

## Research Article

# Homocysteine Serum Level in Relation to Intake of Folate, Vitamins B<sub>12</sub>, B<sub>1</sub>, B<sub>2</sub>, and B<sub>6</sub> and MTHFR c.665C→T Polymorphism among Young Women

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**Abstract**

The study was conducted to assess influence of folate, vitamins B<sub>12</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub> intake, and MTHFR c.665 C→T polymorphism on homocysteine (Hcy) level among 176 female university students. Nutrients intake was assessed by 4-day record method. Serum Hcy, folate and vitamin B<sub>12</sub> levels were assessed in blood morning samples, MTHFR genotype - after DNA extraction. The frequency of TT genotype of MTHFR gene was 13%. Hcy level was higher in TT individuals (9.4μmol/L) than in CC (8.3μmol/L) and CT (8.2μmol/L), while folate level was lower (13.0nmol/L, 16.0nmol/L and 15.4nmol/L, respectively). Serum B<sub>12</sub> was similar in all groups (approx. 274pmol/L). Significant negative correlations between Hcy and folate, B<sub>12</sub> serum levels in all genotype groups were found, with the highest values for TT individuals, except of the relation between B<sub>12</sub> and Hcy. Nutrients intake did not differ among genotype groups. Total folate intake in the whole group of women was low (162μg DFE/d), vitamin B<sub>12</sub> intake amounted 3.1μg/d. Intake of folate and B<sub>1</sub> below EAR concerned 88% and 40% of women, respectively. Folate intake indicated significant negative correlation with Hcy level only in TT subjects. We concluded that folate intake by TT subjects was too low to obtain the same serum Hcy level as in CC and CT subjects despite the same intake of folate, B<sub>1</sub>, B<sub>2</sub> and B<sub>6</sub> vitamins, and slightly higher B<sub>12</sub> intake.

**Keywords:** Folate; Vitamin B<sub>12</sub>; MTHFR polymorphism; Young women; Intake

**Abbreviations**

BMD: Bone Mineral Density; BMI: Body Mass Index; CVD: Cardiovascular Diseases; CAD: Cervical Artery Dissection; DFE: Dietary Folate Equivalent; EAR: Estimated Average Requirement; FAD: Flavin Adenine Dinucleotide; FFQ: Food Frequency Questionnaire; Hcy: Homocysteine; MTHFR: Methylene tetrahydrofolate Reductase; NS: Statistically Non-Significant; NTD: Neural Tube Defects; tHcy: Total Homocysteine

**Introduction**

Nowadays severe vitamin deficiencies seldom occur in developed countries, however suboptimal status is common in some population groups and influences the risk of chronic diseases. Hyperhomocysteinemia (tHcy serum level >15μM/L) may be one of the symptoms of suboptimal intake of some nutrients, as well as may be caused by genetic and life style factors. Among nutritional factors that are best recognized are folate and vitamin B<sub>12</sub>. Both vitamins are important nutrients for many metabolic pathways to maintain adequate homocysteine (Hcy) serum level [1, 2]. They are needed in remethylation Hcy to methionine [3]. Deficiency of either vitamin may lead to serious health problems such as impaired DNA methylation, one of the epigenetic mechanisms to control gene expression [4], megaloblastic anemia [1] or higher risk of dementia, Alzheimer's and Parkinson's diseases [5], carcinogenic processes [6,7]. Folate deficiency among women in childbearing age is the risk

factor of low birth weight and preterm delivery [8], also increases the risk of neural tube defects (NTD) in offspring [9,10].

Elevated serum homocysteine level is also observed in low riboflavin status. Vitamin B<sub>2</sub> is a precursor of Flavin Adenine Dinucleotide (FAD) which is a cofactor of MTHFR. This enzyme interacts with folate in formation of 5-methyltetrahydrofolate, that acts as methyl donor necessary for homocysteine remethylation [11]. It is believed that high folic acid intake may increase riboflavin requirement.

Adequate intake of vitamin B<sub>6</sub> is also important for proper Hcy serum level. Vitamin B<sub>6</sub> takes part in the conversion of tetrahydrofolate to 5, 10-methylenetetrahydrofolate as a cofactor of serine hydroxymethyltransferase [4]. The study conducted in Japan among young women indicated that higher B<sub>6</sub> intake was associated with lower Hcy level [12]. Simultaneously authors indicated that there was statistically significant association between lower Hcy level and higher dairy products intake, as well as lower intake of green and oolong tea. These indicated that the proper proportions of several nutrients and bioactive substances in the diet are very important to maintain the optimal level of Hcy.

Also thiamine was taken into account as a possible nutrient to reduce Hcy level in homocystinuria. It acts as a cofactor of the supposed rate-limiting oxidative decarboxylation in the transamination of methionine. However the results of the study indicated that vitamin B<sub>1</sub> did not lower homocysteine level [13].

The other factor of the risk of mild hyperhomocysteinemia is the genetic mutation of methylenetetrahydrofolate reductase (MTHFR). There are two common polymorphisms: c.665C→T (p.Ala222Val) and c.1286A→C (p.Glu429Ala). Both variants cause that enzyme is thermolabile and has decreased activity what increases folate and B<sub>12</sub> requirements [14, 15].

To maintain adequate homocysteine level is important because it is considered as a risk factor for a number of health problems. However there is no consent between the influence of elevated homocysteine serum level and the risk of several diseases such as cardiovascular diseases. Earlier studies indicated that high Hcy level was linked to the higher risk of cardiovascular diseases [16]. However in the review of the studies conducted among children such relationship was not shown [17]. Experts of Homocysteine Studies Collaboration [18] proposed that higher homocysteine level by patient suffering from CVD is rather a result of a disease than a cause.

Meta-analysis conducted by Luo et al. [19] indicated positive correlations between hyperhomocysteinemia and cervical artery dissection (CAD), as well as between the c.665C→T polymorphism of MTHFR and CAD. It was stated also negative influence on cognitive impairment [20], on bone formation and BMD in children and adolescents [21]. The positive influence of folate and vitamin B<sub>12</sub> status was also detected in other population groups. Literature review indicated that 50 pmol/L increase of serum/plasma vitamin B<sub>12</sub> declined the risk of bone fractures of 4% in the elderly. However the results for folate are not so univocal and depend on gender [22].

The aim of the study was to assess the adequacy of folate, vitamins B<sub>12</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub> intakes (from all nutritional sources: natural food, fortified products and dietary supplements) and their relationship with serum homocysteine level taking into consideration MTHFR c.665 C→T polymorphism.

## Materials and Methods

### Participants

The data presented in this paper were received during the cross-sectional study conducted among 446 young women [23]. All of them were volunteers and university students. From this group individuals for whom MTHFR polymorphism was assessed were taken into consideration in this analysis (n=176). The study protocol was approved by the Ethical Committee of the National Institute of Food and Nutrition in Warsaw, Poland. Each participant signed informed consent and agreement for blood collecting and genetic analysis.

The socio-demographic data and data related to health and life style were collected with the use of a general questionnaire which included questions about age, self-reported health status, self-reported physical activity level, special diet use, smoking habits.

Separate part of the questionnaire concerned dietary supplement usage in the past year. For this work all preparations available on the Warsaw market containing vitamins and/or minerals in the form of tablets, drops, powder etc. were regarded as supplements. Questions included brand and product names, the form (i.e. tablets, capsules, drops etc.), number of doses taken during a day. Information on composition was collected on the basis of producers' declaration on product labels. On the basis of received data amounts of taken nutrients were calculated and individuals were characterized as

supplement users. As a total supplement users were regarded individuals who had taken at least one preparation containing any vitamin and/or mineral even for a short time (i.e. 1 week) during the year before the study.

BMI value was calculated for each individual on the basis of measured weight and height.

### Dietary intake

Data on food and meal consumption were collected by 4-day records during consecutive days with one weekend day. Respondents were asked to report all meals, food products, beverages, fortified products and dietary supplements consumed during those days. Portion sizes were given in grams, milliliters, and if it was not possible in household measures. Household measures were converted to weight measures with the help of „Album of photographs of food products and dishes” [24]. Any food without nutrient added was considered as “natural food”. Folates and vitamins B<sub>12</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub> intakes with natural food were calculated using Polish food composition tables [25].

On the basis of observed intake of energy, protein, methionine, folates and vitamins B<sub>12</sub>, B<sub>1</sub>, B<sub>2</sub>, and B<sub>6</sub> with natural products usual intake was calculated according to procedure described by Carriquiry [26]. Because of the skewed distribution of vitamin intakes (total intake) data were presented as median value and inter-quartile range. It caused the possibility to compare data of vitamins intake with the natural products only and total. Information on current vitamin supplement usage was obtained on the basis of 4-day records.

As our previous surveys indicated that a lot of people in Poland were not conscious that they used fortified products, data on habitual fortified food products intake during the last month were collected by means of the FFQ method. All food intake data were checked with the use of specially prepared for the purpose of this study album of fortified food product photographs available on the Warsaw market. Then 4-day records were corrected appropriately changing not fortified product to fortified if it was needed.

Total vitamin intake was calculated as the sum of observed intake from natural and fortified food and dietary supplements. As the bioavailability of synthetic folic acid (the form added in fortified food and dietary supplements) may differ from that of natural food the conversion to Dietary Folate Equivalents (DFE) was done according to the equation [27]: micrograms of natural food folate + 1.7 (micrograms of folic acid from fortified food + micrograms of folic acid from supplements).

For assessment of adequacy of vitamins intake EAR cut point method was used. Polish EARs for young women equal for folate 320µg, vitamins B<sub>12</sub> 2µg, B<sub>1</sub> 0.9mg, B<sub>2</sub> 0.9mg and B<sub>6</sub> 1.1mg [28]. Results were presented as % of women with intake below EAR.

### Folate, vitamin B<sub>12</sub> status and homocysteine level

Blood samples were collected in the morning after an overnight fasting. The blood was allowed clotting and separated serum was stored at -20°C. Serum total homocysteine level was measured by the radioimmunoassay (Imx analyzer, Abbot Laboratories). Roche Elecsys 2010 electrochemiluminescence immunoassay analyzer was used for measurement of serum folate and serum vitamin B<sub>12</sub>.

### MTHFR gene polymorphism

DNA was extracted from peripheral blood by salting out

**Table 1:** Characteristics of the individuals under the study (n=176).

Parameter	Category	Units	
Age (years)		mean $\pm$ SD range	21.2 $\pm$ 1.5 19 – 26
Economic status <sup>*</sup>	very good good average bad no answer	n (%)	5 (2.8) 53 (30.1) 104 (59.1) 8 (4.5) 6 (3.4)
Health status <sup>*</sup>	very good good average no answer	n (%)	31 (17.6) 120 (68.2) 20 (11.4) 5 (2.8)
Physical activity level <sup>*</sup>	high average low don't know	n (%)	15 (8.5) 118 (67.0) 35 (19.9) 8 (4.5)
Using special diet	yes no no answer	n (%)	21 (11.9) 152 (86.4) 3 (1.7)
BMI (kg/m <sup>2</sup> )		mean $\pm$ SD range	20.7 $\pm$ 2.1 15.2 – 29.4
Smoking	yes	n (%)	17 (9.7)
Total dietary supplements use <sup>*</sup> during a year before the study	yes	n (%)	107 (60.8)
Habitual fortified food intake		n (%)	124 (70.5)

<sup>\*</sup> Self-reported; <sup>\*</sup> Total – all preparations containing any of vitamins, minerals

procedure as described by Miller et al. [29]. For analysis of mutation, PCR reactions were carried out using conditions and primer sequences which have been describe in Arruda et al. [30]. All PCR cycles were performed in a Peltier Thermal Cycler apparatus (PTC-200; M.J.Research, Watertown, MA, USA). Specific PCR products were digested with restriction enzymes according to the manufacturer's instructions and analysed by electrophoresis. All analysis were done in laboratory of the Institute of Mother and Child in Warsaw.

## Statistical Analysis

Statistical analyses were performed with Statistica Stat Soft Pl v. 7.0. For quantitative variable with normal distribution ANOVA test was used, in other cases Kruskal-Wallis test. Because of skewed distribution all blood parameters were ln-transformed before statistical analysis; results are presented after back transformation. For quantitative data Chi<sup>2</sup> test was used. Relationships between status parameters and amounts of vitamins intake were analyzed with the use of Spearman test. P value below 0.05 was considered as statistically significant.

## Results and Discussion

The characteristics of the subjects are shown in Table 1. The average age of women was about 21 years with the range 19-26. Most females estimated their health status as good, economic status and physical activity level as moderate. Almost 12% of students applied special diet, in most cases with reduced energy value. Most of the subjects were slim (85.5%), with BMI amounted approximately 21 kg/m<sup>2</sup>. For only 8.5% of students BMI values were lower than 18.5 kg/m<sup>2</sup> and for almost 8% - higher than 24.9 kg/m<sup>2</sup>. Our group of females was similar to other groups of students in Poland [31,32] while surveys conducted in other population groups showed that overweight and obesity were more prevalent and concerned 17% of teenage girls [33]. In our study smoking was declared by about 10% of students while the data received within Global Adult Tobacco Survey indicated

that the prevalence of daily smokers among adult women is higher and amounts 17.9% [34]. Dietary supplements usage was a common practice among students. About 61% of them took at least one vitamin and/or mineral preparation in the past year. Fortified food products intake was declared by 70% of participants. More detailed analysis indicated that more than 28% of women took dietary supplements and fortified products at the same time. Similarly such practice was also observed among children aged 7-12 years in Warsaw, where 18% of them were administered both products (supplements and fortified food) simultaneously [35].

More detailed information about type of supplemented vitamins are given in Table 2. During the past year about 17% of students chose dietary supplements containing folic acid, 20% - vitamin B<sub>12</sub>, other vitamins B group were used more often. In the day of the study all analyzed supplements were taken by a much lower number of students (Table 3). The slightly less frequency of total supplement usage during the year before the study was also observed by adults in Warsaw (53.3%), while folic acid usage was more prevalent and reached 25% [36].

The frequency of TT genotype of the gene of methylenetetrahydrofolate reductase varies in the world from 3% to 32% [37]. In our study amounted 13%, while 46% of participants had two wild genes (CC) and 41% of women had CT genotype (Table 3). The similar data were obtained in studies on the elderly: Kadziela's

**Table 2:** Characteristics of B vitamin preparations use during a year before the study.

Vitamin	Supplement use Number of individuals (%)
Folate	31 (17.6)
B <sub>12</sub>	36 (20.5)
B <sub>1</sub>	62 (35.2)
B <sub>2</sub>	62 (35.2)
B <sub>6</sub>	68 (38.6)

**Table 3:** Energy, protein and methionine, folates, vitamins B<sub>12</sub>, B<sub>1</sub>, B<sub>2</sub> and B<sub>6</sub>, intakes with a diet by MTHFR c.665C→T genotype by means of 4-day records.

Nutrient	Total	MTHFR genotype			Statistical analysis
		CC	CT	TT	
	% of subjects				Test (p)
100	46.2	40.3	12.5		
Daily intake [Mean ± SD]					ANOVA
Energy (kcal)	1658 ± 199	1658 ± 201	1669 ± 193	1615 ± 210	0.5413
Protein (g)	59.7 ± 5.2	60.1 ± 5.5	59.5 ± 5.0	59.0 ± 4.9	0.6258
Methionine (g)	1.51 ± 0.36	1.55 ± 0.37	1.49 ± 0.34	1.45 ± 0.39	0.4234
Daily vitamins intake with the diet only [Median; Q1-Q3]					K-W <sup>##</sup>
folate (µg DFE <sup>#</sup> )	162 (129-189)	164 (128-198)	162 (133-181)	161 (125-191)	0.7406
vit. B <sub>12</sub> (µg)	3.1 (2.3-4.2)	3.1 (2.3-5.0)	2.9 (2.2-3.9)	3.8 (2.8-4.6)	0.1325
vit. B <sub>1</sub> (mg)	0.87 (0.72-1.03)	0.86 (0.73-1.08)	0.88 (0.70-1.05)	0.88 (0.74-0.94)	0.7593
vit. B <sub>2</sub> (mg)	1.26 (1.09-1.48)	1.26 (1.13-1.52)	1.24 (1.06-1.45)	1.32 (1.17-1.49)	0.4251
vit. B <sub>6</sub> (mg)	1.54 (1.29-1.88)	1.55 (1.24-1.89)	1.59 (1.30-1.94)	1.40 (1.24-1.61)	0.1577
% of subjects with intake with a diet only <EAR					Chi <sup>2</sup>
folate	98.7	97.6	100	100	0.3219
vit. B <sub>12</sub>	13.6	10.8	16.9	13.6	0.5509
vit. B <sub>1</sub>	57.4	56.6	56.4	63.6	0.8175
vit. B <sub>2</sub>	6.8	6.02	7.04	9.09	0.8851
vit. B <sub>6</sub>	14.2	15.7	14.1	8.0	0.7343
Dietary supplements use on days of the study (% of subjects)					Chi <sup>2</sup>
folic acid	8.5	6.0	8.5	18.2	0.1923
vit. B <sub>12</sub>	10.2	6.0	11.3	22.7	0.0664
vit. B <sub>1</sub>	10.2	6.0	11.3	22.7	0.0664
vit. B <sub>2</sub>	10.2	6.0	11.3	22.7	0.0664
vit. B <sub>6</sub>	10.2	6.0	11.3	22.7	0.0664
Total <sup>†</sup> vitamins intake [Median; Q1-Q3]					K-W <sup>#</sup>
folate (µg DFE <sup>##</sup> )	193 (157-257)	189 (151-268)	193 (164-254)	207 (160-285)	0.7609
vit. B <sub>12</sub> (µg)	3.5 (2.6-6.3)	3.4 (2.5-5.2)	3.2 (2.4-4.4)	4.1 (2.9-8.2)	0.1528
vit. B <sub>1</sub> (mg)	1.01 (0.81-1.29)	0.98 (0.79-1.24)	1.02 (0.84-1.31)	1.00 (0.85-1.37)	0.6664
vit. B <sub>2</sub> (mg)	1.40 (1.22-1.76)	1.37 (1.21-1.71)	1.39 (1.21-1.98)	1.66 (1.26-2.49)	0.2342
vit. B <sub>6</sub> (mg)	1.80 (1.42-2.07)	1.75 (1.38-2.17)	1.94 (1.59-2.27)	1.66 (1.32-2.22)	0.0925
% of subjects with total intake <EAR					Chi <sup>2</sup>
folate	87.5	86.8	90.1	81.8	0.5641
vit. B <sub>12</sub>	9.1	10.8	7.0	9.1	0.7157
vit. B <sub>1</sub>	39.2	44.6	32.4	40.9	0.2991
vit. B <sub>2</sub>	2.8	3.6	1.4	4.6	0.6252
vit. B <sub>6</sub>	8.0	7.2	9.9	4.6	0.6836

<sup>#</sup>SD- standard deviation; <sup>†</sup>Inter-quartile range; <sup>#</sup>DFE – Dietary Folate Equivalent; <sup>##</sup> K-W - Kruskal-Wallis test

<sup>††</sup>Total - all sources: diet, preparations containing vitamins and/or minerals, fortified food products.

et al. [38] it amounted 12%, and in Chmurzynska's et. al. [39] on elderly women - 14%. In other European countries the frequency of TT genotype differs. In Germany among adults it amounted 7% [40], in Denmark 8.8% [41]. In North Indian healthy population (18-73 y) was much lower and amounted 3.5% [42]. In the USA the frequency depended on the race-ethnicity population group, and was the highest among Mexican American (20%), then Non-Hispanic white (11.7%), and the lowest in Non-Hispanic black (1.3%) [43].

The analysis of nutritional value of the participants' diet was done in relation to MTHFR genotype (Table 3). Energy intake was at the same level in all genotype groups. Low energy intake explained the fact of being rather slim for most of the women under study. Also protein and methionine intakes were at the same level in all groups. The median folate intake with the diet only was 162 µg DFE/day. After taking into account all sources (i.e. diet, supplements and extra from fortified foods) of this vitamin the median value increased by

about 31 µg FE/day only, and was still much lower than Polish EAR (320 µg DFE/day) for young women [28]. There were no statistically significant differences in folate intake among all the genotype groups. Analysis of the distribution of folate intake with the diet only indicated that for almost all women intake did not exceed EAR level, and the situation was not improved significantly after taking into consideration all sources of this vitamin (87% of all women). The median vitamin B<sub>12</sub> intake with the diet only was 3.1 µg/day, and after taking into account all sources of this vitamin it amounted 3.5 µg/day. There were also no statistically significant differences in B<sub>12</sub> vitamin intake among all the genotype groups and median intake exceeded EAR (2 µg/day) [28]. Other surveys conducted in different population groups in Poland also indicated that vitamin B<sub>12</sub> intake was adequate, while folate intake was too low [44,45]. Median intake of vitamin B<sub>1</sub> with the diet only was slightly lower than EAR value, while for vitamin B<sub>2</sub> and B<sub>6</sub> intakes were above EAR for all the genotype groups. The

**Table 4:** Folate and vitamin B<sub>12</sub> status in relation of the MTHFR c.665C→T genotype.

	Total	MTHFR genotype			ANOVA (p)
		CC	CT	TT	
		mean ± SD			
serum folate (nmol/L)	15.4 ± 1.3	16.0 ± 1.25 <sup>a*</sup>	15.4 ± 1.3 <sup>a</sup>	13.0 ± 1.5 <sup>b</sup>	0.0071
serum vit. B <sub>12</sub> (pmol/L)	275 ± 1.5	276 ± 1.4	276 ± 1.5	264 ± 1.6	0.8706
serum Hcy (µmol/L)	8.36 ± 1.26	8.33 ± 1.27 <sup>ab</sup>	8.11 ± 1.23 <sup>a</sup>	9.35 ± 1.30 <sup>b</sup>	0.0456

\*different letters a, b indicated statistically significant differences on the base of post-hoc analysis with the use of RIR test

**Table 5:** Spearman's correlation coefficients between folate and B<sub>12</sub> status and intake of B group vitamins in relation of the MTHFR c.665C→T genotype.

	Total	MTHFR genotype		
		CC	CT	TT
		serum Hcy		
serum folate	-0.41	-0.36	-0.41	-0.49
serum B <sub>12</sub>	-0.32	-0.26	-0.36	NS
vitamins intake				
dietary B <sub>1</sub>	NS	NS	NS	-0.61
dietary B <sub>2</sub>	-0.16	NS	NS	-0.63
dietary B <sub>6</sub>	NS	NS	NS	-0.51
dietary folate	NS	NS	NS	-0.70
dietary B <sub>12</sub>	-0.18	-0.26	NS	NS
		serum folate		
dietary folate	NS	NS	NS	0.67
		serum B12		
dietary B <sub>12</sub>	NS	NS	NS	NS
		RBC folate		
dietary folate	NS	NS	NS	0.54

NS - statistically non-significant

intake of folate and B<sub>1</sub> vitamin with the diet only below EAR value concerned almost 99% and 60% of examined women, respectively. Taking into account all sources of these vitamins the percentage of subjects with intake below EAR reminded high and concerned about 88% and 40% of individuals, respectively. We can conclude that although using dietary supplements improved the vitamins intake however still high proportion of women realized their requirements below EAR. More detailed analysis indicated that intake below the EAR value for all vitamins discussed in this study concerned only one person (0.6% of all women), then four of vitamins – 3 individuals (1.7%), three – 18 individuals (10.2%), two – 52 individuals (29.5%), one – 83 individuals (47.2%), and none of vitamins – 19 individuals (10.8%). Such situation occurred in spite of the fact that there were statistically significant correlations in all vitamins content in the diet.

Analysis of the folate and B<sub>12</sub> vitamin status indicated that statistically significant differences, taking into account MTHFR genotype, were only for serum folate and Hcy levels. In all genotype groups the average serum folate levels were within the range of references values, but rather close to lower limit, what was probably related to a rather low folate intake. Serum folate level in TT subjects was significantly lower (13.0 nmol/L) than in CC (16.0 nmol/L) and in CT subjects (15.4 nmol/L) in spite of almost the same folate content in the diet (Table 4). The means were lower than 18 nmol/L, the value considered as the lowest limit of the serum level that can optimally prevent birth defects during pregnancy (mean optimal level is 26.1 nmol/L) [46].

Mean vitamin B<sub>12</sub> serum level was also adequate among all genotype groups, however in TT subjects was slightly lower than in

CC and CT subjects (differences not statistically significant).

Mean homocysteine serum level was in all genotype groups below the value of 10 µmol/L, that is considered as the higher limit of optimal range [47]. In TT subjects mean Hcy level was above 9 mol/L, and was higher than in CC (non-significantly) and in CT subjects (significantly) – where we found 8.3 and 8.1 µmol/L, respectively. Almost all subjects had Hcy serum level below 15 µmol/L, only for two individuals the level exceeded this amount (one in CC and one in TT genotype group). Similarly low Hcy serum level was found in women aged 20-74 years (9.7 µmol/L in TT, 8.8 µmol/L in CC and CT subjects) in the Polish National Multicentre Health Survey, while in men was higher (13.1 µmol/L in TT, and 10.2 µmol/L in CC and CT subjects) [48].

Analysis of relations between folate and vitamin B<sub>12</sub> status and serum Hcy levels indicated statistically significant negative correlations, with the highest values for individuals with TT genotype (Table 5). However there was one exception i.e. for the relation between B<sub>12</sub> and Hcy serum levels. In TT subjects this relation was non-significant indicating that the folate status is critical for the proper Hcy level in this subjects. Also folate intake indicated high statistically significant negative correlation with serum Hcy level in TT subjects while such relation was not observed in other genotype groups. Our results indicated that young women with the TT genotype are more sensitive to the folate deficiency in the diet while other vitamins had no such effect. Also in other study conducted among young women with low folate intake it was observed that TT subjects were more sensitive to the improving folate intake, what was measured as DNA methylation [49]. However results of the Polish National Multicentre Health Survey indicated that in women with the TT polymorphism the only factor influencing Hcy level was age, while in CC and CT groups there was observed influence of age, folate and B<sub>12</sub> intake [48].

As limitations of this study we can point the relatively small sample size which limited statistical analysis to MTHFR polymorphism. Moreover we had no possibility to assess neither polymorphisms of other enzymes nor status of other B-group vitamins (B<sub>6</sub> and B<sub>2</sub>).

## Conclusions

In young women taking part in the study, folate intake in TT subjects was too low to reach the same serum Hcy level as in CC and CT subjects despite slightly higher vitamin B<sub>12</sub> intake. Therefore it seemed that young women with the TT genotype were more sensitive to the dietary folate deficiency, while other vitamins had no such effect.

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