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INFLUENCE OF HYPOTHYROIDISM ON LIPID PEROXIDATION, ERYTHROCYTE RESISTANCE AND ANTIOXIDANT PLASMA PROPERTIES IN RABBITS

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The effect of hypothyroidism on some oxidative stress parameters is reported. Moderate hypothyroid state was induced in two groups of female rabbits (3 and 12 months old) by giving 50 mg/kg body weight (BW) of propylthiouracil (PTU) *per os* for 6 days and 20 mg/kg BW of methimazole (MMI) for further 14 days. Serum T_4 and T_3 concentrations decreased by about 38–40 and 32–36%, respectively. The induced hypothyroidism resulted in a significant decrease in the serum concentration of the lipid peroxidation end-product malondialdehyde, as measured by the thiobarbituric-acid assay. Erythrocytes of hypothyroid animals exhibited higher resistance to oxidative stress, while submitted to free radicals generator 2,2'-azo-bis(2-amidinopropane) hydrochloride (AAPH) *in vitro*. Using two detector systems (phospholipid liposomes and deoxyribose), sensitive to either organic or inorganic oxygen radical damage, the ability of euthyroid and hypothyroid rabbit plasma to protect against oxygen radicals was evaluated. The plasma of hypothyroid animals showed about 20% higher ability to protect against iron-binding organic radicals, but about 50% lower chain-breaking antioxidant activity. The antioxidant capacity of plasma against inorganic radicals was not affected by hypothyroidism. In conclusion, the results show that thyroid hormones modulate the free-radical-induced oxidative damage of lipids and that hypothyroidism offers some protection against lipid peroxidation.

Key words: Hypothyroidism, oxidative stress, lipid peroxidation, free radicals

While the biochemical knowledge on free radical metabolism is well documented, there is little information on the endocrine control of oxidative stress. The involvement of thyroid hormones in lipid peroxidation and antioxidant enzyme activities has been studied mainly in liver tissue (Venditti et al., 1997; Huh et al., 1998; Pamplona et al., 1999; Guerrero et al., 1999; Das and Chainy, 2001) but also in heart (Venditti et al., 1997; Lopez-Torres et al., 2000;

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Shinohara et al., 2000; Gredilla et al., 2001), brain (Rahaman et al., 2001), eyes (Bilgihan et al., 1996), thyroid gland (Prem et al., 1998), skeletal muscle (Venditti et al., 1997; Gredilla et al., 2001), erythrocytes (Moreau et al., 1999; Konukoglu et al., 2001) and plasma (Dirican and Tas, 1999). Most of the cited papers concern the relationship between oxidative stress and tissue antioxidant defence systems in hyperthyroidism.

Relatively few papers concerning the influence of hypothyroidism have shown that this condition results in complex effects such as the augmentation of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, reduced and oxidised glutathione and hydrogen peroxide content, and/or a significant decrease of the catalase activity in rat liver mitochondria (Das and Chainy, 2001). Oxidative damage to mitochondrial DNA has been reported also for the mouse heart (Gredilla et al., 2001). The latter indicates that the mitochondrial antioxidant defence system is considerably influenced by hypothyroidism. Pamplona et al. (1999) suggest that thyroid hormones acting as physiologic modulators of tissue oxidative stress and protein degradation are more effective in cytosol, whereas DNA is more protected from oxidative damage. In humans, the low density lipoprotein (LDH) oxidation was shown to be significantly lower in hypothyroid than in hyperthyroid subjects (Constantini et al., 1998).

In the present study we examined the effects of altered thyroid state in 3- and 12-month-old rabbits with experimentally induced hypothyroidism, on lipid peroxidation (the lipid peroxidation end-product malondialdehyde) and on the susceptibility of erythrocytes to oxidative stress. Since iron plays a pivotal role in oxygen radical formation (for a review see Halliwell and Gutteridge, 1990), changes in the iron-binding and iron-oxidising antioxidant protecting ability of hypothyroid rabbit plasma have also been included in the present studies.

Materials and methods

Animals

The experiments were performed on two groups of female White New Zealand rabbits: Group I aged 3 months (n = 28) and Group II aged 12 months (n = 18). Animals were bred in our laboratory, kept at room temperature around 20 °C and exposed to a 10:14 light-dark cycle. They were provided with commercial pellets for rabbits and drinking water *ad libitum.* Rabbits of each age were divided into two groups: euthyroid (control) and hypothyroid (experimental). The hypothyroid induced group received 6-n-propyl-2-thiouracil (PTU; 50 mg/kg BW) *per os* for 6 days and methimazole (MMI; 20 mg/kg BW) for the next 14 days. During pilot observations, these doses of PTU followed by MMI were found to be adequate for inducing hypothyroid state characterised by serum T_4 and T_3 concentrations lowered by about 40 and 35 percent, respectively. Blood was collected with

and without heparin to provide erythrocytes, plasma and serum. All experiments were approved by the Institute Ethical Committee for Experiments on Animals.

Reagents

1,1,3,3-Tetraethoxypropane (MDA, an external standard), trichloroacetic acid, thiobarbituric acid, 6-n-propyl-2-thiouracil (PTU), methimazole (MMI), 2-deoxy-D-ribose, *n*-butanol, ferric chloride, ascorbic acid, and hydrochloric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH) from Polysciences (Warrington, PA, USA).

Iodothyronine determination in serum

The serum levels of thyroxine (T_4) and triiodothyronine (T_3) were measured by radioimmunoassay (Ślebodziński et al., 1982). In this procedure, charcoal methyl cellulose was used for the separation of the free and bound fractions. The procedure detected 2.2 nmol/l T_4 and 0.11 nmol/l T_3 in 0.025–0.05 ml samples of serum. Each sample was determined in triplicate. Mean intra- and inter-assay coefficients of variation were 9.8 and 9.3% for T_4, and 3.4 and 6–7% for T_3.

Assay of thiobarbituric acid reactive substances (TBARS) in serum

The amount of TBARS in serum was determined by the method of Ledwożyw et al. (1986) and expressed in nmoles of MDA/1 ml of serum as described in the previous paper (Brzezińska-Ślebodzińska, 2001).

Erythrocyte susceptibility to free radicals

Susceptibility of erythrocytes to free radicals generated *in vitro* by AAPH was evaluated as indicated by Girodon et al. (1997); for details see Brzezińska-Ślebodzińska (2001). Results were expressed as 50% of maximal haemolysis time (HT_{50} in minutes), using the sigmoidal Boltzmann curve plotted and fitted for each animal by GraphPad Prism computer programme for nonlinear regression.

Organic and inorganic oxygen radicals protection in plasma

The antioxidant protective ability of plasma against organic or inorganic radicals was evaluated as described by Gutteridge and Quinlan (1992). In this method, careful manipulation of the reaction conditions allows to delineate contributions made by iron-binding, iron-oxidising, scavenging and chain-breaking molecules to the antioxidant potential of plasma. Phospholipid liposomes were used as detector molecules for organic radicals and deoxyribose for inorganic radicals. Organic and inorganic radicals were generated depending on amount

and order of addition of ascorbic acid, ferric chloride and hydrogen peroxide (inorganic radicals assay). The oxygen radical damage was evaluated by determination of TBARS according the method described by Gutteridge and Quinlan (1992). Percentage inhibition was calculated relative to the control sample (not containing plasma) to which 100% damage occurred.

For the organic oxygen radicals, the iron-binding, iron-oxidising and chain-breaking antioxidant assay, the bovine brain phospholipid liposomes were prepared according to Gutteridge (1977).

Reactions for the inorganic oxygen radicals, the iron-binding, iron-oxidising and scavenging antioxidant assay was set up in the same way as for organic oxygen radicals, except that deoxyribose was substituted for the phospholipid liposomes and following addition of ascorbic acid and hydrogen peroxide.

TBARS determination (according to Gutteridge, 1977)

Organic oxygen radical damage: after incubation 0.5 ml of 25% (v/v) HCl and 0.5 ml of 1% (w/v) thiobarbituric acid in 50 mM NaOH were added to each tube. The tube contents were mixed and heated at 100 °C for 15 min to develop the pink-colour chromogen. When cool, the chromogen was extracted with 1.5 ml of n-butanol. Phases were separated by centrifugation at 3000 × *g* for 6 min and the absorbance of the upper organic layer, containing chromogen, was measured at 532 nm relative to appropriate blanks.

Inorganic oxygen radical damage: this was performed essentially as described above for organic oxygen radical damage except that 0.5 ml of 2.8% (w/v) trichloroacetic acid was substituted for HCl.

Statistical analysis

The data were analysed for differences by Student's *t*-test. A difference was considered significant at $P < 0.05$.

Results

During the experimental period, the body weight gain of the hypothyroid group (355.3 ± 32.8 g) did not differ significantly from the control (318.2 ± 30.0 g). In the older animals (Group II), the body weight gain increased to 230.0 ± 21.6 g (hypothyroid group) compared to the control 130.0 ± 17.8 g ($P < 0.05$).

The effect of treatment with PTU (1 week) and MMI (2 weeks) on serum T_4 and T_3 concentration is shown in Table 1. Both thyroid hormones decreased significantly ($P < 0.01$–0.001) putting rabbits in a moderate hypothyroid state. The TBARS content in serum, expressed as the lipid peroxidation end-product MDA concentration, decreased significantly ($P < 0.05$) in hypothyroid animals in both groups.

Table 1

Effects of anti-thyroidal treatment (PTU and MMI) on serum T_4, T_3 and TBARS concentrations in two experimental groups of rabbits. Means ± S.E.M.

	Group I		Group II	
	Control n = 14	Experimental n = 14	Control n = 9	Experimental n = 9
T_4 (nmol/l)	41.10 ± 2.05	25.60 ± 1.97***	48.10 ± 5.27	29.10 ± 3.20**
T_3 (nmol/l)	1.44 ± 0.05	0.95 ± 0.04***	1.48 ± 0.04	1.01 ± 0.02***
TBARS (nmol MDA/ml)	9.22 ± 0.26	7.78 ± 0.69*	9.37 ± 0.43	8.04 ± 0.35*

*P < 0.05; **P < 0.01; ***P < 0.001 vs. control group

The time course of the free radical-induced red blood cell haemolysis, fitted by computer analysis, is shown in the Fig. 1. The erythrocytes of each animal in hypothyroid state appeared to be more resistant to free radical damage; the HT_{50} values increased from 142.4 ± 5.35 min (controls) to 162.6 ± 4.47 min (experimental rabbits) in Group I and from 159.6 ± 3.33 min (controls) to 197.4 ± 14.96 min (experimental rabbits) in Group II. These changes were statistically significant (P < 0.05).

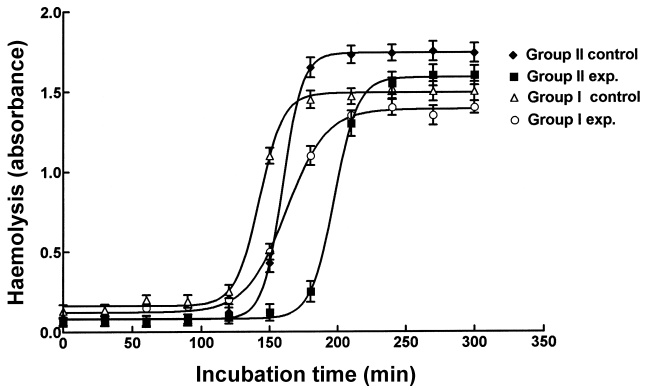

Fig. 1. Erythrocyte haemolysis sigmoidal Bolzmann curves of control (euthyroid) and experimental (hypothyroid) groups of rabbits. Means ± SEM

Results obtained by using two detector systems, sensitive to either organic or inorganic oxygen radical damage, and by evaluating the ability of euthyroid and hypothyroid rabbit plasma to protect against oxygen radicals, are summarised in Table 2. The results showed that animals in hypothyroid state display higher ability to protect against iron-binding organic radicals and lower chain-breaking antioxidant activity. The antioxidant capacity of plasma against inorganic radicals was not affected by hypothyroidism.

Table 2

Antioxidant effects of rabbit plasma on phospholipid and deoxyribose degradation by organic and inorganic oxygen radicals. Values express the percent of oxygen radicals inhibition by plasma. Means ± S.E.M.

	Group I		Group II	
	Control n = 14	Experimental n = 14	Control n = 9	Experimental n = 9
Organic radicals				
Iron-binding	63.8 ± 2.03	82.4 ± 3.64**	74.8 ± 2.08	91.4 ± 2.98**
Iron-oxidising	45.9 ± 1.36	43.1 ± 2.62	46.6 ± 5.47	44.6 ± 3.04
Chain-breaking	16.2 ± 1.84	8.1 ± 1.21*	16.4 ± 0.93	7.2 ± 1.65**
Inorganic radicals				
Iron-binding	86.9 ± 2.42	86.4 ± 3.66	89.9 ± 3.85	96.4 ± 1.80
Iron-oxidising	80.3 ± 1.86	78.3 ± 1.94	83.2 ± 3.50	85.2 ± 3.23
Scavenging	77.1 ± 1.76	74.5 ± 2.50	72.4 ± 1.81	66.8 ± 2.71

*$P < 0.01$; **$P < 0.001$ vs. control

Discussion

Cellular lipid peroxidation consists of an oxidative alteration of polyunsaturated fatty acids present in membrane phospholipids (Kappus, 1985). T_3 administration to rats elicits increases in the lipid peroxidation rate and enhances the peroxide radical generation as found in the rabbit liver mitochondria (Marzoev et al., 1982*a*). Significantly elevated level of hepatic TBARS found in the course of induced hyperthyroidism (Kappus, 1985) is considered to represent a breakdown product of polyunsaturated fatty acids undergoing free radical action. Opposite changes are expected to occur in hypothyroid animals (Marzoev et al., 1982*b*).

In the present work, moderate hypothyroid state was induced by treatment with thyrostatic drugs. Since MMI is an effective inhibitor of thyroid hormone synthesis but does not affect peripheral conversion of T_4 to T_3, the rabbits received both MMI and PTU. PTU not only blocks thyroid hormone synthesis, but unlike MMI, it also inhibits type I iodothyronine deiodinase in liver and kidney, important sites for the peripheral production of T_3 (Leonard and Visser, 1986). The combination of MMI and PTU decreased the T_4 and T_3 concentrations of rabbit serum by about 38–40 and 32–36%, respectively. The results presented in Table 1 indicate that the induced hypothyroidism lowered serum levels of TBARS as compared to the euthyroid controls. Such an effect reflects a decreased rate of oxidative processes at the cellular level in PTU/MMI animals.

Red blood cells (RBC) are particularly sensitive to oxidative damage (Galleano and Puntarulo, 1995), as demonstrated by increasing membrane fragility of the RBC (Stern, 1986; Wagner et al., 1988). In the present study the RBC of hypothyroid rabbits, when subjected to an organic free radical generator (AAPH), displayed an increased resistance to oxidative stress. The half of the maximum haemolysis time (HT_{50}) rose in both experimental groups of rabbits; by about 20 min (Group I, 3-month-old animals) and by about 38 min (Group II, 12-month-old animals) when compared to euthyroid controls ($P < 0.05$). These changes correlate with the lowered peroxidation processes in hypothyroid rabbits, as measured by the TBARS assay (Table 1). The obtained results confirm our earlier observations showing that the plasma antioxidant defence is interrelated with that of the RBC (Brzezińska-Ślebodzińska, 2001). In addition, in agreement with the results of previous studies (Brzezińska-Ślebodzinska, 2001), the erythrocytes of young rabbits appeared to be less resistant to free radical damage than the erythrocytes of older animals ($P < 0.02$).

As the blood plasma contains numerous molecules of high and low molecular weight that are able to react rapidly with organic and inorganic oxygen radicals, two detector systems (phospholipid liposomes and deoxyribose) were used to evaluate the ability of hypothyroid rabbit plasma to inhibit iron-dependent lipid peroxidation. This approach allowed us to delineate the contribution made by iron-binding, iron-oxidising, scavenging and chain-breaking molecules to the antioxidant potential of plasma. Reactive iron can decompose hydrogen peroxide and lipid peroxide to form aggressive oxygen-centred free radicals such as hydroxyl, alkoxyl and peroxyl. Plasma proteins such as transferrin, lactoferrin (Gutteridge et al., 1981) and caeruloplasmin (Gutteridge and Stocks, 1981) have the ability to bind and oxidase iron ions. Albumin binds copper ions and inhibits copper-ion-dependent lipid peroxidation and hydroxyl radical formation (Gutteridge, 1986). Copper ions can attach SH-groups on the membranes of erythrocytes and facilitate their oxidative damage. The present data show that in hypothyroid state a significant increase occurs in the organic iron-binding antioxidant activity of the plasma (Table 2), which may prevent oxygen radical formation. The organic chain-breaking antioxidant potential of plasma appeared to be rather low and decreased in hypothyroidism. Such conditions may be in part caused by the low plasma primary chain-breaking antioxidant – α-tocopherol – content observed concomitantly with hypothyroid state (Constantini et al., 1998; Shinohara et al., 2000). Although the blood plasma of euthyroid rabbits possesses very high inorganic iron-binding, iron-oxidising and scavenging activities (from 77.1% to 86.9% of oxygen radicals inhibition), no alteration in these activities were noticed in hypothyroid rabbits.

The results of the present studies indicate that thyroid hormones modulate the free-radical-induced oxidative damage of lipids and that in hypothyroid state some mechanisms offering protection against lipid peroxidation appear.

References

Bilgihan, K., Bilgihan, A., Diker, S., Ataoglu, O., Dolapci, M., Akata, F., Hasanreisoglu, B. and Turkozkan, N. (1996): Effects of hyper- and hypothyroidism on oxidative stress of the eye in experimental acute anterior uveitis. Acta Ophthalmol. Scand. **74,** 41–43.

Brzezińska-Ślebodzińska, E. (2001): Erythrocyte osmotic fragility test as the measure of defence against free radicals in rabbits of different age. Acta Vet. Hung. **49,** 413–419.

Constantini, F., Pierdomenico, S. D., De-Cesare, D., De-Remigis, P., Bucciarelli, T., Bittolo-Bon, G., Cazzolato, G., Nubile, G., Guagnano, M. T., Sensi, S., Cuccurullo, F. and Mezzetti, A. (1998): Effect of thyroid function on LDL oxidation. Arterioscler. Thromb. Vasc. Biol. **18,** 732–737.

Das, K. and Chainy, G. B. (2001): Modulation of rat liver mitochondrial antioxidant defense system by thyroid hormone. Biochim. Biophys. Acta **27,** 1–13.

Dirican, M. and Tas, S. (1999): Effects of vitamin E and vitamin C supplementation on plasma lipid peroxidation and on oxidation of apolipoprotein B-containing lipoproteins in experimental hyperthyroidism. J. Med. Invest. **46,** 29–33.

Galleano, M. and Puntarulo, S. (1995): Role of antioxidants on the erythrocytes resistance to lipid peroxidation after acute iron overloads in rats. Bioch. Biophys. Acta **1271,** 321–326.

Girodon, F., Blache, D., Monget, A-L., Lombart, M., Brunet-Lecompte, P., Arnaud, J., Richard, M-J. and Galan, P. (1997): Effect of a two-years supplementation with low doses of antioxidant vitamins and/or minerals in elderly subjects on levels of nutrients and antioxidant defense parameters. J. Am. Coll. Nutr. **16,** 357–365.

Gredilla, R., Barja, G. and Lopez-Torres, M. (2001): Thyroid hormone-induced oxidative damage on lipids, glutathione and DNA in the mouse heart. Free Radic. Res. **35,** 417–425.

Guerrero, A., Pamplona, R., Portero-Otin, M., Barja, G. and Lopez-Torres, M. (1999): Effect of thyroid status on lipid composition and peroxidation in the mouse liver. Free Radic. Biol. Med. **26,** 73–80.

Gutteridge, J. M. C. (1977): The measurement of malondialdehyde in peroxidised ox-brain phospholipid liposomes. Analyt. Biochem. **82,** 76–82.

Gutteridge, J. M. C. (1986): Antioxidant properties of the proteins caeruloplasmin, albumin and transferrin. A study of their activity in serum and synovial fluid from patients with rheumatoid arthritis. Biochim. Biophys. Acta **969,** 119–127.

Gutteridge, J. M. C. and Quinlan, G. J. (1992): Antioxidant protection against organic and inorganic oxygen radicals by normal human plasma: the important primary role for iron-binding and iron-oxidising proteins. Biochim. Biophys. Acta **1159,** 248–254.

Gutteridge, J. M. C. and Stocks, J. (1981): Ceruloplasmin: physiological and pathological perspectives. CRC Crit. Rev. Clin. Lab. Med. **14,** 257–329.

Gutteridge, J. M. C., Paterson, S. K., Segal, A. W. and Halliwell, B. (1981): Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. Biochem. J. **199,** 259–261.

Halliwell, B. and Gutteridge, J. M. C. (1990): Role of free radicals and catalytic metal ions in human disease. In: Packer, L. and Glazer, A. N. (eds) Methods in Enzymology Vol. 186: Oxygen Radicals in Biological Systems Part B. Academic Press, New York, pp. 1–85.

Huh, K., Kwon, T. H., Kim, J. S. and Park, J. M. (1998): Role of hepatic xanthine oxidase in thyroid dysfunction: effect of thyroid hormones in oxidative stress in rat liver. Arch. Pharm. Res. **21,** 236–240.

Kappus, H. (1985): Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. In: Sies, H. (ed.) Oxidative Stress. Academic Press, London, pp. 273–295.

Konukoglu, D., Yelke, H. K., Hatemi, H. and Sabuncu, T. (2001): Effects of oxidative stress on the erythrocyte Na^+, K^+ ATPase activity in female hyperthyroid patients. J. Toxicol. Environ. Health A **63,** 289–295.

Ledwożyw, A., Michalak, J., Stępień, A. and Kądziołka, A. (1986): The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. Clin. Chim. Acta **155,** 175–184.

Leonard, J. L. and Visser, T. J. (1986): Biochemistry of deiodination. In: Hennemann, G. (ed.) Thyroid Hormone Metabolism. Marcel Dekker, New York, pp. 189–229.

Lopez-Torres, M., Romero, M. and Barja, G. (2000): Effect of thyroid hormones on mitochondrial oxygen free radical production and DNA oxidative damage in the rat heart. Mol. Cell Endocrinol. **168,** 127–134.

Marzoev, A. I., Kozlov, A. V., Andryuschenko, A. P. and Vladimirov, Y. A. (1982*a*): Activation of lipid peroxidation in the liver mitochondria of hyperthyroid rabbits. Bull. Exp. Biol. Med. **93,** 269–274.

Marzoev, A. I., Kozlov, A. V. and Vladimirov, Y. A. (1982*b*): Inhibition of lipid peroxidation in mitochondria isolated from the liver of hypothyroid rabbits. Bull. Exp. Biol. Med. **93,** 275–280.

Moreau, X., Lejeune, P. J. and Jeanningros, R. (1999): Kinetics of red blood cell T_3 uptake in hypothyroidism with or without hormonal replacement in the rat. J. Endocrinol. Invest. **22,** 257–261.

Pamplona, R., Portero-Otin, M., Ruiz, C., Bellmunt, M. J., Reguena, J. R., Thorpe, S. R., Baynes, J. W., Romero, M., Lopez-Torres, M. and Barja, G. (1999): Thyroid status modulates glycoxidative and lipoxidative modification of tissue proteins. Free Radic. Biol. Med. **27,** 901–910.

Prem, P., Parihar, M. S., Malini, L. and Pradeep, K. G. (1998): Starvation induced hypothyroidism involves perturbations in thyroid superoxide-SOD system in pigeons. Biochem. Mol. Biol. Int. **45,** 73–83.

Rahaman, S. O., Ghosh, S., Mohanakumar, K. P., Das, S. and Sarkar, P. K. (2001): Hypothyroidism in the developing rat brain is associated with marked oxidative stress and aberrant intraneuronal accumulation of neurofilaments. Neurosci. Res. **40,** 273–279.

Shinohara, R., Mano, T., Nagasaka, A., Hayashi, R., Uchimura, K., Nakano, I., Watanabe, F., Tsugawa, T., Makino, M., Kakizawa, H., Nagata, M., Iwase, K., Ishizuki, Y. and Itoh, M. (2000): Lipid peroxidation levels in rat cardiac muscle are affected by age and thyroid status. J. Endocrinol. **164,** 97–102.

Stern, A. (1986): Red cell oxidative damage. In: Sies, H. (ed.) Oxidative Stress. Academic Press, London, UK, pp. 331–349.

Ślebodziński, A. B., Brzezińska-Ślebodzińska, E. and Drews, R. (1982): Reciprocal changes in serum 3,3',5'-triiodothyronine concentration and the peripheral thyroxine inner ring monodeiodination during food restriction in the young pig. J. Endocrinol. **95,** 349–355.

Venditti, P., Balestrieri, M., Di-Meo, S. and De-Leo, T. (1997): Effect of thyroid state on lipid peroxidation, antioxidant defences, and susceptibility to oxidative stress in rat tissues. J. Endocrinol. **155,** 151–157.

Wagner, G. M., Lubin, B. H. and Chiu, D. T-Y. (1988): Oxidative damage to red blood cells. In: Chow, C. K. (ed.) Cellular Antioxidant Defense Mechanisms. CRC Press Inc., Boca Raton, USA, vol. 1, pp. 185–195.