

Original

Association of uricemia with biochemical and dietary factors in human adults with metabolic syndrome genotyped to C677T polymorphism in the methylenetetrahydrofolate reductase gene

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Abstract

It is suggested that hyperuricemia is a marker of cardiovascular risk in human adults with metabolic syndrome (MS). The C677T polymorphism in the gene encoding the enzyme methylenetetrahydrofolate reductase (MTHFR) is associated with hyperuricemia. Data on factors associated with uricemia in human adults with MS genotyped for this polymorphism are lacking. We aimed to investigate the factors associated with uricemia in human adults with MS genotyped for the C677T polymorphism in the *MTHFR* gene. Cross-sectional study was conducted with 63 human adults (24 men and 39 women) with MS. Body weight, body mass index, waist circumference, body fat, glycemia, lipid profile, uricemia, insulinemia, homocysteinemia, plasma folate, erythrocyte folate, blood pressure, smoking, diuretics use, usual dietary alcohol and protein intakes, and the presence of the C677T polymorphism in the *MTHFR* gene were assessed. Hyperuricemia was observed in 16 (25.4%) human adults (10 men and 6 women). In the group, 33% (n = 21) showed the C677T polymorphism, being 19 heterozygous and 2 mutant homozygous. A significant association between hyperuricemia and C677T polymorphism was not verified. Uricemia was positively associated with homocysteinemia (r = 0.43, p < 0.05), triglyceridemia (r = 0.41, p < 0.05), serum concentrations of very-low-density lipoprotein (r = 0.27, p < 0.05) and the habitual alcohol intake (r = 0.37, p < 0.05). However, only homocysteinemia, triglyceridemia, and habitual alcohol intake remained in the final model of linear regression. In human adults with MS genotyped for the C677T polymorphism in the *MTHFR* gene, uricemia was positively associated with homocysteinemia, triglyceridemia and the habitual alcohol intake.

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Key words: Uricemia. Metabolic syndrome. Cardiovascular disease. C677T polymorphism in the *MTHFR* gene.

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ASOCIACIÓN DE LA URICEMIA CON FACTORES BIOQUÍMICOS Y DIETÉTICOS EN HUMANOS ADULTOS CON SÍNDROME METABÓLICO GENOTIPADOS PARA EL POLIMORFISMO C677T EN EL GEN METILENOTETRAHIDROFOLATO REDUCTASA

Resumen

Sugérese que la hiperuricemia sea un factor de riesgo cardiovascular en humanos adultos con síndrome metabólico (SM). El polimorfismo C677T en el gen metileno-tetrahidrofolato reductasa (MTHFR) ha sido asociado con la hiperuricemia. Datos sobre los factores asociados con la uricemia en humanos adultos con SM genotipados para el polimorfismo C677T en el gen *MTHFR* son inexistentes. Se objetivó investigar los factores asociados con la uricemia en individuos con SM genotipados para el polimorfismo C677T. Se ha realizado un estudio transversal con 63 humanos adultos (24 hombres y 39 mujeres). Fueron evaluados peso, altura, índice de masa corporal, circunferencia de la cintura, grasa corporal total, glucemia, uricemia, insulinemia, homocisteinemia, folato plasmático, folato en los eritrocitos, presión arterial, tabaquismo, uso de diuréticos, ingesta habitual de proteínas dietéticas y de alcohol y la presencia del polimorfismo C677T. No fue encontrada asociación significativa entre el polimorfismo C677T y la uricemia. Fue verificado que un 25,4% (n = 16) de los individuos presentaban hiperuricemia (10 hombres y 6 mujeres). Se verificó que un 33% (n = 21) de los individuos presentaron el polimorfismo C677T (19 heterocigotos y 2 homocigotos polimórficos). La uricemia se asoció con los niveles de homocisteína (r = 0,43, p < 0,05), triglicéridos (r = 0,41, p < 0,05) y de lipoproteínas de muy baja densidad (r = 0,27, p < 0,05) y con la ingesta de alcohol (r = 0,37, p < 0,05). Sin embargo, sólo la homocisteinemia, la trigliceridemia y la ingesta habitual de alcohol permanecerán en el modelo final de regresión lineal. En los humanos adultos con SM genotipados para el polimorfismo C677T en el gen *MTHFR*, la uricemia se asoció positivamente con los niveles de homocisteína y triglicéridos y con la ingesta habitual de alcohol.

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Palabras clave: Uricemia. Síndrome metabólico. Enfermedades cardiovasculares. Polimorfismo C677T, en el gen *MTHFR*.

Abbreviations

UA: uric acid.
EF: erythrocyte folate.
PF: plasma folate.
CRF: cardiovascular risk factor.
HDL: high-density lipoprotein.
Hhcy: hyperhomocysteinemia.
HOMA-IR: Homeostatic Model Assessment-Insulin Resistance.
95% CI: 95% confidence interval.
BMI: body mass index.
LDL: low-density lipoprotein.
MTHFR: methylenetetrahydrofolate reductase.
OR: odds ratio.
IR: insulin resistance.
VLDL: very-low-density lipoprotein.

Introduction

The uric acid (UA) is the end product of purines catabolism. The uricemia is the result of the balance among production (liver) and excretion (renal and fecal) of 70% and 30%, respectively. UA, when present in appropriate concentrations, acts as an antioxidant. However, when it is present in high concentrations (hyperuricemia), UA acts as pro-oxidant, contributing to the cardiovascular disease development.¹

In the literature, studies suggest that hyperuricemia is a cardiovascular risk factor (CRF),^{2,3} especially in humans with metabolic syndrome (MS).⁴ However, some studies demonstrated no such relationship,^{5,6} showing that there is no consensus on the recognition of hyperuricemia as CRF.

The high consumption of alcoholic beverages, purine-rich foods and dietary protein, smoking, and the reduced renal excretion, resulting, for example, from diuretics use¹ are among the causes of hyperuricemia. Insulin resistance (IR) has been related to reduced UA renal excretion, which would explain the fact that some authors suggest the hyperuricemia inclusion as a component of MS.⁷

In addition, genetic factors have been associated with hyperuricemia, highlighting the C677T polymorphism in the gene encoding the methylenetetrahydrofolate reductase (MTHFR) enzyme,^{8,9} related with the increasing of plasma homocysteine concentrations (hyperhomocysteinemia-Hhcy), appointed as marker of cardiovascular risk. Data on this relationship in human adults with MS are lacking.

Since hyperuricemia has been identified as CRF, the investigation of uricemia possible determinants in human adults with MS is important in the cardiovascular diseases prevention, which are the leading cause of death in Brazil.¹⁰ Our study aimed to investigate the factors associated with uricemia in human adults with MS genotyped for the C677T polymorphism in the *MTHFR* gene.

Subjects and methods

Study design

A cross-sectional study was conducted from 2002 through 2003, using 63 human adults (24 men and 39 women) with MS, who were recruited among patients of the Nutritional Ambulatory of Hospital Universitário Clementino Fraga Filho of Universidade Federal do Rio de Janeiro, Brazil. The sample included 20 and 59-years old human adults, with no sex or race restriction.

The study sample size was calculated to test the hypothesis of correlation coefficient of about 0.4 different from zero ($H_1: r \neq 0$), with a significance level (alpha) of 0.05 and a test power of 90% (beta = 0.1), based on the formula described by Zar¹¹. Thus, the minimum required sample size was 62 human adults.

The study did not include human adults who were taking vitamin supplements and drugs that interfere with the glucose and lipid metabolism, as well as those with diseases such as gout, diabetes mellitus and kidney disease, as assessed by the history of the present illness.

For MS diagnosis, the criteria established by the International Diabetes Foundation¹² were adopted. Information on sex, age, socio-economic status, medication use, family history, clinical history, and smoking was obtained from medical records and through standard questionnaire developed by the researchers.

Nutrient intake

Usual dietary intake of alcoholic beverages and protein was assessed by a food-frequency questionnaire semi-quantitative.¹³ Nutrient analysis was conducted using the *Food Processor* computer program (Esha Research, Salem, Mass., USA).

Clinical, anthropometrical and body composition measurements

Body mass (kg) and height (m) were measured using the Filizola platform scale and a vertical stadiometer, respectively (Filizola, São Paulo, Brazil).¹⁴ Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).¹⁵ Waist circumference was measured around the shortest trunk circumference, between the lower rib and the iliac crest, using a nonextendable metric tape (Sanny®, São Paulo, Brazil).¹⁶

The percentage of body fat was estimated by the mean of three repeated skinfold thickness (triceps, biceps, subscapular and supriliac)¹⁷ measurements. These measures were made by a single evaluator with scientific caliper (Lange, Cambridge Scientific Industries, Inc., Cambridge, Maryland). Blood pressure was measured using an aneroid sphygmomanometer (Fisiomed, São Paulo, Brazil).¹⁸

Biochemical measurements

After 12 hours of overnight fast, samples of whole blood were collected into tubes with or without ethylene diamine tetra-acetic acid anticoagulant (EDTA) (Vacutainer, Becton Dickinson, USA) to obtain plasma and serum samples, respectively. Aliquots of serum and plasma were separated by centrifugation at 4,000 rpm for 15 minutes at room temperature (Excelsa Baby I, model 206, FANEM®, São Paulo, Brazil). Serum concentrations of glucose, UA, triglycerides, high-density lipoprotein (HDL) and total cholesterol were determined by enzymatic colorimetric method, according to the manufacturer's instructions (CELM®-São Paulo, Brazil and Katal®-Minas Gerais, Brazil), using the spectrophotometer (Beckman DU® 650, USA). Low-density lipoprotein (LDL) was calculated.¹⁹

The serum insulin concentration was determined by radioimmunoassay, according to the manufacturer's instructions (COAT-A-COUNT Insulin®, USA), being used gamma counter equipment (Automatic Gamma Counter 1470, Wallac Wizard TM). IR was estimated by Homeostasis Model Assessment Insulin Resistance (HOMA-IR)²⁰ method. Erythrocyte folate (Folate commercial kit, Diagnostic Products®, USA) and plasma folate (Dualcount commercial kit, Diagnostic Products®, USA) were determined by radioisotope dilution, being used the Gamma Counter equipment (Automatic Gamma Counter 1470 Wizard Wallac TM).

Plasma homocysteine was determined by high-performance liquid chromatography with fluorescence detection.²¹ Analyses were performed in a Shimadzu Liquid Chromatography LC-10 AD (Shimadzu Corporation, Kyoto, Japan) equipped with a fluorescence detector and an LC-18-DB column (150 mm x 4.6 mm id). The mobile phase used was phosphate buffer of mono-potassium (pH 1.95) with 4% acetonitrile.

Analysis of C677T polymorphism in the MTHFR gene

Genomic DNA was extracted from whole blood air dried on special filter paper (Schleicher & Schuel n°. 903, Keene, NH, USA) by resin Chelex method® (Bio-Rad, Rio de Janeiro, Brazil)²². The DNA fragment, containing the 677 C > T variant, was amplified by the polymerase chain reaction (PCR) followed by restriction enzyme digestion analysis.²³ PCR mixture consisted of 75 mM Tris HCl, pH 9.0, 50 mM KCl, 20 mM (NH₄)₂ SO₄, 0.125 mM of each primer (sense: 5'-GAA GCA GGG AGC TTT GAG GCT GAC CT-3' and antisense: 5'-AGT GAT GCC CAT GTC GGT GCA TGC CT'-3'), 0.2 mM dNTPs, 2 mM MgCl₂, 1 U Tth polymerase (Biotools) and genomic DNA in a total volume of 15 L.

Thermal cycling was carried out as follows: initial denaturation at 94°C for 4 min, continued by 35 cycles of 1 min at 94°C, 1 min at 64°C (annealing), 1 min at

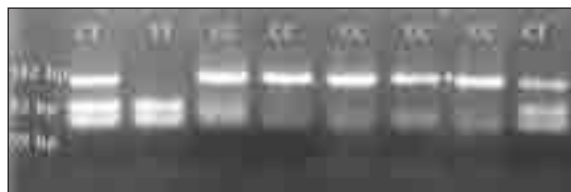


Fig. 1.—Ethidium bromide-stained 3% agarose gel showing PCR products corresponding to a fragment of *MTHFR* gene after digestion with *Taq I*. When the nucleotide 677 substitution C → T was present, it created a restriction site to *Taq I*, resulting in fragments of 59 and 83 base pairs (bp) or remained 142 bp when the normal allele was present. Lines 1 and 8: fragments related to CT genotype (heterozygous); Line 2: fragment related to TT genotype (mutant homozygous); lines 3 to 7: fragments related to CC genotype (normal homozygous).

72°C (extension) and a final extension of 5 min at 72°C. A fragment of 142 base pairs (bp) was obtained and 10 L of PCR product were exposed for 4 h at 65°C to restriction enzyme (*Taq I*) (Promega) and then the digested fragments were observed in a 3% agarose gel. Human adults homozygous (CC) for the *MTHFR* normal allele yielded a fragment of 142-base pair (bp); the heterozygous (CT), a three-fragment pattern of 142, 83 and 59 bp, and the homozygous for the mutation yielded two fragments of 83 and 59 bp (fig. 1).

Statistical analysis

To check the distribution of continuous variables of interest, Kolmogorov-Smirnov adhesion test was performed. The comparisons of mean values were made using the Student's t-test. Data are presented as mean ± standard deviation. The association among uricemia, clinical, anthropometrical, biochemical, and dietary variables was assessed by calculating the Pearson's correlation coefficient.

Additionally, we built a multiple linear regression model, having hyperuricemia as dependent variable. The explanatory variables were selected for the final model, according to the statistical significance achieved in the Pearson's correlation coefficient calculation.

Odds Ratio (OR) and its respective 95% confidence intervals (95% CI) were calculated to investigate the association between hyperuricemia and C677T polymorphism in the *MTHFR* gene. Heterozygous (CT genotype) and mutant homozygous (TT genotype) were grouped together (group CT genotype), because the frequency of TT genotype was low (n = 2; 4%). The Hardy-Weinberg equilibrium was determined by analysis of χ^2 test for all genotypes.²⁴ The association between hyperuricemia and other dichotomous variables (sex: male/female, smoking: yes/no, diuretics use: yes/no; alcoholic beverages intakes: yes/no, and high protein intake: yes/no) was also investigated with OR analysis.

Statistical analysis were conducted, using the statistical package Statistical Package for the Social Sci-

Table I
Clinical, antropometric, body composition and dietary intake data of the human adults¹

Variable	Total
n	63
Age, years	48.6 ± 7.6
Body weight, kg	92.7 ± 20.2
BMI, kg/m ²	33.8 ± 5.4
WC, cm	107.1 ± 11.3
Body fat, %	41.8 ± 6.0
SBP, mmHg	127.6 ± 12.0
DBP, mmHg	87.3 ± 9.4
Proteins, g/day	82.7 ± 27.2

¹Values expressed as average ± standard deviation. WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure.

ences (SPSS) version 11.0. Differences were considered significant at $p < 0.05$.

Ethical

The study protocol was approved by the Research Ethics Committee of Hospital Universitário Clementino Fraga Filho at UFRJ on April 10, 2003 (research protocol n°. 017/03) and conducted according to the Declaration of Helsinki. All human adults were fully informed about all procedures before signing a statement of informed consent prepared in accordance with resolution 196/96 of the National Health Council.

Results

A total of 63 human adults with MS were included in the present study, being 24 (38%) men and 39 (62%) women. Clinical and anthropometrical, body composition, and dietary data are shown in table 1. According to the mean values of BMI and waist circumference, the studied group presented obesity class I,¹⁵ characterized by the visceral fat accumulation.¹² It was verified that 75.8% (n = 47) of the studied group had high protein intake (greater than 1.0 g/kg/day),²⁵ being 18 (38.3%) and 29 (61.7%) men and women, respectively.

In the group, 51.6% (n = 32) of human adults had IR (HOMA-IR > 2.71),²⁶ being 18 women and 14 men. The uricemia did not differ significantly between the sexes, although it was higher in men (6.8 ± 1.8 mg/dL versus 5.2 ± 1.3 mg/dL, $p > 0.05$). The women exhibited less chance for hyperuricemia (OR = 0.25, 95% CI = 0.08-0.84) compared to men. In the group, 16 (25.4%) human adults, being 10 (62.5%) men and 6 (37.5%) women, exhibited hyperuricemia (men > 7.0 mg/dL and women > 6.5 mg/dL).¹ There was no association between uricemia, insulinemia and HOMA-IR index.

Table II
Biochemical data of the human adults¹

Variable	Total
n	63
Glucose, mg/dL	87.8 ± 17.8
Uric acid, mg/dL	5.8 ± 1.7
Triglycerides, mg/dL	241.9 ± 86.7
HDL, mg/dL	35.8 ± 8.4
LDL, mg/dL	181.5 ± 56.6
VLDL, mg/dL	42.1 ± 14.8
Total cholesterol, mg/dL	262.9 ± 61.7
Erythrocyte folate, nmol/L	361.0 ± 150.9
Plasma folate, nmol/L	14.7 ± 9.3
Homocysteine, mol/L	10.0 ± 3.2
Insulin, μU/mL	14.5 ± 8.6
HOMA-IR	3.1 ± 1.9

¹Values expressed as average ± standard deviation.

Table III
Perason's correlation coefficient between uric acid and biochemical and dietary data of the human adults¹

Variable	r	p
Homocysteine	0.43	< 0.05
Triglycerides	0.41	< 0.05
VLDL	0.27	< 0.05
Alcohol	0.37	< 0.05

¹Only the variables that showed significant association were presented.

In reference to the indicators of nutritional folate status, 21% (n = 13) and 38% (n = 24) of human adults, respectively, showed low concentrations of plasma folate (< 7 nmol/L)²⁷ and erythrocytes folate (< 305 nmol/L)²⁷ (table II). Hhcy (homocysteine > 10 mmol/L)²⁸ frequency was 49.2% (n = 31), being 18 women and 13 men.

Among the numerical variables investigated, the uricemia was positively associated with homocysteinemia ($r = 0.43$, $p < 0.05$), triglyceridemia ($r = 0.41$, $p < 0.05$), serum concentrations of very-low-density lipoprotein (VLDL) ($r = 0.27$, $p < 0.05$) and with the habitual alcohol intake ($r = 0.37$, $p < 0.05$) (table III). However, only the variables homocysteinemia, triglyceridemia and habitual alcohol intake remained in the final model of linear regression (table IV).

In this study, 33.3% (n = 21) of the human adults had the C677T polymorphism in the *MTHFR* gene, being 19 heterozygous (CT genotype) and 2 mutant homozygous (TT genotype) (fig. 1). The frequency of genotypes CC, CT and TT was 64% (n = 42), 32% (n = 19) and 4% (n = 2) and the frequency of C and T alleles was 80% and 20%, respectively. *MTHFR* genotype distrib-

Table IV
Multiple linear regression model results with hyperuricemia as the dependent variable

Variable	Coefficiente (β)	95% CI for β	p-value
Homocysteinemia	0.160	0.039-0.280	0.011
Triglyceridemia	0.003	0.001-0.006	0.017
Alcohol	0.050	0.000-1,000	0.053

ution was compatible with the Hardy-Weinberg equilibrium ($\chi^2=0.17$, degrees of freedom = 2, $p > 0.05$).³² A significant association between hyperuricemia and C677T polymorphism in the *MTHFR* gene was not verified (OR = 1.78, 95% CI = 0.55-5.73).

Smoking (1-40 cigarettes/day, medium = 17 cigarettes/day) and diuretics use (mainly thiazides) were reported by 19% (n = 12) and 31.7% (n = 20) of human adults. There was no association between smoking and diuretics use with hyperuricemia.

Discussion

Although there is no consensus in the scientific literature, hyperuricemia has been identified as CRF.^{2,3} This data points to the importance of identifying factors associated with the increasing of uricemia in human adults with MS.

This study, conducted with human adults with MS genotyped for the C677T polymorphism in the *MTHFR* gene, suggests a positive association of uricemia with serum concentrations of VLDL, triglyceridemia, homocysteinemia, and the habitual alcohol intake.

Evidences, such as the frequent presence of hypertriglyceridemia in gouty patients²⁹, reduced urates renal excretion in hyperuricemic and hypertriglyceridemic humans³⁰ and increased urate excretion associated with decreased serum concentrations of VLDL, after body mass reduction,³¹ reinforcing the relationship between lipids and purines metabolisms.

The relationship between uricemia and homocysteinemia in human adults with MS is not fully understood. However, similar to triglyceridemia, it is suggested that genetic factors may be involved. Studies have demonstrated an association between uricemia and C677T polymorphism in the *MTHFR* gene.^{8,9}

The *MTHFR* enzyme is responsible for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the main donor of methyl groups for remethylation reaction of homocysteine to methionine. In presence of C677T polymorphism in the *MTHFR* gene, there is *MTHFR* enzyme lower activity, resulting in Hhcy and 5,10-methylenetetrahydrofolate accumulation that could serve as a substrate for the purines synthesis and therefore, UA.⁸ This fact could explain the relationship between the

uricemia and the C677T polymorphism in the *MTHFR* gene.

In this study, an association of uricemia with the C677T polymorphism in the *MTHFR* gene was not verified. Adequate nutritional folate status, according to the average concentrations of plasma folate (PF) and erythrocyte folate (EF), observed in human adults with the C677T polymorphism in the *MTHFR* gene (PF = 16.4 ± 9.6 nmol/L and EF = $402.1 \pm 118,0$ nmol/L), could have minimized this polymorphism effect on uricemia. Adequate folate levels seem to compensate the effects of *MTHFR* enzyme lower activity in humans with C677T polymorphism in the *MTHFR* gene, thus avoiding the 5,10-methylenetetrahydrofolate³² accumulation.

Yamamoto et al. (2005),³³ in the literature review, described the effects of ethanol on the purines metabolism. According to that, ethanol contributes to the adenosine triphosphate (ATP) rapid degradation in adenosine monophosphate (AMP), which is rapidly converted to UA, as well as it is associated with diuresis increasing, contributing to dehydration and consequent decrease in renal excretion of UA. The diuresis increasing is due to reduced antidiuretic hormone secretion promoted by ethanol. Additionally, ethanol has been associated with increased lactic acid concentrations, which accelerates the UA reabsorption by the proximal tubule.

According to the study of Choi et al. (2004),³⁴ compared to wine and spirits, beer confers a higher risk for gout. In addition to its ethanol content, the beer is the beverage with higher content of purines.³⁶ In our study, 22% of human adults (n = 14) consumed alcoholic beverages, especially beer. Human adults who drank beer had four times more likely to develop hyperuricemia compared to non-drinkers (OR = 4.4, 95% CI = 1.2 - 15.9).

In conclusion, in human adults with MS genotyped for the C677T polymorphism in the *MTHFR* gene, the uricemia was positively associated with homocysteinemia, triglyceridemia, and the habitual alcohol intake. Whereas our results suggest an association between homocysteinemia and uricemia, we underscore the importance to reach of the nutritional recommendations for vitamins that regulate homocysteine metabolism, especially folate, through the food sources ingestion (dark leafy greens, vegetables, greenstuff, citrus fruits and viscera). However, diet alone does not seem to be sufficient to supply the folate needs, requiring folate supplementation, especially in the presence of the C677T polymorphism in the *MTHFR* gene, which has been associated with hyperuricemia. The consumption of alcoholic beverages, especially beer, should also be avoided.

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