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Both Hypothyroidism and Hyperthyroidism Enhance Low Density Lipoprotein Oxidation*

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ABSTRACT

Hypothyroidism is frequently associated with hypercholesterolemia and an increased risk for atherosclerosis, whereas hyperthyroidism is known to precipitate angina or myocardial infarction in patients with underlying coronary heart disease. We have shown previously that L-T_4 functions as an antioxidant *in vitro* and inhibits low density lipoprotein (LDL) oxidation in a dose-dependent fashion. The present study was designed to evaluate the changes in LDL oxidation in subjects with hypothyroidism and hyperthyroidism. Fasting blood samples for LDL oxidation analyses, lipoprotein determinations, and thyroid function tests were collected at baseline and after the patients were rendered euthyroid. The lag phase (mean ± SEM hours) of the Cu^{+2}-catalyzed LDL oxidation in the hypothyroid state and the subsequent euthyroid states were 4 ± 0.65 and 14 ± 0.68 h, respectively ($P < 0.05$). The lag phase during the hyperthyroid phase was 6 ± 0.55 h, and that during the euthyroid phase was 12 ± 0.66 h ($P < 0.05$). The total and LDL cholesterol levels were higher in hypothyroidism than in euthyroidism and were lower in hyperthyroidism than in the euthyroid state. We conclude that LDL has more susceptibility to oxidation in both the hypothyroid and hyperthyroid states. Thus, the enhanced LDL oxidation may play a role in the cardiac disease process in both hypothyroidism and hyperthyroidism. (*J Clin Endocrinol Metab* **82:** 3421–3424, 1997)

IN THE current model of atherosclerosis, oxidative modifications of low density lipoprotein (LDL) by free radicals play a major role in initiation of atherosclerotic lesions (1, 2). Substantial evidence links the hypothyroid state with elevations of total and LDL cholesterol levels and an increased risk for atherosclerosis (3–4). The elevated LDL cholesterol levels in hypothyroidism may occur as a result of increased cholesterol synthesis and absorption (5), decreased hepatic lipase and lipoprotein lipase activities (6), and defects in the receptor-mediated catabolism of LDL (7, 8). The elevation in LDL levels, in turn, may be accompanied by increased formation of oxidized LDL, which may contribute to the enhanced risk for atherosclerosis in these individuals.

In contrast to hypothyroidism, hyperthyroidism is usually associated with low total and LDL cholesterol levels (9). The cardiac abnormalities in this condition, *i.e.* angina and congestive heart failure, are thought to be secondary to the hypermetabolic state. However, in one study in which rats were made experimentally hyperthyroid, it was shown that the hypermetabolic state led to increased production of oxygen free radicals and increased lipid peroxidation (10).

We have shown previously that *in vitro*, L-T_4 functions as an antioxidant and inhibits Cu^{+2}-mediated LDL oxidation in a concentration-dependent fashion (11, 12). We also showed that L-T_4 inhibits LDL oxidation in the presence or absence of thyroglobulin, and the latter functions as an antioxidant in serum as well (12). The present study was designed to evaluate the degree of oxidant stress in subjects with hypothyroidism or hyperthyroidism compared to that when they returned to a euthyroid state.

Subjects and Methods

Subjects

We enrolled patients with either hypothyroidism or hyperthyroidism in the study. Eligibility criteria were age between 21–70 yr, male or female, nonsmokers, and either untreated hypothyroidism [TSH, >10 mU/L; T_4, <45 nmol/L (3.5 μg/dL)] or hyperthyroidism [TSH, <0.04 mU/L; T_4, >142 nmol/L (11 μg/dL)].

Exclusion criteria were 1) any active medical condition, *e.g.* unstable angina, myocardial infarction, or stroke within 6 months; diabetes mellitus; renal or hepatic impairment; or bleeding disorder; 2) taking vitamins A, E, or C; β-carotene; or probucol over the last 3 months; 3) pregnancy, being a prisoner, or being a mentally retarded individual.

All patients gave written informed consent to participate in the study. The study was approved by the institutional review board of Ohio State University. The characteristics of the subjects in the study are shown in Table 1.

Study design

The study was conducted at the Ohio State University Medical Center using an out-patient protocol. All subjects underwent a complete history and physical exam at baseline. Blood samples for measurement of thyroid function (TSH, total T_4, free T_4 index), lipoproteins, and LDL oxidation were drawn at baseline and 8, 16, and 24 weeks; if needed, a final blood sample was drawn when the patient eventually became euthyroid.

After the baseline blood sampling, subjects with hypothyroidism were treated with replacement thyroid hormone therapy, *i.e.* L-T_4, with appropriate dose adjustments based on thyroid function tests. Subjects with hyperthyroidism also underwent treatment with a β-blocker plus

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antithyroid drugs (propylthiouracil or methimazole) and/or radioiodine. All subjects in the study acted as their own controls.

Materials

Malonaldehyde bis(dimethyacetyl) (MDA), trichloroacetic acid, and thiobarbituric acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). Universal agarose film was obtained from Baxter Diagnostics, Scientific Products Division (McGaw Park, IL). Ultracentrifuge tubes were obtained from Seton Scientific (Sunnyvale, CA). Dialysis membranes (12,000–15,000 dalton M_r cut-off) were purchased from Fisher Scientific (Pittsburgh, PA). BSA and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO). Ham's F-10 medium was purchased from Life Technologies (Grand Island, NY).

LDL isolation

Blood (30 mL) was collected in tubes containing EDTA in a final concentration of 1 mg EDTA/mL blood. Plasma was separated by centrifugation at 2000 × *g* for 20 min at room temperature. Gentamicin sulfate (1 mg/25 mL) was added to the plasma. LDL was isolated by sequential ultracentrifugation (13) and was dialyzed for 16 h against 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl and 0.01% EDTA. The protein concentration of LDL was measured by the Lowry method, as modified by Markwell *et al.* (14, 15) The purity of the isolated LDL was assessed by electrophoresis (16).

Resistance of LDL to oxidation

The isolated LDL was incubated in Ham's F-10 medium at 37 C in a humidified environment of 95% air and 5% CO_2 in the presence of 2 μmol/L Cu^{+2}. Samples (0.5 mL) containing 50 μg LDL protein were taken at 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, and 24 h; EDTA was added to a final concentration of 0.1 mmol/L, and the samples were stored at −80 C (12).

The lag phase was defined in this study as the period before an abrupt increase in the rate of oxidation of LDL, *i.e.* at least 3-fold increase in the amount of thiobarbituric acid-reactive substances (TBARS) generated. TBARS represent the amount of lipid peroxides formed as a result of LDL oxidation. The longer the lag phase, the more resistant the LDL is to oxidation. In this study, the data for TBARS concentration were normalized (*i.e.* the 0 value is represented as 1), and the figures represent the x-fold increase in TBARS over time. This calculation was undertaken to allow comparison of data obtained at different times during the study.

Measurement of TBARS

Aliquots (0.5 mL) of the incubation mixture containing 50 μg LDL protein were mixed with 20% trichloroacetic acid (1.5 mL). Thiobarbituric acid (1.5 mL of a 0.67% solution in 0.05 mol/L NaOH) was added with mixing, and the reaction mixture was incubated in a water bath at 80–90 C for 45 min. Samples were centrifuged at 2000 × *g* for 10 min, and the fluorescence of the supernatant was measured using a Hitachi F-2000 spectrofluorometer at excitation and emission wavelengths of 510 and 553 nm, respectively (17). The TBARS concentration in the samples was measured against a MDA standard curve. Results were expressed as nanomoles of MDA per mg LDL protein.

TABLE 1. Baseline profiles of subjects in the study

Baseline	Hypothyroid	Hyperthyroid
No. of subjects	7	7
M/F	1/6	1/6
Age (yr)	41.7 ± 5	42.7 ± 5.3
TSH (mU/L)	82.1 ± 16.1	<0.04
T4 [nmol/L (μg/dL)]	23.2 ± 3.9 (1.8 ± 0.3)	236.8 ± 28.3 (18.4 ± 2.2)

All values shown are expressed as the mean ± SEM.

Measurement of thyroid function tests and lipid profile

Total cholesterol, high density lipoprotein (HDL), and triglyceride concentrations were measured at the clinical chemistry laboratory at the Ohio State Medical Center using Kodak Ektachem Clinical Chemistry slides on the Kodak Ektachem 700 (Eastman Kodak, Rochester, NY), as described by the manufacturer. LDL cholesterol was calculated according to the Friedewald equation: LDL = total cholesterol − HDL − triglycerides/5 (18).

The TSH assay was a second generation assay with a sensitivity of 0.3 mU/L with an interassay coefficient of variation (CV) of 11.1% and an intraassay CV of 5–15% at the lower end of the assay; TSH was measured using a Bayer Immuno-1 instrument (Technicon, Tarrytown, NY). T_4 was also measured using Bayer Immuno-1 instrument, with a sensitivity of 5.14 nmol/L (0.4 μg/dL), an interassay CV of 2.4%, and an intraassay CV of 6.2%.

Statistical analyses

All results in the study were expressed as the mean ± SEM. ANOVA with repeated measures was used to determine any significant differences in the lipid measurements and LDL oxidation parameters between the hypothyroid and euthyroid states as well as between hyperthyroid and euthyroid states. When significance was established at $P < 0.05$, a *post-hoc* Scheffe's F test was used to compare data at different time intervals to the baseline (19).

Results

Lipoprotein profiles

Shown in Table 2 are the lipoprotein profiles in patients before and after achieving euthyroidism. Note there were significant differences in total and LDL cholesterol in the hyperthyroid and hypothyroid states. In hypothyroidism, there was a rise in total and LDL cholesterol, whereas in hyperthyroidism, there was a decrease. HDL cholesterol and triglycerides remained unchanged.

Resistance of LDL to Cu^{+2}-induced LDL oxidation

This was assessed by measuring the duration of lag phase of LDL oxidation.

In hypothyroid subjects (Fig. 1). The lag phases for Cu^{+2}-induced oxidation at baseline and on achieving the euthyroid state were 4 ± 0.65 and 14 ± 0.68 h, respectively. There was a significant increase in the lag phase for LDL oxidation with Cu^{+2}-induced oxidation in the euthyroid state compared to that in the hypothyroid state. This suggests that the LDL is more resistant to oxidation in euthyroidism than in hypothyroidism.

TABLE 2. Lipoprotein profiles in study subjects before and after treatment

	Euthyroid	Hypothyroid	Euthyroid	Hyperthyroid
Total cholesterol	4.95 ± 0.21	6.24 ± 0.64[a]	5.15 ± 0.33	4.08 ± 0.37[a]
Total triglyceride	4.53 ± 1.13	5.30 ± 2.01	3.96 ± 0.86	4.18 ± 0.91
LDL cholesterol	2.85 ± 0.12	3.94 ± 0.56[a]	2.99 ± 0.25	2.07 ± 0.26[a]
HDL cholesterol	1.19 ± 0.10	1.32 ± 0.11	1.37 ± 0.16	1.11 ± 0.12

Values are expressed as millimoles per L ± SEM.
[a] Significantly different from euthyroid subjects $P < 0.05$.

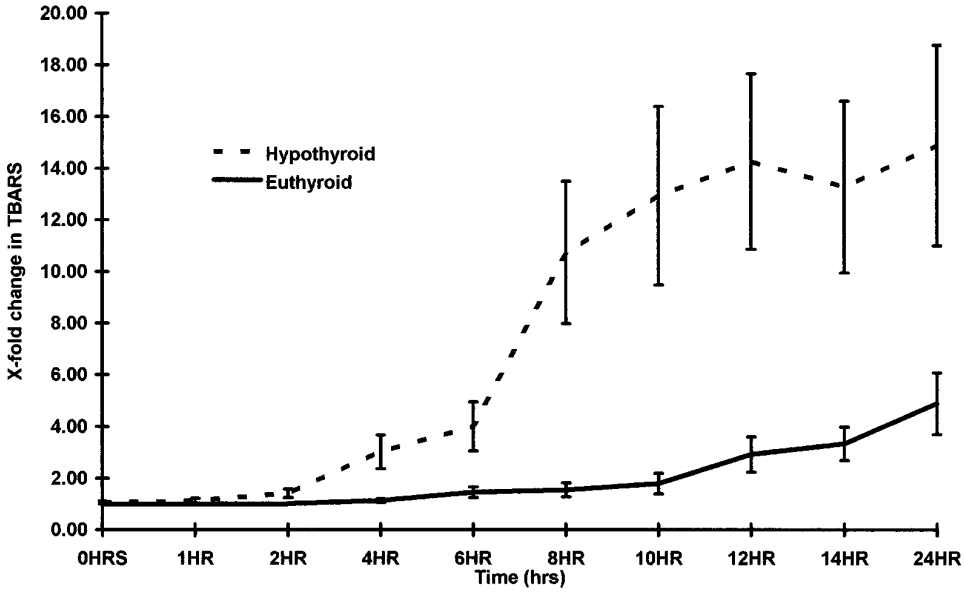

FIG. 1. Plot comparing Cu^{+2}-induced LDL oxidation at various time points during hypothyroidism and the subsequent euthyroid state. The *y*-axis represents the x-fold change (mean ± SEM) in TBARS. Note the significant increase in the lag phase during hypothyroidism.

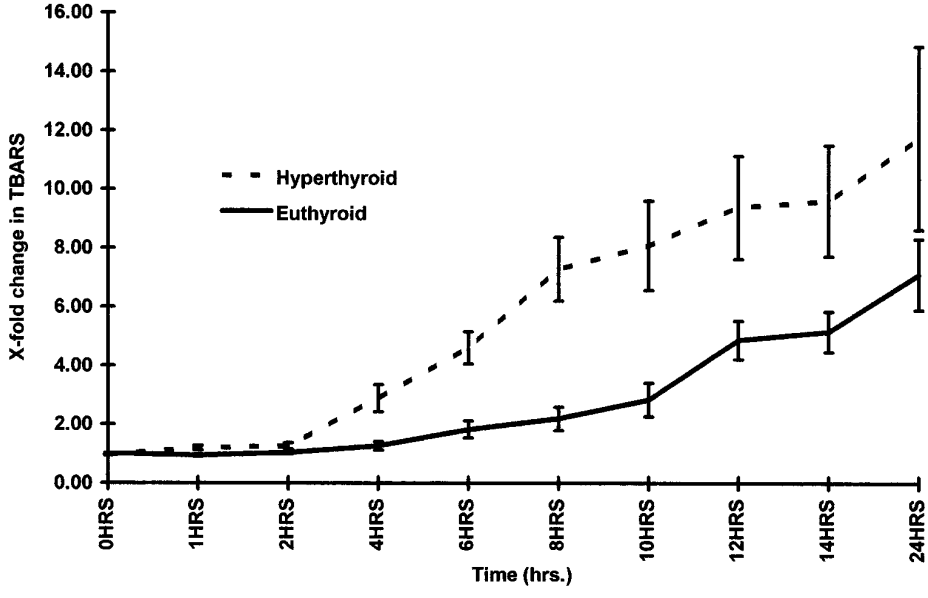

FIG. 2. Plot comparing Cu^{+2}-induced LDL oxidation at various time points during hyperthyroidism and the subsequent euthyroid state. The *y*-axis represents the x-fold change (mean ± SEM) in TBARS. Note that LDL has a lower propensity to oxidation during hyperthyroidism.

We did not find significant differences in the lag phases between hypothyroid patients at baseline and the patients receiving thyroid hormone replacement who had not yet become euthyroid (data not shown).

In hyperthyroid subjects (Fig. 2). The lag phases for Cu^{+2}-induced oxidation at baseline and on achieving the euthyroid state were 6 ± 0.55 and 12 ± 0.66 h, respectively. There was a significant increase in the lag phase for LDL oxidation with Cu^{+2}-induced oxidation in the euthyroid state compared to the hyperthyroid state. This suggests that LDL is more resistant to oxidation in euthyroidism than in hyperthyroidism.

Again there were no significant differences in the lag phases between hyperthyroid patients at baseline and the patients treated with propylthiouracil and/or radioiodine who had not yet become euthyroid (data not shown).

Discussion

This was a clinical study designed to compare the degree of baseline LDL oxidation in hypothyroid or hyperthyroid patients compared to that when subjects achieved a euthyroid state.

In human plasma, thyroid hormones are transported primarily by T_4-binding globulin, T_4-binding prealbumin, and serum albumin. A small fraction (3%) of T_4 is bound to plasma lipoproteins, with a relative distribution of 0.8% to very low density lipoprotein, 6.7% to LDL, and 92% to HDL. T_3 binds to the same proteins, but with a lower affinity (20–21). The T_4-LDL complex is recognized by the LDL receptor, and this interaction provides an additional mode of T_4 entry into the cells (22). Thus, the lipoprotein-bound T_4 could be involved in protecting LDL from oxidation.

Our present study showed that there was a very short lag

phase and a rapid propagation phase of LDL oxidation in the hypothyroid state. When patients were made euthyroid, there was a significant prolongation of the lag phase and a delay in the propagation phase of LDL oxidation. The level of TBARS was also higher in the hypothyroid state than that in the euthyroid state. These data indicate that LDL was more susceptible to Cu^{+2}-mediated oxidation in hypothyroidism, whereas LDL was more resistant to oxidation in euthyroidism. These findings support the *in vitro* data (11, 12).

The increased risk for atherosclerosis in hypothyroidism is thought to be due to the elevated cholesterol levels (9) and hypertension. However, it is possible that oxidative modification of LDL may play a role. In this regard, our present study shows that LDL is also more susceptible to oxidation and forms oxidized LDL. Putatively, there might be less T_4 available to bind to LDL and protect it from oxidation by free radicals, as L-T_4 seems to function as an antioxidant *in vitro* studies.

A more controversial issue is whether subclinical hypothyroidism poses an increased risk for atherosclerosis. Our study involved patients with frank hypothyroidism, but we had two patients whose T_4 levels returned to normal, although the TSH level was still slightly elevated during replacement. These patients also showed enhanced LDL oxidation compared to that in the final euthyroid state. Further, LDL oxidation studies need to be performed to confirm these findings, and they may provide an additional reason to treat patients with subclinical hypothyroidism.

Our results also indicate that LDL oxidation is enhanced in hyperthyroidism compared to euthyroidism, which was unexpected. Further, the lag phases and propagation of LDL oxidation did not seem to be affected by drug therapy as long as the underlying thyroid condition was still present.

However, Fernandez *et al.* (10) were able to show that in experimental hyperthyroid rats, there was increased generation of oxygen free radicals and increased activity of NADH-cytochrome P450 reductase and NADPH oxidases in rat liver microsomes. These effects were accompanied by increased levels of lipid peroxides, suggesting the disruption of polyunsaturated fatty acids by free radical oxidation. A similar mechanism could explain the increased susceptibility of LDL oxidation in hyperthyroid individuals, in whom the increased free radical generation overwhelms the antioxidant factors protecting the LDL.

The significance of enhanced LDL oxidation in hyperthyroid individuals is not clear. Hyperthyroidism is not usually associated with atherosclerosis; any cardiac complications are usually arrhythmia or congestive heart failure secondary to the hypermetabolic state. Hyperthyroidism is known to precipitate angina and/or myocardial infarction in patients with preexisting coronary heart disease, presumably secondary to the increased metabolic demands on the myocardium. However, the increased angina and/or precipitation of myocardial infarction could relate to the oxidative modification of LDL by free radicals, which, in turn, would cause changes in the endothelium-dependent relaxation factor, *etc.* Interestingly, hyperthyroidism reduces the LDL cholesterol concentration without modifying HDL or triglyceride concentrations. It is possible that the effects of LDL oxidation may require a threshold level of LDL to induce atherosclerosis. It is also possible that hyperthyroidism may induce antiatherosclerotic factors (currently unknown) that require further investigation.

In summary, both hypothyroidism and hyperthyroidism are associated with increased susceptibility of LDL to oxidation compared to that in the euthyroid state. Enhanced LDL oxidation may play a role in the cardiac disease process seen in these patients.

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