



## Effect of Folate, Vitamin B<sub>6</sub>, and Vitamin B<sub>12</sub> Intake and *MTHFR* C677T Polymorphism on Homocysteine Concentrations of Renal Transplant Recipients

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### ABSTRACT

Plasma hyperhomocysteinemia (HHcy) is considered a risk factor for chronic allograft dysfunction (CAD), the main cause of functional loss in transplant recipients. Genetic polymorphisms that alter enzymes involved in homocysteine (Hcy) metabolism, such as methylenetetrahydrofolate reductase (*MTHFR*), and vitamin deficiency can result in HHcy. The objectives of this study were to investigate the relationship between HHcy and CAD development, and to evaluate the effect of intake of folate and vitamins B<sub>6</sub> and B<sub>12</sub> as well as *MTHFR* C677T polymorphism on Hcy concentrations. Ninety-eight renal transplant recipients including 48 showing CAD and 50 with normal renal function (NRF), were included in this cross-sectional study. Peripheral blood samples were collected for plasma Hcy quantification by liquid chromatography/sequential mass spectrometry (LC-MS/MS), and for *MTHFR* polymorphism analysis using polymerase chain reaction-restriction fragment length polymorphism. Dietary intake was evaluated using a nutritional questionnaire. HHcy ( $P = .002$ ) and higher mean concentrations of Hcy ( $P = .029$ ) were associated with CAD. An association was observed between HHcy and 677T variant allele in the CAD group ( $P = .0005$ ). There was no correlation between Hcy concentration and folate, vitamin B<sub>6</sub> or vitamin B<sub>12</sub> intake in the CAD group. However, a negative correlation was observed between Hcy concentration and folate intake ( $P = .043$ ), and also between Hcy concentration and vitamin B<sub>6</sub> intake ( $P = .030$ ) in the NRF group. According to our study, HHcy is associated with CAD development. In patients with CAD, *MTHFR* polymorphism seems to have a greater effect on the Hcy concentration than the vitamin intake. Increased folate and vitamin B<sub>6</sub> intakes seem to reduce Hcy concentrations among transplant recipients with NRF, and could contribute to reducing the risk of CAD development.

**P**LASMA hyperhomocysteinemia (HHcy) is considered a risk factor for chronic allograft dysfunction (CAD), the main cause of renal function loss in transplant recipi-

ents.<sup>1</sup> Increased homocysteine (Hcy) concentrations can result from genetic defects that alter enzymes involved in the Hcy re-methylation or trans-sulfuration, and folate,

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**Table 1. Plasma Hcy Concentrations and Insufficient Intake of Folate and Vitamins B<sub>6</sub> and B<sub>12</sub>**

|                                      | Hcy ( $\mu\text{mol/L}$ ) |                 |
|--------------------------------------|---------------------------|-----------------|
|                                      | CAN                       | NRF             |
| Folate intake normal                 | —                         | —               |
| Folate intake insufficient*          | 38.9 $\pm$ 36.4           | 24.4 $\pm$ 26.9 |
| <i>P</i>                             | —                         | —               |
| B <sub>6</sub> intake normal         | 40.4 $\pm$ 38.6           | 24.6 $\pm$ 27.5 |
| B <sub>6</sub> intake insufficient*  | 38.9 $\pm$ 36.8           | 24.5 $\pm$ 27.5 |
| <i>P</i>                             | 0.918                     | 0.918           |
| B <sub>12</sub> intake normal        | 38.9 $\pm$ 36.8           | 24.9 $\pm$ 27.5 |
| B <sub>12</sub> intake insufficient* | 39.1 $\pm$ 35.1           | 24.9 $\pm$ 28.1 |
| <i>P</i>                             | 0.989                     | 0.971           |

\*Intake values below the Estimated Average Requirement (EAR) were considered to characterize insufficient intake.

vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> deficiency, because they also participate in the degradation pathway of this amino acid.<sup>2</sup> The objectives of this study were to investigate the relationship between HHcy and CAD development, and to evaluate the effects of folate and vitamins B<sub>6</sub> and B<sub>12</sub> intake, and *MTHFR* C677T polymorphism on Hcy concentrations in renal transplant recipients.

#### PATIENTS AND METHODS

This study was approved by the National Ethics Commission, Brazil. Ninety-eight renal transplant recipients at least at 12 months were included in this cross-sectional study: 50 subjects had CAD (mean age, 41  $\pm$  10 years, mean time of postoperative follow-up; 5  $\pm$  4 years) and 48 subjects had normal renal function (NRF; mean age, 39  $\pm$  12 years; mean time of postoperative follow-up, 6  $\pm$  4 years). Criteria for inclusion of patients in the CAD group were as follows: serum creatinine values >1.5 mg/dL; creatinine clearance <50 mL/min; and 24-hour proteinuria  $\geq$ 500 mg. Peripheral blood samples were obtained after a 12-hour fast. Plasma Hcy was measured using a liquid chromatography/sequential mass spectrometry (LC-MS/MS) method.<sup>3</sup> HHcy was defined as an Hcy concentration  $\geq$ 15  $\mu\text{mol/L}$ . Genomic DNA was extracted from peripheral blood leukocytes<sup>4</sup> and *MTHFR* C677T polymorphism genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).<sup>5</sup> Dietary intake was evaluated using a validated nutritional questionnaire<sup>6</sup> analyzed by the DIETSYS program. Intake values below the Estimated Average Requirement (EAR)<sup>7</sup> were considered to characterize insufficient intake. Chi-square test, Student *t* test, binary logistic regression, and Spearman correlation coefficients

were used according to the analyzed variable. *P* values  $\leq$  .05 were considered significant.

#### RESULTS

HHcy was frequently observed among patients with CAD (95.8% vs 68%; *P* = .002), as well as higher mean levels of Hcy (38.9  $\pm$  36.4  $\mu\text{mol/L}$  vs 24.5  $\pm$  27  $\mu\text{mol/L}$ ; *P* = .029). An association between HHcy and 677T variant allele was observed in the CAD group (*P* = .0005). Hcy concentrations did not differ among patients with normal or insufficient vitamin intake (Table 1). In the CAD group, no correlation was observed between Hcy and folate intake (*r* = 0.123; *P* = .404), or vitamin B<sub>6</sub> intake; (*r* = 0.249; *P* = .088) or B<sub>12</sub> intake (*r* = 0.202; *P* = .168). However, a discrete negative correlation was evidenced between Hcy and folate intake (Fig. 1A; *r* = -0.288; *P* = .043) and between Hcy and vitamin B<sub>6</sub> intake (Fig. 1B; *r* = -0.307; *P* = .030) in the NRF group, showing a relationship between the increased intake of these nutrients and decreased plasma Hcy.

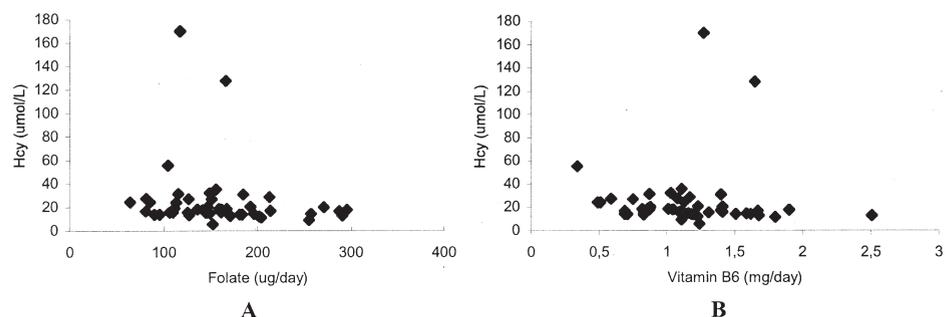
#### DISCUSSION

Our results confirmed an association between HHcy and the risk for CAD development.<sup>8</sup> In the NRF group there was a correlation between increased folate and vitamin B<sub>6</sub> intake with reduction in Hcy concentrations. The absence of this correlation in CAD patients may be due to the fact that subjects with renal failure are less responsive to vitamin therapies; therefore, the concentrations ingested in the diet were not sufficient to normalize Hcy concentrations.<sup>9</sup> In patients with CAD, the *MTHFR* polymorphism seemed to have a greater effect on Hcy concentration than the vitamin intake, once the variant allele was associated with HHcy in this group. According to our study, the increase in folate and vitamin B<sub>6</sub> intake could reduce the Hcy concentrations in transplant recipients with NRF, and could contribute to reducing the risk of CAD development.

#### JUSTIFICATION

The objectives of this study were to evaluate the association between hyperhomocysteinemia and coronary artery disease development, the influence of micronutrient intake by a validated questionnaire (Ribeiro AB, Cardoso. Construção de um questionário de frequência alimentar como subsídio para programas de

**Fig 1.** Folate and vitamin B<sub>6</sub> intake and plasma Hcy concentration in NRF patients. Negative correlation between the following: (A) Hcy concentration and folate intake (*r* = -0.288; *P* = .043); and (B) Hcy concentration and vitamin B<sub>6</sub> intake (*r* = -0.307; *P* = .030).



prevenção de doenças crônicas não transmissíveis. *Rev Nutr* 15: 239; 2002.) and MTHFR polymorphism in plasma homocysteine concentrations among renal transplant recipients. Although the measurements of the folate and vitamins B<sub>6</sub> and B<sub>12</sub> in blood are important, this was not the objective of this work. Furthermore, our institution does not have adequate equipment to perform such analysis; the high cost to do these tests in other specialized centers made this analysis infeasible.

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