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Effect of Thyroxine on Antioxidant Defense System in the Liver of Rats of Different Age

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Summary

The effects of altered thyroid state on the antioxidant defense system in the liver of differently aged rats were examined. Male rats aged 15, 45 and 75 days were treated with L-thyroxine, T_4 (40 μg/100 g body mass, s.c., one dose per day) for 14 days (finally aged 30, 60 and 90 days, respectively). The following antioxidant defense enzymes were measured: superoxide dismutases (both copper zinc, CuZn-SOD and manganese containing, Mn-SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), glutathione reductase (GR), as well as the content of low molecular mass antioxidant glutathione (GSH). The effect of T_4 on antioxidant defense system in the liver differs with respect to age. T_4 treatment decreased CAT and GST activities, as well as the content of GSH in animals aged 60 and 90 days. The same treatment elevated GR activity in rats at 30 days of age, this phenomenon was not observed in older animals. The different response of immature rats to thyroxine compared to older animals could be attributed to the differences in thyroxine metabolism and the developmental pattern. Direct effect of T_4 on mature rats can be considered as a part of its overall catabolic action.

Key words
Antioxidant defense enzymes • Glutathione • Liver • Rats • Thyroxine

Introduction

Normal thyroid gland activity is concerned mainly with energy metabolism in nearly all tissues of the body. Development of the hyperthyroid state in vertebrates elevates basal metabolic rate due to increments in the rate of O_2 consumption in target tissues (Videla 2000) an effect accomplished by both (a) short-term mechanism activating mitochondrial cytochrome c oxidase through 3,5-diiodothyronine signaling and (b) long-term pathway involving changes in nuclear and mitochondrial gene expression through 3,3,5-triiodothyronine signaling. In the latter mechanism respiratory genes may be up-regulated through a ligated thyroid hormone receptor, which binds to a thyroid hormone-responsive element in the promoter regions (Goglia *et al.* 1999). *In vivo*, thyroid hormones are the most important humoral factors involved in setting the basal metabolic rate on a long-term basis in target tissues, such as the liver, heart, kidney and brain. An alteration in the thyroid state has considerable effects on the respiratory activities of rat liver mitochondria. Variations

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of thyroid hormone levels can be one of the main physiological modulators of *in vivo* cellular oxidative stress due to their known effects on mitochondrial respiration (Guerrero *et al.* 1999). These conditions determine a higher consumption of cellular antioxidants (Sies 1986, Fernandez *et al.* 1988, 1991, Huh *et al.* 1998, Giavarotti *et al.* 1998) and inactivation of antioxidant enzymes (Fernandez *et al.* 1988), thus inducing oxidative stress (Sies 1986) with the concomitant increase in hepatic lipid peroxidation and protein oxidation (Videla 2000).

The data concerning changes in the content of low molecular mass antioxidant such as glutathione (GSH) (Asayama *et al.* 1987, Morini *et al.* 1991), as well as the activity of antioxidant defense enzymes (Asayama *et al.* 1987) in different hyperthyroid rat tissues are rather contradictory. This concerns the effects of hyperthyroidism on glutathione peroxidase (GSH-Px) activity in the rat liver. Both a decrease (Asayama *et al.* 1987) and an increase of GSH-Px activity (Morini *et al.* 1991) have been reported.

In this study, we explored the hyperthyroid state and T_4 effects on antioxidant defense system in the liver of rats of different age (30, 60, and 90 days) and the following antioxidant defense enzyme activities were studied: copper zinc containing superoxide dismutase (CuZn-SOD), manganese containing superoxide dismutase (Mn-SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9), glutathione-S-transferase (GST, EC 2.5.1.18), glutathione reductase (GR, EC 1.6.4.2.), as well as the content of low molecular mass antioxidant glutathione (GSH, reduced plus GSSG, oxidized).

Methods

The experiments were carried out on male Mill Hill hybrid hooded rats. Animals were housed from birth up to the 30th day of age with their mothers. Thereafter they were transferred into individual cages (four animals per cage). All animals were held under controlled conditions of illumination (light on: 5:00-17:00h) and temperature (23±2 °C) and were allowed free access to water and food. Starting at the age of 15, 45 and 75 days the animals were treated with L-thyroxine, T_4 (40 μg dissolved in 9 mmol/l NaOH/100 g body mass as suggested by Wooten and Cascarino 1980) during next 14 days, s.c., one dose per day before sacrificing at the final age of 30, 60 or 90 days (n=31). The study was performed using double control group protocol. One control group consisted of non-treated (intact) animals (n=29). The second control group received 9 mmol/l NaOH/100 g body mass, as T_4-treated animals (n=26).

All animals were sacrificed by decapitation always between 8:00-10:00h to avoid any possible rhythmic variations in the antioxidant enzyme level. Immediately after the decapitation, liver was perfused with the cold saline solution (154 mmol/l NaCl) and extracted. Homogenization was performed with a Janke and Kunkel (Staufen, Germany) Ika-Werk Ultra-Turrax homogenizer at 0-4 °C in 0.25 mol/l sucrose, 1 mmol/l EDTA and 0.05 mol/L TRIS-HCl solution, pH 7.4 (Rossi *et al.* 1987, De Waziers and Albrecht 1987). The homogenates were sonicated for 30 s at 10 kHz on ice to release enzymes (Takada *et al.* 1982) and used for determination of the content of total glutathione (GSH+GSSG). The remaining sonicates were centrifuged in Beckman ultracentrifuge (90 min, 85000 x g, 4 °C) and the supernatant was used for determination of antioxidant defense enzyme activities and total protein content. All chemicals were Sigma (St. Louis, MO, U.S.A.) products.

Superoxide dismutase activities (both CuZn-SOD and Mn-SOD) were determined in the supernatant by the epinephrine method (Misra and Fridovich 1972). One unit of SOD activity was defined as the amount of protein causing 50 % inhibition of the autooxidation of adrenaline at 26 °C (Petrović *et al.* 1982). Catalase (CAT) activity was assayed as suggested by Beutler (1982). The activity of glutathione peroxidase (GSH-Px) was measured using t-butyl hydroperoxide as substrate (Paglia and Valentine 1967, modified by Tamura *et al.* 1982). For determination of glutahione-S-transferase (GST) activity, 1-chloro-2,4-dinitro benzene (CDNB) was used as a substrate (Habig *et al.* 1974). The activity of glutathione reductase (GR) was assayed as suggested by Glatzle *et al.* (1974).

All antioxidant defense enzyme activities were expressed per mg of the total tissue proteins (specific activity) and per g of wet tissue mass (total activity) as described by De Quiroga *et al.* (1988).

The content of total GSH (GSH, reduced plus GSSG, oxidized) was measured by enzymatic method suggested by Tietze (1969) as modified by Griffith (1980) and expressed as nmol GSH per g wet mass. Protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a reference.

Statistical analysis was performed using protocols of Hinkle *et al.* (2003). In the experimental

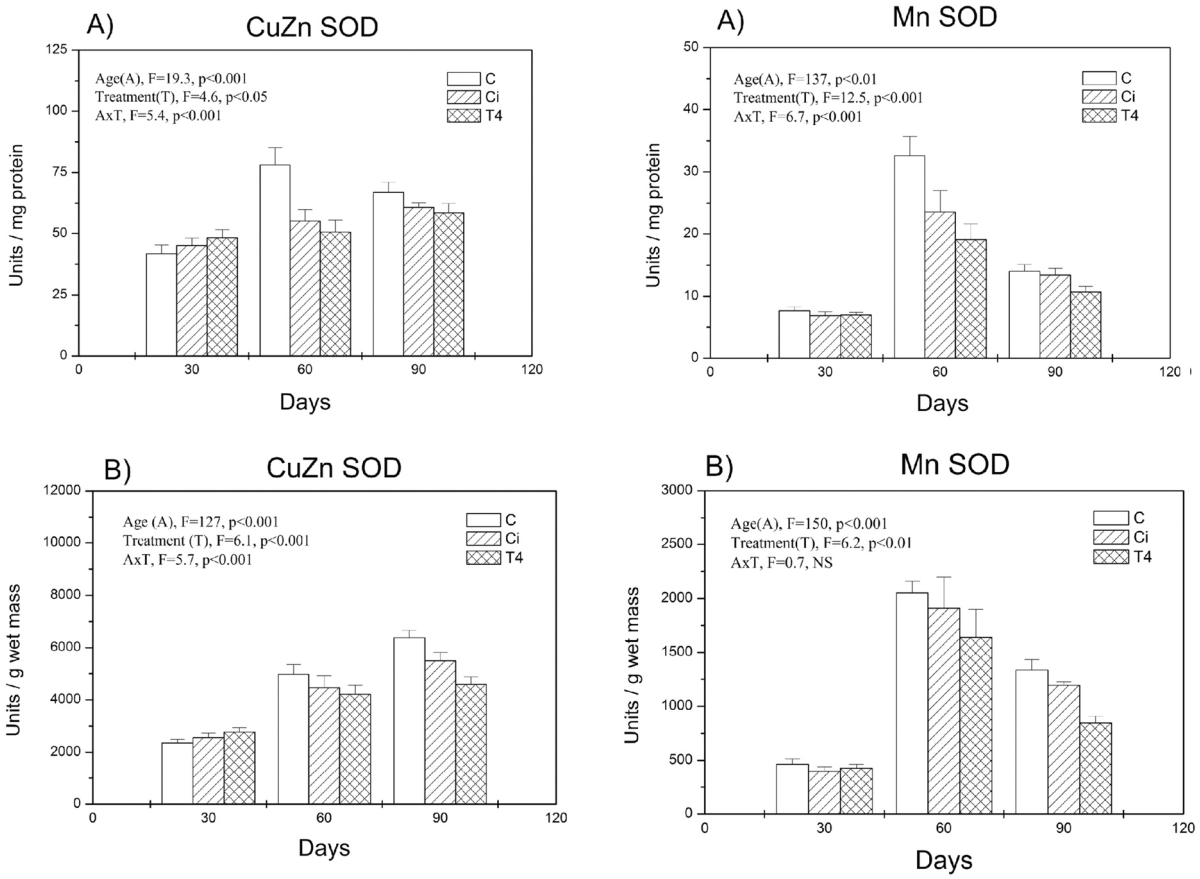

Fig. 1. The activities of copper zinc containing superoxide dismutase (CuZn-SOD) and manganese containing superoxide dismutase (Mn-SOD) in the liver of 30, 60 and 90 days old rats treated with L-thyroxine (T4), internal controls (Ci) and controls (C). Enzyme activities are expressed as specific (A) and as total (B). Data are mean ± S.E.M.

design here the applied procedure was employed on rats of a different age, thereby the effects were statistically analyzed considering two factors: treatment and age using two-way analysis of variance (two-way ANOVA). *Post hoc* comparison of significant ANOVA effects was made using Tukey's honest significant difference (HSD) test.

Results

The activities of antioxidant defense enzyme after T_4 treatment are presented in Figures 1-3 and expressed as specific activity in units per mg protein and as total activity in units per g wet mass. The content of glutathione is shown in Figure 4. In general, it can be assumed that antioxidant defense enzyme activities and the content of glutathione increase during development in rats (significant ANOVA age effect – (A), Figs. 1-4). The activity of antioxidant enzymes is higher in 60- and 90-day-old rats compared to animals aged 30 days. Differences in 60- and 90-day-old rats are less obvious. All antioxidant defense enzymes were affected by T_4. The effect of thyroxine depends on age of animals (ANOVA interaction A x T, Figs 1-3).

Thyroxine decreased CuZn-SOD and Mn-SOD activities in 90-day-old rats (Fig. 1A,B,C,D). There was no effect of T_4 in younger animals. Moreover, thyroxine decreased CAT activity in animals aged 90 days, as well as in 60-day-old animals. There was no effect on 30-day-old animals (Fig. 2A,B). A decrease in GSH-Px activity in T_4-treated rats aged 90 days was found, but only when the activity was expressed per g of wet tissue mass (Fig. 2D). There were no effects in other treated animals (Fig. 2A,B).

A decrease of GST activity (Fig. 3.AB), as well as the content of GSH (Fig. 4) after thyroxine treatment in animals aged 60 and 90 days were observed. However, in 30-day-old rats only decreased GST activity was found. On the other hand, T_4 treatment elevated GR activity (Fig. 3C,D) in rats aged 30 days, but this effect was not found in older animals.

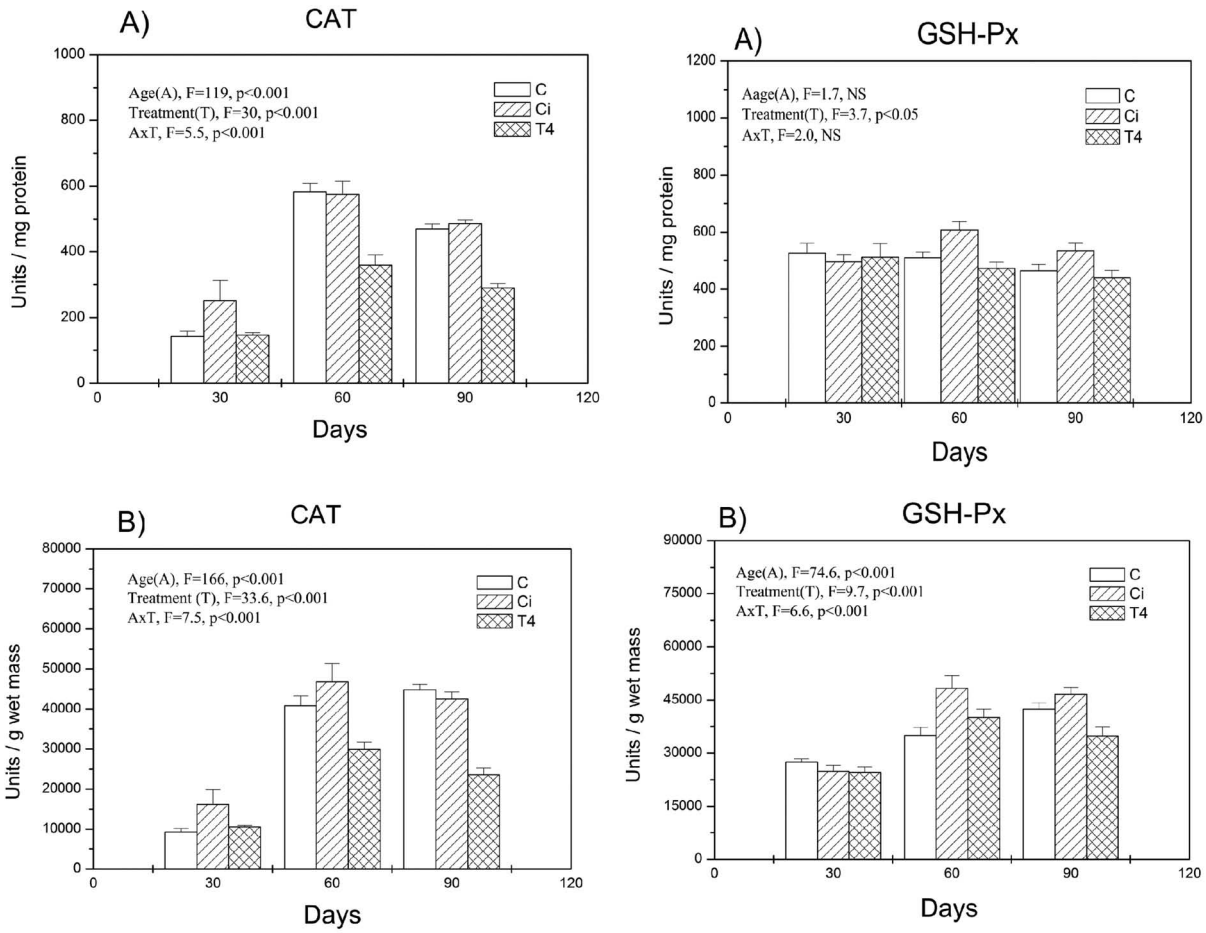

Fig. 2. The activities of catalase (CAT) and glutathione peroxidase (GSH-Px) in the liver of 30-, 60- and 90-day-old rats treated with L-thyroxine (T4), internal controls (Ci) and controls (C). Enzyme activities are expressed as specific (A) and as total (B). Data are mean ± S.E.M.

Discussion

While the literature on free radical metabolism is extensive, there is little information available about endocrine control of tissue oxidative stress. Concerning thyroid hormones, the changes in antioxidant defense enzymes and glutathione metabolism after short- and long-term hormone treatments have been studied (Fernandez *et al.* 1991, 1993, Pereira *et al.* 1994, Guerrero *et al.* 1999).

To the best of our knowledge no previous data about effects of thyroid hormones on the antioxidant defense system on differently aged rats have been published. In the present study, the effects of thyroxine (T_4) on the antioxidant defense system in the liver of differently aged rats are reported. The liver was chosen as a model system for evaluation of thyroid hormone effects on antioxidant defense system since our previous work (Petrović *et al.* 1983) had shown that distribution SOD is the highest in the liver compared to other examined rat tissues. Decrease of SOD and CAT activities and the depletion of GSH content were also found by other authors (Das and Chainy 2001). This pro-oxidant state induced by hyperthyroidism may favor the oxidative deterioration of susceptible biomolecules with consequent alteration or loss of their functions (Fernandez *et al.* 1985, Morini *et al.* 1991).

One of the interesting observations of the present report is the weak effect of T_4 on the antioxidant defense system in the liver of 30-day-old rats. The more pronounced effect of T_4 in adult rats resembled the human response, where the clearance of T_4 and T_3 is more rapid in children than in adults and adequate treatment of hypothyroidism requires relatively larger doses of T_4 in children as compared with adults (Utiger 1995). The only significant changes measured in 30-day-old rats was the increased GR activity. It seems that the elevation of GR activity in 30-day-old rats is adaptive response to overall biochemical and physiological processes within cells rather than direct effect on

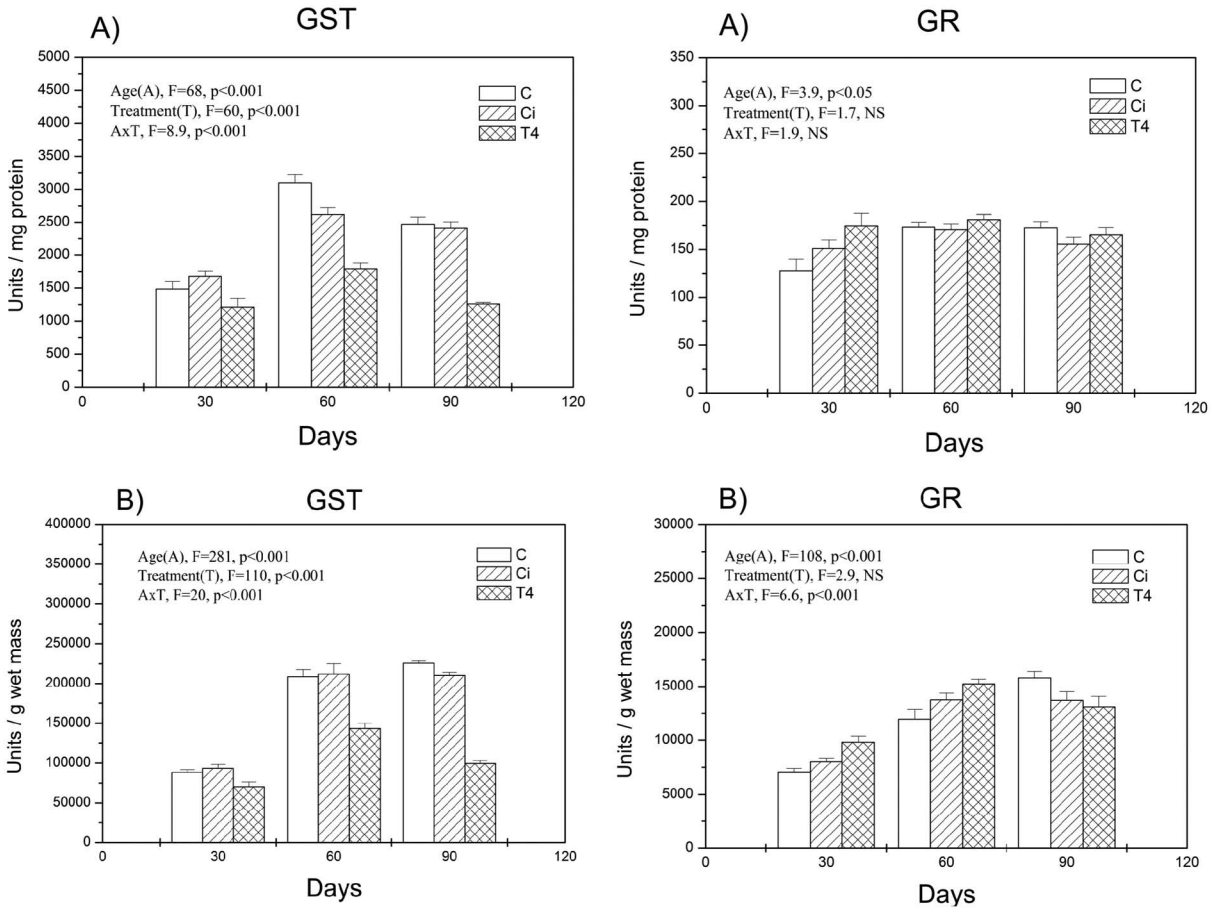

Fig. 3. The activities of glutathione-S-transferase (GST) and glutathione reductase (GR) in the liver of 30-, 60- and 90-day-old rats treated with L-thyroxine (T4), internal controls (Ci) and controls (C). Enzyme activities are expressed as specific (A) and as total (B). Data are mean ± S.E.M.

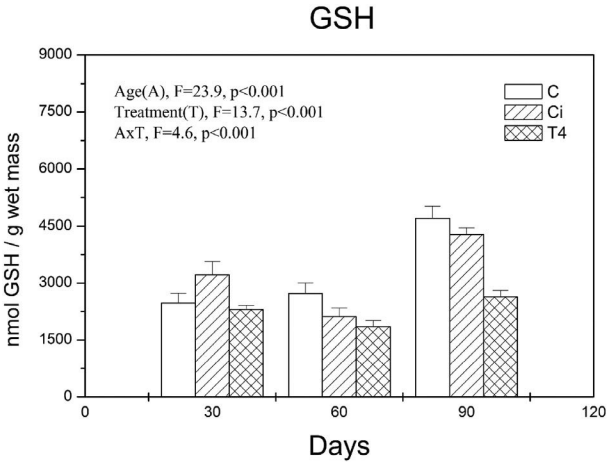

Fig. 4. Glutathione (GSH) content in the liver of 30, 60, or 90-day-old rats treated with L-thyroxine (T4), internal controls (Ci) and controls (C) expressed in nmol GSH per g wet mass. Columns represent mean values and vertical bars are S.E.M.

antioxidative regulatory elements. This suggests increased turnover between oxidized and reduced forms of glutathione and maintaining of stable redox environment. It has been postulated that redox environment obtained by redox couples is one of developmental determinant (Schafer and Buettner 2001). In our previous work, hyperthyroidism induced by T_4 treatment resulted in the augmentation of the SOD level (both CuZn-SOD and Mn-SOD activities) during the development of rats (Saičić *et al.* 2001, 2004) especially in the rat brain (Petrović *et al.* 1982, 1991). Now it is also well known that thyroid hormone in rats during the embryonal, the fetal and early post-natal period of the development (first 15 days after birth) has a direct influence on the normal growth and development of the central nervous system (CNS), but also on other tissues (Utiger 1995).

It was shown that the antioxidant defense system is an endogenous, dynamic system incorporated in homeostatic regulation lead by internal regulatory signals (Blagojević *et al.* 1998). Our results showed the presence of changes at the level of certain antioxidant defense system components (CAT and GST activities and the content of GSH) in 60- and 90-day-old rats. These are in accordance with the results of other authors (Das and

Chainy 2001). The results of our present work that both Mn-SOD and CuZn-SOD activities were unaffected by T_4 treatment are in accordance with the findings of Giavarotti *et al.* (1998), although Seymen *et al.* (1999) reported an elevation. Such a discrepancy may be caused by different experimental conditions. Treatment with T_4 decreased CAT activity in the liver of 60- and 90-day-old rats. On the other hand, T_4 treatment had no significant effects on GSH-Px activity in all examined groups. In the mammalian system, low molecular weight isoenzymes of GST exhibit a similar activity as that of Se-independent GSH-Px which metabolizes organic hydroperoxides (Prohaska 1980). While GST activity in the liver of 60- and 90-day-old rats was decreased, other GSH dependent enzymes such as Se-dependent GSH-Px and GR activities were not altered during the hyperthyroid state (Das and Chainy 2001). Direct effect of T_4 on mature rats might be summarized as a lowering of CAT and GST activities and the content of GSH as a part of its overall catabolic role.

In conclusion, our data indicate that alteration in the thyroid state by T_4 influences the antioxidant defense system in the liver. In this study, we showed that the effect of thyroxine on antioxidant enzyme activities and the content of GSH depends on the age of animals. A part of thyroxine effects can be assigned to its overall catabolic role.

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