E, Andersson A, Brattstrom L, Isaksson A, Israelsson B, Agardh CD (1991) Increased levels of plasma homocysteine are associated with nephropathy, but not severe retinopathy in type 1 diabetes mellitus. Scand J Clin Lab Invest 51: 277–282

Jacobs RL, House JD, Brosnan ME, Brosnan JT (1998) Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. Diabetes 47: 1967–1970

Lanfredini M, Fiorina P, Peca MG, Veronelli A, Mello A, Astorri E, Dall'Aglio P, Craveri A (1998) Fasting and post-methionine load homocyst(e)ine values are correlated with microalbuminuria and could contribute to worsening vascular damage in non-insulin-dependent diabetes mellitus patients. Metabolism 47: 915–921

Lussier-Cacan S, Xhignesse M, Piolot A, Selhub J, Davignon J, Genest J (1996) Plasma total homocysteine in healthy subjects: sex-specific relation with biological traits. Am J Clin Nutr 64: 587–593

Marshall SM (1991) Screening for microalbuminuria: which measurement? Diabetic Med 8: 706–711

Mogensen CE (1999) Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. Diabetologia 42: 263–285

Mudd SH, Levy HL, Skosby F (1995) Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill Inc, New York, pp 1279–1327

Munshi MN, Stone A, Fink L, Fonseca V (1996) Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. Metabolism 45: 133–135

Nagai Y, Takamura T, Nohara E, Yamashita H, Kobayashi K-I (1999) Acute hyperinsulinemia reduces plasma concentrations of homocysteine in healthy men. Diabetes Care 22: 1004

Nelson RG, Tan M, Beck GJ, Bennett PH, Knowler WC, Mitch WE, Blouch K, Myers BD (1999) Changing glomerular filtration with progression from impaired glucose tolerance to Type II diabetes mellitus. Diabetologia 42: 90–93

Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE (1997) Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 337: 230–236

Pietzsch J, Julius U, Hanefeld M (1997) Rapid determination of total homocysteine in human plasma by using N(O,S)-ethoxycarbonyl ethyl ester derivatives and gas chromatography-mass spectrometry. Clin Chem 43: 2001–2004

Pietzsch J, Pixa A (1998) Determination of total homocysteine. Clin Chem 44: 1781–1782

Robinson K, Arheart K, Refsum H, Brattstrom L, Boers G, Ueland P, Rubba P, Palma-Reis R, Meleady R, Daly L, Witteman J, Graham I (1998) The European COMAC Group: Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. Circulation 97: 437–443

Selhub J, Miller JW (1992) The pathogenesis of hyperhomocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 55: 131–138

Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ, O'Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH (1995) Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med 332: 286–291

Taylor LM, Moneta GL, Sexton GJ, Schuff RA, Porter JM (1999) Prospective study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. J Vasc Surg 29: 8–19

Ubbink JB (1997) The role of vitamins in the pathogenesis and treatment of hyperhomocyst(e)inaemia. J Inher Metab 20: 316–325

van den Berg M, Stehouwer CDA, Bierdrager E, Rauwerda JA (1996) Plasma homocysteine and severity of atherosclerosis in young patients with lower-limb atherosclerotic disease. Arterioscler Thromb Vasc Biol 16: 165–171

Wollesen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C (1999) Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. Kidney Int 55: 1028–1035

Yoo JH, Chung CS, Kang SS (1998) Relation of plasma homocyst(e)ine to cerebral infarction and cerebral atherosclerosis. Stroke 29: 2478–2483

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Received May 11, 1999