Homocysteine, nutritional status and insulin in renal transplant recipients

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Abstract
Background. Hyperhomocysteinemia is an independent risk factor for the development of cardiovascular conditions in chronic stable renal-transplant recipients (RTR). Major determinants of plasma total homocysteine (tHcy) in RTR are renal function and folate levels. The data dealing with the possible regulation of the tHcy metabolism by insulin and nutritional status is conflicting in non-transplant populations.

Methods. We examined the relationship between tHcy, insulin and nutritional status in 103 chronic, stable RTR. Demographic, clinical, and biochemical parameters were assessed for each patient.

Results. Mean tHcy was $19.7 \pm 9.2 \text{\mu mol/l}$ (range 8.6–53). The tHcy was strongly related to creatinine clearance ($r_s = 0.55$, $P < 0.0001$). Fasting tHcy levels were negatively related to folate concentrations ($r = -0.32$, $P = 0.01$). There was a positive relationship between tHcy and LDL-cholesterol ($r = 0.34$, $P = 0.03$) and a significant negative correlation between tHcy and insulin ($r = -0.38$, $P = 0.01$). Fasting tHcy concentrations were significantly higher in the lower quartile of insulin concentration than in the upper quartile (27.7 ± 12.7 vs 15.9 ± 9.5, $P = 0.01$). In multivariate analysis, tHcy was associated with serum creatinine ($P = 0.001$), insulin ($P = 0.02$) and folate concentration ($P = 0.03$). Patients with the highest IGF-1 concentration had lower tHcy than patients with the lowest IGF-1 concentration (16.8 ± 5.7 vs 23.3 ± 11 \mu mol/l, $P = 0.01$).

Conclusion. We observed an inverse relationship between insulin and tHcy in chronic, stable RTR.

Keywords: folate concentrations; hyperhomocysteinemia; IGF-1; insulin; nutritional status; renal transplant patients; total homocysteine


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Original Article

Introduction

Homocysteine is a sulphur amino acid formed from methionine during transmethylation, and is either salvaged to methionine by a folate- and cobalamin-dependent re-methylation reaction or directed toward degradation by the vitamin B_{12}-dependent enzyme cystathionine $\beta$-synthase (CBS) [1]. Large studies have demonstrated that moderate hyperhomocysteinemia is an independent risk factor for cardiovascular disease [2]. Stable renal-transplant recipients (RTR) have disproportionately high rates of arteriosclerotic processes [3], and recent reports provide controlled evidence that clinically stable RTR have a high prevalence of hyperhomocysteinaemia [4]. Moreover, our group recently demonstrated that elevated fasting tHcy is an independent risk factor for the development of cardiovascular events in chronic stable RTR [5]. Major determinants of plasma tHcy in RTR are renal function and folate levels, and to a lesser extent vitamin B_{12} and B_{6} concentrations [6]. In non-transplant populations, conflicting data exist as to a possible regulation of the homocysteine metabolism by insulin [7–9]. Nevertheless, there is no report on a relationship between insulin and homocysteine metabolism in RTR. In this study we examined the possible association between tHcy, insulin and nutritional status in RTR.

Subjects and methods

Subjects

The study involved 103 consecutive, chronic, stable RTR. None of the patients received vitamin B supplements, antidiabetic drugs or insulin, and neither did they receive drugs influencing tHcy metabolism (fibrates, trimethoprim).

Demographic (age and gender), clinical (time to transplantation, current immunosuppressive drugs, past history of cardiovascular disease, weight and height) and biological
parameters (serum creatinine concentration, calculated creatinine clearance (Gault–Cockcroft formula), urinary protein excretion, tHcy, folate, cobalamin, pyridoxal-5'-phosphate, insulin, C-peptide, IGF-1, blood glucose and lipid profile) were assessed on the same day for each patient.

**Anthropometric measures**

Weight and height were recorded. Body mass index (BMI) was calculated according to the formula weight (kg)/height² (m²).

**Laboratory methods**

**Homocysteine.** Total plasma Hcy was measured as follows. Blood samples were drawn after an overnight fast. Each blood sample was centrifuged within 15 min of venepuncture, and plasma stored frozen at −20°C. Hcy concentration, the sum of the acid-soluble (that is reduced Hcy, homocystine, disulphide and homocysteine-cysteine mixed disulphide) and protein-bound moieties, was measured by high-performance liquid chromatography (HPLC). This assay includes the following steps: reduction of the sample with tri-n-butylphosphine, precipitations of proteins, alkalinization of the supernatant with sodium borate, derivitization with 7-fluoro-2-oxa-1,3 diazole-4 sulphonate, followed by 8-aminonaphthalene-1,3,6-trisulphonic acid and HPLC separation with fluorescence detection. The normal value of plasma Hcy concentration ranged from 7 to 15 μmol/l. The precision of the assay depends on a coefficient variation <3%.

**Cobalamin, folate, pyridoxal 5'-phosphate.** Cobalamin and folate in plasma were determined by radioassay using purified intrinsic factor and purified folate-binding protein. The normal values for cobalamin and folate were respectively 250 pg/ml and 10±7 ng/ml. Pyridoxal 5'-phosphate levels were measured by HPLC. The normal values ranged from 52 to 145 nmol/l.

**Lipid profile.** Serum total cholesterol, triglyceride and glucose levels were measured by automatic enzymatic methods. HDL was determined after precipitation of the lipoproteins by dextran sulfate. LDL cholesterol was calculated using the Friedewald formula.

**Insulin, C-peptide, IGF-1.** Serum fasting insulin and C-peptide concentrations were measured with AIA-PACK, AIA-PACK IRI and AIA-PACK C-peptide respectively (normal range 6–20 μU/ml for insulin and 0.5–2.2 ng/ml for C-peptide). IGF-1 was measured by a two-site immunoenzymometric assay (IGF-1 IRMA Immunotech). Normal values ranged between 107 and 310 μg/l.

**Statistical methods**

Results are expressed as mean±SD. Triglyceride, insulin, tHcy and IGF-1 were not normally distributed. A Wilcoxon test was used to compare parameters between groups. The Spearman–Pearson test was used to evaluate the relationship between numeric variables. We selected variables with a P value ≤0.05. The selected variables were included in a multivariate model. The significance of variables was tested by multiple regression analysis based on the results of the bivariate correlations. The independence of variables to contribute to the variance of the dependent variable (tHcy) was tested by stepwise multiple regression models. P values <0.05 were considered to be significant. Calculations were performed using Statview 5.

**Results**

**Study population**

The characteristics of the study population are shown in Table 1. All the patients received cyclosporin and prednisone. Eighty-one patients received azathioprine and 15 received mycophenolate mofetil.

**Homocysteine, serum creatinine and vitamin B status**

Fasting tHcy was above normal values in 78% of the study population. The mean tHcy was 19.7±9.2 μmol/l (range 8.6–53). Mean folate concentration was in the low normal range (6.8±3.8 ng/ml) whereas both cobalamin (424±221 pg/ml) and vitamin B₆ (142±41 nmol/l) concentrations were normal.

The tHcy was closely related to creatinine clearance (r=0.55, P<0.0001). Fasting tHcy levels were negatively related to folate concentrations (r=-0.32, P=0.01). There was no relationship between tHcy and cobalamin, or tHcy and vitamin B₆.

**Homocysteine and nutritional status**

Mean BMI was 25.4±4.9 kg/m². Patients with BMIs above the median value (25.6 kg/m²) had tHcy values similar to those with BMIs below the median value. The tHcy did not correlate with either albumin (r=0.20, P=0.1) or BMI (r=-0.15, P=0.26). There was no relationship between tHcy and IGF-1 (r=-0.24, P=0.09). Nevertheless, tHcy levels were significantly lower in patients with IGF-1 concentrations above the median than in patients with IGF-1 concentrations under the median (16.8±5.7 vs

**Table 1.** Demographic, clinical and biological characteristics of the study population (n=103)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48±10</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>69/34</td>
</tr>
<tr>
<td>Transplant duration (months)</td>
<td>86±39</td>
</tr>
<tr>
<td>BMI</td>
<td>25.5±4.8</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>54±16</td>
</tr>
<tr>
<td>Urinary protein excretion (g/day)</td>
<td>0.2±0.3</td>
</tr>
<tr>
<td>LDL (g/l)</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>HDL (g/l)</td>
<td>0.6±0.21</td>
</tr>
<tr>
<td>Triglyceride (g/l)</td>
<td>1.54±0.79</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>5.4±1.1</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>9.6±8</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>197±76</td>
</tr>
<tr>
<td>Folate (ng/ml)</td>
<td>6.8±3.8</td>
</tr>
<tr>
<td>B₁₂ (pg/ml)</td>
<td>424±221</td>
</tr>
<tr>
<td>B₆ (nmol/l)</td>
<td>142±41</td>
</tr>
<tr>
<td>tHcy (μmol/l)</td>
<td>19.7±9.2</td>
</tr>
</tbody>
</table>

Mean BMI was 25.4±4.9 kg/m². Patients with BMIs above the median value (25.6 kg/m²) had tHcy values similar to those with BMIs below the median value. The tHcy did not correlate with either albumin (r=0.20, P=0.1) or BMI (r=-0.15, P=0.26). There was no relationship between tHcy and IGF-1 (r=-0.24, P=0.09). Nevertheless, tHcy levels were significantly lower in patients with IGF-1 concentrations above the median than in patients with IGF-1 concentrations under the median (16.8±5.7 vs
23.3 ± 11 μmol/l, \( P = 0.01 \)). Renal function and folate concentrations were not related to a patient’s IGF-1 level.

**Homocysteine and insulin**

We found a significant negative correlation between tHcy and insulin \((r = -0.38, P = 0.01)\) (Figure 1). Fasting tHcy concentrations were significantly higher in the lower quartile of insulin concentration than in the upper quartile (27.7 ± 12.7 vs 15.9 ± 9.5, \( P = 0.01 \)).

**Homocysteine and lipid profile**

We found a positive relationship between tHcy and LDL-cholesterol \((r = 0.34, P = 0.03)\). This relationship was explained by a significant inverse relationship between LDL-cholesterol and folate concentration \((r = -0.45, P = 0.01)\). The tHcy was related neither to HDL nor to triglyceride.

Variables significantly associated with tHcy (creatinine clearance, folate, insulin and LDL) were entered into the multivariate model. In multivariate analysis, tHcy was associated with creatinine clearance \((P = 0.003)\), folate concentration \((P = 0.03)\) and insulin \((P = 0.02)\).

**Discussion**

The main outcome of our study is the finding of an inverse relationship between tHcy and insulin in RTR. To our knowledge, such a relationship has never been reported in this population. The data on the possible regulation of the tHcy metabolism by insulin is conflicting. It is likely that differences in study populations, especially with respect to insulin resistance, explain the discrepancies between the results. There is some evidence that insulin may decrease tHcy levels. Fonseca et al. [9], using a hyperinsulinaemic–euglycemic clamp, reported that acute hyperinsulinaemia decreases plasma tHcy in non-diabetic but not in type 2 diabetic subjects. These results were confirmed by Nagay et al. [10] who further demonstrated that insulin decreased plasma tHcy in a dose-dependent manner. More recently, Drzewoski et al. [7] also reported an inverse correlation between plasma insulin and tHcy concentrations. Finally, a recent cross-sectional study of non-diabetic subjects demonstrated a significant negative correlation between tHcy and fasting insulin [11]. Interestingly, it has been reported that tHcy significantly decreased in the post-prandial period [12] and that this effect might be mediated by post-prandial insulin secretion.

In contrast, other studies have suggested that insulin might increase tHcy concentrations. Gallistl et al. [8] found a positive relationship between insulin and tHcy concentrations in obese children and adolescents, suggesting that fat-mass associated hyperinsulism may contribute to the impairment of homocysteine metabolism in childhood obesity. Rasmussen et al. [13] also reported a positive relationship between tHcy and BMI, a correlate of hyperinsulinemia and insulin resistance. Nevertheless, it must be noted that hyperinsulinaemia found in insulin resistance is the result of ineffective insulin action. In insulin-resistant patients, the relative defect in insulin may lead to increased tHcy levels [14]. Therefore, we could consider our findings to be in agreement with those results.

Few studies have been conducted in animals. Jacobs et al. [15] first demonstrated an increased activity of trans-sulphuration enzymes and subsequent decreased homocysteine levels in rats with streptozotocin-induced diabetes. More recently, Fonseca et al. [16] examined the effects of hyperinsulinaemia on homocysteine metabolism in rats made insulin resistant. CBS activity was significantly lower and 5,10-methylene tetrahydrofolate reductase (MTHFR) activity was significantly elevated in insulin-resistant rats compared to controls. Fasting plasma insulin correlated significantly and positively with plasma homocysteine and MTHFR activity and negatively with CBS activity. Thus the authors concluded that insulin may be directly involved in the regulation of homocysteine metabolism. Nevertheless, these results are difficult to interpret. Indeed, the coexistence of hyperinsulinaemia and insulin resistance, which is a state of relative insulin deficiency, hampers the conception of a causal relationship between tHcy and insulin. Moreover, the exact mechanism and importance of insulin, insulin resistance and blood glucose in the metabolism of tHcy is difficult to assess, since the same group recently reported that, in an in vitro model, insulin decreased both CBS and MTHFR activities, while glucose increased CBS activity and decreased MTHFR activity [17].

There are complex interactions between nutrition and tHcy metabolism. Lifestyle factors and dietary patterns strongly influence tHcy concentrations, probably through vitamin B intake [18,19], and possibly through obesity-induced insulin resistance [8,9]. In our study, we found lower tHcy levels in patients with the highest IGF-1 concentrations. This difference was observed despite similar renal function, folate levels and insulin concentrations in the two groups. IGF-1 strongly reflects protein nutrition. Some studies have reported higher tHcy levels in vegetarian subjects [19]. This increase in tHcy was primarily due to lower cobalamin levels. The similarity of vitamin B12 concentrations strongly reflects protein nutrition. Some studies have also demonstrated that tHcy is closely related to renal function [21]. Nevertheless, a relationship between renal function, tHcy and insulin is unlikely,
for we have observed no relationship between insulin levels and creatinine clearance. Moreover, both creatinine clearance and insulin were independent determinants of tHcy in the multivariate analysis.

To conclude, we found a negative relationship between insulin and tHcy in chronic, stable RTR. Further studies are required in this population to clarify the roles of insulin and IGF-1 in tHcy metabolism.

References


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