



# Homocysteine, folate, and cobalamin levels in hyperthyroid women before and after treatment

Homocysteina, kwas foliowy i witamina B<sub>12</sub> u kobiet z nadczynnością tarczycy oraz po uzyskaniu eutyreozy

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## Abstract

**Introduction:** Hyperhomocysteinaemia is an independent risk factor for premature atherosclerotic vascular disease and venous thrombosis. Hypothyroidism is associated with mild hyperhomocysteinaemia. The aim of the present study was to assess plasma total homocysteine (tHcy) and its determinants (folate, cobalamin) in hyperthyroid patients before and after treatment.

**Material and methods:** Thirty hyperthyroid and thirty healthy premenopausal women were studied. The hyperthyroid patients were investigated in the untreated state and again after restoration of euthyroidism. The levels of homocysteine, folate, cobalamin, and thyroid stimulating hormone (TSH), free thyroxine (fT<sub>4</sub>), free triiodothyronine (fT<sub>3</sub>), and renal function were measured before and after treatment.

**Results:** In hyperthyroidism, tHcy was lower than in the control group. The serum level of folate was higher and serum cobalamin was lower in the hyperthyroid state. Following antithyroid drug therapy, tHcy significantly increased and folate decreased. The level of cobalamin remained unchanged. Univariate analysis in the hyperthyroid group indicated that tHcy significantly negatively correlated only with fT<sub>3</sub>.

**Conclusions:** Lower homocysteine levels in hyperthyroid state can be explained by the influence of thyroid hormone. High level of folate is only partially responsible for these changes. (*Pol J Endocrinol* 2009; 60 (6): 443–448)

**Key words:** homocysteine, cobalamin, folate, hyperthyroidism

## Streszczenie

**Wstęp:** Podwyższone stężenie we krwi homocysteiny jest niezależnym czynnikiem ryzyka przedwczesnej miażdżycy tętnic oraz zmian zakrzepowo-zatorowych naczyń żylnych. Łagodną hiperhomocysteinemię obserwuje się u osób z niedoczynnością tarczycy. Celem pracy była analiza osoczowych stężeń całkowitej homocysteiny (tHcy, *total homocysteine*), kwasu foliowego oraz witaminy B<sub>12</sub> u chorych kobiet z nadczynnością tarczycy przed i po leczeniu.

**Materiał i metody:** Badaniu poddano 30 pacjentek z nadczynnością tarczycy oraz grupę kontrolną 30 zdrowych premenopauzalnych kobiet. Grupa z nadczynnością tarczycy badana była 2-krotnie, przed leczeniem oraz po uzyskaniu eutyreozy. Oznaczano stężenia osoczowe homocysteiny, kwasu foliowego, witaminy B<sub>12</sub> oraz tyreotropiny (TSH, *thyroid stimulating hormone*), wolnej tyroksyny (fT<sub>4</sub>) i wolnej trójiodotyroniny (fT<sub>3</sub>).

**Wyniki:** W grupie z nadczynnością tarczycy stężenie tHcy było istotnie niższe w porównaniu z grupą kontrolną. Stężenie kwasu foliowego było znacząco wyższe, a witaminy B<sub>12</sub> niższe w grupie kobiet z nadczynnością. Po leczeniu tyreostatykami stężenie tHcy istotnie wzrosło, a kwasu foliowego obniżyło się. Stężenie witaminy B<sub>12</sub> pozostało niezmiennione. Analiza jednoczynnikowa w grupie z nadczynnością tarczycy wykazała, że stężenie tHcy istotnie ujemnie korelowało tylko ze stężeniem fT<sub>3</sub>.

**Wnioski:** Obniżone stężenie homocysteiny w nadczynności tarczycy może być wyjaśnione wpływem hormonów tarczycy. Wysokie stężenia kwasu foliowego tylko częściowo odpowiadają za te zmiany. (*Endokrynol Pol* 2009; 60 (6): 443–448)

**Słowa kluczowe:** homocysteina, witamina B<sub>12</sub>, kwas foliowy, nadczynność tarczycy

## Introduction

Hyperhomocysteinaemia has been identified as a prevalent and strong risk factor of cardiovascular occlusive disease and venous thromboembolism [1–4]. The relationship between homocysteine and cardiovascular disease is dose dependent and independent of other

risk factors [5]. Starting at a plasma total homocysteine (tHcy) concentration level of 10 μmol/L, the risk increases linearly [6]. Elevated plasma tHcy level (> 12 μmol/L) is found in 5–10% of the general population and in up to 40% of patients with vascular disease [7]. Hyperhomocysteinaemia can induce damage of vascular cells directly (endothelial injury, smooth muscle hypertrophy,



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vascular vasomotor function damage) and throughout the process of generating oxidative stress and oxidation of LDL-Ch [8, 9]. Homocysteine (Hcy) induces platelet aggregation and activity of factor V and XII, and inhibits the following: anticoagulant activity, protein C, and antithrombin III [10–12].

Homocysteine is a sulphur-containing amino acid biosynthesized from methionine, an essential amino acid. Homocysteine is an intermediate product in the transfer of activated methyl groups from tetrahydrofolate to S-adenosylmethionine (the reversible remethylation pathway). This reaction is catalyzed by the methionine synthetase and requires vitamin B<sub>12</sub> as a cofactor and 5-methylTHF from folate as a methyl donor. Homocysteine can also be metabolized by irreversible transsulfuration pathway and requires vitamin B<sub>6</sub> as an enzyme cofactor.

Age, gender, life style (coffee, alcohol), and very rarely occurring enzyme function impairment have an impact on Hcy metabolism [13–15]. Other factors influencing plasma tHcy concentration are: vitamin deficiencies, medicaments (methotrexate and other folate antagonists, oral contraceptives, antagonists of vitamin B<sub>12</sub> and B<sub>6</sub>, thiazides, fibrates), and diseases such as: renal failure, hepatic insufficiency, pernicious anaemia, and cancers.

Several studies have demonstrated elevated levels of tHcy in patients with hypothyroidism [16, 17]. Once euthyroidism was achieved with L-thyroxine therapy, tHcy after overnight fasting significantly decreased [18, 19]. In the opposite way: higher plasma tHcy level may be responsible for premature atherosclerosis observed in hypothyroid patients [20]. Even a mild variation of the thyroid hormone (within the normal range) in patients with coronary artery disease may change the result of coronary angiography. Higher concentrations of serum free thyroid hormone were associated with decreased severity of coronary atherosclerosis [21]. A few studies indicated lower tHcy levels in the hyperthyroid state. The heterogeneity of the study population with respect to gender, age, vitamin status, etc probably reduced the impact of this observation.

The aim of this study was to determine plasma tHcy in recently diagnosed hyperthyroid women before and after treatment and to evaluate the potential role of Hcy determinants such as plasma levels of folate, vitamin B<sub>12</sub>, and renal function.

## Material and methods

### Participants

Thirty female study participants were prospectively recruited from subjects referred to our thyroid outpatient clinic. These patients were between 19 and 52 years of age (average age 37.3 ± 9.8 years). Inclusion criteria

were: a newly, non-treated hyperthyroidism and regular menses. Diagnosis of hyperthyroidism was based on clinical examination and basal serum TSH values < 0.3 mU/L and fT<sub>4</sub> > 24 pmol/L or fT<sub>3</sub> > 5.3 pmol/L. The control group consisted of 30 female healthy volunteers between 20 and 44 years of age (average age 31.4 ± 7.7).

Exclusion criteria were: diseases and drugs (folate, vitamin B<sub>12</sub>, and B<sub>6</sub> antagonists, anticonvulsants, thiazides, fibrates) that change plasma Hcy levels; pregnancy, lactation, and taking oral contraception; clinical or history of arteriosclerotic disease; excess of alcohol and coffee consumption and special restrictions of diet. The study protocol was approved by the Regional Ethics Committee. All participants gave their informed consent to participate in this study.

Basic routine blood chemistry tests (including creatinine concentration) were performed for each patient. Serum TSH, fT<sub>4</sub>, fT<sub>3</sub>, vitamin B<sub>12</sub>, folate, and tHcy levels were also measured. Second blood analysis was done after obtaining euthyroid state, after more than 2–3 months of treatment with thiamazol (Thyrozol Merck, Darmstadt, Germany) in individualized doses. Participants were advised not to change their lifestyle.

### Biochemical methods

After physical examination, body mass indexes (BMI) were measured and blood samples were taken at 9:00 a.m. after overnight fasting. The plasma was separated within 20 minutes by centrifugation at 3000 rpm for 5 minutes and stored at –20° C until time of analysis.

Serum fT<sub>4</sub>, fT<sub>3</sub>, and TSH levels were determined by microparticle enzyme immunoassay (MEIA) obtained from Abbott Laboratories (AxSYM analyzer). The normal range for fT<sub>4</sub> was 9–24 pmol/L, for fT<sub>3</sub> it was 2.2–5.3 pmol/L, and for TSH, 0.3–5.0 mU/L. Serum folic acid was determined by MEIA assay (Abbott Laboratories) by IMx analyzer. The reference range was: 2.9–18.7 ng/ml. Vitamin B<sub>12</sub> was determined by chemiluminescent microparticle immunoassay (CMIA) (Abbott Laboratories) with Architect system — reference range 179–1162 pg/ml.

tHcy was determined by fluorescence polarization immunoassay (FPIA); we used a reference range of: < 12 μmol/L for healthy persons and < 10 μmol/L for individuals with high cardiovascular risk [21, 22]. The relative coefficient of variation of this assay varies between 1.4% and 5.2% and the correlation with standard high performance liquid chromatography (HPLC) is high (r = 0.989) [24].

Serum creatinine was measured by an automated enzymatic method, and creatinine clearance (C<sub>cr</sub>) was calculated using the Cockcroft-Gault formula: C<sub>cr</sub> (ml/min) = [(140–age (years))/72 × C<sub>cr</sub>] × weight (kg); for women this value was multiplied by 0.85. This formula has a significant correlation to GFR in the literature [25].

Table I. Characteristics of the study group and control group

Tabela I. Charakterystyka grupy badanej i kontrolnej

Characteristics	Study group	Control group	p value
Total	30	30	–
Age (year)	37.3 ± 9.8	31.4 ± 7.7	0.012
BMI [kg/m <sup>2</sup> ]	22.34 ± 3.1	23.13 ± 5.54	0.517
Coffee [cup/d]	1.2 ± 1.0	2.0 ± 1.4	0.019
Cigarette [piece/d]	0.7 ± 1.7	0.2 ± 0.4	0.160
Multivitamin [% of people]	6.7%	33.3%	0.010
TSH [mU/L]	0.004 ± 0.004	1.32 ± 0.73	< 0.001
fT <sub>4</sub> [pmol/L]	52.49 ± 31.82	15.61 ± 3.98	< 0.001
fT <sub>3</sub> [pmol/L]	17.23 ± 11.07	no date	no date
tHcy [μmol/L]	8.20 ± 1.81	10.81 ± 2.44	< 0.001
Folic acid [ng/ml]	8.65 ± 2.55	5.88 ± 3.09	< 0.001
Vitamin B <sub>12</sub> [pg/ml]	344.86 ± 102.08	420.83 ± 142.07	0.023
Cl <sub>cr</sub> [ml/min]	112.8 ± 30.6	113.1 ± 23.3	0.696

Table II. Characteristics the hyperthyroid group before and after treatment

Tabela II. Charakterystyka chorych z nadczynnością tarczycy przed i po leczeniu

	Hyperthyroid	Euthyroid	p value
BMI [kg/m <sup>2</sup> ]	22.34 ± 3.10	23.33 ± 3.17	< 0.001
TSH [mU/L]	0.004 ± 0.004	0.874 ± 1.524	< 0.001
fT <sub>4</sub> [pmol/L]	52.49 ± 31.82	11.42 ± 5.37	< 0.001
fT <sub>3</sub> [pmol/L]	17.23 ± 11.07	3.87 ± 1.46	< 0.001
tHcy [μmol/L]	8.20 ± 1.81	9.64 ± 2.57	0.004
Folic acid [ng/ml]	8.65 ± 2.55	6.37 ± 1.94	< 0.001
Vitamin B <sub>12</sub> [pg/ml]	344.86 ± 102.08	336.8 ± 137.4	0.335
Cl <sub>cr</sub> [ml/min]	112.8 ± 30.6	84.8 ± 20.9	< 0.001

### Statistical analysis

The data of the hyperthyroid group (before and after treatment) and the control group were determined using Student's paired *t*-test. In the case of non-Gaussian distribution, the original data were transformed to attain normal distribution. After transformation, all of these variables had a normal distribution. Univariate relations between tHcy and other variables are presented as Pearson rank correlations. To assess the simultaneous relation among the various predictors of tHcy, multiple linear regression models were used. The analyses were performed with log-tHcy as the dependent variable. We used the statistical package Statistica 6.0 (StatSoft).

### Results

Table I summarizes the clinical and laboratory data of patients and of the control group. Women with hyperthyroidism had statistically significant lower TSH and

higher fT<sub>4</sub> than the control group. Mean tHcy levels in patients before treatment (8.20 ± 1.81 μmol/L) were significantly lower than in the control group (10.81 ± 2.44 μmol/L, *p* < 0.001). In the patient group, a deficiency of vitamin B<sub>12</sub> was not observed, but the mean value of vitamin B<sub>12</sub> was significantly lower than in the control group (344.86 ± 102.08 pg/ml *v.* 420.83 ± 142.07 pg/ml, *p* = 0.023). No subject had evidence of folate deficiency, although folate was significantly higher in women with hyperthyroidism in comparison with the controls (8.65 ± 2.55 *v.* 5.88 ± 3.09 ng/ml, *p* < 0.001).

After recovery of euthyroidism in fasting state, tHcy significantly increased from 8.20 ± 1.81 to 9.64 ± 2.57 μmol/L (*p* = 0.004), folate significantly decreased from 8.65 ± 2.55 to 6.37 ± 1.94 pg/ml (*p* < 0.001), although vitamin B<sub>12</sub> remained unchanged.

The mean levels of creatinine clearance were not significantly lower in hyperthyroid patients *v.* Controls, and decreased significantly after treatment (Table II).

**Table III.** Correlations between plasma tHcy and other parameters in the hyperthyroid group in univariate analysis**Tabela III.** Korelacje pomiędzy osoczymym stężeniem tHcy i innymi parametrami w grupie z nadczynnością tarczycy (analiza jednoczynnikowa)

Parameter	ln (tHcy)	
	r	p
Age (year)	-0.07	0.674
BMI [kg/m <sup>2</sup> ]	0.12	0.453
Ln (TSH)	0.04	0.799
Ln (fT <sub>4</sub> )	-0.25	0.096
Ln (fT <sub>3</sub> )	<b>-0.28</b>	<b>0.037</b>
Ln (folic acid)	-0.27	0.081
Ln (Vit. B <sub>12</sub> )	-0.27	0.079
Cl <sub>cr</sub>	-0.04	0.798

In univariate analysis, decreased tHcy was significantly associated with high fT<sub>3</sub> levels ( $r = -0.28$ ;  $p = 0.037$ ) (Table III).

In multivariate analysis, decreased fasting tHcy state was associated with low vitamin B<sub>12</sub> levels ( $\beta = -0.29$ ;  $p = 0.042$ ).

## Discussion

In the present study, we found that women with hyperthyroidism had a significantly lower mean tHcy 2.6  $\mu\text{mol/L}$  compared with the control group. Normalization of thyroid hormone levels with antithyroid drug treatment was associated with a significant tHcy increase. In univariate analysis, the serum levels of fT<sub>3</sub> were significant determinants of plasma tHcy. These observations are consistent with the results of Demirbas et al. [26]. They observed that tHcy levels were significantly lower in patients with hyperthyroidism than in healthy subjects ( $11.5 \pm 3.6 \mu\text{mol/L}$  v.  $15.1 \pm 4.5 \mu\text{mol/L}$ ,  $p < 0.05$ ) and increased significantly after treatment. However, no significant correlations were found in correlation analysis. In the largest published study on plasma tHcy and hyperthyroidism (182 patients), normalization of thyroid status was associated with a significant increase of tHcy (8.3 v. 9.2  $p < 0.005$ ); in correlation analysis, a significant correlation with fT<sub>4</sub> was observed [27]. The changes of plasma tHcy in relation to thyroid status may be explained by the influence of thyroid hormones on a variety of biochemical processes in Hcy metabolism, distribution, or clearance [28]. Several experimental studies have shown that the activity of flavoprotein methylene tetrahydrofolate reductase (MTHFR), the enzyme that participates in folate metabolism, is influen-

ced by thyroid status [29]. Hepatic activity of MTHFR is increased in hyperthyroid state and reduced in hypothyroid state. In the reaction catalysed by this enzyme, the methyltetrahydrofolate is formed (it is a methyl — donor in Hcy remethylation catalysed by the methionine synthase). This mechanism could explain the changes in plasma tHcy level observed in thyroid dysfunction and after treatment. The influence of thyroid hormone on MTHFR occurs by the stimulating synthesis of the coenzyme flavin adenine dinucleotide (FAD). FAD protects the MTHFR from inactivation. We investigated the plasma concentration of vitamin B<sub>12</sub> and folic acid (both involved in the remethylation pathway). Vitamin B<sub>12</sub> acts as a cofactor for methionine synthase and folate as a methyl donor in the remethylation of Hcy to methionine. Vitamin deficiency leads to reduced remethylation and elevated plasma tHcy. In our study none of the patients had a vitamin deficiency. In the hyperthyroid group before treatment, the level of folate was higher than in the control group and decreased after treatment. This may be explained by the increased activity of MTHFR in hyperthyroid state. This enzyme produced higher levels of methyltetrahydrofolate — a form marked in the laboratory tests. Ford et al. observed elevated serum folate levels in hyperthyroidism [29]. This finding is in agreement with the results of Nedrebo et al. [27, 31] and Demirbas et al. [26]. Another study demonstrated higher folate levels in a hyperthyroid group compared to a hypothyroid group, a decrease after treatment of hyperthyroid patients, and a positive correlation between tHcy and folate level [19]. We did not find any significant correlation between plasma tHcy and folate in univariate analysis. Conversely, studies on hypothyroid patients found lower folate levels [18, 32] and concluded that tHcy in hypothyroidism is associated with an altered folate status. We did not observe any correlation between tHcy and vitamin B<sub>12</sub> in univariate analysis. Pretreatment levels of vitamin B<sub>12</sub> were inconsiderably lower in hyperthyroid patients, but after treatment were unchanged. Demirbas et al. [26], when studying hyperthyroid patients, did not find any differences in B<sub>12</sub> levels between hyperthyroid and healthy subjects both before and after antithyroid therapy. Nedrebo et al. observed a reduction in plasma vitamin B<sub>12</sub> following antithyroid therapy [27]; however, in another study they found no relation to thyroid status [31]. In hypothyroid patients, others have demonstrated reduced [33, 34] or unchanged levels of vitamin B<sub>12</sub> [19, 35].

The following mechanism for alterations of tHcy levels is the renal function. Thyroid hormones influence renal blood flow, glomerular filtration rate (GFR), and active tubular transport [36, 37], and are related to the severity of thyroid dysfunction. Several studies have demonstrated reduced serum creatinine levels and en-



hanced GFR in hyperthyroidism, and its normalisation after achieving euthyrosis [38, 39]. Even a mild reduction in GFR leads to increased levels of Hcy. There are reports of a positive correlation between tHcy and serum creatinine concentrations [40]. The clearance of Hcy plays a major role in kidney metabolism of this amino acid, and renal excretion of Hcy is negligible [41]. Unexpectedly, in our study hyperthyroid patients had creatinine clearance similar to the control group. After treatment we observed its significant decrease. In univariate analysis, this parameter was not correlated with tHcy.

The important elements of our findings are prospective, longitudinal design and homogeneous population. We ruled out the other main factors associated with hyperhomocysteinaemia. Our patients were young women (mean age 37.3 + 9.8 yrs), with regular menses, who led healthy lives, and were without other diseases. In adults, tHcy is usually about 2  $\mu\text{mol/L}$  higher in men than in women. From puberty to old age, tHcy increases 3–5  $\mu\text{mol/L}$  in both sexes. After menopause, tHcy gradually rises and reaches the level of men. Sex-related differences are explained by the effects of oestrogen status [42, 43]. Concentrations of tHcy are different in the follicular and luteal phase of the menstrual cycle [44, 45]. Therefore, the differences in menstrual status may affect tHcy levels and obscure the results. In our study we did not take into account the phase of the cycle because hyperthyroidism can be the cause of the anovulation.

In conclusion, our study confirms previous observations, showing that the hyperthyroid status is associated with decreased plasma tHcy concentrations. Free triiodothyronine is an independent determinant of plasma tHcy. Higher folate levels in hyperthyroidism only partially explain the changes in tHcy.

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