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# THE EFFECTS OF HYPERTHYROIDISM ON LIPID PEROXIDATION, ERYTHROCYTE GLUTATHIONE AND GLUTATHIONE PEROXIDASE

## EFEKTI HIPERTIROIDIZMA NA LIPIDNU PEROKSIDACIJU, GLUTATION I GLUTATION-PEROKSIDAZU U ERITROCITIMA

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**Summary:** The aim of this study was to determine if lipid peroxidation, glutathione, and glutathione peroxidase levels can be effected by hyperthyroidism. Twenty-three subjects with hyperthyroidism (18 females/5 males), and 19 euthyroid subjects (11 females/8 males) were examined in this study. Plasma and erythrocytes malondialdehyde (MDA), erythrocytes glutathione (GSH) and glutathione peroxidase (GSH-PX) were measured. Results show that an increase in lipid peroxidation was observed in the hyperthyroid patients ($p< 0.001$). This was accompanied by a decrease in glutathione and glutathione peroxidase in the same subjects ($p< 0.001$). The results suggest that hyperthyroidism has some effects on lipid peroxidation and free radical scavengers.

**Keywords:** hyperthyroidism, lipid peroxidation, erythrocytes glutathione, glutathione peroxidase

**Kratak sadržaj:** Cilj studije bio je da se odredi da li nivoi lipidne peroksidacije, glutationa i glutation-peroksidaze mogu biti izazvani hipertiroidizmom. Dvadeset pet subjekata sa hipertiroidizmom (18 žena/5 muškaraca) i 19 subjekata sa zdravom štitnom žlezdom (11 žena/8 muškaraca) ispitano je u okviru studije. Malondialdehid (MDA), glutation (GSH) i glutation-peroksidaza (GSH-PX) izmereni su u eritrocitima. Rezultati pokazuju da je kod pacijenata sa hipertiroidizmom primećen porast lipidne peroksidacije ($p<0,001$). Ovo je praćeno sniženjem glutationa i glutation-peroksidaze kod istih subjekata ($p<0,001$). Rezultati ukazuju da hipertiroidizam ima izvesne efekte na lipidnu peroksidaciju i lovce slobodnih radikala.

**Ključne reči:** hipertiroidizmom, lipidna peroksidacija, eritrocitni glutation, glutation peroksidaza

### Introduction

A free radical is any species capable of independent existence that contains one or more unpaired electrons or a molecule with an unpaired electron in an outer valence shell e.g. oxygen-centered compound (R-O) (1). Oxygen-free radicals or so-called reactive oxygen species (ROS) have important effects on the pathogenesis of tissue damage in several pathologic conditions (2). Many free radicals have been discovered and described by Haber & Weiss (1934) such as superoxide, hydrogen peroxide, and hydroxyl radical. Baeyer & Villiger (1901) discovered peroxynitrite, while the discovery of superoxide dismutase (SOD) was made by McCord & Fridovich (1969) (3). Many biochemical compounds, namely nucleic acids, amino acids, proteins, lipids, lipoproteins, carbohydrates, and macromolecules of collagen tissue, can be damaged irreversibly or reversibly by free radicals. ROS accumulate in tissues due to intracellular and extracellular processes.

There are different antioxidant systems against free radicals. The GSH and GSH-PX system, either one of them, removes free radicals from the environment (4). Expression of antioxidants and pro-oxidant enzymes changes in some diseases such as cancer, e.g. decrease of Mg and Cu/Zn SOD, catalase and glutathione peroxidase-1 can occur, while cyclooxygenase-2 and nitric oxide synthase-2 can be increased (1). Many diseases poorly metabolize hydrogen peroxide; for this reason, the $H_2O_2$ concentration is commonly increased in pathologic human cells (2, 5). Clinical and experimental studies showed an elevated

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free radical level in hyperthyroidism. Hyperthyroidism is commonly due to antibodies called thyroid stimulating immunoglobulins that can attack the TSH receptors on the thyroid gland causing it to be stimulated (autoimmune disease) which also may result in goiter. The aim of this study is to investigate the relation between lipid peroxidation (MDA), GSH and GSH–PX in hyperthyroidism.

## Materials and Methods

Twenty-three hyperthyroid patients (18 females/5 males, mean age 37.00 ± 14.50 years, BMI 23.10 ± 3.36 kg/m$^2$), and 19 euthyroid subjects (11 females/8 males, 34.55 ± 9.43 years, BMI, 24.12 ± 5.25 kg/m$^2$) participated in this study. None of them smoked cigarettes or were taking medications. T3, T4, TSH, plasma MDA, erythrocyte MDA, erythrocyte GSH and GSH-PX levels were measured for both groups. A blood sample was collected from each subject while fasting, and serum was frozen at –20 °C until analysis for T3, T4, and TSH. LKT31 (DPC, USA, 1997) was used for T3 and its normal range is 1.25–2.74 nmol/L; LKT41 (DPC, USA, 1997) was used for T4 and its normal range is 57.9–160.8 nmol/L; LKTR1 (DPC, USA, 1995) was used for TSH and its normal range is 0.4–4 IU/mL. To measure GSH and GSH-PX levels, blood samples (10 mL) were obtained in heparinized tubes, and centrifuged at 2000 rpm for 10 minutes.

Plasma was separated and the buffer coat was discarded. Erythrocytes were washed with a cold sterile 9 g/L sodium chloride three times after 1/10 dilutions. Lipid peroxidation was assayed by measurements of malondialdehyde (MDA) generation. One volume of plasma or washed erythrocyte was mixed thoroughly with two volumes of stock solution of 15% trichloroacetic acid (w/v), 0.375% thiobarbituric acid (w/v), and 0.25 mol/L hydrochloric acid (w/v). The combination of the sample and the stock solution was heated for 30 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 3200 rpm for 15 min. The absorbance of the clear supernatant was determined at 535 nm and MDA concentrations were calculated using 1.50 x $10^5$ $M^{-1}$ $cm^{-1}$ as plasma coefficient (6).

Erythrocyte GSH levels were determined according to the method of Beutler et al. (7) using metaphosphoric acid for protein precipitation and 5,5′-dithio–2 nitrobenzoic acid for color development at 412 nm.

GSH-PX activity was determined by the method of Paglia and Valentine (8). Enzyme activity was determined from the oxidation of NADPH in the presence of $H_2O_2$ as substrate and monitored spectrophotometrically at 340 nm. One unit enzyme activity was defined as 1 μmol/L NADPH oxidized per minute. Activity was expressed as U/g Hb. Results were evaluated by independent T test and expressed as mean ± standard deviation (SD).

## Results

Age, BMI, Hb% and levels of cholesterol, triglycerides, T3, T4 and TSH in subjects with hyperthyroidism and controls are shown in *Table I*, while plasma MDA, erythrocyte MDA, erythrocyte GSH, and GSH-PX levels in subjects with hyperthyroidism,

**Table I** Results of BMI, Hb%, Cholesterol, Triglyceride, $T_3$, $T_4$ and TSH in hyperthyroid and euthyroid subjects.

| Parameters | Hyperthyroidism (n=23) | Controls (n=19) | P |
|---|---|---|---|
| Age (year) | 37.0 ± 14.5 | 34.5 ± 9.4 | > 0.05 |
| BMI (kg/m$^2$) | 23.1 ± 3.3 | 24.1 ± 5.2 | > 0.05 |
| Hb % | 12.3 ± 0.5 | 12.8 ± 0.4 | > 0.05 |
| Cholesterol (mmol/L) | 4.8 ± 0.5 | 4.9 ± 0.7 | > 0.05 |
| Triglyceride (mmol/L) | 2.2 ± 0.5 | 2.3 ± 0.7 | > 0.05 |
| T3 (nmol/L) | 5.5 ± 2.8 | 2.0 ± 0.5 | < 0.001 |
| T4 (nmol/L) | 197.4 ± 77.0 | 104.7 ± 18.7 | < 0.001 |
| TSH (IU/mL) | 0.02 ± 0.0 | 1.0 ± 0.6 | < 0.001 |

**Table II** Results of Plasma & Erythrocyte MDA and erythrocytes GSH & GSH-PX in hyperthyroid and euthyroid subjects.

| Parameters | Hyperthyroidism (n=23) | Controls (n=19) | P |
|---|---|---|---|
| Plasma MDA (nmol/mL) | 4.0 ± 2.0 | 2.3 ± 1.1 | < 0.001 |
| Erythrocyte MDA (nmol/g Hb) | 235.0 ± 53.1 | 129.6 ± 54.7 | < 0.001 |
| Erythrocyte GSH (nmol/g Hb) | 6.6 ± 1.4 | 10.3 ± 1.2 | < 0.001 |
| GSH-PX (U/g Hb) | 8.9 ± 1.8 | 20.0 ± 1.0 | < 0.001 |

and controls are shown in *Table II*. When the results of the two groups were compared, plasma and erythrocyte MDA levels were significantly higher in the patient group than in the control group ($p< 0.001$). Erythrocyte GSH and GSH-PX levels were also significantly lower in the patient group than in the control group ($p<0.001$). T3, T4 and TSH levels were different in these groups.

## Discussion

It has been reported that there is a relation between hyperthyroidism and deteriorations of free radical and antioxidant systems that increase lipid peroxidation (9). In aerobic cells, active oxygen species are generated as byproducts of oxidative metabolism in mitochondria. Hyperthyroidism leads to an enhancement of the metabolic rate and, more specifically, of the oxidative metabolism (10). In previous studies different interpretations were given. It was demonstrated that thyroxin decreased the concentration of the products of lipid peroxidation in animal experiments (11, 12). However, Fernandez et al. (13) showed that the products of lipid peroxidation were increased in rats that were given triiodothyronine. Dumitriu et al. (14) found high plasma MDA levels in hyperthyroidic patients as opposed to the control group. Costantini et al. (15) demonstrated that hyperthyroidism can stimulate lipid peroxidation. Venditti et al. (16) investigated the effects of hyperthyroidism on lipid peroxidation in rats. They reported that hyperthyroidism increased the products of lipid peroxidation in several tissues. Iangalenko et al. (17) found that lipid peroxidation was increased in hyperthyroid patients. There was a correlation between lipid peroxidation and thyroid iodine uptake. Mano et al. (18) suggested that lipid peroxidation and GSH-PX were increased in the thyroid tissue in hyperthyroidism. Asayama et al. observed that lipid peroxide was increased in the heart and soleus muscles and GSH-PX was decreased in all tissues of hyperthyroid rats, and in contrast, no adverse reaction mediated by active oxygen species was found in hypothyroid rat tissues. Venditti et al. (19) showed that lipid peroxide was increased, but the antioxidant system was not affected in hyperthyroid rats. Asayama and Kato (20) showed that the damaging effect of lipid peroxidation was increased, diminishing antioxidant enzymes in experimental hyperthyroidism.

We determined that the level of lipid peroxidation products was higher in the hyperthyroidism group than control levels; however, GSH and GSH-PX were significantly lower than control levels. So, this study confirms some previous studies.

In conclusion, there is an important relation between hyperthyroidism and free radical/antioxidant systems.

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## Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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