Free Rad. Res., Vol. 35, pp. 417–425
Reprints available directly from the publisher
Photocopying permitted by license only

© 2001 OPA (Overseas Publishers Association) N.V.
Published by license under
the Harwood Academic Publishers imprint,
part of Gordon and Breach Publishing,
a member of the Taylor & Francis Group.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

Thyroid Hormone-induced Oxidative Damage on Lipids, Glutathione and DNA in the Mouse Heart

RICARDO GREDILLA, GUSTAVO BARJA and MÓNICA LÓPEZ-TORRES*

Department of Animal Biology II (Animal Physiology), Faculty of Biology, Complutense University, Madrid 28040, Spain

Accepted by Prof. H.E. Poulsen

(Received 14 July 2000; In revised form 4 December 2000)

Oxygen radicals of mitochondrial origin are involved in oxidative damage. In order to analyze the possible relationship between metabolic rate, oxidative stress and oxidative damage, OF1 female mice were rendered hyper- and hypothyroid by chronic administration of 0.0012% L-thyroxine (T_4) and 0.05% 6-*n*-propyl-2-thiouracil (PTU), respectively, in their drinking water for 5 weeks.

Hyperthyroidism significantly increased the sensitivity to lipid peroxidation in the heart, although the endogenous levels of lipid peroxidation were not altered. Thyroid hormone-induced oxidative stress also resulted in higher levels of GSSG and GSSG/GSH ratio. Oxidative damage to mitochondrial DNA was greater than that to genomic DNA. Hyperthyroidism decreased oxidative damage to genomic DNA. Hypothyroidism did not modify oxidative damage in the lipid fraction but significantly decreased GSSG and GSSG/GSH ratio and oxidative damage to mitochondrial DNA.

These results indicate that thyroid hormones modulate oxidative damage to lipids and DNA, and cellular redox potential in the mouse heart. A higher oxidative stress in the hyperthyroid group is presumably neutralized in the case of nuclear DNA by an increase in repair activity, thus protecting this key molecule. Treatment with PTU, a thyroid hormone inhibitor, reduced oxidative damage in the different cell compartments.

Keywords: Thyroid status, heart, mouse, DNA damage, oxidative stress, oxygen free radicals, oxygen consumption, 8-oxodG, glutathione, lipid peroxidation

Abbreviations: dG, deoxyguanosine; 8-oxodG, 8-oxo-7,8-dihidro-2′deoxyguanosine; GSH, reduced glutathione; GSSG, oxidized glutathione; PTU, 6-*n*-propyl-2-thiouracil; T_4, L-thyroxine; TBARS, thiobarbituric acid reactive substances

INTRODUCTION

Mitochondria are considered the main source of reactive oxygen species in healthy tissues. These species oxidatively damage biological macromolecules including proteins, lipids and DNA and contribute to the development of pathologies and possibly aging.[1] Among those molecular targets, oxidative damage to DNA seems specially important, since repair of all

*Corresponding author. Tel.: 34-91-3944986. Fax: 34-91-3944935.

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

other biologically relevant macromolecules is finally dependent on DNA-encoded information. The heart continuously generates oxygen radicals *in vivo*, due to its high density of active mitochondria which supply ATP for cardiac contraction. Moreover, the heart, like muscle tissues in general, has particularly low levels of antioxidants, and its postmitotic character makes more difficult the repair of tissue damage. If the mitochondrial rate of oxygen radical production were directly related to the rate of mitochondrial oxygen consumption, thyroid hormones could be used as physiological modulators of *in vivo* cellular oxidative stress. An alteration in the thyroid state considerably affects the respiratory activities of rodent heart mitochondria, hyperthyroidism increasing[2,3] and hypothyroidism decreasing such activities.[2,4]

Besides, the literature on free radical metabolism is extensive but there is little information about the endocrine control of tissue oxidative stress. Concerning thyroid hormones, studies about oxidative damage almost exclusively deal with lipid peroxidation, antioxidant enzymes or glutathione.[5–7] DNA damage resulting from continuous exposure to oxygen radicals includes strand breaks, DNA-protein crosslinks, and modified bases. A modification of the rate of free radical attack can change the levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a mutagenic and well known marker of oxidative DNA damage.[8] In the present study we have measured 8-oxodG levels in heart genomic and mitochondrial DNA of hyperthyroid and hypothyroid mice. No previous information about the effect of thyroid hormones on oxidative damage to DNA has been published. In order to study the possible relationship between metabolic rate and oxidative stress, we have also estimated oxidative damage in the soluble and lipid fraction by quantifying GSSG and GSSG/GSH ratio and *in vivo* and *in vitro* lipid peroxidation after chronic manipulation of tissue oxygen consumption by treatment with thyroid hormones and their inhibitors. The oxygen consumption of the whole animal and heart cytochrome c oxidase activity were also analyzed as indicators of basal and maximum aerobic capacity in order to ascertain the effectiveness of the hormonal treatments. Rectal temperatures and serum hormone levels were also quantified with the same purpose.

MATERIALS AND METHODS

Chemicals

All reagents used were purchased from Sigma Chemicals (Sigma, St. Louis, MO).

Animals and Treatments

Twelve-week-old female OF1 mice were obtained from Iffa-Creddo (Lyon, France). They were maintained at $22 \pm 2\,°C$, 12:12 (light:dark) cycle, $50 \pm 10\%$ relative humidity and fed *ad libitum*. Hypothyroid mice were treated with 0.05% 6-*n*-propyl-2-thiouracil (PTU) in their drinking water for 5 weeks. Hyperthyroid mice were treated with 0.0012% T_4 in their drinking water for 5 weeks also. Rectal temperature was monitored throughout the experimental period.

After treatment the animals were sacrificed by decapitation and a blood sample was obtained. Hearts and sera were stored at $-80\,°C$ until determination. The thyroid status of all animals was confirmed by measurement of serum T_4 concentration by RIA.

Oxygen Consumption and Cytochrome c Oxidase Activity

The oxygen consumption (VO_2) of individual animals at rest was measured by closed-circuit respirometry at $22 \pm 1\,°C$ before the beginning and at the end of the treatments.[9]

Cytochrome c oxidase activity was measured as an indicator of maximum aerobic capacity in the heart tissue, following the oxidation of cytochrome c at 550 nm and 30 °C.[10]

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

Lipid Peroxidation

Endogenous *in vivo* heart lipid peroxidation was measured by a thiobarbituric acid (TBA) test specially adapted to tissue extracts.[11] The sensitivity to *in vitro* peroxidation was estimated by incubating heart homogenates with 0.4 mM ascorbate and 0.05 mM $FeSO_4$ for 6 h at 37 °C before performing the TBA assay. This incubation time ensures that the lipid peroxidation process has reached saturation in this particular sample. The TBA assay was performed in the presence of 0.07 mM butylhydroxytoluene (BHT) to prevent artefactual peroxidation during the heating step.[12] The concentration of lipid peroxidation products was estimated spectrophotometrically at 535 nm. The results were expressed as nanomoles of malondialdehyde (MDA) per g of tissue.

Glutathione System

Heart samples were homogenized in cold 5% TCA with 0.01 N HCl, and total glutathione was measured by a spectrophotometric recycling assay[13] in the presence of 5–5′-dithiobis (2-nitrobenzoic acid), NADPH and glutathione reductase at 412 nm. GSSG was assayed by the same method after derivatization of GSH with 12.5 mM *N*-ethylmaleimide (NEM) followed by alkaline hydrolysis of NEM[14] with direct control of pH with a micropHmeter. NEM was present in the homogenization buffer to avoid GSH artifactual oxidation. GSH values were obtained by subtracting GSSG from total glutathione.

Isolation of Mitochondrial and Genomic DNA

Genomic DNA was isolated, after SDS treatment of heart samples, by chloroform extraction and ethanol precipitation.[8] Mitochondrial DNA was isolated by the method of Latorre *et al.*[15] with some modifications.[16,17] The mitochondrial DNA preparations were free of nuclear DNA as tested by agarose gel electrophoresis and staining with ethidium bromide. Before digestion, the amount of mtDNA was determined in a small aliquot by measuring the fluorescence after binding of the Hoechst 33256 dye.

nDNA and mtDNA Digestion

Isolated genomic and mitochondrial DNA were digested to deoxynucleoside level by incubation at 37 °C with 5 units of nuclease P1 (in 20 μl of 20 mM sodium acetate, 10 mM $ZnCl_2$, 15% glycerol, pH 4.8) during 30 min, and 1 unit of alkaline phosphatase (in 20 μl of 1 M Tris-HCl, pH 8.0) for 1 h.[8]

8-oxodG and dG Assays in nDNA and mtDNA

The concentrations of 8-oxodG and deoxyguanosine (dG) were measured by HPLC with on line electrochemical and ultraviolet detection respectively. The nucleoside mixture was injected into a reverse-phase Beckman Ultrasphere ODS column (5 μm, 4.6 mm × 25 cm), eluted with 2.5% acetonitrile in 50 mM phosphate buffer pH 5. The amount of deoxynucleosides injected in the HPLC was higher than the minimum needed to avoid potential artifacts due to injection of small quantities of deoxynucleosides in the HPLC system.[17,18] A waters 590 pump at 1 ml/min was used. 8-oxodG was detected with an ESA Coulochem II electrochemical coulometric detector (ESA, Inc. Bedford, MA) with a 5011 analytical cell run in the oxidative mode (225 mV/10nA), and dG was detected with a Biorad 1806 UV detector at 254 nm. Peak areas of dG and 8-oxodG (Sigma, three level calibration) standards were measured during each HPLC run.

Statistical Analyses

Data were statistically analyzed with Student's *t*-tests. The 0.05 level was selected as the point of minimal statistical significance in every comparison.

RIGHTSLINK

RESULTS

Treatments

Rectal temperature was monitored throughout the experimental period; it showed a clear increase in the hyperthyroid group and a decrease in hypothyroid animals (Figure 1). These results agree with the T_4 serum concentrations analyzed by RIA (hypothyroid: 19.47 ± 1.09 ng/ml; euthyroid: 58.94 ± 3.47 ng/ml; hyperthyroid: 402.84 ± 6.00 ng/ml).

Oxygen Consumption and Cytochrome c Oxidase Activity

In order to estimate basal and maximum aerobic capacity, the oxygen consumption of the whole animal at rest and the heart cytochrome c oxidase activity were measured. Oxygen consumption greatly increased in the hyperthyroid group after 5 weeks of treatment (Table I). Heart cytochrome c oxidase activity also increased in the hyperthyroid animals (33.18 ± 6.1; $p < .05$) and did not show significant changes in the hypothyroid group (24.13 ± 2.7). The trend to decreased levels of oxygen consumption found in the hypothyroid animals did not reach statistical significance (Table I).

Lipid Peroxidation

Endogenous levels of lipid peroxidation were not changed by the hormonal and PTU treatments (hypothyroid: 127.9 ± 9.2 nmol of MDA/g tissue; euthyroid: 124.3 ± 9.6 nmol of MDA/g tissue; hyperthyroid: 125.8 ± 6.6 nmol of MDA/g tissue). The sensitivity to lipid peroxidation significantly increased in the hyperthyroid group and was not modified by the PTU treatment (Figure 2).

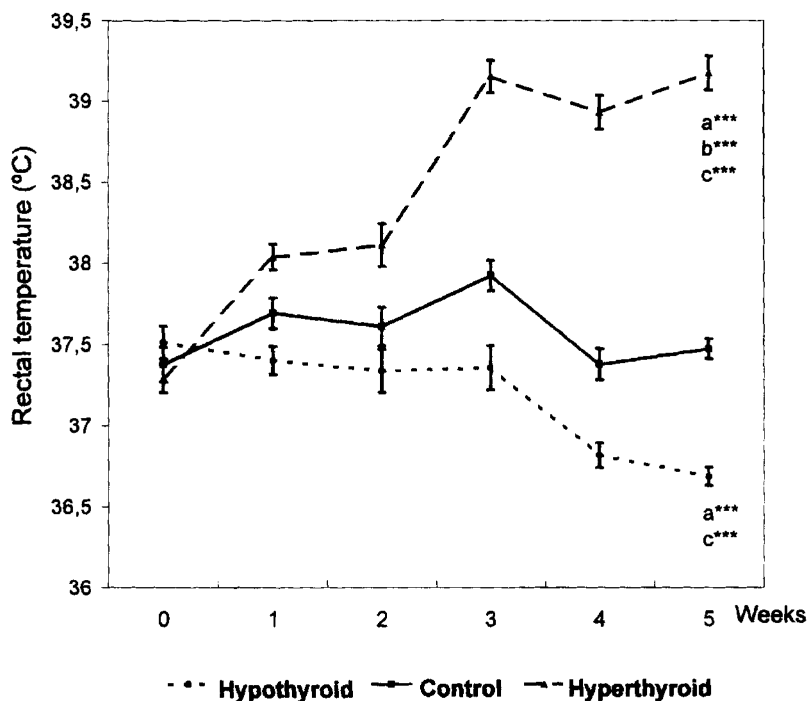


FIGURE 1 Rectal temperature throughout the experimental period. Data are means ± SEM from 15 to 17 animals. Significant difference with control group (a); between hyper- and hypothyroid groups (b); compared with the initial value within the same group (c). *** $p < .001$.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

RIGHTSLINK

TABLE I Basal metabolic rate and heart cytochrome c oxidase in mice differing in thyroid status

	Hypothyroid	Control	Hyperthyroid
Oxygen consumption (ml O_2/g.h)			
Initial	2.95 ± 0.11	3.03 ± 0.06	3.34 ± 0.21
Final	2.77 ± 0.14	3.00 ± 0.06	5.35 ± 0.11[a,b,***]
Cytochrome c oxidase activity (nmol/min/mg tissue)	24.13 ± 2.65	20.14 ± 1.53	33.18 ± 6.10[a,*]

Values are Means ± S.E.M. from 5 to 6 animals. [a] significant difference with control group. [b] significant difference between hypo and hyperthyroid groups. *$p < .05$; ***$p < .001$.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

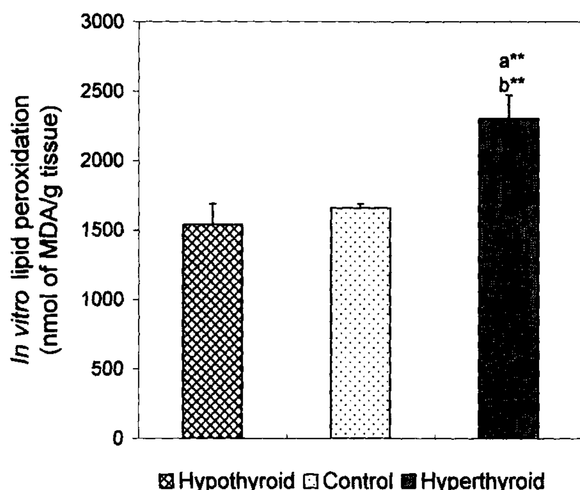


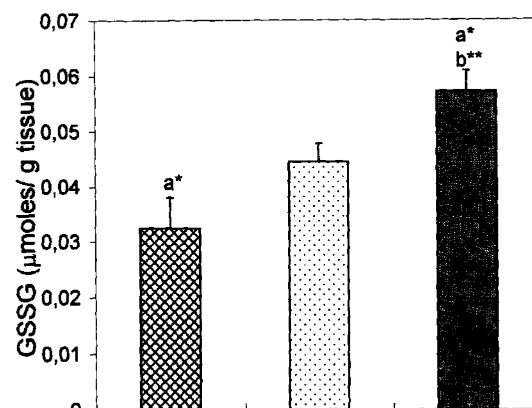
FIGURE 2 *In vitro* lipid peroxidation levels in the mice heart. Data are means ± SEM from 5 to 7 animals. Significant difference with control group (a); between hyper- and hypothyroid groups (b). ** $p < .01$.

Glutathione System

Reduced glutathione did not change as a function of the thyroid status (hypothyroid: 1.19 ± 0.12 μmol/g tissue; euthyroid: 1.09 ± 0.11 μmol/g tissue; hyperthyroid: 1.12 ± 0.14 μmol/g tissue). GSSG and GSSG/GSH ratio significantly decreased in the hypothyroid animals and GSSG significantly increased in the hyperthyroid group (Figure 3).

Mitochondrial and Genomic Oxidative DNA Damage

Hypothyroid animals showed significantly lower levels of 8-oxodG in mitochondrial DNA than hyperthyroid and control animals (Table II).



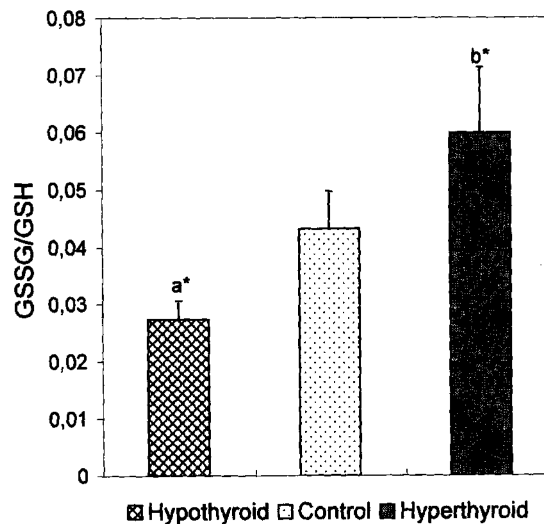


FIGURE 3 Oxidized glutathione and GSSG / GSH ratio in the mice heart. Data are means ± SEM from 5 to 7 animals. Significant difference with control group (a); between hyper- and hypothyroid groups (b). * $p < .05$, ** $p < .01$.

The 8-oxodG levels of genomic DNA were significantly lower in the hyperthyroid than in the other two groups (Table II). 8-oxodG values

RIGHTSLINK

TABLE II Oxidative damage in mitochondrial and genomic DNA in the heart of mice differing in thyroid status

	Hypothyroid	Control	Hyperthyroid
mtDNA (8-oxodG / 10^5 dG)	$5.22 \pm 0.71^{a,*}$	6.88 ± 0.51	$7.20 \pm 0.52^{b,*}$
nDNA (8-oxodG / 10^5 dG)	2.69 ± 0.32	2.52 ± 0.35	$1.28 \pm 0.16^{a,b,**}$

Values are Means ± S.E.M. from 5 to 8 animals. [a] significant difference with control group. [b] significant difference between hypo and hyperthyroid groups. $*p < .05$; $**p < .01$.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

were significantly higher in mitochondrial than in genomic DNA in the three groups (Table II).

DISCUSSION

In this study, hyper- and hypothyroidism were achieved by T_4 and PTU administration, respectively, in the drinking water. This kind of administration minimizes the large diurnal variations in plasma hormone levels that take place when daily injections are used to maintain hyperthyroidism,[19] although it is less potent[20] and requires a longer period of treatment. The measurements of rectal temperature and hormone levels indicate that a hyper- and hypothyroid state have been obtained. As expected, hyperthyroidism resulted in a greater (almost two fold) oxygen consumption than in control animals. Hyperthyroid mice also showed increased heart cytochrome c oxidase activity than control and hypothyroid animals. The increase in oxygen consumption observed in the hyperthyroid group was even greater than that previously described[21] in one of the few studies available in mice. This larger increase is probably due to the longer period of treatment used in our case. Similar changes in cytochrome c oxidase activity have been previously observed in hyperthyroidism.[22] Both parameters, oxygen consumption and cytochrome c oxidase activity, confirm the effectiveness of the hormonal treatment to increase basal and maximum aerobic capacity in the hyperthyroid state. The PTU treatment did not modify oxidative metabolism in the mice heart, in agreement with previous reports in mice and rat liver.[2,23] These results contrast with those obtained in the rat heart,[2,24] although in this last study hypothyroidism was achieved by combining thyroidectomy with the administration of an anti-thyroid drug. Therefore, cytochrome c oxidase activity seems to be dependent on thyroid hormones in a tissue or species-specific fashion.

In the present work treatment with PTU did not modify either endogenous levels of lipid peroxidation or tissue sensitivity to lipid peroxidation processes. Very few studies have analyzed the relationship between hypothyroidism and lipid peroxidation in mice. Similar results have been described by others in various rat tissues[2,6] and by our laboratory in mice liver.[23] Treatment with T_4, in contrast, increased heart sensitivity to lipid peroxidation although the endogenous levels of lipid peroxides were not altered by the hyperthyroid state. The results of available studies about the relationship between hyperthyroidism and lipid peroxidation processes are not very homogenous. Results similar to those found here have been described in rat[2] and mice liver,[23] but increases of lipid peroxidation have been found in rat heart and muscle.[2,25,26] Thus, hyperthyroidism seems to have a different effect depending upon the type of hormonal treatment, the tissue, or the animal species studied.

On the other hand, our data about sensitivity to lipid peroxidation showed an increased susceptibility in the hyperthyroid animals. It has been found that thyroid hormones change the lipid composition of mice liver[21,23] and various rat tissues.[27,28] In fact, changes in lipid composition appear to be a well established feature of altered thyroid states.[29] These changes could

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

explain the increased sensitivity to lipid peroxidation of hyperthyroid animals. Hyperthyroidism increases the ratio 20:4n-6/18:2n-6, probably due to an increase in δ-6 desaturase activity.[27,29,30] Sensitivity to lipid peroxidation increases as a function of the number of double bonds present in the fatty acids. Thus, an increase in fatty acid double bond number can be responsible for the increased susceptibility to lipid peroxidation observed in the hyperthyroid animals.

The decrease in GSSG and GSSG/GSH in hypothyroid animals and the increase in GSSG in the hyperthyroid ones found in this study, are consistent with a role of thyroid hormones in the determination of heart oxidative stress. Concerning GSH, different authors have observed decreases in rat liver[7,31] and heart[26] after thyroid hormonal treatment. Nevertheless, we have not found changes in the case of mice liver.[23] It seems that the effect of thyroid hormones on GSH levels, also depends upon the tissue under study.

In agreement with previous studies in rodent liver[32–35] and human brain,[36] oxidative damage in mitochondrial DNA was greater than in genomic DNA in the present investigation. The scarcity of studies on the topic and some methodological artifacts have led to question this difference;[37,38] but the present work and recent results from our laboratory show that it also takes place in other organs and species.[17,35]

The main explanation for the greater oxidative damage to mitochondrial in relation to nuclear DNA relies on its localization near the principal free radical production site, the mitochondrial respiratory chain. Besides, genomic DNA is better protected by associated proteins, and the most reactive free radical species, like $OH^{\bullet}$, can not diffuse far away and will unspecifically react with biological molecules situated near their sites of generation. A lack of efficient repair of oxidative damage in mitochondrial DNA, previously postulated as another explanation, has been discredited by recent investigations.[38–40] Analogously to what happens in genomic DNA, repair of oxidative damage is very active in mitochondrial DNA. This indicates that the higher oxidative damage observed in mitochondrial DNA, in relation to genomic DNA, must be mainly due to the presence of a higher rate of oxygen free radical attack to DNA in the mitochondria, rather than to differences in the repair of oxidative DNA damage between the two cell-compartments. The heart of hypothyroid animals showed a decrease in oxidative damage to mitochondrial DNA together with a decrease in GSSG/GSH ratio. These results correlate with the decrease in mitochondrial free radical production found by us in the hypothyroid state in the rat heart.[41] On the other hand, hyperthyroidism did not increase oxidative damage to mitochondrial DNA or GSSG/GSH ratio. The lack of increase in 8-oxodG in mitochondrial DNA in the hyperthyroid state is probably due to a very effective protection of this molecule. It is known that increases in oxidative stress induce increases in repair of DNA oxidative damage.[42]

Oxidative damage to genomic DNA also showed modifications as a function of thyroid state. In the hyperthyroid group, a lower oxidative damage in relation to control animals was observed that could be attributed to the higher rate of repair rate of DNA damaged by oxidative stress.[43,44] Thus, the decrease in oxidative damage to genomic DNA observed in the hyperthyroid group can be due to a supercompensation of oxidative stress. Similarly, rat heart nuclear DNA does not show increased oxidative damage after exercise,[45] in spite of an increased mitochondrial oxygen consumption and free radical production.

In summary, treatment with PTU, a thyroid hormone inhibitor, reduced oxidative damage in the different cell compartments in agreement with the lower free radical production previously found in the hypothyroid rat heart.[41] On the other hand, the higher oxidative stress observed in the hyperthyroid animals is presumably neutralized, in the case of DNA, by an

RIGHTSLINK

increase in repair activity which protects this key molecule.

Acknowledgements

This work was financially supported by grant n° 96/1253 from the National Research Foundation of the Spanish Ministry of Health (FISss).

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

References

[1] D. Harman (1994) Aging: prospects for further increases in the functional life span. *Age*, **17**, 119–146.
[2] K. Asayama, K. Dobashi, H. Hayashibe, Y. Megata and K. Kato (1987) Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. *Endocrinology*, **121**, 2112–2118.
[3] M. Moreno, A. Lann, A. Lombardi and F. Goglia (1997) How the thyroid controls metabolism in the rat: different roles for triiodothyronine and diiodothyronines. *Journal of Physiology*, **505**, 529–538.
[4] R.J. Stevens, M.L. Nishio and D.A. Hood (1995) Effect of hypothyroidism on the expression of cytochrome c and cytochrome c oxidase in heart and muscle during development. *Molecular and Cellular Biochemistry*, **143**, 119–127.
[5] T. Mano, R. Sinohara, Y. Sawai, N. Oda, Y. Nishida, T. Mokuno, M. Kotake, M. Hamada, R. Masunaga, A. Nakai and A. Nagasaka (1995) Effects of thyroid hormone on coenzyme Q and other free radical scavengers in rat heart muscle. *Journal of Endocrinology*, **145**, 131–136.
[6] P. Venditti, M. Balestrieri, S. Di Meo and T. De Leo (1997) Effect of thyroid state on lipid peroxidation, antioxidant defenses, and susceptibility to oxidative stress in rat tissues. *Journal of Endocrinology*, **155**, 151–157.
[7] V. Fernández, K. Simizu, S.B.M. Barros, L.A. Azzalis, R. Pimentel, V.B.C. Junqueira and L.A. Videla (1991) Effects of hyperthyroidism on rat liver glutathione metabolism: related enzymes activities, efflux, and turnover. *Endocrinology*, **129**, 85–91.
[8] S. Loft and H.E. Poulsen (1999) Markers of oxidative damage to DNA: antioxidants and molecular damage. *Methods in Enzymology*, **300**, 166–184.
[9] G. Barja de Quiroga, R. Pérez-Campo and M. López-Torres (1990) Antioxidant defenses and peroxidation in liver and brain of aged rats. *Biochemical Journal*, **272**, 247–250.
[10] L. Smith (1955) Spectrophotometric assay of cytochrome oxidase. In *Methods of biochemical analysis* (ed. D. Gick), Wiley-Interscience, New York, pp. 427–434.
[11] M. Uchiyama and M. Mihara (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analitical Biochemistry*, **86**, 271–278.
[12] H. Nohl, L. Gille, K. Schönheit and Y. Liu (1996) Conditions allowing redox-cycling ubisemiquinone in mitochondria to establish a direct redox couple with molecular oxygen. *Free Radical Biology and Medicine*, **20**, 207–213.
[13] F. Tietze (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione; applications to mammalian blood and other tissues. *Analitical Biochemistry*, **27**, 502–522.
[14] P. Sacchetta, D. Di Cola and G. Federici (1986) Alkaline hydrolysis of *N*-ethylmaleimide allows a rapid assay of glutathione disulfide in biological samples. *Analitical Biochemistry*, **154**, 205–208.
[15] A. Latorre, A. Moya and A. Ayala (1986) Evolution of mitochondrial DNA in *Drosophila Suboscura*. *Proceedings of the National Academy of Sciences of USA*, **83**, 8649–8653.
[16] J.G. Asunción, A. Millan, R. Pla, L. Bruseghini, A. Esteras, F.V. Pallardo, J. Sastrê and J. Viña (1996) Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. *FASEB Journal*, **10**, 333–338.
[17] G. Barja and A. Herrero (2000) Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB Journal*, **15**, 312–318.
[18] K.B. Beckman and B.N. Ames (1996) Detection and quantification of oxidative adducts of mitochondrial DNA. *Methods in Enzymology*, **264**, 442–453.
[19] J.M. Connors and G.A. Hedge (1980) Feedback effectiveness of periodic *vs* Constant triiodothyronine replacement. *Endocrinology*, **106**, 911–917.
[20] J. Gross and R. Pitt-Rivers (1953) 3,5,3′- Triiodothyronine. 2. Physiological activity. *Biochemical Journal*, **53**, 652–656.
[21] N. Desphande and A.J. Hulbert (1995) Dietary w6 fatty acids and the effects of hyperthyroidism in mice. *Journal of Endocrinology*, **144**, 431–439.
[22] I. Schmehl, S. Luvisetto, M. Canton, F. Gennari and G.F. Azzone (1995) Nature of respiratory stimulation in hyperthyroidism: the redox behaviour of cytochrome c. *FEBS Letters*, **375**, 206–210.
[23] A. Guerrero, R. Pamplona, M. Portero-Otín, G. Barja and M. López-Torres (1999) Effect of thyroid status on lipid composition and peroxidation in the mouse liver. *Free Radical Biology and Medicine*, **26**, 73–80.
[24] M.L. Nishio, O.I. Ornatsky and D.A. Hood (1995) Effects of hypothyroidism and aortic constriction on mitochondria during cardiac hypertrophy. *Medicine and Science in Sports and Exercise*, **27**, 1500–1508.
[25] K. Asayama, K. Dobashi, H. Hayashibe and K. Kato (1989) Vitamin E protects against thyroxine-induced acceleration of lipid peroxidation in cardiac and skeletal muscles in rats. *Journal of Nutrition Science and Vitaminology*, **35**, 407–418.
[26] P. Venditti, T. De Leo and S. Di Meo (1998) Antioxidant-sensitive shortening of ventricular action potential in hyperthyroid rats is independent of lipid peroxidation. *Molecular and Cellular Endocrinology*, **142**, 15–23.
[27] F.H. Faas and W.J. Carter (1981) Fatty-acid desaturation and microsomal lipid fatty-acid composition in experimental hyperthyroidism. *Biochemical Journal*, **193**, 845–852.
[28] D. Raederstorff, C.A. Meler, U. Moser and P. Walter (1991) Hypothyroidism and thyroxin substitution affect the n-3 fatty acid composition of rat liver mitochondria. *Lipids*, **26**, 781–787.
[29] F.L. Hoch (1988) Lipids and thyroid hormones. *Progress in Lipid Research*, **27**, 199–270.

RIGHTSLINK

[30] M.D. Brand, D. Steverding, B. Kadenbach, P.M. Stevenson and R.P. Hafner (1992) The mechanism of the increase in mitochondrial proton permeability induced by thyroid hormones. *European Journal of Biochemistry*, **206**, 775–781.
[31] K. Huh, T.-H. Kwon, J.-S. Kim and J.M. Park (1998) Role of the hepatic xanthine oxidase in thyroid dysfunction: effect of thyroid hormones in oxidative stress in rat liver. *Archives in Pharmacology Research*, **21**, 236–240.
[32] C. Richter, J.W. Park and B.N. Ames (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proceedings of the National Academy of Sciences of USA*, **85**, 6465–66467.
[33] M.H. Chung, H. Kasai, S. Nishimura and B.P. Yu (1992) Protection of DNA damage by dietary restriction. *Free Radical Biology and Medicine*, **12**, 523–525.
[34] M. Suter and C. Richter (1999) Fragmented mitochondrial DNA is the predominant carrier of oxidized DNA bases. *Biochemistry*, **38**, 459–464.
[35] A. Herrero and G. Barja (1999) 8-oxo-deoxyguanosine levels in heart and brain mitochondrial and nuclear DNA of two mammals and three birds in relation to their different rates of aging. *Aging Clinical and Experimental Research*, **11**, 294–300.
[36] P. Mecocci, U. MacGarvey, A.E. Kaufman, D. Koontz, J.M. Shoffner, D.C. Wallace and M.F. Beal (1993) Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Annuals in Neurology*, **34**, 609–616.
[37] K.B. Beckman and B.N. Ames (1998) The free radical theory of aging matures. *Physiological Reviews*, **78**, 547–581.
[38] R.M. Anson, D.L. Croteau, R.H. Stierum, C. Filburn, R. Parsell and V.A. Bohr (1998) Homogenous repair of singlet oxygen-induced DNA damage in differentially transcribed regions and strands of human mitochondrial DNA. *Nucleic Acids Research*, **26**, 662–668.
[39] V.A. Bohr and G.L. Dianov (1999) Oxidative DNA damage processing in nuclear and mitochondrial DNA. *Biochimie*, **81**, 155–160.
[40] N.C. Souza-Pinto, D.L. Croteau, E.K. Hudson, R.G. Hansford and V.A. Bohr (1999) Age-associated increase in 8-oxo-deoxyguanosine glycosylase/AP lyase activity in rat mitochondria. *Nucleic Acids Research*, **27**, 1935–1942.
[41] M. López-Torres, M. Romero and G. Barja (2000) Effect of thyroid hormones on mitochondrial oxygen free radical production and DNA oxidative damage in the rat heart. *Molecular and Cellular Endocrinology*, (in press).
[42] R. Yamaguchi, T. Hirano, S. Asami, A. Sugita and H. Kasai (1996) Increase in the 8-hydroxyguanine repair activity in the rat kidney after the administration of a renal carcinogen, ferric nitriloacetate. *Environmental Health Perspectives*, **104**, 651–653.
[43] V.I. Grishko, W.J. Driggers, S.P LeDoux and G.L. Wilson (1997) Repair of oxidative damage in nuclear DNA sequences with different transcriptional activities. *Mutation Research*, **384**, 73–80.
[44] A.Y. Antipenko and Y.N. Antipenko (1994) Thyroid hormones and regulation of cell reliability system. *Advances in Enzyme Regulation*, **34**, 173–198.
[45] S. Asami, T. Hirano, R. Yamaguchi, Y. Tsurudome, H. Itoh and H. Kasai (1998) Effects of forced and spontaneous exercise on 8-hydroxydeoxyguanosine levels in rat organs. *Biochemistry Biophysics Research Communications*, **243**, 678–682.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

RIGHTSLINK