

## OXIDATIVE STRESS IN EXPERIMENTAL HYPOTHYROIDISM: EFFECT OF VITAMIN E SUPPLEMENTATION

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### Abstract

*Oxidative stress may result from either overproduction of free radicals or from insufficiency of several antioxidant defence systems.*

*Thyroid hormones have well-known effects on mitochondrial oxygen consumption, but data about how hypothyroidism affects oxidative stress are controversial.*

**The aim of the study** was to investigate the oxidant and antioxidant status in propylthiouracil-induced hypothyroidism and the effect of vitamin E supplementation on this experimental model.

**Materials and Methods.** Thirty male Wistar rats were used in the study. Hypothyroidism was induced by administering Propylthiouracil (5mg/100g animal/day) for 30 days.

Plasma was used to determine malondialdehyde (MDA), carbonyl proteins, SH groups, glutathione (GSH) and superoxide dismutase (SOD) while MDA, carbonyl proteins, SH groups and GSH were determined from the thyroid gland.

**Results.** In plasma and thyroid homogenate of hypothyroid rats, the lipid peroxidation did not differ significantly from the control group. Carbonyl proteins levels increased significantly in serum and thyroid tissue of hypothyroid animals as compared to the control group. Antioxidant parameters SH groups, GSH and SOD levels did not show any significant changes.

Vitamin E supplementation significantly increased carbonyl proteins levels and decreased SH, GSH and SOD levels compared with the Propylthiouracil treated group.

**Conclusions.** Our study suggests an elevated reactive oxygen species which may be a result of increased oxidative stress.

**Keywords:** oxidative stress, antioxidant capacity, vitamin E, hypothyroidism.

## STRESUL OXIDATIV ÎN HIPOTIROIDISMUL EXPERIMENTAL: EFECTUL SUPLIMENTĂRII CU VITAMINA E

### Rezumat

*Stresul oxidativ poate rezulta fie din producția crescută a radicalilor liberi fie din insuficiența diferitelor sisteme de apărare antioxidantă.*

*Hormonii tiroidieni au efecte bine cunoscute asupra consumului de oxigen mitochondrial, dar datele despre modul în care hipotiroidismul afectează stresul oxidativ sunt controversate.*

**Scopul studiului** a fost de a investiga statusul oxidant și antioxidant în hipotiroidismul indus de propiltiouracil și efectul suplimentării cu Vitamina E în acest model experimental.

**Material și metodă:** Am luat în studiu 30 de șobolani masculi, rasa Wistar. Hipotiroidismul a fost indus prin administrare de Propiltiouracil (5mg/100g animal/zi) timp de 30 de zile.

*S-au determinat din plasmă malondialdehida (MDA), proteinele carbonilate, grupările SH, glutathionul (GSH) și superoxid-dismutaza (SOD), iar din tiroidă MDA, proteinele carbonilate, grupările SH și GSH.*

**Rezultate.** Peroxidarea lipidică, în plasma și omogenatul tiroidian al șobolanilor cu hipotiroidism nu a suferit modificări semnificative față de lotul martor. Proteinele carbonilate au crescut semnificativ în serul și țesutul tiroidian al animalelor cu hipotiroidism față de grupul de control. Parametrii antioxidanți: GSH, SOD și grupările SH nu au indicat modificări semnificative. Suplimentarea cu Vitamina E, a crescut semnificativ nivelurile proteinelor carbonilate și a scăzut nivelurile grupărilor SH, GSH și SOD în comparație cu grupul tratat cu Propiltiouracil.

**Concluzii.** Studiul nostru arată o creștere a speciilor reactive ale oxigenului care poate fi un rezultat al stresului oxidativ crescut.

**Cuvinte cheie:** stres oxidativ, capacitate antioxidantă, vitamina E, hipotiroidism.

## Introduction

Oxidative stress may result from either over-production of reactive oxygen species (ROS) or from failure of the antioxidant defense systems [1]. ROS have a high reactivity potential, therefore they are toxic and can lead to oxidative damage in cellular macromolecules such as proteins, lipids, and DNA [2]. The antioxidant defense system includes both enzymatic and non-enzymatic components. The antioxidant enzymes comprise superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT). Non-enzymatic small molecules that act as antioxidants include vitamins E and C, glutathione (GSH), thiol groups (SH) and coenzyme Q.

Thyroid hormones regulate oxidative metabolism and thus play an important role in free radical production. On the other hand, they regulate protein, vitamin and antioxidant enzyme synthesis and degradation [3]. It is well known that thyroid hormone biosynthesis is an oxidative biochemical reaction which depends on the formation of peroxides [4]. One of the major effects of thyroid hormones is to increase mitochondrial respiration which results in increased generation of reactive oxygen species (ROS) [5]. A recent study [6] found a positive association between thyroid hormones in excess and lipid peroxides correlated by linear regression which clearly suggest induction of oxidative stress.

In hypothyroidism, a decrease in free radical production is expected because of the metabolic suppression brought about by the decrease in thyroid hormone levels [3,7,8].

Recent studies have shown an increased production of reactive oxygen species in hypothyroidism [9,10]. The effect of hypothyroidism on the antioxidant enzymes has been investigated in several tissues, but the results are rather controversial. In some cases, the change of antioxidant

enzyme activity seems to be tissue specific [11,12]. On the other hand, within a single tissue, the response of the antioxidant enzymes to hypothyroidism is not always similar [13,14].

Vitamin E is a potent lipid soluble antioxidant in biological systems with the ability to directly quench free radicals and function as membrane stabilizer [15].

Antioxidants treatments might be helpful in reducing the oxidative damage due to hypothyroidism.

This study aims at investigating oxidative stress parameters, antioxidant status markers and their response to vitamin E supplementation in experimental hypothyroidism.

## Materials and Methods

### Animals

White male Wistar rats, weighing between 220 and 240 g, were purchased from The Iuliu Hatieganu University of Medicine and Pharmacy biobase. Upon arrival, the animals were allowed to acclimatize for two weeks in the Department of Physiology biobase. All animals were kept under the same environmental conditions, at a room temperature of  $23 \pm 1^\circ\text{C}$ , with an artificial lighting cycle (lights on 08.00-20.00 h) and water ad libitum.

They were divided into 3 groups of 10 animals each: group 1 – controls, group 2 – animals treated with Propylthiouracil (5mg/100g animal /day), for 30 days and group 3 – Propylthiouracil treated rats protected with 10 mg/animal/day of vitamin E administered intramuscularly, for 30 days. The Propylthiouracil quantity dissolved in 2 ml of milk was administered by gavage in the morning on an empty stomach.

The experimental procedures used in this study met the guidelines of the Animal Care and The Iuliu Hatieganu University of Medicine and Pharmacy Ethics Committee approved the study.

### Thyroid tissue preparation

Thirty days into the experiment, blood was collected

from the retroorbital sinus and the rats were sacrificed by cervical dislocation following ether anaesthesia.

The thyroids were immediately dissected out and placed into ice-cold isolation medium. Tissue homogenates were used for analytical procedures.

### Analytical procedures

The oxidative stress and antioxidant status parameters were determined in the Oxidative Stress Laboratory of the Department of Physiology from the Iuliu Hatieganu University of Medicine and Pharmacy.

The lipid peroxides level was assessed by fluorescence according to the Conti and Moran method [16], based on the reaction between malondialdehyde, the marker of lipid peroxidation and thiobarbituric acid, measured spectrophotometrically at 534nm. Concentration values of MDA were expressed as nmol/ml based on specific calibration curves.

Protein oxidation was determined through the estimation of carbonyl groups photometrically with dinitrophenylhydrazine according to the Reznick method [17] and expressed as nmol per mg of protein (nmol/mg protein).

The thiol content of samples was determined with dithionitrobenzoic acid (DTNB), according to the Hu method [18]. The results were expressed as nmol SH per milligram of protein (nmol/mg protein).

Fluorescence was used to determine the glutathione (GSH) values [18]. The results were expressed as micromoles per litre ( $\mu\text{mol/l}$ ).

Superoxide dismutase (SOD) activity of the samples was evaluated using the Flohe method [19] and expressed as U SOD per milligram of protein (U/mg protein).

Serum free-thyroxine ( $\text{FT}_4$ ) concentrations were measured with an enzyme immunoassay kit (EIAgen Free  $\text{T}_4$  Kit, Adaltis Italia).

### Statistical analysis

Data are given as mean  $\pm$  standard deviation (S.D.). Statistical analysis were performed using the one-way ANOVA and paired T-test for normally distributed samples, and the Kruskal-Wallis and Wilcoxon tests for data which were not normally distributed. A level of  $P < 0.05$  was accepted as statistically significant.

### Results

Significantly low  $\text{FT}_4$  ( $p < 0.001$ ) values were observed in the Propylthiouracil administered group as compared with the control group (Table 1).

In plasma and thyroid tissue of the hypothyroid rats, the MDA levels did not differ significantly from euthyroid values ( $p > 0.05$ ) (Table 1,2).

We found that carbonyl proteins levels were significantly higher ( $0.99 \pm 0.27$ ,  $p < 0.05$ ) in plasma, and the thyroid tissue ( $1.99 \pm 0.61$ ,  $p < 0.05$ ) of the Propylthiouracil treated rats, as compared with the control group.

Vitamin E supplementation increased significantly the carbonyl proteins levels as compared with the hypothyroid rats (Table 1,2).

Thiol groups (SH), superoxide dismutase (SOD) and glutathione (GSH) levels in the hypothyroid group did not differ significantly from the control group.

Administration of Vitamin E to hypothyroid rats resulted in a significant decrease in plasma antioxidant status parameters (SH, SOD, GSH) levels as compared with the Propylthiouracil treated rats (Table 1).

### Discussion

Our results show a general lack of significant changes in levels of lipid peroxidation (MDA) in serum and thyroid tissue of hypothyroid rats. This is in line with the results of Venditti et al. [20] who showed that in all tissues of hypothyroid rats, the malondialdehyde (MDA) levels did not differ significantly from euthyroid values. Mano

**Table 1.** Plasma lipid peroxides (MDA), protein carbonyls (PC), antioxidant status parameters and  $\text{FT}_4$  values in the experimental groups (mean  $\pm$  SD).

	Control group	Hypothyroid group	Hypothyroid + vitamin E group
MDA(nmol/ml)	1.51 $\pm$ 0.23	1.42 $\pm$ 0.21	1.36 $\pm$ 0.29
PC(nmol/mg protein)	0.69 $\pm$ 0.17	0.99 $\pm$ 0.27 <sup>a*</sup>	1.53 $\pm$ 0.71 <sup>b</sup>
SH(mmol/l)	0.27 $\pm$ 0.02	0.26 $\pm$ 0.04	0.15 $\pm$ 0.03 <sup>b*</sup>
GSH( $\mu\text{mol/l}$ )	65.71 $\pm$ 15.06	52.07 $\pm$ 23.15	13.45 $\pm$ 8.51 <sup>b*</sup>
SOD(U/g protein)	6928.94 $\pm$ 1031.47	7718.47 $\pm$ 930.13	5897.72 $\pm$ 359.41 <sup>b</sup>
FT <sub>4</sub> (ng/dl)	1.48 $\pm$ 0.21	0.75 $\pm$ 0.13 <sup>a</sup>	0.53 $\pm$ 0.48

<sup>a</sup> Significant ( $p < 0.001$ ) versus control group; <sup>a\*</sup> Significant ( $p < 0.05$ ) versus control group; <sup>b</sup> Significant ( $p < 0.05$ ) versus hypothyroid group; <sup>b\*</sup> Significant ( $p < 0.001$ ) versus hypothyroid group.

**Table 2.** Lipid peroxides (MDA), protein carbonyls (PC) and antioxidant status parameters in the thyroid gland (mean  $\pm$  SD).

	Control group	Hypothyroid group	Hypothyroid+vitamin E group
MDA(nmol/ml)	0.30 $\pm$ 0.06	0.22 $\pm$ 0.06	0.23 $\pm$ 0.10
PC(nmol/mg protein)	1.42 $\pm$ 0.32	1.99 $\pm$ 0.61 <sup>a</sup>	2.29 $\pm$ 0.49
SH(mmol/l)	0.02 $\pm$ 0.008	0.10 $\pm$ 0.04	0.05 $\pm$ 0.02 <sup>b</sup>
GSH( $\mu\text{mol/l}$ )	93.34 $\pm$ 10.54	61.61 $\pm$ 12.83	73.24 $\pm$ 19.55

<sup>a</sup> Significant ( $p < 0.05$ ) versus control group; <sup>b</sup> Significant ( $p < 0.05$ ) versus hypothyroid group.

et al. [21] found that the concentration of lipid peroxides, determined indirectly by the measurement of thiobarbituric acid reactants, did not change in hypothyroid rats when compared with the euthyroid animals. Dariyerli et al. [22] showed that there is no statistically significant difference found between hypothyroid and control groups in the lipid peroxidation indicator MDA. The results of Yilmaz et al. [23], who reported increased plasma, liver and muscle MDA levels in hypothyroid rats contradict our findings. Sarandol et al. [10] observed increased lipid peroxidation in plasma, liver, heart and muscle of Propylthiouracil treated rats reflecting an enhanced oxidative status in hypothyroidism. This conflicting findings are thought to be due to different study materials in several animal models [12].

In our study we found that carbonyl proteins levels were significantly increased in plasma, and the thyroid tissue of the Propylthiouracil treated rats, suggesting the presence of oxidative stress in hypothyroidism. This is in agreement with Nanda et al. [24] who found significantly higher carbonyl proteins levels in plasma of hypothyroid patients compared to their respective controls.

The mechanism of increased oxidative stress in hypothyroidism is controversial. Although most of the studies did not suggest it, an insufficient antioxidant defence system is thought to be a factor.

Antioxidant status parameters, namely thiol groups (SH), superoxide dismutase (SOD) and glutathione (GSH) levels did not differ significantly in serum, and the thyroid tissue of the hypothyroidism-induced rats in comparison to the control group.

GSH is endogenously synthesized in the liver and is the first line of defence against pro-oxidant stress [25]. This antioxidant molecule is one of the main parts of the cellular endogenous antioxidant systems. It exerts its antioxidant function by donating electrons to radicals and changing to its oxidized form, which is subsequently reduced by the enzyme glutathione reductase [26].

In contrast with our results, Das et al. [13] have reported increased GSH levels in the mitochondria of hypothyroid rat liver, while the results of Sarandol et al. [10] who didn't observed any significant changes in GSH levels in the liver and kidney tissues of hypothyroid rats agree with our findings.

The organism can defend itself against the effects of oxidative stress by increasing SOD activity as a protection mechanism, but we did not observe any alteration in the serum and thyroid tissue of the hypothyroid rats. This is in line with the results of Messarah et al. [27] who observed no difference in SOD levels between hypothyroid rats and controls. On the contrary, Das et al. [13] found increased SOD activity in the liver of hypothyroid rats.

Venditti et al. [12] have showed that antioxidants are not affected in the same manner in different tissues of hypothyroid rats; some of them increase, while several decrease or remain unchanged. The physiological state of

the thyroid gland, the dose and the duration of treatment are also of a major influence on antioxidants enzymes.

Data on the effects of vitamin E supplementation on thyroid hormone levels are limited. In our study, vitamin E supplementation significantly increased plasma and thyroid tissue protein carbonyls levels and decreased the levels of plasma antioxidant markers SH, GSH and SOD in the Propylthiouracil treated group compared with the only Propylthiouracil treated rats. Significantly low levels of the SH groups ( $p < 0.05$ ) were found in thyroid homogenates of the Propylthiouracil supplemented group as compared with the only Propylthiouracil treated rats. This could be explained by the relative doses of vitamin E administered as compared with the study of Sarandol [10] which were not enough to suppress the oxidative stress in hypothyroid rats. For the first time in the literature, Erdamar et al. [9] showed that the level of vitamin E was significantly increased in patients with hypothyroidism, which might be due to an adaptation against oxidative stress provoked by hypothyroidism.

Although it has been suggested that the hypometabolic state is associated with a decrease in oxidative stress, literature data are controversial, revealing an individuality of antioxidant status in relation to tissue properties and responsiveness.

These findings may add some information to the literature in this field, in which a definite conclusion has yet to be reached.

## Conclusions

1. The present study suggests an increased oxidative stress in hypothyroid state.
2. Hypothyroidism did not show protection against oxidative stress.
3. No protective effects of vitamin E in the dosage administered, on oxidative stress induced by hypothyroidism were detected.

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