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INCREASED LIPID PEROXIDATION IN HYPERTHYROID PATIENTS: SUPPRESSION BY PROPYLTHIOURACIL TREATMENT

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Plasma and urinary levels of thiobarbituric acid reactive substances (TBAR) were determined in 24 hyperthyroid patients, 19 hypothyroid subjects, 35 controls, and 17 hyperthyroid patients before and after propylthiouracil (PTU) treatment (400 mg/day for 2–3 months), as indexes of lipid peroxidation. These measurements were carried out together with t-butyl hydroperoxide (t-BHP)-induced oxygen uptake and visible chemiluminescence in erythrocytes as functional tests related to the antioxigenic capacity of cells. Hyperthyroid patients exhibited increased levels of plasma and urinary TBAR compared to controls. Erythrocyte suspensions from hyperthyroid patients showed, compared to controls, higher rates of oxygen consumption with shorter induction periods upon addition of t-BHP, together with 142% and 75% increases in basal and t-BHP-induced chemiluminescence, respectively. Levels of TBAR in untreated hyperthyroid patients in plasma (16.2 $\pm$ 1.3 pmol/mg of protein) and urine (15.9 $\pm$ 1.5 nmol/mg of creatinine) were decreased after PTU treatment (Plasma, 9.5 $\pm$ 0.7, $p < 10^{-4}$; urine, 7.8 $\pm$ 0.9, $P < 10^{-5}$) to values not significantly different from those of the control group (plasma, 10.3 $\pm$ 0.6; urine, 7.9 $\pm$ 0.7). Compared to control, elevated rates of oxygen uptake induced by t-BHP, basal and t-BHP-induced chemiluminescence in erythrocyte suspensions from untreated hyperthyroid patients were reverted to normal by PTU, while decreased induction period (T_0) values were enhanced. Determination of these lipid peroxidative parameters in hypothyroid patients revealed no significant changes over control values, excepted t-BHP-induced chemiluminescence in erythrocytes that was diminished.

These data indicate that hyperthyroidism is associated with a pro-oxidant condition characterized by an enhancement in circulating and urinary lipid peroxidative indexes, which is suppressed by PTU treatment. It is suggested that this condition might reflect an oxidative stress at cellular level in tissues which are target for thyroid hormone action with a calorigenic response.

KEY WORDS: Hyperthyroidism, lipid peroxidation, propylthiouracil

INTRODUCTION

Thyroid calorigenesis in vertebrates is a functional state characterized by a significant increment in the basal metabolic rate, which is related to an enhancement in the rate of oxygen consumption of various target tissues including the liver[1,2]. Thyroid hormone-induced hepatic respiration seems to be primarily linked to active cation transport via sodium-potassium pump adaptation, coupled to higher rates of mitochondrial oxidative phosphorylation[3,4]. In addition to this view, recent studies by our

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group have pointed out that the calorigenic effect of T_3 on the liver is accompanied by a respiratory component involving the univalent reduction of oxygen at the microsomal level[2]. In fact, T_3 treatment resulted in increased rates of superoxide radical (O_2^-) generation by rat liver microsomal fractions, concomitant with an enhancement in the activity of NADPH oxidase, an enzymatic activity known to be associated with O_2^- production[2]. Oxygen-related free radicals can readily initiate the process of lipid peroxidation[5], in agreement with the increases in the thiobarbituric acid reactive substances (TBAR) content and the spontaneous chemiluminescence found in the liver of T_3-treated rats[2]. Lipid peroxidation is an oxygen-dependent and highly destructive process that is able to alter a number of essential biomolecules, with the concomitant loss of biochemical functions of cells and their structural integrity[5]. From this point of view, this process has been considered as a major mechanism of liver injury caused by several xenobiotics and has been observed to be associated with various genetic and acquired diseases[5,6].

In view of these observations, the present work was undertaken in order to evaluate: a) the lipid peroxidative status of subjects with thyroid dysfunction, as an indication of oxidative stress at the cellular level related to changes in metabolic activity, and b) the influence of the antithyroid agent propylthiouracil (PTU) on the oxidative status of hyperthyroid patients. For this purpose, TBAR levels were determined in plasma and urine samples from hyperthyroid patients and were compared to those found in euthyroid subjects and in a hypothyroid group. TBAR determinations were carried out together with measurements of t-butyl hydroperoxide (t-BHP)-induced oxygen uptake and visible chemiluminescence in erythrocyte suspensions, indexes known to be related to both the lipid peroxidative rate and the antioxygenic capacity of cells[7,8]. These indexes were also measured in a hyperthyroid group before and after treatment with PTU.

MATERIALS AND METHODS

Patients

This study comprised 78 subjects (73 women and 5 men) referred to the Unit of Endocrinology of the San Juan de Dios Hospital, Santiago, between 1985 and 1986.

TABLE I

Serum T_3, T_4 and TSH levels in hypothyroid patients, hyperthyroid patients before and after propylthiouracil (PTU) treatment, and control subjects*

		T_3 (ng/dl)	T_4 (μg/dl)	TSH (μU/ml)
A.-	Control group	131.2 ± 5.4 (33)	9.0 ± 0.3 (34)	2.8 ± 0.2 (28)
	Hypothyroid patients	N.D.+	3.3 ± 0.6 (18)	25.7 ± 2.8 (19)
	Hyperthyroid patients	325.3 ± 37.6 (24)	17.0 ± 1.0 (24)	1.4 ± 0.2 (15)
B.-	Hyperthyroid patients before PTU treatment	359.0 ± 50.1 (17)	17.9 ± 1.3 (17)	N.D.+
	Hyperthyroid patients after PTU treatment	139.0 ± 19.7 (17)	7.2 ± 0.8 (17)	N.D.+

*Results are expressed as means ± SE with the number of sujects in parentheses. Hyperthyroid patients in (B) were studied before and after treatment with 400 mg of PTU/day for 2–3 months. Normal ranges in the authors' laboratory are: 65–185 ng/dl for T_3, 4.5–11.5 μg/dl for T_4, and 0.9–5.5 μU/ml for TSH.

+ N.D. not determined.

Ages ranged from 18 to 80 years with an average of 42. Subjects with cardiac failure, thyroid storm or prestorm, hemolytic anemia, immunologic alterations, previous hepatic disease or positive HBsAg, drug hypersensitivity, alcohol or drug abuse history, cigarette smoking, and any other concomitant disease, were not included. A complete clinical history, a physical examination and laboratory tests, including serum levels of T_3, T_4 and TSH were performed. Serum T_3, T_4 and TSH levels were determined with RIA kits (Amersham, Arlington Heights,IL, USA) in samples from each subject run in duplicate. Intra-assay variation was 3.2% at 3.1 μg/dl and 3.3% at 13.5 μg/dl for T_4, 3.7% at 33 ng/dl and 2.5% at 290 ng/dl for T_3, 4% at 2.0 μU/ml and 4.5% at 18.1 μU/ml for TSH. Between-assay variation was 4.0% for T_4, 4.2% for T_3, and 7.1% for TSH. The assay sensitivity limit was 2.1 μg/dl for T_4, 9.0 ng/dl for T_3, and 0.5 μU/ml for TSH. Subjects were classified as hypothyroid (n = 19), hyperthyroid (n = 24) or euthyroid (n = 35) (control group) according to clinical criteria and their respective hormones levels in serum (Table I). From the initial hyperthyroid group (n = 24), a second evaluation was carried out in 17 patients after PTU treatment (40 mg/day for 2–3 months) (Table I); the antithyroid agent was withdrawn at least 16 h before sampling (PTU half-life of 2 h)[9] An informed consent was obtained from all subjects and the investigation was approved by the Committee of Ethics, Culture and History of the Faculty of Medicine, University of Chile.

Lipid peroxidative indexes

Blood and urine samples were obtained on admission and were processed immediately. Blood samples were obtained with heparinized syringes and the cell count was determined. Plasma was obtained by centrifugation of blood aliquots at 2500 × g for 10 min at 4°C and its protein content was measured[10]. Urine was collected under standard conditions, centrifuged at 3000 × g for 10 min at 4°C and the creatinine content was determined[11]. For the measurement of TBAR in plasma and urine, 1 ml of sample was deproteinized with 30% w/v trichloroacetic acid prior to color development with 0.67% w/v thiobarbituric acid and measurement at 535 nm[12]. Results are expressed as amount of TBAR per mg of plasma or per mg of creatinine.

Blood samples were diluted 1:450 with a solution containing 140 mM NaCl and 10 mM sodium phosphate buffer pH 7.4 for the determination of t-BHP-induced oxygen uptake and chemiluminescence[7,8]. Oxygen consuption was measured polarographically[13] in 5 ml of erythrocyte suspensions at 37°C as shown in Figure 1A. The relevant parameters to be analyzed are the induction period (T_0), after addition of 0.75 mM t-BHP, and the initial rate of oxygen consumption that follows it (Figure 1A). Measurements of visible chemiluminescence in 5 ml of red blood cell suspensions were performed in a Beckman LS-315OP liquid scintillation counter in the out-of-coincidence mode at 25°C[14] as presented in Figure 1B. Readings were taken every 0.35 min for the determination of backgrounds (vials alone) or the light emitted by the cell suspensions without additions (basal chemiluminescence) (Figure 1B). After the addition of 0.75 mM t-BHP, readings were taken every 5.15 min until maximal light emission was elicited (t-BHP-induced chemiluminescence) (Figure 1B). Results obtained are expressed as cpm/10^6 cells.

All chemicals used were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Results are expressed as means ± SE. When groups were compared Student's t-test for unpaired data was used. Analysis of correlations were carried out on the preprogrammed Monroe 1930 statistic calculator (Ditton Industries, Morristown, N.J., USA).

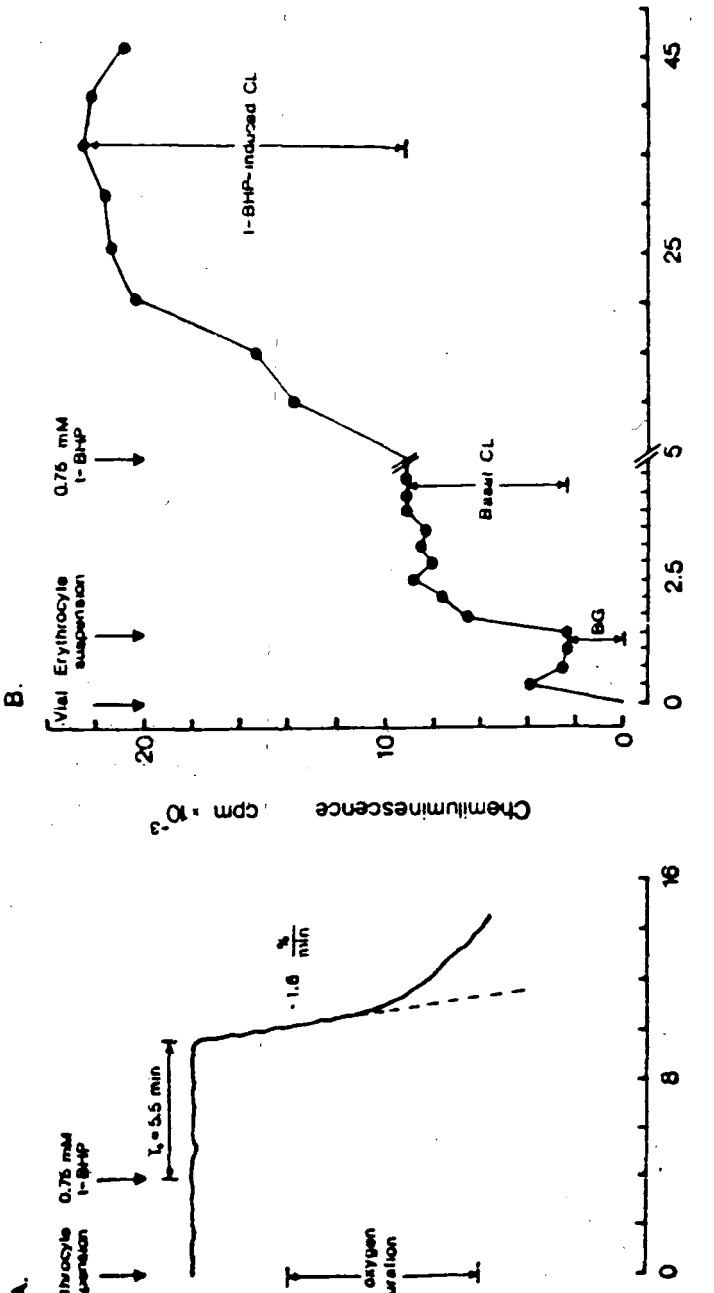

FIGURE 1 (A) Oxygen uptake after the addition of 0.75 mM t-butyl hydroperoxide (t-BHP) to an erythrocyte suspension (1:450) of a normal volunteer. T_0 corresponds to the induction peroid and the number adjacent to the trace indicates the initial decrease in oxygen tension per min (20.5 nmol/10^6 cells/h; amount of cells used: 10.6 × 10^6 cells/ml of suspension). (B) Basal and t-BHP (0.75 mM)-induced chemiluminescence (CL) in an erythrocyte suspension (1:450). Experimental conditions were as described in the Materials and Methods section. BG, background.

TABLE II

Plasma and urine content of thiobarbituric acid reactive substances (TBAR) and functional tests related to the antioxygenic capacity of erythrocytes, in samples from patients with thyroid dysfunction and control subjects*

Parameters		Control group (n = 35)	Hypothyroid patients (n = 19)	Hyperthyroid patients (n = 24)
A.-	TBAR			
	Plasma (pmol/mg of protein)	10.3 ± 0.5	9.4 ± 0.8	15.3 ± 1.2[b]
	Urine (nmol/mg of creatinine)	7.9 ± 0.7	8.3 ± 0.6	17.3 ± 2.0[a]
B.-	Functional tests			
	t-BHP-induced oxygen uptake+ (nmol/10^6 cells/h)	32.2 ± 1.5	28.7 ± 1.7	55.7 ± 2.7[a]
	Induction time (T_0) (min)	5.5 ± 0.4	5.2 ± 0.2	3.5 ± 0.2[b]
	Basal chemiluminescence (cmp/10^6 cells)	60 ± 8	48 ± 9	145 ± 10[a]
	t-BHP-induced chemiluminescence+ (cpm/10^6 cells)	597 ± 62	391 ± 44[d]	1046 ± 113[c]

*Results are expressed as means ± SE with the number of subjects in each group in parentheses. Significance studies with respect to the control group: [a]$P < 10^{-5}$, [b]$P < 10^{-4}$, [c]$P < 0.001$, [d]$P < 0.05$.

+ Oxygen uptake and chemiluminescence were induced by 0.75 mM t-butyl hydroperoxide (t-BHP), as shown in Figure 1.

RESULTS AND DISCUSSION

Data presented in this work indicate that hyperthyroidism is associated with a pro-oxidant condition characterized by an elevation of lipid peroxidative indexes. In fact, hyperthyroid patients exhibited higher plasma and urinary TBAR levels than controls (Table IIA), an index considered to represent breakdown products of cellular polyunsaturated fatty acids undergoing free-radical oxidation[5]. Plasma TBAR levels were significantly correlated with those of serum T_4 in the whole group ($r = 0.50$; $P < 10^{-5}$); however, no correlation was found between these parameters in the hyperthyroid group. This would indicate that the magnitude of the elicited oxidative stress does not depend on the severity of hyperthyroidism. TBAR measurements in plasma and urine were carried out together with functional tests related to the free-radical reactions of lipid peroxidation, assayed in erythrocyte suspensions (Figure 1). When exposed to t-BHP, red blood cells consume oxygen after an induction period (T_0) (Figure 1A) and emit visible light (Figure 1B), processes set in by free radicals arising from the scission of the hydroperoxide by hemoglobin[7,8]. In this system, T_0 is related to the intracellular protective antioxidant mechanisms[8,15] while oxygen uptake is attributed to lipid peroxidation because of its suppression by free-radical scavengers[8] and the anaerobic nature of the erythrocyte metabolism. Data shown in Table IIB strongly suggest that erythrocytes from hyperthyroid patients are more susceptible to t-BHP-induced oxidative stress than those of control subjects. This view is supported by the lower T_0 values found, in addition to the enhanced rates of oxygen consumption and chemiluminescence induced by t-BHP (Table IIB), which were found to be significantly correlated ($r = 0.49$; $P < 10^{-5}$). Basal chemiluminescence exhibits a similar tendency (Table IIB). Photoemission is related to the free-radical oxidation reactions of lipid peroxidation and is due to the decay of the excited

species, formed in the process, to ground state[16]. In agreement with these findings, hyperthyroidism has been shown to be associated with a significant enhancement in red blood cell glucose-6-phosphate dehydrogenase[17] and catalase[18] activities compared to control values. These alterations can be interpreted as adaptive mechanisms against thyrotoxicosis-induced oxidative stress, condition which might result in shortened half lives of erythrocytes[19] and associated erythropoiesis stimulation[20].

The contention that the lipid peroxidative status of subjects is influenced by their thyroid gland activity is further supported in this work by the studies in hyperthyroid patients before and after PTU treatment, which elicited a reduction of serum T_3 and T_4 levels to values comparable to those in the control group and within normal ranges (Table I). In fact, PTU was found to suppress the oxidative stress observed in hyperthyroidism, as it completely reversed the increased circulating and urinary TBAR levels (Figure 2) and the lipid peroxidation-related functional tests (Figure 3) to control values, with serum levels of T_3 ($r = 0.51$; $P < 0.005$) and T_4 ($r = 0.60$;

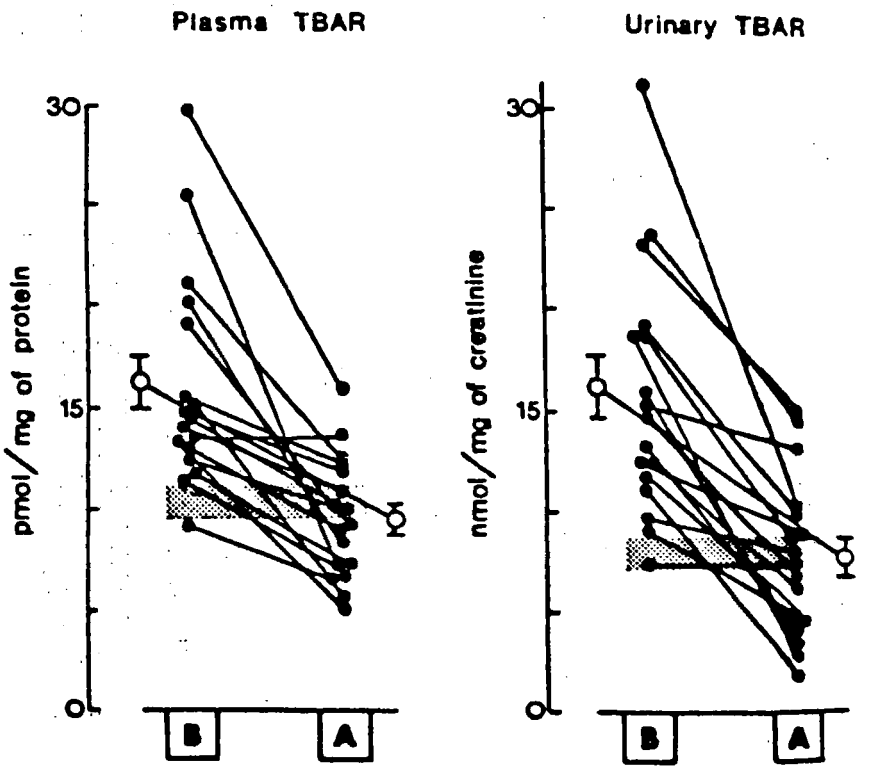

FIGURE 2 Plasma and urine content of thiobarbituric acid reactive substances (TBAR) in hyperthyroid patients before (B) and after (A) PTU treatment (400 mg/day for 2–3 months) and control subjects. Individual (●) and means ± SE (○) values in hyperthyroid patients ($n = 17$) are shown, with shaded areas representing means ± SE for control subjects ($n = 35$) (Table IIA). Significance calculated by comparing hyperthyroid patients before and after PTU treatment: $P < 10^{-4}$ (plasma TBAR), $P < 10^{-5}$ (urinary TBAR). All values in untreated hyperthyroid patients were significantly different from control values ($P < 10^{-4}$), while those in hyperthyroid patients treated with PTU and controls were comparable ($P > 0.05$).

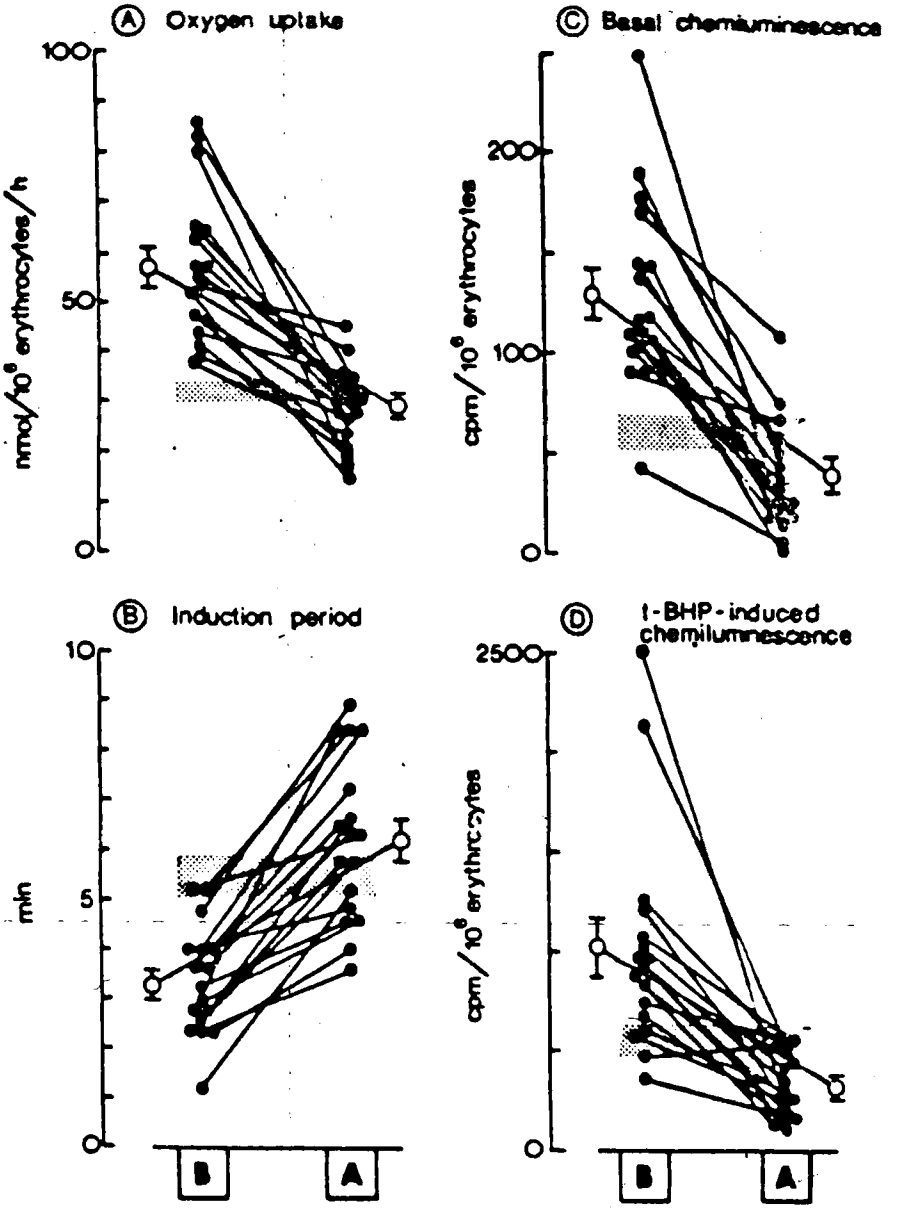

FIGURE 3 Oxygen uptake and its induction period (T_0), basal and t-butyl hydroperoxide (t-BHP)-induced chemiluminescence (CL) in erythrocyte suspensions from hyperthyroid patients before (B) and after (A) PTU treatment (400 mg/day for 2–3 months) and control subjects. Individual (●) and mean ± SE (○) values in hyperthyroid patients (n = 17) are shown, with shaded areas representing means ± SE for control subjects (n = 35) (Table IIB). Significance calculated by comparing hyperthyroid patients before and after PTU treatment: $P < 10^{-3}$ (t-BHP-CL), $P < 10^{-5}$ (oxygen uptake), $P < 10^{-6}$ (T_0 and basal-CL). All values in untreated hyperthyroid patients were significantly different from controls ($P < 10^{-3}$), while those in hyperthyroid patients treated with PTU and controls were comparable ($P > 0.05$).

$P < 0.001$) being correlated with the respective plasma TBAR levels in patients before and after PTU treatment. Moreover, the adaptive response to thyroid hormone-induced oxidative stress, represented by increased catalase activity in erythrocytes from hyperthyroid patients, diminished in parallel with serum levels of thyroid hormones during antithyroid therapy with thiamazole[18]. The suppressive effect of PTU on the lipid peroxidative status observed in hyperthyroidism could primarily be ascribed to the attainment of the euthyroid state. This can be achieved through the ability of PTU to reduce thyroid gland activity by inhibition of thyroid hormones synthesis and/or by a diminution in the peripheral deiodiantion of T_4 into T_3.[21] Thus, the oxygen demand of target tissues will decrease [1] with the consequent diminution of oxygen utilization in reactions generating active species ($O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$)[2], which initiate lipid peroxidation, or in the process itself[5,6,16]. Alternatively, PTU can directly act through two mechanisms of action. Firstly, PTU can substitute for reduced glutathione (GSH) as a substrate in glutathione-S-transferase catalyzed reactions[22]. This would lead to a GSH-sparing effect together with an elevation in the intracellular content of the tripeptide over baseline levels, as found in rat liver[23]. An enhanced availability of GSH at cellular level would imply an increased antioxidant capacity, as GSH participates in the catabolism of toxic hydroperoxides formed in lipid peroxidation[24] and can act as a direct free-radical scavenger[25]. In line with this view, a T_0 (After PTU)/T_0 (Before PTU) ratio of 2.00 ± 0.19 ($n = 17$) is obtained in erythrocyte suspensions supplemented with t-BHP (from data in Figure 3). This indicates the relative increase in the duration of the lag phase under the influence of PTU treatment and reflects a greater resistance of erythrocytes to lipid peroxidation. Secondly, PTU has recently been reported to exert a direct inhibitory effect on oxygen radical formation in human neutrophils, at therapeutic concentrations[26]. Similar findings were reported for methimazole, the active metabolite of the antithyroid drug carbimazole, assessed in resting and stimulated monocytes[27].

The assessment of the lipid peroxidative status in hypothyroid patients revealed no significant changes in the measured indexes compared to controls, with the exception of t-BHP-induced chemiluminescence in erythrocyte suspensions that was decreased ($P < 0.05$) (Table II). Since lipid peroxidation is related to oxygen metabolism of tissues[5,24], which, in turn, is known to be correlated to the basal metabolic rate (BMR) of the individual[1], the lack of significant reduction in the lipid peroxidative status in hypothroidism probably reflects an impairment of other biochemical processes which contribute to lipid peroxidation, in addition to changes in BMR.

In conclusion, human hyperthyroidism was found to be associated with an enhanced lipid peroxidative status which was suppressed by PTU treatment. This state might reflect an oxidative stress at cellular level, particularly in tissues which are target for thyroid hormone action with a calorigenic response, such as the liver[2,28]. An increased lipid peroxidation could conceivably play a role in the diminution of liver GSH levels[29] and in the development of non specific histological hepatic changes and alterations in liver laboratory tests described among hyperthyroid patients[29,30]. Furthermore, thyroid hormone-induced oxidative stress could lead to an enhanced susceptibility of the liver to exogenous or endogenous aggresion involving a free-radical mediated mechanism. This view is supported by studies showing an increased sensitivity of the liver to anoxia[31], chloroform[32] or carbon tetrachloride[33] in experimental hyperthyroidism, with PTU exerting a protective effect against acetaminophen[23,34] or experimental alcohol-induced hepatotoxicity in the euthyroid rat[35].

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