

UTJECAJ PREHRAMBENOG MODELA I POLIMORFIZMA C677T METILENTETRAHIDROFOLAT REDUKTAZE NA RAZINU HOMOCISTEINA U PLAZMI MEĐU ZDRAVIM VEGETARIJANCIMA I OMNIVORAMA

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INFLUENCE OF DIETARY PATTERN AND METHYLENTETRAHYDROFOLATE REDUCTASE C677T POLYMORPHISM ON THE PLASMA HOMOCYSTEINE LEVEL AMONG HEALTHY VEGETARIANS AND OMNIVORES

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Summary

Elevated total plasma homocysteine level (Hcy) is associated with physiological and dietary factors as well as the genetic defect of enzymes involved in Hcy metabolism. The objectives of the study were to examine (1) differences between healthy vegetarian and omnivorous subjects in relation to biochemical parameters, prevalence of the MTHFR (methylentetrahydrofolate reductase) T/T genotype, and the plasma Hcy level, and (2) the effects of the MTHFR C677T polymorphism and dietary pattern on the plasma Hcy level. In 47 vegetarian and 53 omnivorous subjects the plasma level of Hcy, folate, vitamin B₁₂, glucose, total cholesterol, triglycerides, HDL and LDL-cholesterol and creatinine were measured. MTHFR C677T polymorphisms were analyzed using the PCR-RFLP method. Obtained results have shown that vegetarians had lower vitamin B₁₂, total cholesterol, LDL-cholesterol and creatinine status. The plasma Hcy level was higher among vegetarians compared with omnivore subjects (14.10±6.69 vs. 10.49±2.41 μmol/L) and negatively correlated with vitamin B₁₂ status and folate. The plasma Hcy level did not differ between the given MTHFR C677T genotypes among either vegetarians or omnivores. Unlike the MTHFR C677T polymorphism, the effect of dietary pattern on plasma Hcy level was confirmed. It could be concluded that vegetarians tend to have lower vitamin B₁₂ status and a higher plasma Hcy level. The MTHFR 677C/T polymorphism has no effect on plasma Hcy level, in contrast to dietary pattern which indicates the importance of adequate vitamin B₁₂ and folate status in bypassing the mutation.

Key words: folate, homocysteine, MTHFR C677T polymorphism, vegetarian diet, vitamin B₁₂

Abbreviations: HDL - High Density Lipoprotein-cholesterol, LDL—Low Density Lipoprotein-cholesterol, Hcy – homocysteine, MTHFR- methylentetrahydrofolate reductase

Introduction

A review of epidemiological reports has established a high plasma homocysteine (Hcy) level (hyperhomocysteinemia, hHcy) as a risk factor for cardiovascular diseases, cerebrovascular diseases, dementia-type disorders, and osteoporosis-associated fractures (Maron and Loscalzo, 2009). Also, important meta-analyses on the connection of hHcy with type 2 diabetes and

mental disorders such as schizophrenia and depression have recently been published (Huang et al., 2013; Nishi et al., 2014).

It has been found that inadequate nutrition is associated with hHcy. Insufficient vitamin B₁₂, B₆ and folate intake, as well as low ω-3 polyunsaturated fatty acids (ω-3 PUFA) status, are associated with elevation of the plasma Hcy level (Huang et al., 2012; Li, 2013; Stabler and Allen, 2004). Lower levels of those nutrients are com-

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monly found among vegetarians. An excessively energy restrictive and monotonous vegetarian diet may result in nutrient deficiency, mainly in fat and saturated fatty acids (SFA), heme iron (Fe^{2+}), zinc, vitamin A, B₁₂, D and especially in ω -3 PUFA. Even though morbidity and mortality are generally lower in vegetarians than omnivores (Appleby et al., 2001), vitamin B₁₂, folate and Hcy status could be better thus lowering the risk of hHcy associated diseases.

Besides by nutritional deficiencies, hHcy can also be genetically induced. One of the significant genetic factors contributing to hHcy is MTHFR (methylentetrahydrofolate reductase) C677T polymorphism. When the MTHFR C677T polymorphism is accompanied by nutritional deficiencies the effect on the plasma Hcy level is even more accentuated.

The aim of this study was to examine the differences between healthy vegetarian and omnivorous subjects in relation to biochemical parameters, the prevalence of the MTHFR T/T genotype, and the plasma Hcy level, and its association with the B12 and folate status. In the second step, the effects of the methylentetrahydrofolate reductase (MTHFR) C677T polymorphism and the dietary pattern on the plasma Hcy level between healthy vegetarian and omnivorous subjects were examined.

Subjects and methods

Subjects

The examined group consisted of 100 healthy subjects between 18 and 69 years of age. Among them, 47 were vegetarians and 53 omnivores. Regarding vegetarian subgroups, 46 vegetarian subjects were lacto-vegetarians and one was vegan. Subjects with history of hormonal intake or other medical treatments as well as present chronic diseases were excluded to eliminate the possible effect on blood nutrient levels.

Methods

All the molecular analyses were done at the Laboratory for Molecular Diagnostics at the Department of Pathology, University of Rijeka. The procedures were approved by the medical ethics committee of the University Hospital Center Rijeka and Faculty of Medicine at the

University of Rijeka and in accordance to the World Medical Association Declaration of Helsinki and the Nürnberg codex regarding ethical principles for medical research. All subjects gave consent to participate in the study, being motivated by general health, Hcy, vitamin B₁₂ and folate status feedback. Fundamental bioethical principles (autonomy, beneficence, nonmaleficence and justice) as well as privacy and protection of personal data were assured throughout the whole study.

Dietary pattern data

Self-reported meat-eating habits were the basis for categorizing the subjects into the vegetarian or omnivorous group, regardless of the length, types or levels of the vegetarian diet. The vegetarian dietary patterns were determined according to reported eating of no meat for at least two years. Furthermore, the type and level of vegetarianism was not specified.

Biochemical analyses

Several hematological and biochemical parameters were observed. Hematological parameters were obtained from whole blood samples while glucose, total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, creatinine, total Hcy, folate and vitamin B₁₂ were obtained from plasma or serum. Routine hematological parameters were detected on a hematological analyzer Cell Dyn 3700 (Abbott, Illinois, USA), biochemical parameters on an analyzer COBAS 6000 (Roche, Mannheim, Germany) while Hcy (Architect Homocysteine Reagent, Abbott, Illinois, USA), folate (Architect Folate Reagent, Abbott, Illinois, USA) and vitamin B₁₂ levels (Architect B₁₂ Reagent, Abbott, Illinois, USA) were measured using the chemiluminescence method (CMIA) on an automated immunoassay analyzer Architect (Abbott Laboratories, Illinois, USA). For C677T MTHFR polymorphism analysis, DNA from peripheral blood leukocytes was isolated with a NucleoSpin Blood kit (Machery-Nagel, Duren, Germany), according to the manufacturer's recommendations. Target fragment of MTHFR gene (198 base-pairs) was amplified by polymerase chain reaction (PCR). MTHFR C677T genotype was determined by

digestion of a PCR product with the restriction enzyme Hinf I, as recommended by the manufacturer (Takara, Illinois, Japan) and the obtained DNA fragments were separated on a 3% agarose gel and visualized by ethidium bromide dye.

Statistical analysis

Data were presented as mean (and standard deviation, SD) and percentages. The one-way analysis of variance and the Chi-square (χ^2) test were used for comparisons between the groups. To determine the frequency of MTHFR C677T polymorphism, the Hardy-Weinberg equilibrium was used. A χ^2 analysis was used to test the significant differences in the prevalence of MTHFR C677T genotypes among vegetarian and omnivores. Pearson correlation coefficients were used to assess the correlations between the vitamin B12, folate and the plasma Hcy level.

To test differences in the plasma Hcy levels between the vegetarian and omnivorous subjects regarding all three MTHFR C677T genotypes, the one-way analysis of variance and nonparametric Kruskal-Wallis test were performed. The factorial ANOVA was performed to compare means across two independent variables (MTHFR C677T genotypes and dietary pattern) in the Hcy level. The differences were considered significant at the 5% level in this study and data were interpreted with a 1% and 5% confidence level. The SPSS software 19.0 was used in the statistical analyses.

Results and discussion

The demographic characteristics, together with hematological and biochemical parameters of both of the dietary pattern groups and total population, are listed in Table 1.

Table 1. Characteristics of the subjects in total and two subgroups

Variable	Total population ¹ (n = 100)	Vegetarians ¹ (n=47)	Omnivores ¹ (n=53)	P ²
Age (y)	42.8±11.9	42.3±11.7	43.3±12.2	
Male (n)	27	16	11	
Female (n)	73	31	42	
B ₁₂ (pmol/L)	281.50±117.00	237.21± 117.81	320.77± 102.16	0.001
Folate (g/mL)	8.70±4.70	8.17± 4.04	9.16± 5.21	0.295
Hcy (μmol/L)	12.19±5.21	14.10± 6.69	10.49± 2.41	0.001
Glucose (mmol/L)	4.88±0.48	4.88±0.53	4.89±0.44	0.922
Cholesterol (mmol/L)	5.08±1.30	4.58±1.46	5.53±0.94	0.001
Triglycerides (mmol/L)	0.94±0.37	0.90±0.38	0.98±0.35	0.277
HDL (mmol/L)	1.58±0.41	1.52±0.35	1.63±0.46	0.164
LDL (mmol/L)	3.11±0.96	2.76±0.99	3.43±0.81	0.001
Creatinine (μmol/L)	73.16±13.42	69.83±12.23	76.11±13.84	0.019
Iron (μmol/L)	15.72±6.75	15.76±7.20	15.76±6.40	0.950
UIBC (μmol/L)	46.20±11.61	47.35±12.89	45.18±10.36	0.353
TIBC (μmol/L)	61.37±8.65	63.00±8.41	59.92±8.67	0.074
Hcy>15 μmol/L (%)	19	15	4	0.002
MTHFR T/Tgenotype (%)	13	4	9	0.209
¹ Values are means±SD, or percentages. UIBC - Unsaturated iron binding capacity; TIBC - Total iron binding capacity				
² ANOVA or chi-square test between vegetarians and omnivores				

In this predominantly female population, 47% consumed a vegetarian diet. Vegetarians statistically had a lower vitamin B₁₂ level and a higher Hcy level than omnivores. A statistically significantly higher prevalence of hHcy among vegetarians was shown, while, in total, 19% of subjects had hHcy (Hcy level >15 µmol/L). The folate level was lower among vegetarians but did not differ significantly between the two groups, as was expected. A statistically significant dif-

ference was found among vegetarians having lower total cholesterol, LDL-cholesterol and creatinine levels, compared with omnivores.

The MTHFR C677T genotype distribution was compatible with Hardy-Weinberg equilibrium (Table 2). The prevalence of the MTHFR C677T T allele frequency is 13%, which corresponds to an average of 12.5%, as well as C/C and C/T genotype.

Table 2. MTHFR C677T genotype distribution according to dietary pattern

MTHFR C677T	f _{ob}	f _{ex}	(f _{ob} -f _{ex}) ² /f _{ex}	Vegetarian n (%)	Omnivores n (%)	P ¹
T/T	13	11.90	0.101	4 (8.3)	9 (17.3)	0.209
C/T	43	45.20	0.107	22 (47.9)	21 (38.5)	0.897
C/C	44	42.90	0.028	21 (43.8)	23 (44.2)	0.469
Total	100	100.00	0.236	47 (100)	53(100)	0.431

f_{ob} - observed frequency; f_{ex} - expected frequency by Hardy-Weinberg equilibrium; ¹Chi-square test

As shown in Table 2, there was a higher frequency of homozygous MTHFR 677T/T mutation carriers in the omnivore group (17.3% vs 8.3%) and a higher frequency of heterozygous MTHFR 677C/T mutation carriers in the vegetarian group (47.9% vs 38.5%), but no signifi-

cant difference in prevalence of the MTHFR genotypes regarding dietary pattern has been found. The plasma Hcy level was not significantly different between the vegetarian and omnivorous subjects regarding all three MTHFR genotypes (Table 3.).

Table 3. Plasma Hcy level according to the MTHFR polymorphism genotype in vegetarians and omnivores

MTHFR genotype	Homocysteine level (µmol/L)			P ²
	Vegetarian	Omnivores	Total	
T/T type	17.08±9.71 ¹	10.07±2.16	12.23±6.17	0.209
C/T type	13.95±6.34	10.35±2.25	12.19±5.08	0.469
C/C type	13.69±6.69	10.79±2.69	12.17±5.16	0.897

¹Data are expressed as mean±S.D.; ²Statistical significance was analyzed by one-way analysis of variance and checked with the nonparametric Kruskal-Wallis test.

As shown in Table 4, the vegetarian diet has significant effect on the plasma Hcy level (F(1.94)=13.58, p<0.001), while the effects of MTHFR

genotype (F(2.94)=0.383, p>0.05) and the interaction of vegetarian diet and MTHFR genotype (p=0.471) were not significant.

Table 4. Factorial ANOVA for the effect of vegetarian diet and MTHFR genotype on the plasma Hcy level

	SS	df	MS	F	p	Eta
MTHFR	18.86	2	9.44	0.383	0.683	0.008
Vegetarian	334.92	1	334.92	13.58	0.000	0.126
MTHFR/ Vegetarian	37.49	2	18.74	0.76	0.471	0.016
Error	2318.60	94	24.67			
Total	17542.92	100				

MTHFR genotype did not correlate with the plasma Hcy level (Table 5.). Significant inverse correlations were found between plasma Hcy and vitamin B₁₂ status ($r = -0.559$ among

vegetarian, $r = -0.411$ among omnivores), and between plasma Hcy and folate ($r = -0.420$ among vegetarian, $r = -0.502$ among omnivores).

Table 5. Pearson correlation coefficients for MTHFR genotype, B₁₂ and folate in the subgroups and total population

Parameters	Homocysteine level (μmol/L)		
	Vegetarian	Omnivores	Total
MTHFR C677T	-0.105	0.114	-0.004
B ₁₂ (pmol/L)	-0.559**	-0.411**	-0.548**
Folate (g/mL)	-0.420**	-0.502**	-0.387**

Statistical significance level * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

This is a study investigating the influence of both dietary pattern and MTHFR C677T polymorphism on the plasma Hcy level among healthy vegetarians and omnivores. Results have confirmed the association of the vegetarian diet with an elevated plasma Hcy level, but not with MTHFR C677T polymorphism.

The importance of these findings lies in the fact that an elevated plasma Hcy level is an independent risk factor for cardiovascular diseases. Unfortunately, a detailed list of diseases associated with a Hcy level higher than 15 μmol/L is much longer: coronary artery disease, stroke, fracture, venous thrombosis, retinal artery and vein occlusion, nonarteritic anterior ischemic optic neuropathy, abdominal aortic aneurysm, diabetes and diabetes induced peripheral neuropathy and nephropathy, cancer, depression, schizophrenia, Parkinson's and Alzheimer's diseases, as well as inflammatory bowel diseases. pregnancy complications and poor pregnancy outcomes (Nishi et al., 2014; Wu and Wu, 2002).

Our results have shown that vegetarians had a higher plasma Hcy level than omnivores (14.10±6.69 vs 10.49±2.41 μmol/L). As mentioned above, the cut-off value for defining hHcy is 15 μmol/L and 20 μmol/L for the elderly (Refsum et al., 2004). Based on that criterion, 31.91% of our vegetarian and 7.55% of our omnivorous subjects had hHcy (in total 19%). We also had 3 vegetarian and 7 omnivorous subjects older than 60 years but none of them had Hcy levels higher than 20 μmol/L. If taken into ac-

count that some European societies define the 12 μmol/L as a cut-off value, our vegetarian subjects indeed are at risk of developing hHcy associated diseases (Stanger et al., 2003). According to data from Slovakia and Austria, 29% and 53% of vegetarians, respectively, had Hcy levels higher than 15 μmol/L (Krajcovicova-Kudlackova et al., 2000; Majchrzak et al., 2006). Chinese scientists came to a similar conclusion examining 103 vegetarian men whose average Hcy level was 13.99±4.63 μmol/L. According to them, the prevalence of hHcy (plasma Hcy ≥14 μmol/L) in vegetarians is 26.47%, in contrast to 13.28% seen in an omnivore group (Huang et al., 2013). Furthermore, a study of vegetarian families conducted in India on 300 subjects from 119 families showed that hHcy (>15 μmol/L) was present in 75% of male and 35% of female family members (Deshmukh et al., 2010).

There are several environmental and disease-related factors considered to raise the plasma Hcy level, such as lack of B vitamins, ω-3 PUFA, alcoholism, smoking, kidney disease, hypothyroidism, estrogen deficiency and certain medications (Huang et al., 2012; Li, 2013; Maron and Loscalzo, 2009). However, elevation in plasma Hcy levels among healthy individuals is generally caused by two factors: nutritional deficiencies of vitamin cofactors, and genetic defects in key enzymes involved in Hcy metabolism (Lovričević et al., 2004).

A vegetarian diet and the Hcy level

The World Health Organization (2012) states that vitamin B₁₂ and folate deficiency, together with iron, iodine and vitamin A deficiency, is recognized as a global public healthcare problem. Vitamin B₁₂ and folate deficiency are primarily the result of inadequate dietary intake. Individuals who regularly do not consume fruits rich in folate, green leafy vegetables, legumes, enriched cereals and breads are at risk of developing folate deficiency, while those excluding or reducing the intake of animal source foods are at risk from developing vitamin B₁₂ deficiency (de Benoist, 2008).

In the Eastern part of the world, vegetarianism is a long-known traditional dietary pattern, while in Western countries many people turn to vegetarianism to achieve better health. A diverse and planned vegetarian diet is considered to be appropriate and can provide all the nutrients needed. Depending on the type of vegetarian diet and the ability to plan and design their diet properly, people can either enjoy health benefits or be at risk of the previously mentioned diseases. For instance, hematological and biochemical parameters from our study showed that vegetarians (mainly lacto-vegetarians) had significantly lower total cholesterol ($p < 0.001$), LDL-cholesterol ($p < 0.001$) and creatinine ($p < 0.019$). On the other hand, a poorly designed vegetarian diet usually resulted in the lack of vitamin B₁₂, folate and ω -3 PUFA, all being nutrients important for Hcy level regulation (Li, 2013).

Results from our study confirmed that vegetarians have a lower vitamin B₁₂ and folate level. Regarding folate status, there was no statistically significant difference between the two groups, even though vegetarians in average did have a lower folate level than omnivores (8.17 ± 4.04 g/mL vs 9.16 ± 5.21 g/mL). A particularly low folate level is a risk factor for many diseases associated with Hcy status. Folate (tetrahydrofolate) is a methyl donor needed for the conversion of sufficient amounts of plasma Hcy back to methionine. A decrease of folate level below 4 ng/mL causes Hcy to accumulate in the blood, leading to hHcy (WHO, 2012). Almost three decades ago, Kang et al. (1987) found that a folate level below 4 ng/mL, found in 65% of the subjects,

was related to Hcy accumulation (Kang et al., 1987).

In addition to folate being an essential substrate in the reaction of Hcy transmethylation, vitamin B₁₂ is a cofactor of transmethylation enzyme i.e. methionine synthase. Vitamin B₁₂ deficiency impairs Hcy metabolism and leads to its accumulation in the plasma. An elevated Hcy level was found in more than 95% of individuals with clinically and laboratory confirmed vitamin B₁₂ deficiency (Selhub et al., 2007).

Vitamin B₁₂ deficiency is considered a global public health problem (Allen, 2009). Borderline values for defining deficiency differ from one study to another, ranging between 150 – 350 pmol/L. Together with different sensitivity and specificity of serum vitamin B₁₂ tests (13% - 75% and 45% - 100%), this is the reason why epidemiological data on vitamin B₁₂ deficiency are inconsistent (Willis et al., 2011). Last year's review revealed that 5% - 7% of young people, 10% - 30% of people older than 65, 63% lacto and lacto-ovo vegetarians and over 86% vegans were vitamin B₁₂ deficient. The review also presumes that vitamin B₁₂ deficiency data were understated (Groeber et al., 2013). The average vitamin B₁₂ level in the group of our subjects was 237.21 ± 117.81 pmol/L among vegetarians and 320.77 ± 102.16 pmol/L among omnivores. Another Croatian study on vitamin B₁₂ status among vegetarians found a slightly higher vitamin B₁₂ level (271.7 pg/mL in average) (Štalić, 2009). In addition, a study from four Indian states demonstrated that 49% of the subjects had less than 220 pg/L vitamin B₁₂, and 30% of those subjects had hHcy (>15 μ mol/L). Based on their results, they concluded that individuals with an optimum vitamin B₁₂ and folate status generally have a low Hcy level, even in an hHcy related risk genotype (Sukla et al., 2012). Our results showed strong inverse correlation of both vitamin B₁₂ and folate status, but not the MTHFR C677T polymorphism, with plasma Hcy level.

MTHFR C677T polymorphism and the Hcy level

MTHFR is an enzyme that facilitates the conversion of Hcy to methionine. When the most com-

mon genetically inherited mutation at nucleotide 677 (C677T) occurs, the MTHFR enzyme activity becomes impaired and can result in increasing the Hcy level by 25%. Homozygous T/T genotype is an independent risk factor for hHcy (Maron and Loscalzo, 2009). The prevalence of MTHFR C677T gene mutation varies between population groups. A Croatian study from 2004, performed on a sample of 228 subjects, found 9.21% homozygous (T/T), 44.74% heterozygous (C/T) and 46.05% wild type mutation (C/C) carriers (Lovričević et al., 2004). Our study showed a similar distribution, finding 13% total homozygous (T/T), 43% heterozygous (C/T) and 44% wild type (C/C) carriers. A South-East Bosnian study on 207 healthy subjects found 11.11% homozygous (T/T) and 44.44% heterozygous (C/T) carriers. All of these results are in correlation with mutation frequency for the European population.

Fodinger et al. (2000) found the association of MTHFR C677T polymorphism with elevated plasma Hcy. In contrast to these findings, our study did not confirm that association; there was no statistically significant difference in the Hcy level between different genotypes either among vegetarians or omnivores. Vegetarian homozygous (T/T) carriers indeed did have higher Hcy levels but without the expected statistical significance. Lea et al. (2009) from Australia had similar findings, showing no statistically significant effect of the T/T genotype on plasma Hcy level (Lea et al., 2009). A possible explanation could be related to the small sample size (group of 50). The effect of MTHFR C677T genotype on the plasma Hcy level is dependent on the folate status and is smaller in areas like Croatia and Australia with folate fortification, compared with low folate regions like Asia (Holmes et al., 2011).

Overall, it was shown that MTHFR C677T polymorphism alone, as well as the interaction of MTHFR C677T polymorphism with vegetarian diet, had no effect on plasma Hcy level. On the other hand, a vegetarian diet had a strong and statistically significant influence and effect on plasma Hcy level. Therefore, a lower level of vitamin B12 and the folate status in vegetarians are indicators that supplementation is needed in

order to bypass methylation and influence on genetic variations of the MTHFR enzyme essential in Hcy metabolism.

Conclusion

Vegetarians had lower vitamin B₁₂ and higher Hcy levels than omnivores. The lower vitamin B₁₂ and folate status was correlated with higher plasma Hcy levels. The MTHFR C677T genotype was not associated with plasma Hcy level either among vegetarians or omnivores. Nevertheless, the MTHFR C677T polymorphism had no effect on plasma Hcy level. To conclude, the effects of a vegetarian diet on plasma Hcy level are evident and indicate the significance of adequate vitamin B₁₂ and folate status in bypassing the MTHFR C677T polymorphism. The limitations of this study are the sample size and the absence of data on dietary intake of vitamin B₁₂ and folate. Further clinical trials on a larger number of participants are recommended.

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UTJECAJ PREHRAMBENOG MODELA I POLIMORFIZMA C677T METILENTETRAHIDROFOLAT REDUKTAZE NA RAZINU HOMOCISTEINA U PLAZMI MEĐU ZDRAVIM VEGETARIJANCIMA I OMNIVORAMA

Sažetak

Povišena razina homocisteina u plazmi (Hcy) povezana je s fiziološkim i prehrambenim čimbenicima, kao i genetskim defektom enzima koji su uključeni u metabolizam Hcy. Ciljevi ove studije bili su (1) utvrditi razlike između zdravih vegetarijanca i omnivora u odnosu na biokemijske parametare, prevalenciju MTHFR genotipa i razinu Hcy, i (2) utvrditi učinke polimorfizma metilentetrahidrofolat reduktaze (MTHFR) C677T i načina prehrane na razinu Hcy u plazmi. U 47 vegetarijanaca i 53 omnivora izmjerena je razina Hcy, folata, vitamina B12, glukoze, ukupnog kolesterola, triglicerida, HDL i LDL kolesterola i kreatinina u plazmi. Polimorfizam MTHFR C677T analiziran je pomoću PCR-RFLP metode. Dobiveni rezultati su pokazali da su vegetarijanci imali niži vitamin B12, ukupni kolesterol, LDL-kolesterol i status kreatinina. Razina Hcy u plazmi bila je veća kod vegetarijanaca u usporedbi s omnivorima ($14,10 \pm 6,69$ vs $10,49 \pm 2,41$ $\mu\text{mol/L}$) i negativno je korelirala sa statusom vitamina B12 i folatom. Razina plazme Hcy nije bila različita u odnosu na genotipove MTHFR C677T, ni među vegetarijancima ni omnivorima. Za razliku od MTHFR C677T polimorfizma, potvrđen je utjecaj načina prehrane na razinu Hcy u plazmi. Može se zaključiti da vegetarijanci obično imaju niži status vitamina B12 i višu razinu Hcy u plazmi. Polimorfizam MTHFR C677T nema utjecaja na razinu Hcy plazme, za razliku od prehrambenog uzorka koji ukazuje na važnost adekvatnog vitamina B12 i statusa folata u zaobilaženju mutacije.

Ključne riječi: homocistein, MTHFR C677T polimorfizam, vegetarijanska prehrana, vitamin B12,