CELL BIOCHEMISTRY AND FUNCTION
Cell Biochem Funct (2011)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/cbf.1765

Thyroid hormone—induced haemoglobin changes and antioxidant enzymes response in erythrocytes

A. S. R. Araujo[1], F. E. R. Seibel[1], U. O. Oliveira[1], T. Fernandes[1], S. Llesuy[2], L. Kucharski[1] and A. Belló-Klein[1]*

[1]*Cardiovascular Physiology Laboratory, Physiology Department, Basic Health Sciences Institute at Federal University of Rio Grande do Sul, Porto Alegre, Brazil*
[2]*Catedra de Quimica General y Inorgánica. Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Buenos Aires, Argentina*

Thyroid hormones modulate haemoglobin and reactive oxygen species (ROS) production, leading to antioxidant changes. This study evaluated the antioxidant response to ROS in erythrocytes in hypothyroid and hyperthyroid rats. Wistar rats were divided into four groups: control; hyperthyroid (T_4-12 mg l^{-1} in drinking water); sham operated (simulation of thyroidectomy); and hypothyroid (thyroidectomized). Four weeks after, blood was collected and haemoglobin and T_4 levels, lipid peroxidation (LPO), protein oxidation, superoxide dismutase (SOD), catalase (CAT) , glutathione S-transferase (GST) and glutathione peroxidase (GPx) activities, and total radical antioxidant potential (TRAP) were measured. SOD, CAT and GST immunocontent was evaluated. Haemoglobin levels were increased in hyperthyroid erythrocytes. LPO and carbonyls were augmented (65% and 55%, respectively) in hyperthyroid and reduced (31% and 56%, respectively) in hypothyroid group. SOD and CAT activities have not changed, as well as CAT immunocontent. TRAP was diminished in both hyperthyroid and hypothyroid groups (36% and 37%, respectively). GST activity and immunocontent, as well as GPx activity, were increased in hyper and hypothyroid rats. The data suggest that thyroid hormone changes determine ROS concentration changes and decrease of some antioxidant defences that would lead to a compensatory answer of the GST and GPx enzymes, which could be consider as credible biomarkers. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS—hypothyroidism; hyperthyroidism; blood-biomarkers; GST; GPx; oxidative damage

INTRODUCTION

Thyroid hormones are involved in the regulation of basal metabolic state and in oxidative metabolism.[1] The variation in its levels plays an important role in the physiological modulation of the mitochondrial respiration process *in vivo*.[2] An elevation in thyroid hormone levels, such as in hyperthyroidism, could lead to increased oxygen consumption and subsequent reactive oxygen species (ROS) generation. ROS concentration increase may be implicated in several pathological conditions.[3]

On the other hand, hypothyroidism is a disease because of a diminished thyroid hormone synthesis, resulting from thyroid gland dysfunction. Physiologic alterations generally occur because of the hypometabolic state induced by hypothyroidism.[4] The depression of basal metabolism is associated with decreased mitochondrial oxygen consumption and less ROS generation,[5] resulting in decreased lipid peroxidation and protein oxidation, as well as diminished antioxidant defences in heart tissue.[6]

Reactive oxygen species demonstrate important chemical reactivity, because they may react with cellular macromolecules and lead to oxidative damage to proteins, lipids and DNA.[7] It has been reported the important role of antioxidant defence system in the control of ROS levels. Two basic antioxidant systems can be highlighted, the enzymatic (superoxide dismutase-SOD, glutathione-S-transferase-GST, glutathione peroxidase-GPx and catalase-CAT), as well as non-enzymatic defence molecules (reduced glutathione-GSH, ascorbate, vitamin E and flavonoids).[8] However, when ROS generation exceeds the antioxidant capacity, the oxidative stress can develop, leading to cellular potential damage.[9]

Some tissues are very susceptible to ROS, such as blood[10] because of its high levels of polyunsaturated fatty acid, iron and oxygen.[11] Furthermore, thyroid hormones may induce changes in the iron metabolism and haemoglobin synthesis control, as well as systemic alterations in oxygen consumption and oxidative metabolism, which may have impact on the redox environment of hematopoietic tissues.[5,12] Therefore, there is a pronounced clinical interest of measuring blood oxidative stress through some oxidative biomarkers, which it can be considered as minimally invasive measurements.[13]

There are several evidences in literature suggesting blood components as biomarkers of thyroid hormones-induced

*Correspondence to: Adriane Belló-Klein, Cardiovascular Physiology Laboratory, Physiology Department, Basic Health Sciences Institute at Federal University of Rio Grande do Sul, Rua Sarmento Leite, 500. CEP: 90050-170 - Porto Alegre - RS - Brasil. E-mail: belklein@ufrgs.br

Received 17 November 2010
Revised 22 March 2011
Accepted 12 April 2011

Copyright © 2011 John Wiley & Sons, Ltd.

oxidative stress.[14] However, there is a paucity of information attaining the mechanisms involved in the modulation of redox balance and antioxidants in erythrocytes under different thyroid hormones levels, especially concerning protein expression of antioxidants. Thus, the purpose of the present study is to determinate potential blood biomarkers that could represent thyroid hormone-dependent alterations in antioxidant defences and oxidative damage, in rats subjected to the experimental hyperthyroidism and hypothyroidism.

MATERIAL AND METHODS

Animals

Male Wistar rats (250 ± 50 g) were obtained from the Central Animal House of the Universidade Federal do Rio Grande do Sul (UFRGS), Brazil. Animals were housed in plastic cages (four animals in each) and received water and pelleted food *ad libitum*. The animals were maintained under standard laboratory conditions (controlled temperature of 21 °C, 12 h light/dark cycle). Animals were weighed weekly to follow body weight gain during the time course of the experimental protocol (28 days). All animals were carefully monitored and maintained in accordance with ethical recommendations of the Brazilian Veterinary Medicine Council and Brazilian College of Animal Experimentation, and the experimental protocol was approved by UFRGS Animal Care Committee (nº 17333).

Experimental groups and protocol

Animals were divided in four groups ($n = 6$/each) as follows: (1) **Control** (receiving water *ad libitum*); (2) **Hyperthyroid** [receiving L-thyroxine (T_4) (12 mg l^{-1} in drinking water)][15]; (3) **Sham operated** (rats were subjected to the simulation of thyroidectomy); and (4) **Hypothyroid** (rats were thyroidectomized).[6]

Thyroxine concentration

Blood samples were collected, through the right carotid artery cannulated with a PE 50 catheter, and immediately centrifuged at 1000 × g for 10 min. Serum thyroxine concentration was estimated by chemiluminescence (CL) using the Immulite 2000 kit (Biomedical Technologies, Inc., Stoughton, MA, USA) at Weinmann Clinical Analysis Laboratory.

Red blood cells preparation

Trunk blood samples were collected into tubes containing heparin as anti-clotting agent. After centrifugation at 1000 × g for 10 min, between 0–4 °C (Sorval RC 5B-rotor SM24, Du Pont Instruments, EUA), plasma was removed. Erythrocytes were washed three times with saline solution (1 : 1 v/v), and the obtained packed cells were resuspended. The prepared red blood cells samples were taken for assay of oxidative stress parameters and antioxidant enzymes.[10]

Determination of haemoglobin concentration

Haemoglobin concentration was detected by the Drabkin reagent through the conversion of haemoglobin to cyanomethemoglobin, measured against a standard curve at 545 nm[16] and expressed in mg ml^{-1}.

Oxidative damage measurements

Lipid peroxidation (LPO) was measured by the *tert*-butyl hydroperoxide-initiated CL assay.[17] *Tert*-butyl hydroperoxide may react with Fe^{+2}, yielding alkoxyl free radical. The production of both peroxyl and alkoxyl free radicals is able to initiate chain reactions upon polyunsaturated membrane phospholipids, eventually leading to photoemission.[18] The assay was carried out with an LKB Rack Beta Liquid Scintillation Spectrometer 1215 (LKB Producter AB, Bromma, Sweden) in the out-of-coincidence mode at room temperature. Erythrocytes were placed in low-potassium vials at a protein concentration of 0·5–1·0 mg of protein ml^{-1} in a reaction medium consisting of 120 mmol l^{-1} KCl, 30 mmol l^{-1} phosphate buffer (pH 7·4). Measurements were started by the addition of 3 mmol l^{-1} *tert*-butyl hydroperoxide, and the data expressed as counts per second per milligramme of protein.

Protein oxidation was performed by carbonyl assay. Plasma samples were incubated with 2,4 dinitrophenylhydrazine (10 mmol l^{-1}) in 2·5 mol l^{-1} HCl solution for 1 h at room temperature, in the dark. Samples were vortexed every 15 min. Then 20% trichloroacetic acid (TCA) (w/v) solution was added in tube samples, left in ice for 10 min and centrifuged for 5 min at 1000 × g, to collect protein precipitates. Another wash was performed with 10% TCA. The pellet was washed three times with ethanol:ethyl acetate (1 : 1; v/v). The final precipitates were dissolved in 6 mol l^{-1} guanidine hydrochloride solution, left for 10 min at 37 °C, and read at 360 nm.[19] The results were expressed as picomole per milligramme protein.

Determination of antioxidant defences

Superoxide dismutase activity, expressed as units per milligramme of protein, was based on the inhibition of superoxide radical reaction with pyrogallol.[20] CAT activity was determined by following the decrease in 240-nm absorption of hydrogen peroxide (H_2O_2). It was expressed as picomoles of H_2O_2 reduced per minute per milligramme of protein.[21] GST activity, expressed as nanomols per milligramme of protein, was measured by the rate of formation of dinitrophenyl-S-glutathione at 340 nm.[22] GPx activity was measured by following nicotinamide adenine dinucleotide phosphate (NADPH) oxidation at 340 nm, and results were expressed as nanomols of peroxide/hydroperoxide reduced per minute per milligramme of protein.[23]

Total radical-trapping antioxidant potential (TRAP), which indicates total antioxidant capacity present in plasma, was also screened. TRAP was measured via CL using 2,2'-azo-bis(2-amidinopropane), a source of alkyl peroxyl free radicals, and luminol. Luminescence was measured in a liquid scintillation counter in the out-of-coincidence mode,

Copyright © 2011 John Wiley & Sons, Ltd.

and results were expressed in millimoles per litre of Trolox.[24]

Western Blot analysis

Erythrocytes were used for measuring antioxidant enzyme concentrations. Electrophoresis and protein transference were performed as described elsewhere.[15,25] The membranes were processed for immunodetection using sheep anti-Cu/Zn SOD polyclonal antibody, rabbit anti-CAT polyclonal antibody and rabbit anti-GST polyclonal antibody as primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). The bound primary antibodies were detected using rabbit anti-sheep or goat anti-rabbit horseradish peroxidase-conjugate secondary antibodies, and membranes were revealed by CL. The autor-radiographs generated were quantitatively analysed with an image densitometer (Imagemaster VDS CL, Amersham Biosciences Europe, IT). The molecular weights of the bands were determined by reference to a molecular weight marker standard (RPN 800 rainbow full range Bio-Rad, CA, USA). The results from each membrane were normalized through Ponceau red method.[26]

Determination of protein concentration

Protein concentration was measured by the Lowry method,[27] using bovine serum albumin as a standard.

Statistical analysis

Data were expressed as mean ± SD and compared by one-way ANOVA followed by the Student-Newmann–Keuls test. Values of $P < 0{\cdot}05$ were considered significant.

RESULTS

Hyperthyroidism and hypothyroidism development and haemoglobin levels

At the end of the 4 weeks post-treatment period, serum thyroxine levels (in nanogrammes per millilitre) were significantly increased in hyperthyroid (19·7 ± 0·9) as compared with control (3·9 ± 0·6) group ($P < 0{\cdot}05$). Hypothyroid group exhibited thyroxine levels diminished (0·17 ± 0·04) as compared with their respective controls (3·2 ± 0·7) ($P < 0{\cdot}05$). Haemoglobin concentration (in milligrammes per millilitre) was increased in hyperthyroid erythrocytes (58·5 ± 3·0) as compared with the control (44·3 ± 2·7), hypothyroid (40·2 ± 1·0) and sham (45·0 ± 3·2) groups ($P < 0{\cdot}05$).

Oxidative damage and antioxidant non-enzymatic defences in plasma

Hyperthyroid plasma demonstrated increased protein oxidation by 55% as compared with the control group (Table 1) ($P < 0{\cdot}05$). Plasma oxidative damage, evaluated by carbonyl assay, demonstrated to be reduced by 56% in hypothyroid as compared with the sham group (Table 1) ($P < 0{\cdot}05$). Carbonyl levels were minor (by ~64%) in hypothyroid as compared with hyperthyroid group (Table 1) ($P < 0{\cdot}05$).

Total antioxidant capacity (TRAP) in plasma (in $mmol\,l^{-1}$) was significantly decreased in hyperthyroid (by ~36%) as compared with the control group ($P < 0{\cdot}05$). Hypothyroid group also exhibited diminished TRAP (by ~ 37%) as compared with its respective control ($P < 0{\cdot}05$) (Table 1).

Oxidative damage and antioxidant enzyme activities in red blood cells

Hyperthyroid red blood cells demonstrated increased lipid peroxidation levels (evaluated by CL) by 65% as compared with the control group (Table 2) ($P < 0{\cdot}05$). Erythrocytes CL demonstrated to be reduced by 31% in hypothyroid as compared with the sham group (Table 2) ($P < 0{\cdot}05$). CL levels were minor in hypothyroid (by ~ 60%) as compared with hyperthyroid group (Table 2) ($P < 0{\cdot}05$).

Antioxidant enzyme activities were evaluated in both hyperthyroid and hypothyroid groups in red blood cells. SOD and CAT activities, enzymes that detoxify superoxide anion and hydrogen peroxide, respectively, were not significantly different among experimental groups (Table 2). In red blood cells of hyperthyroid and hypothyroid group, GST and GPx activities were significantly increased (by ~25% and ~30%, respectively) as compared with its respective control groups (Table 2) ($P < 0{\cdot}05$).

Antioxidant enzymes immunocontent

Protein levels of antioxidant enzymes were analysed by Western blot technique. In red blood cells, SOD protein expression decreased by 40%, in hypothyroid as compared with the sham group ($P < 0{\cdot}05$) (Figure 1). However, SOD immunocontent did not change in hyperthyroid group (Figure 1). CAT immunocontent did not change (Figure 2). Protein expression of GST was increased in hyperthyroid and hypothyroid (by ~40% and ~30%, respectively) as compared with the control group ($P < 0{\cdot}05$) (Figure 3).

Table 1. Oxidative damage and non-enzymatic antioxidant defences in plasma, after 4-week treatment with thyroxine and/or thyroidectomy

	Control	Hyperthyroid	Sham	Hypothyroid
Carbonyl assay ($pmol\,mg^{-1}prot$)	225 ± 22	347 ± 48*	245 ± 15	173 ± 23**
Total antioxidant capacity ($mmol\,l^{-1}$)	3000 ± 130	1913 ± 108*	2985 ± 359	1890 ± 200*

Data have been shown as mean ± standard deviation from six animals in each group.
*Significantly different from the control and sham groups ($P < 0{\cdot}05$).
**Significantly different from the control, sham and hyperthyroid groups ($P < 0{\cdot}05$).

Copyright © 2011 John Wiley & Sons, Ltd.

Table 2. Oxidative damage and antioxidant enzyme activities in red blood cells, after 4-week treatment with thyroxine and/or thyroidectomy

	Control	Hyperthyroid	Sham	Hypothyroid
Lipid peroxidation (cps mg prot 10^5)	163 ± 25	267 ± 28*	161 ± 19	147 ± 21**
Superoxide dismutase (U mg^{-1} prot)	5·1 ± 1·1	4·8 ± 0·8	4·9 ± 1·5	4·1 ± 0·9
Catalase (pmol mg^{-1} prot)	42106 ± 2795	50602 ± 616	40302 ± 529	34209 ± 1956
Glutathione -S- transferase (nmol mg^{-1} prot)	33·0 ± 5·1	51·1 ± 2·7*	37·0 ± 3·9	53·5 ± 6·1*
Glutathione peroxidase (nmol mg^{-1} prot)	31·1 ± 7·3	54·0 ± 5·2*	41·2 ± 6·4	60·3 ± 7·6*

Data have been shown as mean ± standard deviation from six animals in each group.
*Significantly different from the control and sham groups ($P < 0·05$).
**Significantly different from the control, sham and hyperthyroid groups ($P < 0·05$).

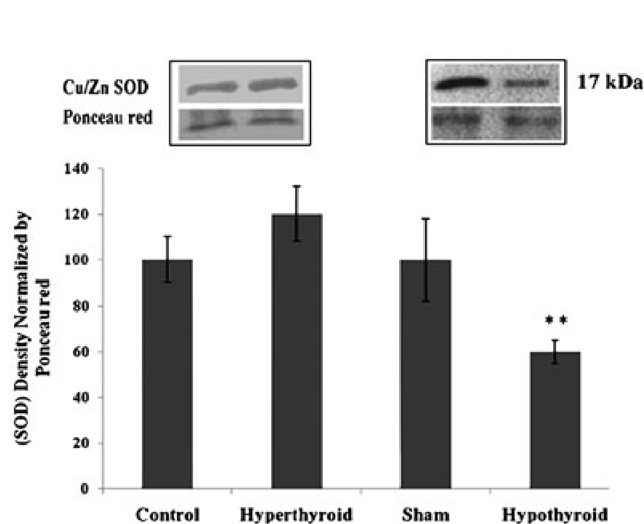

Figure 1. Superoxide dismutase (SOD) antioxidant enzyme immunocontent (one representative gel of five Western blot experiments, showing two bands for each experimental group) in erythrocytes. Data have been shown as mean ± standard deviation from six animals in each group.**Significantly different from the control and sham groups ($P < 0·05$)

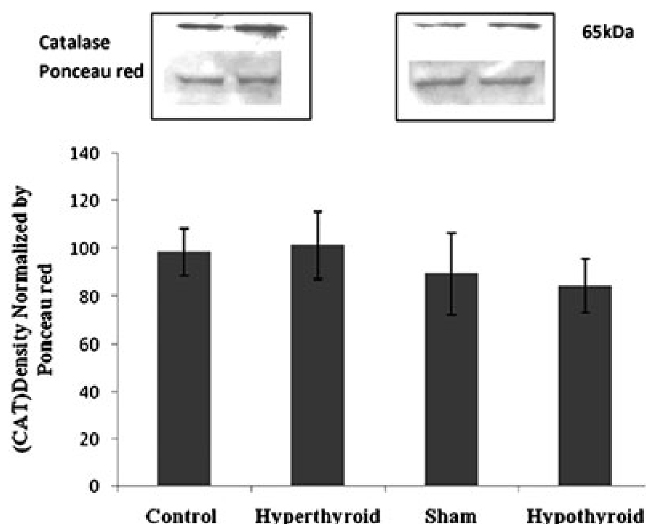

Figure 2. Catalase (CAT) antioxidant enzyme immunocontent (one representative gel of five Western blot experiments, showing two bands for each experimental group) in erythrocytes. Data have been shown as mean ± standard deviation from six animals in each group.

DISCUSSION

The major finding of this study was to demonstrate that erythrocytary GST and GPx arise as efficient biomarkers of the antioxidant defences reduction and/or oxidative injury induced by thyroid hormones variation. Moreover, the increased activity of these enzymes may contribute to the diminished oxidative damage in hypothyroid group. In parallel, in hyperthyroid, besides GPx and GST have been also increased (in response to the higher haemoglobin and oxidative metabolism levels), the antioxidant defences were overwhelmed, and the oxidative damage was installed.

Thyroidectomy was performed as an effective experimental model to induce hypothyroidism,[6] because it provides a decreased thyroid hormone levels and avoids possible interference of the drugs alternatively used, such as propylthiouracil,[28] which could interfere in oxidative stress measurements because of its sulphydril moiety. In parallel, T_4-induced hyperthyroidism in drinking water was also incisive to sustain thyroid hormone levels elevated in this study.[15]

Thyroid hormone level variations play a key role in the modulation of oxidative stress in erythrocytes. In fact, our results demonstrated an increased LPO and protein oxidation when T_4 levels were elevated. On the other hand, in hypothyroid group, oxidative damage has been shown to be reduced in red blood cells, represented by diminished LPO and protein oxidation. These antagonic results could reflect the difference in metabolic state between hyperthyroid and hypothyroid groups.[2] Moreover, thyroid hormones could control iron metabolism and haemoglobin synthesis in erythrocytes.[12] In fact, our study demonstrated increased levels of haemoglobin associated to hyperthyroidism. It has been known that ROS generation can involve transition metals, such as in the Fenton reaction that produces hydroxyl radical formation in the iron presence *in vitro*.[9] However, the occurrence of this reaction *in vivo* is widely debated in literature, in order that its occurrence was better

Copyright © 2011 John Wiley & Sons, Ltd.

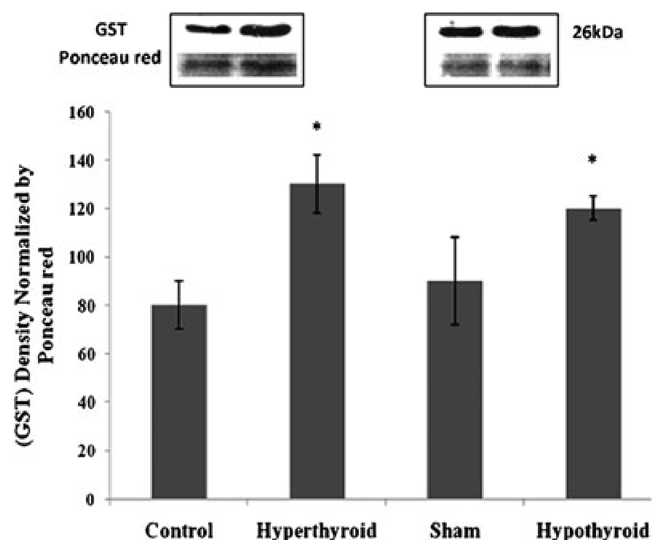

Figure 3. Glutathione S-transferase (GST) antioxidant enzyme immunocontent (one representative gel of five Western blot experiments, showing two bands for each experimental group) in erythrocytes. Data have been shown as mean ± standard deviation from six animals in each group. *Significantly different from the control, sham and hypothyroid groups ($P < 0{\cdot}05$).

demonstrated in iron overload situations.[29] Nevertheless, Fenton reaction was also reported to occur *in vivo*, through a synergism between ascorbic acid and metalloporphyrin.[30] On the other hand, although the hypothyroid erythrocytes demonstrated decreased oxidative damage, it was not evidenced that haemoglobin level changes. This may be explained by a concomitant reduction of plasmatic volume.[31] Because erythrocytes lack of mitochondria, the ROS production relative to oxidative metabolism is not meaningful. Nevertheless, thyroid hormones may influence directly the oxidative metabolism of other tissues, which would promote changes in ROS production, and thus affecting the redox environment where erythrocytes circulate. In order to characterize if oxidative stress condition is present, it is important the evaluation not only of the oxidative damage but also the response of the antioxidant systems. Thus, we evaluated some antioxidants.

Total radical antioxidant potential levels and SOD immunocontent were reduced in hypothyroid group. According to literature, it has been described an important decrease in some non-enzymatic antioxidants, such as coenzyme Q9 and vitamin C, as well as other important cofactors of the antioxidant system, such as Cu^{2+} and Zn^{+} in thyroidectomized rats, explaining the TRAP and SOD reduction observed in hypothyroid, respectively.[32,33] Plasma TRAP levels were also noticed to be reduced in hyperthyroid rats, as it was previously described in other tissues.[34] In our study, however, SOD and CAT activities were not changed in the analysed groups. This non-responsive enzymatic profile could also contribute to T_4-induced oxidative damage in hyperthyroid erythrocytes. Indeed, the hyperthyroid have higher oxygen consumption, with increased ROS formation, without a proportional increase in the enzymatic activity, except for GST and GPx, and also exhibiting less non enzymatic antioxidants. This environment is prone to produce oxidative damage, seen through the increased lipid and protein oxidation. In this scenario, GST and GPx enhancement in the hyperthyroid animals seems to be not enough to counteract oxidative challenge and damage is installed. On the other hand, in the hypothyroid, GST and GPx take part of a counter-regulatory mechanism capable to avoid oxidative damage. These enzymes are involved in organic peroxides, H_2O_2 and xenobiotics detoxification.[35]

In summary, both enzymes have shown same profile of activity, indicating that these enzymes involved with GSH metabolism are relevant blood biomarkers in the situation where there is not only decreased antioxidant defences (as hypothyroidism—where SOD and TRAP are diminished) but also increased oxidative injury (as hyperthyroidism—where lipid peroxidation and oxidized protein where elevated).

Thus, parameters analysed in erythrocytes in this study, especially GST and GPx, could be useful in the clinical screening of oxidative profile variations involved with thyroid hormone dysfunctions, as very important biomarkers.

FUNDING

This work was supported by the Secretaria de Ciência e Tecnologia do Estado do Rio Grande do Sul, CNPq, CAPES and FAPERGS, Brazilian Research Agencies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

ACKNOWLEDGEMENTS

CNPq and FAPERGS, Brazilian Research Agencies supported this study. We would like to thank the Weinmann Clinical Analysis Laboratory for performing the thyroid hormone measurements.

REFERENCES

1. Klein I, Danzi S. Thyroid disease and the heart. *Circulation* 2007; **116**: 1725–1735.
2. Venditti P, Bari A, Di Stefano L, Di Meo S. Tri-iodothyronine treatment differently affects liver metabolic response and oxidative stress in sedentary and trained rats. *J Endocrinol* 2008; **197**: 65–74.
3. Erdamar H, Demirci H, Yaman H, *et al*. The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. *Clin Chem Lab Med* 2008; **46**: 1004–1010.
4. Gravina FS, da Silveira CK, de Assis AM, *et al*. Experimental hypothyroidism inhibits delta-aminolevulinate dehydratase activity in

Copyright © 2011 John Wiley & Sons, Ltd.

neonatal rat blood and liver. *Exp Biol Med (Maywood)* 2007; **232**: 1021–1026.
5. Venditti P, Di Meo S. Thyroid hormone-induced oxidative stress. *Cell Mol Life Sci* 2006; **63**: 414–434.
6. Araujo ASR, Silva de Miranda MF, de Oliveira UO, *et al*. Increased resistance to hydrogen peroxide-induced cardiac contracture is associated with decreased myocardial oxidative stress in hypothyroid rats. *Cell Biochem Funct* 2010; **28**: 38–44.
7. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; **39**: 44–84.
8. Halliwell B. Lipid peroxidation, antioxidants and cardiovascular disease: how should we move forward? *Cardiovasc Res* 2000; **47**: 410–418.
9. Halliwell B. Biochemistry of oxidative stress. *Biochem Soc Trans* 2007; **35**: 1147–1150.
10. Jacob MH, da R Janner D, Jahn MP, Kucharski LC, Bello-Klein A, Ribeiro MF. Age-related effects of DHEA on peripheral markers of oxidative stress. *Cell Biochem Funct* 2010; **28**: 52–57.
11. Aliciguzel Y, Ozdem SN, Ozdem SS, *et al*. Erythrocyte, plasma, and serum antioxidant activities in untreated toxic multinodular goiter patients. *Free Radic Biol Med* 2001; **30**: 665–670.
12. Cinemre H, Bilir C, Gokosmanoglu F, Bahcebasi T. Hematologic effects of levothyroxine in iron-deficient subclinical hypothyroid patients: a randomized, double-blind, controlled study. *J Clin Endocrinol Metab* 2009; **94**: 151–156.
13. Repetto M, Reides C, Gomez Carretero ML, Costa M, Griemberg G, Llesuy S. Oxidative stress in blood of HIV infected patients. *Clin Chim Acta* 1996; **255**: 107–117.
14. Videla LA, Sir T, Wolff C. Increased lipid peroxidation in hyperthyroid patients: suppression by propylthiouracil treatment. *Free Radic Res Commun* 1988; **5**: 1–10.
15. Araujo AS, Ribeiro MF, Enzveiler A, *et al*. Myocardial antioxidant enzyme activities and concentration and glutathione metabolism in experimental hyperthyroidism. *Mol Cell Endocrinol* 2006; **249**: 133–139.
16. Drabkin DL. The standardization of hemoglobin measurement. *Am J Med Sci* 1949; **217**: 710.
17. Gonzalez-Flecha B, Llesuy S, Boveris A. Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle. *Free Radic Biol Med* 1991; **10**: 93–100.
18. Llesuy SF, Milei J, Gonzalez Flecha BS, Boveris A. Myocardial damage induced by doxorubicins: hydroperoxide-initiated chemiluminescence and morphology. *Free Radic Biol Med* 1990; **8**: 259–264.
19. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. In Methods Enzymol, Packer L (ed.). Serie 233; Academic Press: San Diego, 1994; 357–363.
20. Marklund SL. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. *Biochem J* 1984; **222**: 649–655.
21. Aebi H. Catalase in vitro. In: Methods Enzymol, Colowick SP, Kaplan NO (eds.). Serie 105; Academic Press: San Diego, 1984; 121–126.
22. Mannervik B, Guthenberg C. Glutathione transferase (human placenta). In: Methods Enzymol, Jacoby W (ed.). Serie 77; Academic press: New York, 1981; 231–235.
23. Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 1984; **105**: 114–121.
24. Lissi E, Pascual C, Del Castillo MD. Luminol luminescence induced by 2,2'-Azo-bis(2-amidinopropane) thermolysis. *Free Radic Res Commun* 1992; **17**: 299–311.
25. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680–685.
26. Klein D, Kern RM, Sokol RZ. A method for quantification and correction of proteins after transfer to immobilization membranes. *Biochem Mol Biol Int* 1995; **36**: 59–66.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
28. Sewerynek E, Swiercznska-Machura D, Lewinski A. Effect of propylthiouracil on the level of Schiff's bases in tissues of rats on diet with different doses of potassium iodide. *Neuro Endocrinol Lett* 2006; **27**: 595–599.
29. Aruoma OI, Bomford A, Polson RJ, Halliwell B. Nontransferrin-bound iron in plasma from hemochromatosis patients: effect of phlebotomy therapy. *Blood* 1988; **72**: 1416–1419.
30. Tian J, Peehl DM, Knox SJ. Metalloporphyrin synergizes with ascorbic acid to inhibit cancer cell growth through fenton chemistry. *Cancer Biother Radiopharm* 2010; **25**: 439–448.
31. Das KC, Mukherjee M, Sarkar TK, Dash RJ, Rastogi GK. Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. *J Clin Endocrinol Metab* 1975; **40**: 211–220.
32. Alturfan AA, Zengin E, Dariyerli N, *et al*. Investigation of zinc and copper levels in methimazole-induced hypothyroidism: relation with the oxidant-antioxidant status. *Folia Biol (Praha)* 2007; **53**: 183–188.
33. Das K, Chainy GB. Thyroid hormone influences antioxidant defense system in adult rat brain. *Neurochem Res* 2004; **29**: 1755–1766.
34. Araujo AS, Schenkel P, Enzveiler AT, *et al*. The role of redox signaling in cardiac hypertrophy induced by experimental hyperthyroidism. *J Mol Endocrinol* 2008; **41**: 423–430.
35. Martin NJ, Collier AC, Bowen LD, *et al*. Polymorphisms in the NQO1, GSTT and GSTM genes are associated with coronary heart disease and biomarkers of oxidative stress. *Mutat Res* 2009; **674**: 93–100.

Copyright © 2011 John Wiley & Sons, Ltd.