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## The impact of thyroid activity variations on some oxidizing-stress parameters in rats

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### Abstract

The effect of the thyroid activity on the formation of lipid peroxidation and on liver and heart antioxidant enzyme activities was investigated in Wistar rats. Hypothyroidism and hyperthyroidism conditions were induced for five weeks by the administration of 0.05% benzythiouracile (BTU) and L-thyroxine sodium salt (0.0012%), in drinking water, respectively. No significant effect was observed on the rates of both lipid peroxidation and the vitamin E in hepatic and cardiac tissues of hypothyroidism rats compared to the controls, contrary to the hyperthyroidism rats, which expressed a pronounced increase. The increased glutathione peroxidase activity in rats suffering from hyperthyroidism was associated with a fall of the reduced glutathione in the homogenate and a marked increase in the glutathione reductase activity. An increase in superoxyde dismutase and catalase activities was also recorded in hyperthyroidism. Our results explain the thyroid activity variation in relation to the lipid peroxidation and the tissular contents of the enzymatic and the non-enzymatic antioxidants. To conclude, our results show the occurrence of a state of oxidizing stress in relation to hyperthyroidism. **To cite this article:** M. Messarah et al., *C. R. Biologies 330 (2007)*.

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### Résumé

**Impact des variations de l'activité thyroïdienne sur quelques paramètres du stress oxydant chez les rats.** L'étude de l'effet des variations de l'activité thyroïdienne sur la peroxydation lipidique et sur l'activité antioxydante hépatique et cardiaque chez des rats de souche Wistar est menée par l'observation d'une hypothyroïdie provoquée par administration du benzylthiouracile (BTU) (0,05%) et d'une hyperthyroïdie provoquée par administration de l'hormone L-thyroxinate de sodium (0,0012%) dans l'eau de boisson, sur une durée de cinq semaines. Aucune incidence significative sur les taux de la peroxydation lipidique et de la vitamine E n'est observée au niveau des tissus hépatiques et cardiaques chez les rats souffrant d'une hypothyroïdie par rapport aux témoins, contrairement au lot hyperthyroïdien, qui a exprimé une augmentation. L'activité glutathion peroxydase, augmentée chez le lot hyperthyroïdien par rapport au témoin, s'accompagne d'une diminution du niveau du glutathion réduit dans l'homogénat des tissus et d'une augmentation hautement significative de l'activité de la glutathion réductase. Une augmentation de l'activité du superoxyde dismutase et de la catalase chez le lot hyperthyroïdien est aussi observée. Nos résultats expliquent la variation

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de l'activité thyroïdienne en relation avec la peroxydation lipidique et le contenu tissulaire en anti-oxydants enzymatiques et non enzymatiques. En conclusion, notre étude montre l'installation d'un état de stress oxydant en relation avec l'hyperactivité thyroïdienne. **Pour citer cet article : M. Messarah et al., C. R. Biologies 330 (2007).**

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**Mots-clés :** Antioxydant ; Cœur ; Foie ; Rat ; Stress oxydatif ; Thyroïde

## 1. Introduction

Among the most important functions of the thyroid gland in various mammal tissues, we distinguish its capacity to increase the basal metabolic rate, particularly that of mitochondria. The latter are known to be the main sites of oxygen free radical (ROS) production in healthy tissues because of their leak from the mitochondrial respiratory chain. These (ROS) would lead to oxidative damage to biological macromolecules, including lipids, proteins and DNA [1,2]. However, the thyroid gland function could be either inhibited or activated, therefore altering several biochemical and physiological processes, such as cell metabolism and oxidizing-stress parameters [3–7]. Thus, hypothyroidism is known to slow down the speed of cellular metabolism, resulting in the reduction of free-radical formation, accompanied by a fall in the level of formed peroxides [8]. On the other hand, hyperthyroidism is characterised by an increasing cellular metabolic rate, and thus an increased amount of free radicals [3,6] and an increase in peroxides levels, and either an increase [9] or a decrease [4, 10] in antioxidant enzymes, such as glutathione peroxidase.

Most substances undergoing auto-oxidation are polyunsaturated fatty acids that are present in cell membranes, which are subjected to a chain of peroxidative reactions [11–13]. These biochemical changes bring about an imbalance in the nervous system functions [14], cell haemolysis and disturbances in cell metabolism, leading to cell death. In fact, the cell contains a variety of substances capable of scavenging the free radicals, protecting them from harmful effects. Among the most known enzymatic antioxidants, we notice glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST), while the most known non-enzymatic antioxidants are glutathione (GSH), vitamin E,  $\beta$ -carotene, ascorbate and coenzyme Q [15,16].

The aim of the present study has been to investigate the effect of altering the thyroid gland activity on the oxidative stress parameters in both the liver and the heart. Accordingly, hypothyroidism and hyperthy-

roidism have been induced in Wistar rats to elucidate the possible changes in the levels of vitamin E, malondialdehyde (MDA), hydroperoxides (HPs), reduced glutathione (GSH), catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase.

## 2. Materials and methods

Male Wistar rats of eight weeks of age were obtained from the breeding centre of the Central Pharmacy of Tunis, weighing of  $103 \pm 8$  g. Food (standard diet, supplied by the 'Société industrielle des concentrés', SICO, Sfax, Tunisia), and water were available ad libitum.

The animals were acclimated for one week under the same laboratory conditions of photoperiod (12-h light: 12-h dark cycle), a minimum relative humidity of 40% and room temperature  $23 \pm 2^\circ\text{C}$ . The rats were randomly divided into three groups of eight males, each as follows:

- *group 1*, comprising eight individuals and serving as control;
- *group 2*, whose individuals received 0.05% BTU in drinking water for five weeks to induce hypothyroidism;
- *group 3*, whose rats were treated with 0.0012% of L-thyroxine sodium salt in their drinking water for five weeks to induce hyperthyroidism.

The total rat body weights were daily recorded throughout the experimental period. The rats were sacrificed by decapitation. Their hearts and livers were removed and weighed after the removal of the surrounding connective tissues, and then they were placed in a buffer solution of TBS (Tampon buffer saline, 50 mM Tris, 150 mM NaCl, pH = 7.4) at a temperature of  $4^\circ\text{C}$ . One gram of each organ was homogenised in 2 ml of TBS in an ultrasound homogeniser. Homogenates were centrifuged at 9000 rpm for 15 min at  $4^\circ\text{C}$ , and the supernatants were divided into aliquots and stored at  $-20^\circ\text{C}$  before being used. Blood samples were immediately collected before being centrifuged at 3000 rpm for 15 min; plasma were then separated and stored at  $-20^\circ\text{C}$  before being used for hormones assays.

T<sub>3</sub> and T<sub>4</sub> were determined by radioimmunoassay (commercial RIA Kit, CIS International, France), as described by López-Torres et al. (2000) [17].

MDA concentrations were estimated according to the method of Esterbauer et al. [18], while hydroperoxides were determined following the method of Heath and Tappel [19]. On the other hand, vitamin E was evaluated using an HPLC procedure, as described by Lang et al. [20]. The reduced glutathione content was estimated using a colorimetric technique, as mentioned by Weckbeker and Corey [21], while the glutathione peroxidase activity was measured following the method of Flohe and Gunzler [22].

The glutathione peroxydase activity was expressed by the quantity of oxidized GSH having disappeared, while that of glutathione reductase was determined following the method of Horn [23]. The activity of catalase was measured according to the method of Aebi [24], whereas that of superoxide dismutase was achieved by the nitrobluetetrazolium test (NBT), according to the method of Asada et al. [25]. Protein contents of various samples were determined by the method of Bradford [26], using bovine serum albumin as a standard.

The results were expressed as mean values  $\pm$  SEM. Comparisons between mean values were made using the one-way analysis of variance (ANOVA), followed by the Student–Newmann–Keuls test when necessary.

### 3. Results

Changes in T<sub>3</sub> concentrations are illustrated in Table 1. T<sub>3</sub> underwent a highly significant increase ( $p \leq 0.01$ ) in the hyperthyroid rats compared to the hypothyroid and the control ones, whereas in hypothy-

Table 1

Changes in body weight (g), heart and liver relative weight (g/kg) and T<sub>3</sub> and T<sub>4</sub> concentrations (ng/ml). Results are expressed as mean  $\pm$  SEM ( $n = 8$  in each group)

Parameters studied	Control	Hypothyroidism	Hyperthyroidism
Body weight (g)	212.5 $\pm$ 6.38	160.53 $\pm$ 5.14**	141.46 $\pm$ 4.57***##
HRW (g/kg)	3.64 $\pm$ 0.05	3.20 $\pm$ 0.027	4.1 $\pm$ 0.04#
LRW (g/kg)	53.03 $\pm$ 4.6	41.68 $\pm$ 2.7**	48.83 $\pm$ 3.61*##
T <sub>3</sub> (ng/ml)	0.83 $\pm$ 0.03	0.35 $\pm$ 0.09**	9.25 $\pm$ 0.3***###
T <sub>4</sub> (ng/ml)	41.98 $\pm$ 3.6	10.95 $\pm$ 0.88***	20.70 $\pm$ 1.17***##

HRW: Heart Relative Weight.

LRW: Liver Relative Weight.

Significant differences: \* Compared to controls (\*:  $p \leq 0.05$ ;

\*\* :  $p \leq 0.01$ ; \*\*\*:  $p \leq 0.001$ ).

# Hyperthyroidism vs. hypothyroidism (#:  $p \leq 0.05$ ;

##:  $p \leq 0.01$ ; ###:  $p \leq 0.001$ ).

roid animals the concentrations were remarkably reduced ( $p \leq 0.01$ ) compared to those of untreated animals. However, T<sub>4</sub> plasma levels did show a significant decrease ( $p \leq 0.01$ ) in both hyperthyroidism and hypothyroidism, even though the decrease was more pronounced in the latter case ( $p \leq 0.001$ ) in comparison with the controls.

Changes in total body weight are also shown in Table 1. The total body weight showed a pronounced reduction ( $p \leq 0.01$ ) in rats of both treated groups. Besides, a significant rise ( $p \leq 0.01$ ) in mean heart weights of hyperthyroidism was recorded, compared only to the case of hypothyroid rats, while a significant decrease ( $p \leq 0.01$ ) in mean liver weights of both hypothyroid and hyperthyroid rats was noted compared to the controls.

Lipid peroxidation products such as malondialdehyde and hydroperoxide concentrations did not show any significant change as regards the hearts and livers in the hypothyroid individuals compared to controls (Tables 1 and 2), whereas those of hyperthyroid rats were

Table 2

Effect of thyroid status on lipid peroxidation and antioxidant enzyme activities in the rat liver. Results are expressed as mean  $\pm$  SEM ( $n = 8$  in each group)

Parameters studied	Control	Hypothyroidism	Hyperthyroidism
Malondialdehyde (nmol/mg protein)	1.41 $\pm$ 0.10	1.07 $\pm$ 0.08	3.26 $\pm$ 0.11**##
Hydroperoxides (nmol/g tissue)	1.44 $\pm$ 0.07	1.24 $\pm$ 0.23	2.2 $\pm$ 0.20**##
Vit E (nmol/g tissue)	36.33 $\pm$ 3.23	32.06 $\pm$ 1.25	44.29 $\pm$ 3.66**##
Reduced glutathione (nmol/mg protein)	62.45 $\pm$ 4.62	71.96 $\pm$ 1.83*	51.53 $\pm$ 2.59**##
SOD (units/mg protein)	18.18 $\pm$ 1.75	20.89 $\pm$ 0.78	29.48 $\pm$ 1.24**#
GR (IU/mg protein)	1.97 $\pm$ 0.06	1.03 $\pm$ 0.07*	3.8 $\pm$ 0.71**##
GPx (units/mg protein)	0.45 $\pm$ 0.02	0.78 $\pm$ 0.04*	1.22 $\pm$ 0.08**#
Catalase (IU/mg protein)	26.40 $\pm$ 2.97	25.26 $\pm$ 1.58	33.56 $\pm$ 2.48*#

Hydroperoxides (HPs) were measured as nmol NADPH/min/g tissue; GSH: nmol/mg protein; SOD: represents the amount of enzyme that inhibits the oxidation of NBT by 50%/mg protein; GPx:  $\mu$ mol GSH/min/mg protein; GR: nmol NADPH oxidised/min/mg protein; CAT:  $\mu$ mol H<sub>2</sub>O<sub>2</sub>/min/mg protein.

Significant differences: \* Compared to controls (\*:  $p \leq 0.05$ ;

\*\* :  $p \leq 0.01$ ).

# Hyperthyroidism vs. hypothyroidism (#:  $p \leq 0.05$ ;

##:  $p \leq 0.01$ ).

Table 3

Effect of thyroid status on lipid peroxidation and antioxidant enzyme activities in heart muscle rat. Results are expressed as mean  $\pm$  SEM ( $n = 8$  in each group)

Parameters studied	Control	Hypothyroidism	Hyperthyroidism
Malondialdehyde (nmol/mg protein)	0.73 $\pm$ 0.04	0.55 $\pm$ 0.054	1.38 $\pm$ 0.05 <sup>**##</sup>
Hydroperoxides (nmol/g tissue)	0.58 $\pm$ 0.04	0.52 $\pm$ 0.03	0.92 $\pm$ 0.04 <sup>**##</sup>
Vit E (nmol/g tissue)	41.3 $\pm$ 1.67	40.09 $\pm$ 2.4	51.46 $\pm$ 5.60 <sup>*#</sup>
Reduced glutathione (nmol/mg protein)	46.29 $\pm$ 2.56	58.50 $\pm$ 4.24	35.36 $\pm$ 3.20 <sup>**##</sup>
SOD (units/mg protein)	19.4 $\pm$ 2.26	21.7 $\pm$ 2.01	28.60 $\pm$ 2.34 <sup>**#</sup>
GR (IU/mg protein)	1.14 $\pm$ 0.07	0.69 $\pm$ 0.05 <sup>*</sup>	1.83 $\pm$ 0.06 <sup>**##</sup>
GPx (units/mg protein)	0.29 $\pm$ 0.02	0.65 $\pm$ 0.07 <sup>*</sup>	0.97 $\pm$ 0.03 <sup>**#</sup>
Catalase (IU/mg protein)	17.02 $\pm$ 3.14	13.16 $\pm$ 2.45	30.94 $\pm$ 2.08 <sup>**##</sup>

Hydroperoxides (HPs) were measured as nmol NADPH/min/g tissue; GSH: nmol/mg protein; SOD: represents the amount of enzyme that inhibits the oxidation of NBT by 50%/mg protein; GPx:  $\mu$ mol GSH/min/mg protein; GR: nmoles NADPH oxidised/min/mg protein; CAT:  $\mu$ mol H<sub>2</sub>O<sub>2</sub>/min/mg protein.

Significant differences: \* Compared to controls (\*:  $p \leq 0.05$ ; \*\*:  $p \leq 0.01$ ).

# Hyperthyroidism vs. hypothyroidism (#:  $p \leq 0.05$ ; ##:  $p \leq 0.01$ ).

significantly higher ( $p \leq 0.01$ ) than those of controls (Tables 1 and 2). Finally, vitamin E levels underwent a significant increase ( $p \leq 0.01$ ) in hyperthyroid rats, compared to those of the controls (Tables 1 and 2).

Changes in GSH concentrations, GPx, GR, SOD and CAT activities are illustrated in Tables 2 and 3. A significant reduction ( $p \leq 0.01$ ) in GSH levels accompanied by an increase ( $p \leq 0.01$ ) of GPx activity in hyperthyroid rats was observed compared to those of controls.

The GR activity of hyperthyroid rats was remarkably higher ( $p \leq 0.01$ ) than that recorded in hypothyroid animals and even more pronounced ( $p \leq 0.05$ ) than that of the controls.

On the other hand, SOD and CAT activities in hyperthyroid rats did undergo a significant increase ( $p \leq 0.01$ ) compared to that recorded in both hypothyroid and control rats.

#### 4. Discussion

The preliminary results arising from the current investigation reveal that thyroid hormones affect the effectiveness of antioxidants in the rat hearts and livers. Most if not all the studied biochemical parameters related to the oxidizing stress are in favour of such a hypothesis.

The overall changes in the thyroid hormones profile reflect the observed decline in the total body weight, as well as the relative weights of hearts and livers. Nonetheless, the present results show that thyroxine depletion (treatment with BTU) did induce a loss in body weight. Such an observation does not agree with some

previous studies where body gain has been reported to occur under such conditions, even though few studies confirm our present results [17,27–29]. Moreover, the altered levels of T<sub>3</sub> and T<sub>4</sub> might be considered as a sound argument in the induction of either hypo- or hyperthyroidism.

Besides, hyperthyroidism leads to a rise in oxygen consumption, inducing in turn, an increase in the level of oxidative stress (singlet oxygen <sup>1</sup>O<sub>2</sub>) in the heart and in the skeletal muscles [4,17], as well as in the liver [10, 29,30]. Consequently, the formation of lipid peroxidations and hydroperoxides increases.

The high increase in the level of MDA and hydroperoxides in hyperthyroidism compared to those of both hypothyroidism and controls might be due to the possible changes in the cellular respiration of target tissues, which are undoubtedly related to any alteration in the thyroid function, knowing the major role of the thyroid hormones in the control (acceleration) of the mitochondrial respiration rate [1,2,28]. From a biochemical point of view, the provoked hyperthyroidism, and via a variety of mechanisms, mitochondrial respiratory chain activity is altered, leading to an increase in electrons transfer from the respiratory chain through the acceleration of the cellular metabolism rate, resulting in the increased generation of superoxide (O<sub>2</sub><sup>•-</sup>) at the site of ubiquinone [7]. Superoxide radicals can lead to the formation of many other reactive species, including hydroxyl radicals (OH<sup>•</sup>), which can readily start the free-radical process of lipid peroxidation [3,6].

It is worth mentioning that the inhibition of the thyroid gland activity leads not only to cellular metabolism

inhibition, but also to other negative effects, such as a reduction of the effectiveness of antioxidants that have a crucial role in tissue protection [4,5].

Since vitamin E has a hydrophobic character, it adheres to cell-membrane fatty acids and lipoproteins. Vitamin E is also known to have a phenolic function with an antioxidant capacity. Therefore, such a vitamin protects and prohibits the propagation of lipid peroxidation, arising from the oxidative stress. Besides, the observed increase in vitamin E concentrations in rats suffering from hyperthyroidism, might be due to an adaptation against the oxidative stress provoked by the thyroid hyperactivity. Such results are in total agreement with other findings [29–33].

GSH is a tripeptide with a broad spectrum of effects. It is involved in several biological activities including neutralisation of reactive oxygen species (ROS), detoxification of xenobiotics, lipid peroxidations, and the hydroperoxides issued from the respiratory chain [33,34]. The reduction in GSH levels in hyperthyroid rats reflects its consumption through the oxidative stress, associated with the increase in GPx activity, ensuring the elimination of lipid peroxidations. This does not only confirm the main role of the thyroid hormones in regulating the oxidative stress in target cells, but is also in agreement with that of Morini et al. [9], where an increase in GPx activity in hyperthyroid rats was observed. In contrast, Asayama et al. [4] and Fernandez et al. [10] reported a significant reduction in GPx activity of rats suffering from hyperthyroidism. The difference in GPx enzyme activity was probably due to the age (eight weeks) of the rats used in the current investigation. The physiological state of the thyroid gland, the dose and the duration of treatment are also of a major influence on antioxidants enzymes. It was reported in previous studies that the level of lipid peroxidation in the heart was affected by both the age and the state of the thyroid gland, in hyperthyroid rats [35]. From another point of view, the above-mentioned effects might involve an accumulation of superoxide anion that inhibits CAT activity, giving rise to hydrogen peroxide ( $H_2O_2$ ) concentrations [1].

The reduction in GR activity in hypothyroid rats seems to be due to the lack of the reduced equivalents of NADPH coenzyme, necessary for the activity of glucose-6-phosphate dehydrogenase. A key enzyme in the pentose phosphate pathway in in-vivo conditions, since GR activity is dependent on the availability of these reducing equivalents [33]. Similar observations have been reported in recent studies carried out on rabbits [36]. Yet, there has been an increase in the activity of GR in the hearts and livers of hyperthyroid rats. This accounts for the accumulation of toxic peroxides. Since

GSH is widely involved in oxido-reduction reactions, in the presence of the GPx, it oxidizes GSH to GSSG, affecting the natural ratio of GSH/GSSG and, consequently, GR interferes in maintaining this ratio through the neo-formation of GSH.

The acquired results reveal also a marked increase in SOD activity in hyperthyroidism, indicating the presence of oxidative stress due to the increasing mitochondrial oxidation rate, characterised by an overproduction of superoxide anion. The latter is known for its harmfulness to the cell membrane. The SOD is also known for its role in transforming ( $O_2^-$ ) into inorganic hydroperoxide ( $H_2O_2$ ), which will, in turn, be reduced by both CAT and GPx enzymes [1,35,37]. Accordingly, an increase in CAT activity in the homogenates of hyperthyroid rats is noted. This accelerates the speed of the formation of superoxides and the renewal of  $H_2O_2$  quantity (substrate of CAT), which increases CAT activity until the dismutation of hydrogen peroxide [33, 34,38]. Both SOD and CAT function together in a way linked to the dissociation and formation of  $H_2O_2$ , and their activities are adjusted by their variation in the thyroid gland's activity.

In the case of the thyroid gland inhibition, one might expect a fall in cellular respiration and, by analogy, it does not have an effect on the SOD activity, showing the possible effect of thyroid hormones in the determination of the antioxidant enzyme levels. Similar assumptions have already been made by other authors [33,35].

To conclude, it is assumed from the obtained results that the formation of lipid peroxidation in tissues depend on the physiological state of the thyroid gland, where the administration of an antithyroid drug does not affect peroxide products, whereas the treatment with exogenous thyroxine leads to their increase.

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