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Homocysteine and chronic diabetic complications

HOMOCYSTEINE

The word "homocysteine" was used first by Du Vigneaud and his coworkers over 65 years ago when they discovered this compound and provided a definitive proof that it had the structure of a thiol 4-carbon α amino acid: $HSCH_2CH_2CH(NH_2)COOH$. The abbreviation "tHcy" refers to the totality of homocysteine present after the quantitative reductive cleavage of all disulfide bonds in a sample. The abbreviation "fHcy" may be used to signify free Hcy (alternatively termed non-protein bound Hcy or acid soluble Hcy). The abbreviation "bHcy" designates bound homocysteine, i.e. homocysteine bound to protein by disulfide linkage (11).

Homocysteine, a sulphur-containing amino acid, is a product of methionine metabolism. It lies at the intersection of two major pathways: remethylation and trans-sulphuration. During the first pathway, homocysteine is remethylated in a process that requires methyltetrahydrofolate as a cosubstrate (methyl donor). This chain of reactions requires an adequate supply of folate and vitamin B_{12} and functional integrity of the enzymes methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MS). In the reaction of trans-sulphuration, homocysteine is irreversibly transformed to cystathionine in a process requiring the vitamin B_6 derivative (pyridoxal 5-phosphate) as a cofactor. This reaction is catabolized by cystathionine β-synthase (CBS).

The plasma concentration of tHcy is determined by several factors, both genetic and acquired. The most important is: (1) genetic defects in the genes encoding for enzymes of homocysteine metabolism; CBS, MTHFR, or any of the enzymes participating in the synthesis of methylated vitamin B_{12}. Of these disorders, defects in the gene encoding for CBS, lead to the classic form of congenital homocysteinuria, which leads to extreme plasma tHcy level and homocysteinuria in homozygous patients or mild-moderate hyperhomocysteinemia in heterozygous subjects. Less severe hyperhomocysteinemia may also be due to a genetic variant of an enzyme, such the thermolabile variant, a mutation in the gene encoding MTHFR that has recently been described. This mutation (C677T) results in the substitution of an alanine residue by valine, rendering the enzyme both thermolabile and less active, which leads to an increase in tHcy levels especially in low folate conditions (2). Aging, which could lead to an increase in levels of plasma tHcy, perhaps as a result of an age-dependent reduction of enzymatic metabolism or alteration of the renal function (3). Gender: men have higher tHcy levels than women, probably due to greater muscle mass or different hormone patterns, levels in post-menopausal women approximate those in men (4). Deficiency of nutritional factors such as folate, vitamin B_{12} and vitamin B_6, which appear to have a close negative correlation with tHcy levels (5). Finally, renal dysfunction, which is intimately related to hyperhomocysteinemia (3).

HOMOCYSTEINE LEVELS IN DIABETIC PATIENTS

Consensus regarding plasma tHcy level in diabetes has not been achieved. Probably the impairment of renal function appears to be the principal modulator of the homocysteine levels. The homocysteine concentration is different in patients with different chronic diabetic complications.

There are two groups of chronic diabetic complications: vascular lesions (*angiopathia diabetica*) and nerve lesions (*neuropathia diabetica*). *Angiopathia diabetica* is divided into microangiopathy (retinopathy and nephropathy) and macroangiopathy (coronary artery disease, cerebral stroke, peripheral artery disease).

A few studies found no significant differences in fasting plasma tHcy levels between patients with type 2 diabetes and healthy subjects (8). The other showed that tHcy concentration is low or normal in insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) patients, except when nephropathy or impaired renal clearance is present (3). Hofmann et al. and Chico et al. in contrast found elevated tHcy in NIDDM patients, but in both studies, the vast majority of diabetic patients with fasting elevated tHcy presented nephropathy (3,5,12).

In patients with diabetes mellitus type 1, microvascular and macrovascular complications and neuropathy are found to be increased in those with hyperhomocysteinemia. In patients with diabetes mellitus type 2 a higher prevalence of macrovascular complications in diabetic patients with hyperhomocysteinemia is associated with a higher prevalence of renal disease. Agullo-Ortuno et al. found a relationship between high Hcy levels and prevalence of macroangiopathy, retinopathy and nephropathy in the type 1 diabetic patients, which has not been observed in type 2 diabetic patients. As a result, they consider plasmatic Hcy a complication-risk indicator in type 1 DM, and they recommend its use together with already established biochemical parameters in the control of the evolution of the disease. Moreover, patients with hyperhomocysteinemia have hypertension and dyslipemia (2). Multivariate regression analyses have shown an independent relationship between homocysteine and macrovascular complications (14).

Abnormally low levels of plasma tHcy might also be an effect of abnormal homocysteine metabolism in diabetics due to endogenous or exogenous factors. Since insulin has profound effects on amino acid metabolism, it has been suggested that an acquired defect in homocysteine metabolism may occur in diabetic patients. Some authors studied the effect of acute hyperinsulinemia on plasma tHcy concentrations and demonstrated that plasma tHcy concentrations were not regulated by acute hyperinsulinemia. Additionally, the other authors found that there was no indication that insulin or sulfonylureas alter tHcy metabolism. In contrast, metformin may induce vitamin B_{12} malabsorbtion and thereby increase the serum tHcy level. Nevertheless, some authors have not found an important effect of metformin on serum tHcy levels in subjects with NIDDM (3).

HOMOCYSTEINE AND NEPHROPATHY

Homocysteine levels in human diabetic patients appear to depend on the presence or absence of nephropathy. Diabetic patients with elevated creatinine levels (an indicator of kidney dysfunction) tend to exhibit an increase in plasma homocysteine (8,12,13). This is consistent with studies that show that the kidney is an important organ in the removal of plasma homocysteine. On the other hand, type 1 patients with normal creatinine levels have decreased plasma homocysteine. The reason for such decreased homocysteine levels is unknown, but the regulation of homocysteine metabolism by insulin could afford an explanation. The decrease in plasma homocysteine levels was associated with an increase in the enzymatic activities of the hepatic transsulfuration pathway. Normalization of these parameters after insulin treatment emphasizes the importance of insulin in homocysteine metabolism. The mode of insulin action requires further study (13).

Microalbuminuria, an early marker of endothelial dysfunction and a strong indicator of the risk of future renal dysfunction, as well as nephropathy, has been reported to be associated with plasma tHcy level in patients with diabetes (5,8,12). However, Emoto et al. recently found no association of microalbuminuria with plasma tHcy levels in patients with type 2 diabetes (8). Patients with type 2 diabetes with proteinuria more commonly have hyperhomocysteinemia than control subjects, and plasma tHcy levels in subjects with renal failure were markedly increased (5).

HOMOCYSTEINE AND RETINOPATHY

An increased plasma homocysteine level is an important risk factor for vascular disease in the general population. However, the role of hyperhomocysteinemia in the development of type 2 diabetic retinopathy is still unknown (14). Diabetic retinopathy is a major vascular complication leading frequently to blindness (16).

Some authors investigated the association of the methylenetetrahydrofolate reductase (MTHFR) gene polymorphism with diabetic retinopathy in patients with NIDDM (16) and IDDM (18). Neugebauer et al. found that the frequency of the mutated allele causing an alanine to valine substitution in MTHFR was significantly higher in the patients with NIDDM and diabetic retinopathy compared with the NIDDM patients without retinopathy. Change of MTHFR genotype may contribute to the development of diabetic retinopathy and possibly help explain the concomitant progression of other diabetic complications in patients with diabetic retinopathy (16). Vacarro et al. showed that the allelic frequency of the C667T mutation in MTHFR gene was similar in the group of patients with no retinopathy and nonproliferative diabetic retinopathy (NPDR), but was significantly higher in the patients with proliferative diabetic retinopathy (PDR) as compared with those with no retinopathy. The authors suggest a relationship between PDR and plasma homocysteine levels independent of some obvious confounders and coexisting conditions associated with the elevation of plasma homocysteine or retinopathy (18). Based on these data, a role for homocysteine in the development of PDR can be hypothesized. This hypothesis is also supported by the results of *in vitro* experiments showing a synergistic effect of plasma homocysteine and hyperglycemia in inducing cell damage in the vascular endothelium (12).

Abdella et al. have not found any association between hyperhomocysteinemia and retinopathy (1). But some authors suggested that hyperhomocysteinemia maybe a risk factor for the development and progression of type 2 diabetic retinopathy. They found a significantly higher plasma tHcy level in PDR group than in background diabetic retinopathy (BDR) or nonproliferative diabetic retinopathy group (18).

HOMOCYSTEINE AND NEUROPATHY

The data indicate that homocysteine is independently associated with the prevalence of diabetic neuropathy in a collective of type 2 diabetic patients. Hyperhomocysteinemia and non-insulin-dependent diabetes mellitus (NIDDM) are both associated with premature vascular disease. Microvascular ischaemia may be a risk factor for diabetic sensorimotor peripheral neuropathy (DSPN) and diabetic autonomic neuropathy (DAN). Cohen et al. suggested that hyperhomocysteinemia may be a risk factor for DAN but not for DSPN. This relationship may be related to differential small fiber injury. Further studies are needed to investigate this relationship between tHcy and diabetic autonomic neuropathy specifically whether treatment of hyperhomocysteinemia may modify diabetic autonomic neuropathy (7). Abdella et al. have not found any association between hyperhomocysteinemia and neuropathy (1). A larger, prospective study would be desirable to clarify the role of homocysteine in the pathogenesis of diabetic neuropathy.

HOMOCYSTEINE AND CARDIOVASCULAR DISEASE

Patients with diabetes mellitus have two- to sixfold increase in the prevalence of cardiovascular disease compared to nondiabetic subjects. Epidemiological data show that diabetes mellitus is synergic with other conventional risk factors such as lipids, smoking and hypertension (3). Over the last decade, other multiple factors have been identified as potential contributors for cardiovascular disease. As shown by observational studies, an increase in total plasma homocysteine is one of these new factors, along with systemic inflammation, coagulation factors, oxidative stress, ventricular hypertrophy and various dyslipidaemia subtypes (9).

Homocysteine is a risk factor in the patogenesis of atherosclerosis, which is the most frequent cause of coronary heart disease, peripheral arterial disease, and the main cause of stroke (4). High plasma homocysteine level has been associated with increased risk for coronary heart disease (CHD) events in nondiabetic individuals, especially in those with previously diagnosed CHD (6, 17). In persons with type 2 diabetes mellitus, the association between homocysteine level and cardiovascular disease may be stronger than that in nondiabetic individuals, but no large prospective studies have examined the relationship between homocysteine level and CHD mortality in persons with type 2 diabetes (17). The prevalence and secondary cardiovascular risk is higher in patients with diabetes type 2 than in those with diabetes type 1 (14). Furthermore, it also suggests that DM may interact with homocysteine conferring a multiplicative risk of CHD (3).

In type 2 diabetes, homocysteine was associated with the angiographic severity of peripheral arterial disease (PAD), but neither the genotypes nor vitamin levels contributed to this association (6).

Homocysteine generates oxygen radicals (superoxide anion and hydrogen peroxide) that are known to produce vasoconstriction. Hypertension is a common problem in individuals with diabetes mellitus. It is possible that hypertension in diabetic patients may be due to increased levels of plasma homocysteine. The studies indicated that the level of plasma homocysteine is elevated in hypertensive diabetic patients. Hyperhomocysteinemia may be involved in the induction and sustaining of hypertension in diabetic patients (15). Van Guldener et al. tried to explain the relationship between homocysteine and blood pressure: increased arterial stiffness, endothelial dysfunction with decreased availability of nitric oxide, low folate status, and insulin resistance. But, so far, no evidence has been provided that these mechanisms are operative in humans (10).

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SUMMARY

Homocysteine is a sulphur aminoacid with a free thiol group which is not present in dietary protein. This aminoacid is an intermediate in the metabolism of methionine. The plasma concentration of tHcy is determined by several factors: genetic defects, aging, gender, deficiency of folate, vitamin B_{12} and vitamin B_6, renal dysfunction. The purpose of this article was to explore the relationship between plasma levels of homocysteine and chronic diabetic complications. Consensus regarding plasma tHcy level in diabetes has not been achieved. The homocysteine concentration is different in patients with different chronic diabetic complications. Homocysteine level in human diabetic patients appear to depend on the presence or absence of nephropathy. Diabetic patients with elevated creatinine levels tend to exhibit an increase in plasma homocysteine. An elevated plasma homocysteine level is an important risk factor for vascular disease. Some authors suggest that hyperhomocysteinemia is a risk factor for the development and progression of diabetic retinopathy, neuropathy and cardiovascular diseases. The studies found a higher plasma tHcy level in proliferative and nonproliferative diabetic retinopathy than in the control group. In diabetic neuropathy hyperhomocysteinemia is probably a risk factor for diabetic autonomic neuropathy but not for diabetic sensorimotor peripheral neuropathy. Homocysteine is a risk factor in the patogenesis of atherosclerosis, which is the most frequent cause of coronary heart disease, peripheral arterial disease, and the main cause of stroke. Patients with diabetes mellitus have two- to sixfold increase in the cardiovascular disease incidence rate compared to nondiabetic subjects. The data show that hyperhomocysteinemia may be a new risk factor for cardiovascular disease. Nevertheless, it is necesary to perform more prospective and intervention studies to clarify the independent risk of homocysteine and thus easy alternative treatments.

Homocysteina a przewlekłe powikłania cukrzycy

Homocysteina należy do aminokwasów zawierających siarkę, z wolną grupą tiolową, która nie jest obecna w białku dostarczanym z pożywieniem. Aminokwas ten jest związkiem pośrednim w metabolizmie metioniny. Poziom homocysteiny w osoczu zależy od wielu czynników: defektów genetycznych, wieku, płci, niedoboru kwasu foliowego, witaminy B_{12} i witaminy B_6, niewydolności nerek. Celem pracy była ocena związku pomiędzy poziomem homocysteiny w osoczu i przewlekłymi powikłaniami cukrzycy. Jak dotąd nie został osiągnięty konsensus dotyczący poziomu homocysteiny w osoczu. Zawartość homocysteiny w osoczu jest inna w zależności od różnych przewlekłych powikłań cukrzycy. Poziom homocysteiny u pacjentów z cukrzycą jest inny w zależności od obecności bądź nieobecności nefropatii. Pacjenci z cukrzycą z wysokim poziomem kreatyniny mają tendencję do wysokiego poziomu homocysteiny. Podwyższony poziom homocysteiny jest natomiast ważnym czynnikiem ryzyka chorób naczyniowych. Niektórzy autorzy suge-

rują, że hiperhomocysteinemia jest czynnikiem ryzyka rozwoju i progresji retinopatii cukrzycowej, neuropatii cukrzycowej i chorób sercowo-naczyniowych. Badania wykazały wyższy poziom homocysteiny w proliferacyjnej i nieproliferacyjnej retinopatii cukrzycowej niż w grupie kontrolnej. W neuropatii cukrzycowej hiperhomocysteinemia jest prawdopodobnie czynnikiem ryzyka cukrzycowej neuropatii autonomicznej, ale nie obwodowej neuropatii czuciowo-ruchowej. Homocysteina jest czynnikiem ryzyka w patogenezie miażdżycy tętnic, która jest najczęstszą przyczyną choroby wieńcowej, chorób tętnic obwodowych i główną przyczyną udaru mózgu. Pacjenci z cukrzycą mają od 2- do 6-krotnie zwiększoną zachorowalność na choroby sercowo-naczyniowe w porównaniu z osobami bez cukrzycy. Dane wskazują na to, że hiperhomocysteinemia może być nowym czynnikiem ryzyka chorób sercowo-naczyniowych. Konieczne jest jednak przeprowadzenie bardziej prospektywnych i dokładnych badań, aby wyjaśnić rolę homocysteiny i w ten sposób ocenić przydatność jej stosowania w leczeniu.