

Hyperthyroidism is Associated with Increased Aortic Oxidative DNA Damage in a Rat Model

KONSTANTINOS G. MOULAKAKIS^{1,2}, MARIA V. POULAKOU¹, KOSMAS I. PARASKEVAS^{1,5}, ISMENE DONTAS¹, IOANNIS S. VLACHOS¹, DIMITRIOS P. SOKOLIS³, THEODOSIOS DOSIOS⁴, PANAGIOTIS E. KARAYANNACOS³ and DESPINA PERREA¹

¹Laboratory for Experimental Surgery and Surgical Research, and ⁴Department of Thoracic Surgery, Athens University Medical School, Athens;

²Department of Vascular Surgery, Red Cross General Hospital, Athens;

³Center for Experimental Surgery, Foundation of Biomedical Research, Academy of Athens, Athens, Greece

⁵Department of Clinical Biochemistry (Vascular Disease Prevention Clinic) and Academic Department of Surgery, Royal Free Hospital and Royal Free University College London, London, U.K.

Abstract. *Background:* Hyperthyroidism is associated with increased oxidative stress and oxygen free radical production. Oxygen free radicals are implicated in several signalling pathways leading to vascular pathology. The present study evaluates the extent of aortic oxidative stress in experimental hyperthyroidism. *Materials and Methods:* Chronic hyperthyroidism was induced in 20 male Wistar rats; another 20 animals served as controls. Oxidative damage to lipids and genomic DNA was assessed by measuring serum and aortic wall 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) levels (a mutagenic marker of oxidative DNA damage), as well as serum ceruloplasmin, malondialdehyde (MDA) and lipids. *Results:* Hyperthyroid animals had significantly higher values of serum ceruloplasmin (11.27 ± 1.16 vs. 9.58 ± 1.17 mg/dl), MDA (5.34 ± 1.32 vs. 0.64 ± 0.53 nmol/ml) and 8-oxo-dG (33.91 ± 9.63 vs. 17.56 ± 4.44 ng/ml) compared with controls ($p < 0.001$ for all associations). Aortic 8-oxo-dG levels were elevated in the thyrotoxic compared with the control group (13.01 ± 2.38 vs. 4.09 ± 1.27 ng/ml, respectively; $p < 0.001$). 8-Oxo-dG measurements in aortic rings and in serum were positively correlated in the hyperthyroid rats (Pearson's correlation coefficient = 0.66; $p = 0.007$). *Conclusion:* Hyperthyroidism is associated with increased oxidative stress in the aortic wall. The animal model we describe has provided some preliminary data regarding the effect of hyperthyroidism on the vascular system. Verification of our results and further

exploration of our animal model may help determine the association between oxidative DNA damage with functional changes of the vascular wall, such as endothelial function and vascular nitric oxide signalling.

Thyroid hormones have both direct and indirect effects on the heart and the vascular system. Hyperthyroidism induces a hyperdynamic cardiovascular state characterized by high cardiac output with low systemic vascular resistance, a faster heart rate, enhanced left ventricular systolic and diastolic function, and increased prevalence of supraventricular tachyarrhythmias (1, 2). The basis for these effects is the ability of thyroid hormones to act not only on cardiac myocytes, but also on vascular smooth muscle cells and endothelium (1, 2).

Studies on animal models have documented that thyroid hormones exert considerable effects on cellular respiratory activities and that hyperthyroidism increases such activities (3-5). Oxidative stress plays a crucial role in several aspects of vascular biology. Oxygen free radicals are implicated in various signaling pathways leading to vascular pathology (6, 7). Oxidative stress is associated with increased vascular endothelial permeability; in addition it promotes leukocyte adhesion. These effects are coupled with alterations in endothelial signal transduction and redox-regulated transcription factors (8).

In the present study, chronic hyperthyroidism was induced in an animal model. The aim of this study was to evaluate the oxidant status of the aorta in experimental thyrotoxicosis.

Materials and Methods

Animal preparation and induction of hyperthyroidism. The experiments were carried out on 40 male Wistar rats weighing 250-300 g each. Rats were housed individually in the Laboratory for Experimental Surgery and Surgical Research, Athens University Medical School, in a controlled environment at $20 \pm 2^\circ\text{C}$, in cages

Correspondence to: Despina N. Perrea, Director, "N. S. Christeas" Laboratory for Experimental Surgery and Surgical Research, Athens University Medical School, 15 Agiou Thoma Str, Athens 115 27, Greece. Tel: +30 210 7462501, Fax: +30 210 7462539, e-mail: dperrea@med.uoa.gr

Key Words: Oxidative stress, hyperthyroidism, aorta, ceruloplasmin.

with European standards (Tecniplast), 55% relative humidity, central ventilation (15 air changes/h) and an artificial 12-h light-dark cycle. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" (9). The experimental protocol was approved by the Institutional Animal Care and Use Committee of Athens University Medical School and the Veterinary Directorate of the Athens Prefecture (permit number K/53/31.01.03). After one week of adaptation to the new environment, the animals were randomly divided into two experimental groups each consisting of 20 animals (hyperthyroid (Group A) and age-matched controls (Group B)). Access to food was unrestricted for both groups.

Severe hyperthyroidism was induced in the animals forming the hyperthyroid group by administration of 0.0036% L-thyroxine (T4) (L-thyroxine sodium salt pentahydrate; Sigma Chemicals, St. Louis MO, USA; Cat. Nr. T-2501) in their drinking water for 8 weeks. This solution was prepared daily. The daily consumption of water was measured for each animal during the study period. The T4 dose was determined according to the literature (8). This route of administration requires a long period of treatment but minimizes the large diurnal variations in plasma hormone levels that occur when daily injections are used to maintain hyperthyroidism. It also avoids puncture-related stress (10). The T4 dose used was able to induce hyperthyroidism, as confirmed by serum hormonal tests, as well as by clinical and necropsy findings.

Clinical examination of the animals of both groups during the entire study period included twice weekly measurement of body weight (Navigator Balance, Ohaus Corporation, USA), rectal temperature and evaluation of specific signs, namely protracted diarrhea and hyperkinesis. Necropsy findings included the animal's body weight, left ventricular weight and estimation of left ventricular weight/body weight ratio.

Animal sacrifice and tissue preparation. All animals of both groups were sacrificed 8 weeks after the initiation of the study. Each animal was sedated with a combination of an intramuscular injection of ketamine (90 mg/kg) and xylazine (5 mg/kg). After weighing the animal, the abdominal wall was opened through a midline incision, the inferior vena cava was dissected and blood was withdrawn with a heparinized syringe. Blood samples were centrifuged at 3000 xg for 15 min. The serum aliquots were stored at -80°C until analyzed. Following the cessation of respiration and heart function, the thoracic cavity was opened through a median sternotomy. The heart was excised, washed of blood and weighed. The descending aorta from the left subclavian artery to the diaphragm, with the surrounding loose periaortic tissue, was excised with extreme care to avoid damage to the aortic wall. A complete 2-3 mm-long aortic ring at the level of the second intercostal artery was carefully excised. For genomic DNA isolation and determinations, the aortic ring was quick-frozen in liquid nitrogen and kept at -80°C until analysed. Animals in the control group were treated in the same manner.

Thyroid status. The diagnosis of thyroid function abnormality was determined on the basis of a full clinical examination and serum hormonal tests. Serum total triiodothyronine (tT3), free triiodothyronine (fT3) and total thyroxine (tT4) were measured every 14 days during the 8 weeks of treatment until the day of sacrifice. Serum tT3 and fT3 concentrations were measured by the

microparticle enzyme immunoassay (Abbott autoanalyzer IMX System; Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL 60064, USA). Serum tT4 concentrations were determined by a fluorescence polarization immunoassay (Abbott autoanalyzer IMX System).

Blood samples were collected at the same time (9:00 p.m.) for every measurement.

Serum lipid measurement. High-density lipoprotein (HDL)-cholesterol was isolated by precipitating chylomicrons, very low-density lipoprotein (VLDL)-cholesterol and low-density lipoprotein (LDL)-cholesterol by adding phosphotungstic acid and magnesium ions to the samples. Serum cholesterol and HDL-cholesterol were determined enzymatically by the cholesterol oxidase peroxidase-amidopyrine (CHOD-PAP) method using a commercially available kit (Biosis, Hellas). Serum triglycerides were measured by the enzymatic glycerol-3-phosphate-oxidase peroxidase-amidopyrine (GPO-PAP) method using a commercially available kit (Biosis). LDL-cholesterol was estimated by the Friedewald formula.

Lipids were measured at the end of the study following animal sacrifice.

Ceruloplasmin measurement. Serum ceruloplasmin was measured using the immunoturbidimetric assay on the Technicon RA-XT analyzer (Aptec, Belgium). Reagents, standards and controls were provided by the manufacturer. Ceruloplasmin was measured at the end of the study following animal sacrifice.

Malondialdehyde (MDA) measurement. Oxidative stress was monitored by determining MDA (end-product of lipid peroxidation) as evaluated by reaction with thiobarbituric acid (11). MDA was measured at the end of the study following animal sacrifice.

The samples were preserved in tubes with anticoagulant and antioxidative agent (butylated hydroxytoluene 0.2% in methanol) to measure 2-thiobarbituric acid (TBA) reactive substances (TBARS). The plasma was separated by low-speed centrifugation (3500 xg for 15 min). A 0.5 ml aliquot of each sample was added to a tube containing 3 ml of 0.05 N HCl and mixed. In each tube, 1 ml of 46 mmol/l 2-TBA was added. The tubes were boiled for 30 min and allowed to cool. These mixtures were then added to tubes containing 4 ml methanol/butanol solution (ratio 3:17) and mixed. After centrifugation at 2500 xg for 20 min, 1.5 ml of each supernatant was taken and the absorbance at 535 nm was measured. The TBARS were calculated as MDA equivalents, using freshly diluted MDA-bis-1,1,3,3-tetraethoxypropane as the standard. MDA was prepared by hydrolysis of 1,1,3,3-tetraethoxypropane with concentrated H_2SO_4 .

Quantitative measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG). Genomic DNA was extracted from aortic rings using the Genomic DNA Purification Kit (Macherey-Nagel, Germany). DNA was digested with nuclease P1, phosphodiesterase I and alkaline phosphatase to yield free deoxynucleosides. The amount of 8-oxo-dG (an oxidation damage marker) was measured using a competitive enzyme-linked immunosorbent assay (ELISA) (Highly Sensitive 8-OhdG ELISA Kit, Japan Institute for the Control of Aging, Fukuroi, Japan).

Serum 8-oxo-dG levels were measured using a competitive ELISA (New 8-OhdG ELISA Kit, Japan Institute for the Control of Aging, Fukuroi, Japan).

Table I. Thyroid hormones values, clinical and necropsy findings in hyperthyroid and control animals, 2 and 8 weeks after initiation of the study. Values are expressed as Mean \pm SD.

	At 2 weeks		At 8 weeks (end of study)	
	Hyperthyroid (n=20)	Control (n=20)	Hyperthyroid (n=17)	Control (n=19)
Total T4 (ng/ml)	343.5 \pm 78.4 [†]	39.31 \pm 24.1	478.2 \pm 107.2 [†]	41.1 \pm 23.2
Total T3 (ng/ml)	2.99 \pm 0.9 [†]	0.24 \pm 0.08	3.17 \pm 0.7 [†]	0.38 \pm 0.09
Free T3 (pg/ml)	15.7 \pm 6.4 [†]	0.57 \pm 0.7	20.40 \pm 4.2 [†]	0.63 \pm 0.28
Temperature range ($^{\circ}$ C)	38.2–39.1 [‡]	36.9–37.4	39.2–39.8 [‡]	37.0–37.5
Heart weight (g)	-	-	1.62 \pm 0.3 [†]	1.03 \pm 0.24
Body weight (g)	260 \pm 14 [†]	330 \pm 8	228 \pm 12 [†]	448 \pm 14
LVW/BW	-	-	2.8 \pm 0.1 [†]	1.8 \pm 0.08

LVW: Left ventricular weight; BW: body weight; [†] p <0.001, [‡] p <0.05.

Statistical analysis. Results are expressed as mean \pm SD. The SPSS 12 (SPSS Inc. Chicago, Illinois, USA) program was used for analyses, including linear and power regressions. The results of the hyperthyroid group were compared with those of controls using one-way ANOVA (applying robust tests of equality of means: Welch and Brown-Forsythe) and the non-parametric Mann-Whitney test. Correlations between quantitative normally distributed parameters were assessed with Pearson's two-way test or Spearman's rho. A p -value less than 0.05 was considered significant.

Results

Determination of the thyroid status. The thyroid status of the two groups of animals is shown in Tables I and II. Compared with the control group, animals receiving T4 became markedly thyrotoxic (p <0.001) by day 15, as manifested by serum thyroid hormone levels (Table I); these levels remained elevated until the end of the study. Three rats in the experimental group died 46, 48 and 50 days, respectively, after initiation of treatment with T4, while 1 rat in the control group died during blood sampling.

In order to ascertain the effectiveness of the experimental model, rectal temperature was measured before, on the 2nd, 5th and 8th week of the 8-week treatment period. Mean temperature increased in the hyperthyroid group while it remained unchanged in euthyroid animals. On the 5th week, rectal temperature was significantly higher in the hyperthyroid compared with the euthyroid group (39.0 \pm 0.2 vs. 37.4 \pm 0.1 $^{\circ}$ C, respectively; p <0.001). Baseline values of thyroid hormones were similar in groups. Hyperthyroid animals presented with increased heart rate, protracted diarrhea and reduced final body weight compared with the control group (228 \pm 12 vs. 448 \pm 14 g, respectively; p <0.001) (Table I). The thyrotoxic

Table II. Oxidative stress variables and plasma lipid levels in hyperthyroid and control animals at the end of the study. Values are expressed as Mean \pm SD.

Oxidative stress variables	Hyperthyroid (n=17)	Control (n=19)	p
Aortic ring 8-oxo-dG (ng/ml)	13.01 \pm 2.38	4.09 \pm 1.27	<0.001
Serum 8-oxo-dG (ng/ml)	33.91 \pm 9.63	17.56 \pm 4.44	<0.001
Serum ceruloplasmin (mg/dl)	11.27 \pm 1.16	9.58 \pm 1.17	<0.001
Plasma MDA (nmol/ml)	5.34 \pm 1.32	0.64 \pm 0.53	<0.001
Lipid profile (Serum)	Hyperthyroid (n=17)	Control (n=19)	p
Total cholesterol (mg/dl)	49.4 \pm 7.4	97.3 \pm 18.4	<0.001
Triglycerides (mg/dl)	85.0 \pm 40.0	67.3 \pm 14.6	0.319
LDL-C (mg/dl)	21.3 \pm 6.2	41.1 \pm 14.2	<0.001
HDL-C (mg/dl)	32.2 \pm 4.8	42.8 \pm 9.8	<0.001

8-oxo-dG: 8-oxo-7, 8-dihydro-2'-deoxyguanosine, MDA: Malondialdehyde, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol.

status was confirmed by autopsy findings, namely left ventricular hypertrophy (indicated by increased left ventricular weight/body weight ratio).

Lipid peroxidation and ceruloplasmin changes. The two groups differed significantly in all parameters of lipid peroxidation and in the lipid profile. Plasma MDA levels were significantly elevated in the thyrotoxic compared with the control group (5.34 \pm 1.32 vs. 0.64 \pm 0.53 nmol/ml, respectively; p <0.001). In the hyperthyroid group, total serum cholesterol (49.4 \pm 7.4 vs. 97.3 \pm 18.4 mg/dl), LDL (21.3 \pm 6.2 vs. 41.1 \pm 14.2 mg/dl) and HDL cholesterol levels (32.2 \pm 4.8 vs. 42.8 \pm 9.8 mg/dl) were significantly lower compared with normal rats (for all comparisons, p <0.001). Ceruloplasmin levels were significantly elevated in the hyperthyroid compared with the control group (11.27 \pm 1.16 vs. 9.58 \pm 1.17 mg/dl, respectively; p <0.001).

Oxidative DNA damage. Serum and aortic ring 8-oxo-dG levels were higher in the thyrotoxic compared with the control group (33.91 \pm 9.63 vs. 17.56 \pm 4.44 ng/ml and 13.01 \pm 2.38 vs. 4.09 \pm 1.27 ng/ml, respectively; p <0.001) (Figure 1). Serum and aortic ring 8-oxo-dG levels were positively correlated in the hyperthyroid animals (r =0.66, p =0.007) (Figure 2).

Discussion

Oxidative stress and reactive oxygen species are thought to be involved in the pathogenesis of cardiovascular diseases (12). All vascular cells, namely endothelial cells, smooth muscle cells and fibroblasts, generate reactive oxygen species.

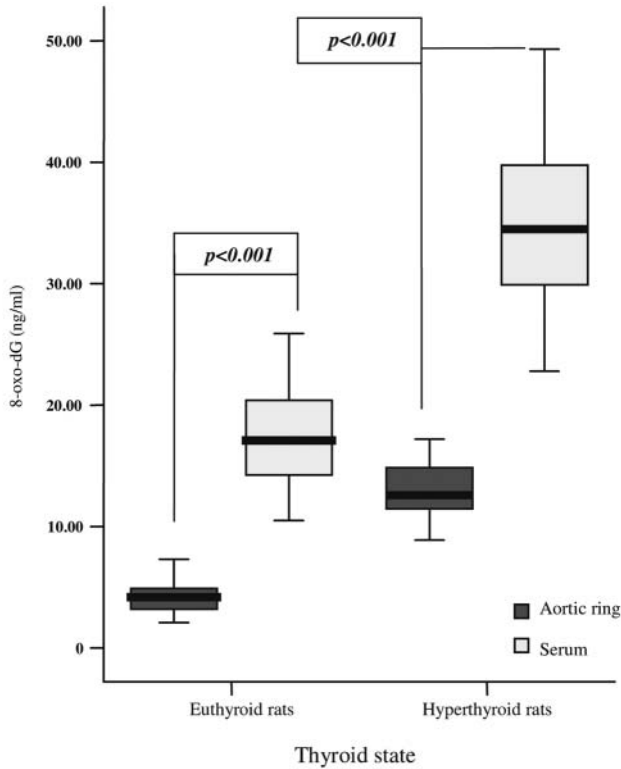


Figure 1. Box and Whisker plot of serum and aortic ring 8-oxo-dG levels for hyperthyroid and euthyroid animals.

A thyrotoxic state was achieved as confirmed by serum T3 and T4 levels, as well as by clinical and pathological findings. We showed that hyperthyroidism increases lipid peroxidation. This finding is in agreement with previous reports indicating that the hypermetabolic state in hyperthyroidism is associated with increases in free radical production and lipid peroxide levels (13, 14) This study provides evidence that hyperthyroidism is associated with oxidative stress on DNA in the vascular wall and particularly in the descending aorta. 8-Oxo-dG, one of the oxidatively modified DNA bases, is a typical marker of oxidative stress. In our study, serum and aortic ring 8-oxo-dG levels (expressing the oxidant status in genomic DNA) were significantly higher in the hyperthyroid group compared with controls. Furthermore, serum and aortic 8-oxo-dG measurements correlated positively ($r=0.56$, $p<0.05$) in the hyperthyroid animals. An issue that remains to be resolved in future trials is which of the two markers (serum or aortic 8-oxo-dG) rises first. Verification of the hypothesis that serum levels rise first and are then followed by aortic levels could provide a useful early marker of oxidative tissue damage.

Experimental studies have demonstrated that thyroid hormones modulate oxidative damage to lipids and DNA, as

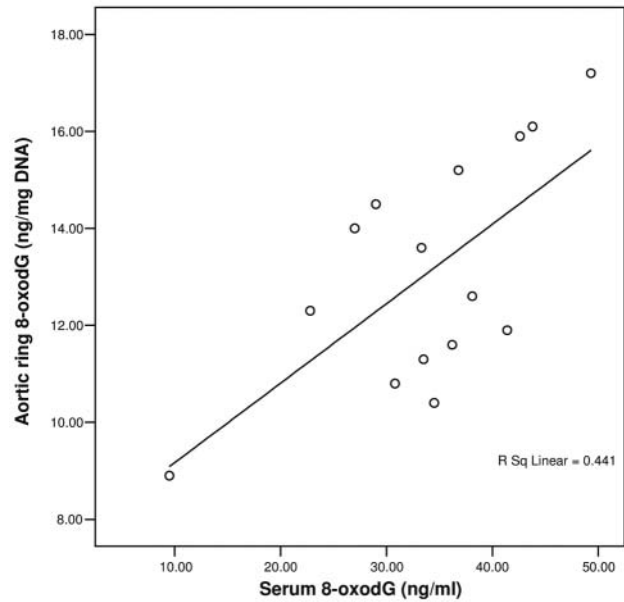


Figure 2. Correlation between serum and aortic ring 8-oxo-dG measurements in the hyperthyroid rats ($r=0.66$, $p=0.007$).

well as cellular redox potential (15). Information about the endocrine control of tissue oxidative stress concerning thyroid hormones is scarce and focuses primarily on lipid peroxidation and the glutathione system (13, 14, 16). Previous investigations indicate that DNA seems to be better protected *in vivo* from oxidative stress than other macromolecules such as lipids and proteins, probably due to its ability for self-repair and its vital function (17, 18). This relationship was not observed in our study as expressed using 8-oxo-dG, a well-known mutagenic marker of oxidative DNA damage (19, 20).

The clinical significance of the accumulation of damage in the nuclear DNA of vascular wall cells has not yet been fully determined. Observations in various target tissues have indicated a direct association of *in vivo* 8-oxo-dG formation with pathological processes, including inflammatory diseases and cancer (21-23).

Oxygen free radicals have an important role in various signalling mechanisms which lead to vascular pathology (6, 7). Several enzymes expressed in vascular tissue contribute to the production and efficient degradation of reactive oxygen species. Each of these systems has specific mechanisms controlling oxidase activity, as well as certain roles in physiological or pathophysiological regulation. Based on these observations, various studies have proposed a role for oxidative stress in the pathogenesis of vascular pathology in both animals and humans (6-8, 24, 25).

In our study, serum ceruloplasmin levels were significantly higher in the hyperthyroid animals reflecting a

greater oxidant stress. Ceruloplasmin is a copper-binding protein with well-documented antioxidant properties. A recent report revealed its capacity to promote vasculopathic effects that include lipid oxidation, negation of nitric oxide bioactivity and endothelial cell apoptosis (26). Through these effects, elevated circulating ceruloplasmin levels have been associated with an increased risk for cardiovascular disease.

Our study has several limitations. Firstly, the animal model we describe may not necessarily represent identical alterations in humans. Nevertheless, this is a useful and easily reproducible model for the study of oxidative stress developing in the vascular system. Verification of our results and further development of our model may help us understand the effect of hyperthyroidism on the vascular system, namely the association between oxidative DNA damage and functional changes of the vascular wall (*i.e.* endothelial function and vascular nitric oxide signalling). Moreover, blood glucose was not measured in this study. There is evidence that hyperglycemia is associated with increased oxidative stress (27, 28). On the other hand, hyperthyroidism is also associated with elevated blood glucose levels (29, 30). Based on literature data, this elevation is not marked (29, 30). Nevertheless, whether this rise in blood glucose participates in the oxidative stress measured in our study is not known. Further studies should investigate this issue.

Conclusion

This study provides evidence of increased oxidative stress in DNA of the descending aorta in hyperthyroidism. Hyperthyroidism was associated with increased oxidative DNA damage on the aortic wall and elevated circulating ceruloplasmin levels. The animal model we describe may prove useful in understanding the physiological alterations inflicted on the vascular system by excessive thyroid hormone production. This model also represents oxidative stress in the absence of raised LDL-cholesterol levels. This model may therefore provide an opportunity to evaluate the antioxidant effect of statins (31) without a concomitant substantial fall in LDL-cholesterol levels.

Further investigation attempting to correlate oxidative DNA damage with functional changes of the vascular tissue (such as endothelial function and vascular nitric oxide signalling) may elucidate the clinical significance of oxidative DNA damage of the aortic wall.

Acknowledgements

This work was supported by funds from the Laboratory for Experimental Surgery and Surgical Research, Athens University Medical School, Athens, Greece.

References

- 1 Klein I and Ojamaa K: Thyroid hormone and the cardiovascular system. *N Engl J Med* 344: 501-509, 2001.
- 2 Ojamaa K, Klemperer JD and Klein I: Acute effects of thyroid hormone on vascular smooth muscle. *Thyroid* 6: 505-512, 1996.
- 3 Asayama K, Dobashi K, Hayashibe H, Megata Y and Kato K: 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, 1987.
- 4 Moreno M, Lanni A, Lombardi A and Goglia F: How the thyroid controls metabolism in the rat: different roles for triiodothyronine and diiodothyronines. *J Physiol* 505: 529-538, 1997.
- 5 Gredilla R, Lopez Torres M, Portero-Otin M, Pamplona R and Barja G: Influence of hyper- and hypothyroidism on lipid peroxidation, unsaturation of phospholipids, glutathione system and oxidative damage to nuclear and mitochondrial DNA in mice skeletal muscle. *Mol Cell Biochem* 221: 41-48, 2001.
- 6 Lounsbury KM, Hu Q and Ziegelstein RC: Calcium signaling and oxidant stress in the vasculature. *Free Radic Biol Med* 28: 1362-1369, 2000.
- 7 Wassmann S, Wassmann K and Nickenig G: Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. *Hypertension* 44: 381-386, 2004.
- 8 Connors JM and Hedge GA: Feedback regulation of thyrotropin by thyroxine under physiological conditions. *Am J Physiol* 240: E308-313, 1981.
- 9 Clark JD, Gebhart GF, Gonder JC, Keeling ME and Kohn DF: Special report. The 1996 Guide for the Care and Use of Laboratory Animals. *ILAR J* 38: 41-48, 1997.
- 10 Lum H and Roebuck KA: Oxidant stress and endothelial cell dysfunction. *Am J Physiol Cell Physiol* 280: C719-741, 2001.
- 11 Buege JA and Aust SD: Microsomal lipid peroxidation. *Methods Enzymol* 52: 302-310, 1978.
- 12 Wassmann S, Wassmann K and Nickenig G: Regulation of antioxidant and oxidant enzymes in vascular cells and implications for vascular disease. *Curr Hypertens Rep* 8: 69-78, 2006.
- 13 Fernandez V, Barrientos X, Kipreos K, Valenzuela A and Videla LA: Superoxide radical generation, NADPH oxidase activity, and cytochrome P-450 content of rat liver microsomal fractions in an experimental hyperthyroid state: relation to lipid peroxidation. *Endocrinology* 117: 496-501, 1985.
- 14 Asayama K, Dobashi K, Hayashibe H and Kato K: Vitamin E protects against thyroxine-induced acceleration of lipid peroxidation in cardiac and skeletal muscles in rats. *J Nutr Sci Vitaminol (Tokyo)* 35: 407-418, 1989.
- 15 Gredilla R, Barja G and Lopez-Torres M: Thyroid hormone-induced oxidative damage on lipids, glutathione and DNA in the mouse heart. *Free Radic Res* 35: 417-425, 2001.
- 16 Fernandez V, Simizu K, Barros SB, Azzalis LA, Pimentel R, Junqueira VB and Videla LA: Effects of hyperthyroidism on rat liver glutathione metabolism: related enzymes' activities, efflux, and turnover. *Endocrinology* 129: 85-91, 1991.
- 17 Draper HH, Agarwal S, Nelson DE, Wee JJ, Ghoshal AK and Farber E: Effects of peroxidative stress and age on the concentration of a deoxyguanosine-malondialdehyde adduct in rat DNA. *Lipids* 30: 959-961, 1995.

- 18 Prieme H, Loft S, Nyssonen K, Salonen JT and Poulsen HE: No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7, 8-dihydro-2'-deoxyguanosine excretion in smokers. *Am J Clin Nutr* 65: 503-507, 1997.
- 19 Loft S and Poulsen HE: Markers of oxidative damage to DNA: antioxidants and molecular damage. *Methods Enzymol* 300: 166-184, 1999.
- 20 Aust AE and Eveleigh JF: Mechanisms of DNA oxidation. *Proc Soc Exp Biol Med* 222: 246-252, 1999.
- 21 Stevnsner T, Thorslund T, de Souza-Pinto NC and Bohr VA: Mitochondrial repair of 8-oxoguanine and changes with aging. *Exp Gerontol* 37: 1189-1196, 2002.
- 22 Loft S and Poulsen HE: Cancer risk and oxidative DNA damage in man. *J Mol Med* 74: 297-312, 1996.
- 23 Cooke MS, Evans MD, Dizdaroglu M and Lunec J: Oxidative DNA damage: Mechanisms, mutation, and disease. *FASEB J* 17: 1195-1214, 2003.
- 24 Landmesser U, Spiekermann S, Dikalov S, Tatge H, Wilke R, Kohler C, Harrison DG, Hornig B and Drexler H: Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. *Circulation* 106: 3073-3078, 2002.
- 25 Wolin MS, Gupte SA and Oeckler RA: Superoxide in the vascular system. *J Vasc Res* 39: 191-207, 2002.
- 26 Shukla N, Maher J, Masters J, Angelini GD and Jeremy JY: Does oxidative stress change ceruloplasmin from a protective to a vasculopathic factor? *Atherosclerosis* 187: 238-250, 2006.
- 27 Haidara MA, Yassin HZ, Rateb M, Ammar H and Zorkani MA: Role of oxidative stress in the development of cardiovascular complications in diabetes mellitus. *Curr Vasc Pharmacol* 4: 215-227, 2006.
- 28 Wright E Jr, Scism-Bacon JL and Glass LC: Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. *Int J Clin Pract* 60: 308-314, 2006.
- 29 Fukuchi M, Shimabukuro M, Shimajiri Y, Oshiro Y, Higa M, Akamine H, Komiya I and Takasu N: Evidence for a deficient pancreatic beta-cell response in a rat model of hyperthyroidism. *Life Sci* 71: 1059-1070, 2002.
- 30 Ikeda T, Mokuda O, Tominaga M and Mashiba H: Glucose intolerance in thyrotoxic rats: role of insulin, glucagon, and epinephrine. *Am J Physiol* 255: E843-849, 1988.
- 31 Ceriello A: Controlling oxidative stress as a novel molecular approach to protecting the vascular wall in diabetes. *Curr Opin Lipidol* 17: 510-518, 2006.

Received February 26, 2007

Revised April 26, 2007

Accepted May 18, 2007