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## FEVER INDUCED OXIDATIVE STRESS: THE EFFECT ON THYROID STATUS AND THE 5'-MONODEIODINASE ACTIVITY, PROTECTIVE ROLE OF SELENIUM AND VITAMIN E.

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The thyroid hormones metabolism is considerably altered in many pathological processes including fever. Experiments performed on rabbits ( $n=62$ ) showed that increase in the rectal temperature by  $1^{\circ}\text{C}$  (after turpentine oil sc injections) decreased 5'-monodeiodinase activity, the enzyme responsible for deiodination of thyroxine to the most active thyroid hormone 3,3',5-triiodothyronine ( $T_3$ ), in the liver by 25% and in the kidney by 20%. Triiodothyronines concentration in serum decreased during fever from  $1.57 \pm 0.12$  to  $0.52 \pm 0.02$  nmol $T_3$ /l and from  $0.17 \pm 0.01$  to  $0.07 \pm 0.02$  nmol r $T_3$ /l. The increase in the body temperature intensified lipid peroxidation processes (malondialdehyde level increased from 1.2 times in kidney, and 1.4 times in the liver homogenates to 1.6 times in serum). The antioxidants (vitamin E and selenium) supplementation decreased lipid peroxidation processes during fever and partly restored the 5'-monodeiodinase activity. The present study confirmed our previous observations *in vitro* that lipid peroxidation (free radical formation) influences the 5'-monodeiodinase activity in tissues and alters the thyroid hormones metabolism.

**Key words:** oxidative stress, 5'-monodeiodinase, thyroid, vitamin E, selenium

### INTRODUCTION

Oxidative stress results when production of reactive metabolites of oxygen exceeds their safe disposal by antioxidant mechanisms (1). Electrons which fail to reach the terminal enzyme of the transport chain may initiate problems by partially reducing oxygen to reactive molecules (free radicals) which are potentially toxic (2). Anything which increases metabolic rate or activity of oxidative enzymes could increase number of electrons transferred, amount of oxygen consumed, and production of superoxide free radical ( $\text{O}_2^{\cdot-}$ ) (3). Superoxide may be converted into more reactive free radicals, mostly into

extremely reactive hydroxyl radical ( $\bullet\text{OH}$ ). Free radicals have been implicated in many pathological processes, including ischemia, inflammation and malignancy, and alter the activity of some membrane-bound tissue enzymes.

Thyroxine ( $\text{T}_4$ ) is a prohormone requiring 5'-monodeiodination to produce the most active thyroid hormone 3,3',5-triiodothyronine ( $\text{T}_3$ ). 5'-Deiodination of  $\text{T}_4$  occurs in practically all tissues of the body and the reaction is catalysed by the family of enzymes known as the iodothyronine deiodinases. The liver, kidney and muscle supply more than 80% of plasma  $\text{T}_3$  (4). The works of Huang et al. (5) and Brzezińska-Ślebodzińska & Pietras (6) showed that free radicals may influence 5'-monodeiodinase activity (5'-MD), and indirectly reduce plasma  $\text{T}_3$  level.  $\text{T}_3$  is involved in the modulation of protein, lipid and carbohydrate metabolism and play important role in every stage of mammalian growth and development. The activity of tissue-specific regulatory factor, such as monodeiodinases are integrated with the secretory activities and feedback regulation of the thyroid gland to maintain hormonal homeostasis.

Fever increases production of free oxygen radicals by cells of the reticulo-endothelial system (7), increases the lipid peroxidation parameters and decreases the cell antioxidant system (8). In this study we have examined whether the free radicals generated during fever may influence the liver and kidney iodothyronine 5'-MD activity. As the most important mechanism of tissue damage by free radicals is lipid peroxidation, we have measured the by-product of lipid peroxidation — malondialdehyde (MDA) and the protective role of antioxidants, vitamin E and selenium, on the thyroid status in rabbits with increased temperature of the body.

## MATERIAL AND METHODS

### *Materials and reagents*

The 28 days old ( $n=62$ ) New Zealand White rabbits were divided into six groups receiving: sc injection of saline — control group I, three sc doses of turpentine oil ( $1\mu\text{l/g BW}$ ) at 3 days intervals — group II, vitamin E ( $0.5 \text{ U/g BW}$  im injection at 3 days intervals) — group III, turpentine oil plus vitamin E (like on groups II and III) — group IV, selenium (sodium selenite  $0.2 \mu\text{g Se/g BW}$  single intraperitoneal injection, 10 days before they were killed) — group V, turpentine oil plus selenium (like on groups II and V) — group VI.

The rectal temperature of each animal (RT) was measured with accuracy to  $0.05^\circ\text{C}$ , once daily, by means of temperature recorder (Type 28, Ellab, Copenhagen).

For *in vitro* study, animals were lightly anaesthetised with ether, blood was taken by cardiac puncture and after decapitation; the liver and kidney were dissected out and frozen at  $-20^\circ\text{C}$ .

All experiments were performed with acceptance of the Institute Ethical Committee for Experiments on Animals.

1,1,3,3-Tetraethoxypropane (MDA), sodium selenite were purchased from Sigma Chemical Co. (St. Louis, MO, USA), vitamin E ( $\alpha$ -tocopherol acetate) from Merck (Darmstadt, Germany) and tri-chloroacetic acid (TBA) was purchased from Loba Feinchemie (Fischamend, Germany).

## Tissue preparation

The liver and kidney were homogenized in 4 vol. (w/v) of ice-cold 0.2 M Tris-HCl buffer containing 0.25 M sucrose and 5 mM EDTA (pH 9.0) for 5'-MD determination and 0.2 M Tris-HCl buffer containing 0.25 M sucrose (pH 7.4) for thiobarbituric acid reactive substances (TBARS) determination and centrifuged at  $10000 \times g$  for 30 min. The supernatant was used for incubation. Protein concentration in tissue homogenate supernatant was estimated by the method of Lowry *et al.* (9) using bovine serum albumin as standard.

## 5'-Monodeiodinase determination

The 3,3',5'-triiodothyronine (reverse  $T_3$ ,  $rT_3$ ), the biologically less active product in  $T_4$  metabolism, is widely used as a substrate for 5'-MD determination. The measurement of 5'-MD activity was based on release of  $^{125}I^-$  from  $(^{125}I)rT_3$  according to method of Leonard & Rosenberg (10) in modification of Jack *et al.* (11). The  $(^{125}I)rT_3$  labelled in the outer ring only, specific activity 976–1260  $\mu Ci/\mu g$ , purchased from NEN (Du Pont — NEN, Belgium), was purified by Sephadex LH-20 chromatography immediately before assay (12). The  $(^{125}I)rT_3$  fraction was dried under nitrogen and dissolve in 0.1 M potassium phosphate buffer (pH 7.0). The assay mixture contained in total volume of 120  $\mu l$ : 0.1 M potassium phosphate buffer (pH 7.0), 1 mM EDTA, 10 mM DTT, tissue homogenate supernatant (5  $\mu g$  protein) and between 50 000–70 000 cpm  $(^{125}I)rT_3$  mixed with unlabelled  $rT_3$  to a final concentration of about 400 nmol/l. The assay mixture was incubated at 37°C for 2 min (blank value) and 12 min (sample). Reactions were terminated by addition of 0.5 ml of ice-cold horse serum followed by 0.5 ml of 10% (w/v) TCA. Each sample was determined in triplicate. After centrifugation for 20 min at  $2000 \times g$ , radioactivity of free  $^{125}I^-$  in 0.5 ml of supernatant was measured. The radioactivity of the blank tube was subtracted from that in the sample tube and the results expressed in pmol  $I^-$  liberated/mg protein  $\times$  min.

## Iodothyronine determination in serum

The  $T_3$  and  $rT_3$  levels in serum were measured by radioimmunoassay (13). In this procedure charcoal methyl cellulose for the separation of the free and bound fractions was used. The procedure detected 0.11 nmol/l  $T_3$  and  $rT_3$  in 0.025–0.05 ml samples of serum. Each sample was determined in triplicate. Mean intra- and inter-assay coefficient of variation were 3.4 and 6–7% for  $T_3$ , and 6.5 and 10% for  $rT_3$ .

## Assay of TBARS

The amount of TBARS in serum and tissue homogenate supernatant was determined by the method of Ledwozyw *et al.* (14). In short 0.25 ml of sample was mixed with 1.25 ml of 1.22 M TCA in 0.6 M HCl and allowed to stand for 15 min. To this mixture 0.75 ml of thiobarbituric acid solution was added (obtained by dissolving 500 mg of thiobarbituric acid in 6 ml 1 M NaOH and then adding 69 ml  $H_2O$ ) and thereafter heating for 30 min in 100°C. After cooling to room temperature 2 ml of n-butanol was added and the mixture was shaken vigorously for 3 min and centrifuged 20 min at  $2000 \times g$ . The organic layer was removed and its absorbance was measured at 532 nm. The results were expressed as nmoles of MDA/1 ml of sample using MDA as standard.

## Statistical analysis

The Duncan's new multiple range test was applied. Statistical significance was defined as  $P < 0.05$ . Values were given as means  $\pm$  S.E.M.

## RESULTS

The turpentine oil injection increased the rectal temperature (RT) by about  $1^\circ\text{C}$  in the experimental groups II, IV (Fig. 1) and group VI — not shown in the Figure. The increase in the RT, showed some variations, was kept for the whole observation period.

As shown in the Fig. 2, during the period, serum triiodothyronine ( $T_3$  and  $rT_3$ ) concentrations decreased significantly from  $1.57 \pm 0.12$  (control) to  $0.52 \pm 0.02$  nmol  $T_3/l$  (group II;  $P < 0.001$ ) and from  $0.17 \pm 0.01$  (control) to  $0.07 \pm 0.02$  nmol  $rT_3/l$  (group II;  $P < 0.001$ ). Supplementation with vitamin E (group IV) and selenium (group VI) did not exert any protected effect on the decreased serum  $T_3$  and  $rT_3$  levels.

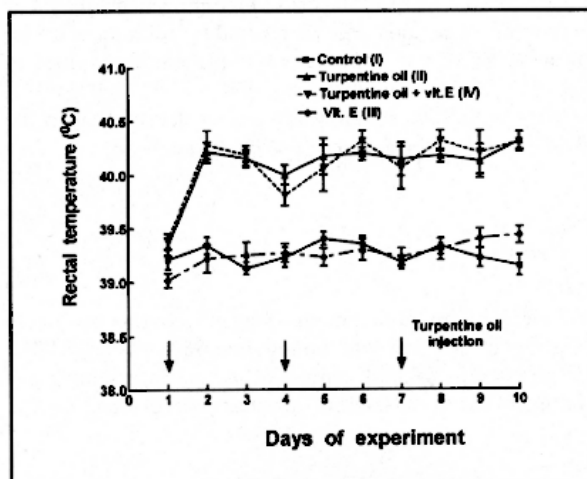


Fig. 1. Changes in RT (mean  $\pm$  S.E.M.) relative to treatment. The Roman numbers in parenthesis denote experimental groups, 8—10 animals in each.

The induced fever caused a significant reduction in the  $5'$ -MD activity, from  $33.4 \pm 1.62$  to  $24.9 \pm 0.74$  pmol  $I^-/\text{mg protein} \times \text{min}$  in the liver ( $P < 0.001$ ) and from  $48.8 \pm 1.15$  to  $38.5 \pm 1.31$  pmol  $I^-/\text{mg protein} \times \text{min}$  in the kidney ( $P < 0.001$ ) homogenate. In the turpentine oil receiving group (II),  $5'$ -MD activity decreased by 25% in the liver and by 20% in the kidney (Fig. 3). Selenium administration (group VI), but not vitamin E, restored completely the enzyme activity in both examined tissues.

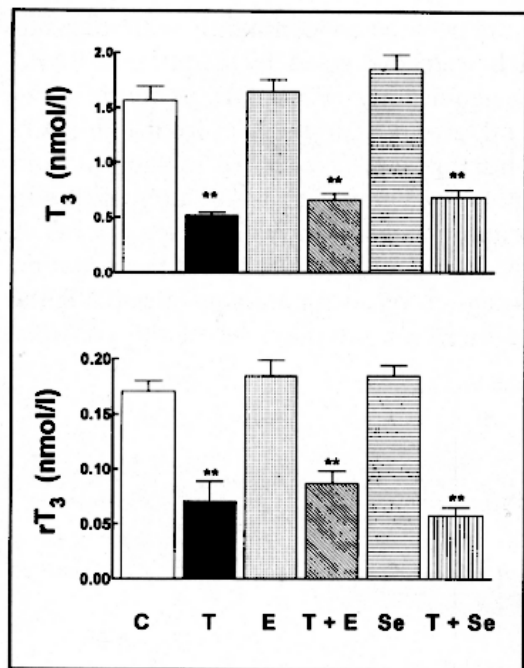


Fig. 2. Serum  $T_3$  and  $rT_3$  concentrations in control (C; group I), turpentine oil (T; group II), vitamin E (E; group III), turpentine oil + vitamin E (T+E; group IV), selenium (Se; group V) and turpentine oil + selenium (T+Se; group VI) receiving groups.

\*\* $P < 0.001$  vs control.

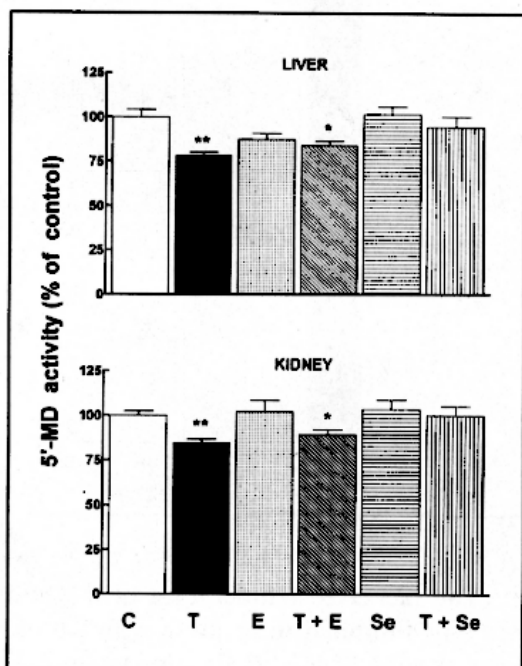


Fig. 3. 5'- Monodeiodinase activity in the liver and kidney homogenates. The 5'-MD activity in the control group was taken as 100%.

\* $P < 0.01$ , \*\* $P < 0.001$  vs control.

The increase in the body temperature occurred concomitantly with intensified lipid peroxidation processes which were expressed by a rise in TBARS formation (Fig. 4). MDA increased significantly ( $P < 0.001$ ) in serum from  $5.5 \pm 0.13$  (control) to  $8.6 \pm 0.20$  nmol/ml (group II), in the liver from  $18.6 \pm 0.39$  (control) to  $25.4 \pm 0.42$  nmol/ml of homogenate (group II), in kidney from  $33.8 \pm 0.65$  (control) to  $39.7 \pm 0.81$  nmol/ml of homogenate. Vitamin E (group III) and selenium (group V) supplementation significantly decreased the MDA level in kidney ( $P < 0.001$ ) and diminished the fever induced lipid peroxidation processes. In the turpentine oil and selenium receiving animals (group VI) the lipid peroxidation in the liver and kidney was at the level of the controls.

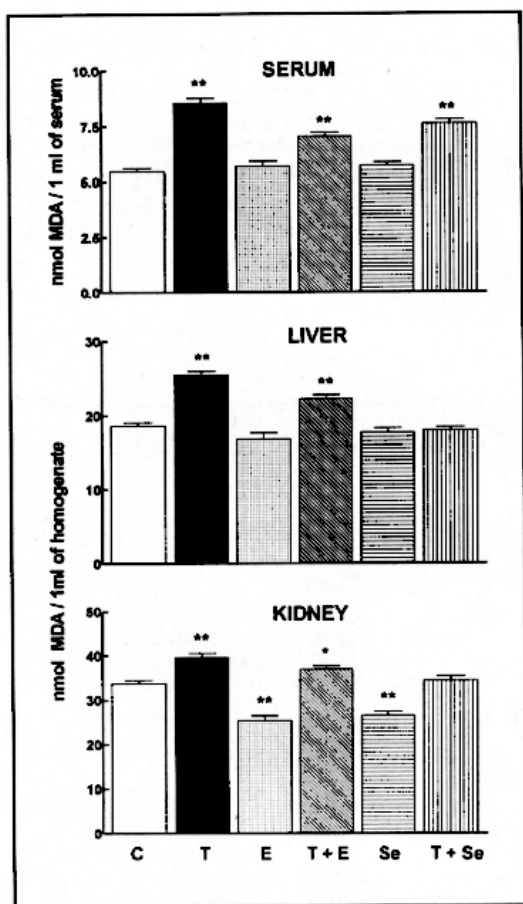


Fig. 4. TBARS concentrations in serum and the liver and kidney homogenates. \* $P < 0.01$ , \*\* $P < 0.001$  vs control.

The increased temperature of the body in turpentine oil receiving groups not only inhibited the body weight gain but in the groups II and VI decreased their weight (Table I). In the vitamin E or selenium supplemented groups

(III and IV) the body weight gain of the rabbit did not differ from the controls (group I).

Table 1. The body weight (g) gain or loss in rabbits after 10 days of experimental period. Means  $\pm$  SEM; 9–11 animals in each group.

GROUPS	INITIAL WEIGHT	FINAL WEIGHT	WEIGHT GAIN or LOSS
Control	637.2 $\pm$ 39.0	876.1 $\pm$ 57.5	+ 238.9 $\pm$ 26.3
Turpentine oil	684.4 $\pm$ 35.6	670.0 $\pm$ 25.8	- 14.4 $\pm$ 21.8
Vitamin E	723.5 $\pm$ 32.9	962.0 $\pm$ 38.5	+ 238.5 $\pm$ 15.1
Turp. + vit. E	581.1 $\pm$ 25.2	590.0 $\pm$ 17.0	+ 8.9 $\pm$ 13.1
Selenium	658.0 $\pm$ 46.2	892.0 $\pm$ 71.2	+ 234.0 $\pm$ 34.9
Turp. + Selenium	700.8 $\pm$ 37.9	685.8 $\pm$ 34.6	- 15.0 $\pm$ 22.2

## DISCUSSION

The present study confirmed our previous observations *in vitro* (6) that free radicals influence 5'-MD activity and may cause a reduction in the extrathyroidal T<sub>3</sub> generation from T<sub>4</sub>. The results of our previous *in vitro* studies (6) suggest that oxygen radicals, hydrogen peroxide and hydroxyl radicals may be involved in the inhibition of the 5'-MD activity. The intraperitoneal injection of bacterial endotoxin (lipopolysaccharide; LPS), used by some authors to induce fever (15,16,17), gives only a short time effect. In our experiments turpentine oil, injected in rabbits three times at 3 days intervals, caused the increase of rectal temperature by about 1°C for the period of 10 days (Fig. 1). According to Huang et al (18), fever induced by the turpentine oil injection changes the thyroid hormones metabolism which closely resemble those observed by Chopra et al. (19) in patients with nonthyroidal illnesses (19).

Several new findings suggest an inhibition of hepatic and thyroidal 5'-MD activity by proinflammatory cytokines (20). Depend on animal species, type of cytokine, the cytokines either elevates, decreases or unaltered 5'-MD activities (for review see 21, 22).

Observed in this work significant increase in the TBARS level in serum and tissue homogenates during fever indicate that free radicals can influence the 5'-MD activity by inducing the lipid peroxidation processes. Besides, the increased lipid peroxidation in the liver and kidney microsomes might imply the peroxidative breakdown of endoplasmic reticulum membrane and alterations in its enzymic, physical and structural changes. It cannot be excluded that lipid peroxidation is an ancillary process, occurring parallel to another process which directly influences the 5'-MD, for example the lipid hydroperoxides, generated by the free radicals *in situ*, influence the 5'-monodeiodinase activity.



The decrease of serum  $T_3$  concentration during fever probably results from the lowered the 5'-MD activity, mainly in the liver. The  $T_3$  formed by the liver 5'-MD is transferred into the circulation; kidney contribute at a minor extent to the circulating  $T_3$ . Biologically inert 3,3',5'-triiodothyronine (reverse triiodo-thyronine;  $rT_3$ ), the metabolite resulting from 5-deiodination of  $T_4$  (5-deiodination activity, 5-MD) reflects a bioinactivating pathway of  $T_4$  metabolism (23). It appears in blood serum mainly during fetal and perinatal period. The serum  $rT_3$  concentration in rabbit is 10 times lowered compared to serum  $T_3$  (Fig. 2) and decreases significantly during fever. It is possible that free radicals produced during fever impair both (5'-MD and 5-MD) deiodinases.

Vitamin E is the most important lipid soluble antioxidant in membranes (24) and blood plasma (25) which stabilizes hydroxyl radical by giving up an electron, becoming itself a radical but with reactivity too low to continue the chain reaction. In our experiment, vitamin E supplementation decreased the peroxidation processes during fever (by 10 to 20%), but it did not protect the 5'-MD against free radicals damage. Somewhat, better protection against free radicals during fever was noted in animals supplemented with selenium. Selenium restored the 5'-MD activity in the liver and kidney completely, and decreased the peroxidation processes in liver and kidney to the control value.

The results of Beckett et al (26) show that selenium has a crucial role in the control of thyroid hormone homeostasis. The protective role of selenium could be accounted for activity of the iodothyronine 5'-monodeiodinases which are selenoproteins, with a single selenium molecule as selenocysteine at its active site (27, 28). Selenocysteine appears of critical importance for the 5'-MD catalytic activity. Selenium can protect thyroid hormone metabolism while its deficiency may result in a marked decrease in 5'-MD activity, both in the liver and the kidney (29).

The present study confirmed our previous observations *in vitro* that lipid peroxidation (free radical formation) influences the 5'-monodeiodinase activity in tissues and alters the thyroid hormones metabolism.

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