Neuroendocrinology Letters Vol.26 No.5, October 2005
 ISSN 0172–780X **www.nel.edu**

ORIGINAL ARTICLE

Changes in plasma homocysteine levels of rats with experimentally induced hypothyroidism and hyperthyroidism

Yusuf Özkan [1], Emir Dönder[2], Hasan Güney[2] & Gıyaseddin Baydaş[3]
Fırat University School of Medicine, Department of
[1]Endocrinology, [2]Internal medicine, [3]Physiology, Elazig, Turkey.

Correspondence to: Dr. Yusuf ÖZKAN
Fırat University School of Medicine,
Fırat Medical Center Department of Endocrinology
23200-Elazig, TURKEY

Submitted: April 20, 2005 *Accepted: July 27, 2005*

Key words: **experimental hypothyroidism; experimental hyperthyroidism; homocysteine; rat**

Neuroendocrinol Lett 2005; **26**(5):536–540 **PMID:** 16264404 NEL260505A18

Abstract

INTRODUCTION: It is claimed in a limited number of studies carried out on human beings that plasma homocysteine levels increased in hypothyroid patients and decreased in hyperthyroid patients.
OBJECTIVE: The aim of this study is to determine total plasma homocysteine, thyroid function tests, vitamin B12, folic acid and lipid levels and to explore the relations among them in rat models with induced hypothyroidism and hyperthyroidism with a view to investigating whether hypothyroid and hyperthyroid rat models could represent human hypothyroidism and hyperthyroidism models.
MATERIAL AND METHOD: The study included 30 male Wistar Albino species rats with a mean weight of 200–250 g. Rats were randomly divided into 3 groups as 1) hypothyroid group, 2) hyperthyroid group and 3) control group. Hypothyroidism was induced by adding 10 mg/kg/day propylthiouracil to rats' drinking water for 30 days. In order to induce hyperthyroidism, rats were administered 10 μg/100 g L-thyroxin ampule via intraperitoneal route for 10 days.
RESULTS: We found that total plasma homocysteine level of the hypothyroid group was significantly lower than those of the control group ($p<0.05$) and the hyperthyroid group ($p<0.001$). Total plasma homocysteine level of the hypothyroid group was found insignificantly higher than that of the control group ($p>0.05$) and significantly higher than that of the hyperthyroid group ($p<0.001$). We established a significant and positive correlation between total plasma homocysteine level and thyroid hormone levels. We did not identify a significant relation between total plasma homocysteine level and serum folic acid and serum vitamin B12 levels.
CONCLUSION: Our findings are different from the findings reported in human hypothyroidism and hyperthyroidism studies. We believe that hypothyroid and hyperthyroid rat models cannot represent human hypothyroidism and hyperthyroidism models.

Introduction

Homocysteine is an intermediate metabolite that is found in intracellular and extracellular fluids and that contains sulphur. It is produced during the enzymatic demethylation of methionine, an essential amino acid [1, 2]. At present, high plasma homocysteine level is regarded a primary and independent risk factor in the etiopathogenesis

of coronary heart disease and cerebrovascular diseases [3–6].

Total plasma homocysteine levels may increase due to physiological reasons like advanced age and male sex and in case of various pathological conditions. Such lifestyle components as sedentary living, smoking, alcohol and coffee can bring about changes in total plasma homocysteine levels [7, 8]. Chronic inflammatory diseases like hypothyroidism, rheumatoid arthritis and psoriasis, intestinal diseases causing malabsorption, various malignancies, diabetes mellitus, renal failure, vitamin deficiencies (folic acid, B12, B6, B2) and some medications can also increase total plasma homocysteine levels [8, 9].

Several studies conducted on human beings showed that thyroid hormone levels affected total plasma homocysteine levels [10–13]. Total plasma homocysteine levels increase in hypothyroidism and decrease in hyperthyroidism. Increased serum total cholesterol and homocysteine potentiate atherogenesis in hypothyroid patients. Besides, it was demonstrated in some studies that thyroid hormone replacement therapy reduced total plasma homocysteine levels in hypothyroid patients [14–16].

The aim of the present study is to examine total plasma homocysteine levels in rats with induced hypothyroidism and hyperthyroidism, to compare the results of the rat model with the results of human hypothyroidism and hyperthyroidism models and to explore whether rat model can be applied to human hypothyroidism and hyperthyroidism model in terms of total plasma homocysteine levels.

Material and method

Selection of subjects

Thirty male wistar albino rats weighting of 200–250 g were used in this study. The rats were kept in a well-lighted setting, in cages which were cleaned daily. Temperature of the room where the rats were kept was maintained at about 20–22 °C. The rats were randomly distributed to 3 groups. Each group contained 10 rats and 5 rats were placed in each cage.

Group 1 (hypothyroidism group, n=10): 10 mg/kg/day propylthiouracil (PTU) (Sigma) was added to the drinking water of the rats in this group for one month to induce hypothyroidism [17]. Group 2 (hyperthyroidism group, n=10): 10 μg/100 g L-Thyroxin ampule was administered to rats via intraperitoneal route for 10 days to induce hyperthyroidism [17]. Group 3 (control group, n=10): 0.5 ml saline was injected intraperitoneal to the control rats every day for 30 days.

The rats in all three groups were decapitated after one month. Blood samples collected from the animals were put into glass tubes. In order to separate the serum, the samples were centrifuged for 10 minutes at 5000 rpm. The serums were kept in a deep freezer at –60 °C until being analysed to determine the levels of homocysteine, thyroid hormones, serum lipids, vitamin B12 and folic acid.

Homocysteine measurement

Serums that were kept in ependorf tubes at –60 °C were dissolved in room temperature. Homocysteine was measured using DIAZYME made commercial kit (Diazyme Laboratories P.O. Box 85608 San Diego U.S.A.) in ELP-40 washer (U.S.A.) and EL x 800 registerer (U.S.A.) according to ELISA method. Free T3 (fT3), free T4 (fT4), total T3 (TT3) and total T4 (TT4) levels were determined with Chemilusence method in E170 (Japan) equipment using Roche made commercial kits in order to evaluate thyroid functions of all three groups.

Serum vitamin B12 and folic acid levels of the three groups were determined using DPC made commercial kits in LKB WALLAC 1261 Multigramma (Finland) gamma counter according to RIA method.

Total cholesterol, triglyceride, LDL cholesterol, HDL cholesterol and VLDL cholesterol levels of all three groups were measured using Olympus made commercial kits with Olympus AU 600 (Japan) equipment, using spectrophotometer method.

Table1: Serum thyroid hormone, vitamin B12, folic acid and total homocysteine levels (mean ± SD) values in the control, hypothyroid and hyperthyroid group.

	Control Group (n = 10)	Hypothyroid Group (n = 10)	Hyperthyroid Group (n = 10)
fT3 (pg/mL)	2,20 ± 0,43	0,54 ± 0,12*	4,82 ± 0,84[a,2]
fT4 (ng/dL)	2,41 ± 0,74	0,08 ± 0,002*	5,62 ± 0,66[a,2]
TT3 (ng/dL)	109,11 ± 16,82	39,77 ± 4,44*	157,58 ± 35,26[a,2]
TT4 (μg/dL)	4,22 ± 0,98	0,72 ± 0,04*	7,09 ± 0,77[a,2]
Vitamin B12 (pg/mL)	1320,00 ± 173,12	1242,85 ± 143,14	1503,75 ± 162,73[1]
Folic acid (ng/mL)	11,81 ± 1,66	11,56 ± 1,63	13,50 ± 1,43
Homocysteine (nmol/mL)	18,06 ± 9,09	6,37 ± 2,62*	19,29 ± 7,01[2]

*:p<0.001 Comparison of the hypothyroidism group with the control group
a:p<0.001 Comparison of the hyperthyroidism group with the control group
1:p<0.05 Comparison of the hypothyroidism group with the hyperthyroidism group
2:p<0.001 Comparison of the hypothyroidism group with the hyperthyroid group

Table 2: Serum total cholesterol, triglyceride, LDL cholesterol, HDL cholesterol and VLDL cholesterol levels (mean ± SD) values in the control, hypothyroid and hyperthyroid group.

	Control Group (n = 10)	Hypothyroid Group (n = 10)	Hyperthyroid Group (n = 10)
Total cholesterol (mg/dL)	50,10 ± 5,38	65,90 ± 7,15*	45,60 ± 9,72[2]
Triglyceride (mg/dL)	58,00 ± 9,60	75,05 ± 13,84*	47,20 ± 13,45[2]
LDL cholesterol (mg/dL)	17,06 ± 6,63	27,84 ± 8,17*	15,80 ± 7,46[1]
HDL cholesterol (mg/dL)	21,23 ± 6,52	23,03 ± 4,92	23,26 ± 3,71
VLDL cholesterol (mg/dL)	11,65 ± 1,91	15,03 ± 2,77*	9,45 ± 2,70[1]

*:p<0.001 Comparison of the hypothyroidism group with the control group
1:p<0.05 Comparison of the hypothyroidism group with the hyperthyroidism group
2:p<0.001 Comparison of the hypothyroidism group with the hyperthyroidism group

Statistical analysis

The data obtained from the study were uploaded to "SPSS 10.00 for Windows" software. Variance analysis test was conducted. Level of significance was set at p<0.05.

Results

Serum free T3 (fT3), free T4 (fT4), total T3 (TT3) and total T4 (TT4) levels were lower in the hypothyroid group and higher in the hyperthyroid group, when compared to the control group (*Table 1*).

Total homocysteine level in the hypothyroidism group was lower than that in the control group (p<0.01). Total homocysteine level in hyperthyroidism group was insignificantly higher than that in the control group (p>0.05) and significantly higher than that in the hypothyroidism group (p<0.001) (*Table 1*).

Serum vitamin B12 level was found insignificantly lower in the hypothyroidism group than in the control group (p>0.05). Serum vitamin B12 level in the hyperthyroidism group was insignificantly higher than that in the control group (p>0.05) and significantly higher than that in the hypothyroidism group (p<0.05) (*Table 1*).

Serum folic acid level in the control group was insignificantly higher than that in the hypothyroid group (p>0.05). Serum folic acid level in the hyperthyroidism group was found insignificantly higher than those in the control (p>0.05) and hypothyroidism groups (p>0.05) (*Table 1*).

Mean and standard deviation values of the serum total cholesterol, triglyceride, LDL cholesterol, HDL cholesterol and VLDL cholesterol levels and the comparison of the groups in terms of these values are presented in *Table 2*.

There was a significant correlation between total homocysteine level and serum fT4 level (r=0.65, p<0.001) and serum fT3 level (r=0.592, p<0.001).

No significant correlation was found between serum folic acid level and total homocysteine level (r=0.184, p>0.05). The correlation between serum folic acid level and serum fT3 (r=0.603, p<0.05) and serum fT4 (r=0.586, p<0.05) levels was significant.

The correlation between serum vitamin B12 level and serum sT4 level was significant (r=0.598, p<0.001). There was no significant correlation between serum vitamin B12 level and total homocysteine level (r=0.232, p>0.05) and serum fT3 level (r=0.596, p>0.05).

Discussion

Changes in thyroid hormone levels are one of the major determiners of total plasma homocysteine level. Most of the data indicating how thyroid hormone levels affect homocysteine metabolism are based on human studies [10–14]. Studies with hypothyroid and hyperthyroid human models suggest that thyroid hormone levels influence serum folic acid and vitamin B12 levels and play a determining role in homocysteine metabolism [18–21].

Diekman [11], Hussein [12], Nedrebo [13], Catargi [14], Morris [16] and Chris-Crain et al. [22] all found that serum folic acid levels were significantly lower and total plasma homocysteine level was significantly higher in hypothyroid patients in comparison to the control group and hyperthyroid patients. The same researchers reported that vitamin B12 levels decreased or did not change in hypothyroidism.

Nedrebo et al. [13] found that total plasma homocysteine level in the hypothyroidism group was significantly higher than those in the control and hyperthyroidism groups and that the level in the hyperthyroidism group was similar to that in the control group. In addition, serum folic acid was significantly higher in the patients comprising the hyperthyroidism group than in both the hypothyroid patients and the controls. The researchers who established higher serum cholesterol levels in the hypothyroid patients than the individuals in the other two groups stated that hyperhomocysteinemia together with hypercholesterolemia accelerated atherogenesis in these patients. In our study, we found total plasma homocysteine level in the hypothyroid group significantly lower than those in the control (p<0.05) and hyperthyroid groups (p<0.001).

Catargi et al. [14] compared and contrasted 40 hypothyroid patients, of whom 14 had autoimmune thyroid and 26 had thyroid carcinoma, with the control

group made up of 26 healthy individuals. They established higher total homocysteine levels in hypothyroid patients than the individuals in the control group. As total homocysteine level in the hypothyroid group had a positive relation with TSH and a negative relation with folic acid, the researchers pointed to TSH as a strong indicator of total plasma homocysteine, irrespective of age, folic acid, vitamin B12 and creatine. It was found in this study that thyroid hormone was incapable of restoring total homocysteine level in hypothyroid patients and it was noted that thyroid hormone replacement and folic acid combination treatment could be more useful in hypothyroid patients with low folic acid levels.

In this study, we did not measure the TSH level. The reason for this is that our aim was not to monitor total plasma homocysteine levels in subclinical hypothyroidism and hyperthyroidism, but to evaluate total plasma homocysteine levels after the induction of plain hypothyroidism and hyperthyroidism in rats. Measurement of thyroid hormone levels unmistakably indicated the development of hypothyroidism and hyperthyroidism. The lipid abnormalities we observed in hypothyroidism are parallel to the results noted by Catargi [14], but we did not observe the negative relation between serum folic acid level and total plasma homocysteine level as reported in hypothyroidism.

Lien et al. [15] stated that hypothyroid patients had higher total homocysteine levels than healthy controls and hyperthyroid patients. Total plasma homocysteine level in hyperthyroid patients was found similar to that in the controls. We, on the other hand, found total plasma homocysteine level of the hypothyroid group significantly lower than those of the control group ($p<0.001$) and hyperthyroid group ($p<0.001$). We established a positive relation between serum sT4 level and total plasma homocysteine level. Our findings were not consistent with the findings of the researchers who studied human beings [11, 13, 14, 16].

Morris et al. [16] studied the relation between hypothyroidism and, hypercholesterolemia and hyperhomocysteinemia in the American society and found a positive relation between hypothyroidism and, hypercholesterolemia and hyperhomocysteinemia. They attributed the insignificant decrease they observed in homocysteine level in hypothyroidism to low creatine levels resulting from increased GFR. They stated in the concerned study that in the American society 90% of hypothyroid people had hypercholesterolemia and hyperhomocysteinemia, whereas this rate fell down to 31% in individuals who did not have hypothyroidism.

In the present study we found significantly higher serum total cholesterol levels in the hypothyroid group, in comparison to control and hyperthyroid groups. Although our results concerning serum total cholesterol levels are parallel to those of Morris et al. [16], our results pertaining to thyroid hormones and total plasma homocysteine level are inconsistent with the results of these researchers.

The only literature study examining how changes in thyroid hormone levels affect homocysteine metabolism in rats with experimentally induced hypothyroidism is the one by Rene et al. [23]. The researchers observed that, as opposed to hypothyroid humans, hypothyroid rats had low total plasma homocysteine levels. They found that the rate of decrease in total plasma homocysteine levels was 30% in rats that were administered PTU and 50% in rats that underwent thyroidectomy. They saw that after thyroid hormone was given to thyroidectomized rats, homocysteine levels returned to normal values and found that hypothyroid rats had high hepatic transsulphurization enzymes. Therefore, the researchers suggested that hypothyroid rat model was not congruous with hypothyroid human model. Our findings are consistent with the positive relation Rene et al. [23] found between sT3, sT4, TT3, TT4 and total plasma homocysteine level. However, unlike Rene et al. [23], we did not identify a relation between total plasma homocysteine level and serum folic acid and vitamin B12 levels.

Studies including hypothyroid individuals [11–13, 22, 24] claim that the increase in total plasma homocysteine level is caused mainly by the decreases observed in serum folic acid and vitamin B12 levels in these patients. Although serum folic acid and vitamin B12 levels are affected by thyroid hormone levels in humans, they are not solely responsible for the change in homocysteine metabolism in hypothyroidism and hyperthyroidism. Besides, rat metabolism is different from human metabolism in various respects. Rene et al. [23] established a positive relation between thyroid hormone level and total plasma homocysteine level in their hypothyroid rat model and attributed this relation to the increase in hepatic transsulphurization enzymes of the hypothyroid rats. In the present study, we could not find a relation between serum folic acid and vitamin B12 level and total plasma homocysteine level.

In conclusion, our findings are different from the findings obtained in human hypothyroidism and hyperthyroidism studies. We believe that hypothyroid and hyperthyroid rat models cannot be representative of human hypothyroidism and hyperthyroidism models. We think that factors other than serum folic acid and vitamin B12 levels may be indicative in terms of homocysteine metabolism in hypothyroid and hyperthyroid rats.

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