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NOTE

Lipoprotein(a) Concentration in Subclinical Hypothyroidism Before and After Levo-Thyroxine Therapy

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Abstract. Subclinical hypothyroidism is a frequent disorder in populations and has been shown to be a risk factor for coronary heart disease (CHD). Less is known about the contribution of lipoprotein (a) [Lp(a)] to the development of CHD in this disorder. Therefore this study was designed to evaluate Lp(a) and other lipoprotein concentrations before and after L-T4 therapy in 20 patients with subclinical hypothyroidism and 20 normal healthy subjects matched for sex, age and BMI. In the basal state of subclinical hypothyroidism, a significant increase in total cholesterol, LDL-cholesterol and apolipoprotein (apo) B concentrations was observed in patients compared with those in the control group. The mean Lp(a) concentration before treatment was 163 ± 15 mg/L. This is slightly but not significantly higher than those in the control group (131 ± 15 mg/L). Treatment of subclinical hypothyroidism with a low dose of L-T4 (25 μg daily) for 3 months after restoration of euthyroidism led to decreases in levels of Lp(a) from 163 mg/L to 126 mg/L (23% reduction, $P<0.001$), total cholesterol from 5.5 mmol/L to 5.1 mmol/L (7% reduction, $P<0.001$), LDL-cholesterol from 4.14 mmol/L to 3.63 mmol/L (12%, $P<0.001$), and apo B from 98 mg/dL to 86 mg/dL (12% reduction, $P<0.05$), but triglyceride, HDL-cholesterol and apo A-I concentrations were unchanged. These data suggest that L-T4 replacement therapy in patients with subclinical hypothyroidism has beneficial effects on the lipid profile since L-T4 replacement therapy lowered the concentrations of Lp(a) and other atherogenic lipid particles.

Key words: Subclinical hypothyroidism, Lipoprotein(a), Levo-thyroxine therapy

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SUBCLINICAL hypothyroidism is a frequent disorder in populations [1] and has been shown to be a risk factor for coronary heart disease (CHD) [2, 3]. Alterations in the lipid profile observed in subclinical hypothyroidism may explain the higher prevalence of CHD [4]. Thyroid hormones regulate lipid synthesis, mobilization and catabolism affecting both lipoprotein and hepatic lipase enzymes and modify low density lipoprotein (LDL) receptor activity [5, 6].

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Lipoprotein(a) [Lp(a)] is a cholesterol-rich lipoprotein and its concentration in plasma varies widely among individuals [7, 8]. The Lp(a) lipid composition is similar to that of LDL, but the protein composition is different, consisting of two major proteins, apolipoproteins (apo) B 100 and apo (a) attached to each other by a disulfide bridge [9]. Lp(a) is closely correlated with a high risk for CHD [10, 11]. Metabolism of Lp(a) is largely unknown; its sites of degradation and the factors affecting its plasma levels are not fully understood. Diets and most drugs that decrease LDL cholesterol levels do not substantially alter Lp(a) levels [12] except the use of neomycin in combination with nicotinic acid [13].

Recently, several reports have been published

examining the plasma concentrations of Lp(a) in clinically overt hypothyroidism [14–18], but conflicting results have been obtained concerning the response of Lp(a) levels to thyroid hormone substitution in overt hypothyroidism. Some studies reported a slight decrease [14, 15], while others reported no significant change [16–18] in Lp(a) levels after treatment. But to our knowledge only one study [19] has evaluated the effect of Levothyroxine (L-T4) therapy on Lp(a) in subclinical hypothyroidism and found no significant change in Lp(a) levels after treatment. We therefore aimed to investigate the effects of thyroid hormone substitution on Lp(a) and other lipoprotein profiles in patients with subclinical hypothyroidism.

Materials and methods

Twenty subjects (16 women and 4 men, aged 22–53, mean: 41.3 ± 2.1) with subclinical hypothyroidism diagnosed on the basis of normal serum free triiodothyronine (FT3) (normal range: 3.4–7.2 pmol/L) and free thyroxine (FT4) (normal range: 9.4–25 pmol/L) and high serum TSH (> 6.5 mU/L) concentrations were studied before and 3 months after the initiation of L-T4 (25 μg/day) therapy when patients were euthyroid. Each patient's thyroid function was tested on at least 3 occasions 8 weeks apart and maintained in this stable state before treatment for at least 4 months for exclusion of the temporary TSH increase. They were compared with 20 euthyroid healthy subjects matched for age (40.2 ± 2.4), sex (16 women and 4 men) and body mass index (BMI) (24.5 ± 0.6 kg/m^2). None had symptoms suggestive of hypothyroidism or a history of thyroid disease.

The great majority of the patients (16 of 20) had Hashimato's thyroiditis and 4 patients had a history of radioiodine therapy (4–10 years previously) for thyrotoxicosis. None of the patients or controls was acutely ill at the time of sampling or had a history of CHD, and all of the study subjects had normal baseline electrocardiograms. Patients with diabetes mellitus, renal failure, nephrotic syndrome, paraproteinemia, familial hypercholesterolemia, a history of alcohol abuse, hepatic and malignant disease and also taking oral contraceptives or antihypertensive drugs (e.g., beta-blockers) were excluded from the study. All of the patients were instructed to maintain their usual diets, physical activities and smoking habits.

From all patients and controls, venous blood samples were drawn for lipid and thyroid function tests after an overnight fast before and at 3 months after L-T4 replacement therapy. Sera were stored −40 °C until analysis and in order to minimize assay variation, all samples from a given individual were run in the same assay. Total serum cholesterol and triglyceride levels were determined on a Technicon RA-1000 autoanalyser by using enzymatic colorimetric methods with kits provided by Boehringer-Mannheim (Mannheim, Germany). High density lipoprotein (HDL)-cholesterol was measured, after phosphotungustic acid-magnesium precipitation at very low density (VLDL) and LDL by the CHOD-PAP method (as for cholesterol plus Reagent Set HDL Precipitant (Boehringer Mannheim Diagnosticum, Mannheim, Germany). LDL-cholesterol was calculated using the Friedewald formula [20]. Apo A-I and apo B levels were determined by an immunoturbidimetric method with Sera Pak kits (Bayer Diagnostics, Tournai, Belgium).

Lp(a) concentrations were quantified by electroimmunodiffusion with a Hydragel kit from Sebia (Issy Les Moulineaux, France). The intraassay coefficients of variation (CVs) for serum cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, apo A-I, apo B and Lp(a) were 1.8%, 2.3%, 3.5%, 3.6%, 3.0% and 7.6%, respectively.

Serum TSH levels were measured by immunoradiometric assay (Johnson & Johnson Clinical Diagnostics Ltd., Amersham, UK); intraassay CV was 3.4% at 1.0 IU/L. FT3 and FT4 concentrations were measured by radioimmunoassay with commercial kits (Amerlex-MAB, Amersham UK). The FT3 and FT4 assay had an intraassay CV of 4.3% at 5.93 pmol/L and 3.3% at 12.81 pmol/L, respectively. All patients gave their written consent for this study and the protocol was approved by the Local Ethical Committee of Gülhane School of Medicine.

Statistical analysis of data were tested by paired or unpaired Student's *t*-test. The relationships between the Lp(a) and other variables were evaluated by Spearman's rank correlation test. All values have been expressed as the mean ± SEM, and *P* values less than 0.05 are considered significant.

Results

Thyroid function tests of patients and controls are shown in Table 1. FT3 and FT4 levels before and after L-T4 treatment were within the normal range, both in patient and control groups, whereas basal TSH levels were significantly higher in subclinical hypothyroid patients ($P<0.001$) than in those in the control group but became normal after adjustment with L-T4 therapy. During the L-T4 therapy mean FT3 and FT4 levels increased but the changes were not significantly different from the pretreatment values.

Comparison of lipid results in the basal state of subclinical hypothyroidism with those in the control group revealed a significant increase ($P<0.05$, respectively) in total cholesterol, LDL-cholesterol and apo B levels in patients, whereas Lp(a) levels, although slightly increased, were not significantly different from the controls (Table 2). Individual Lp(a) levels before and after treatment are shown in Fig. 1. As a result of L-T4 therapy, mean total cholesterol ($P<0.001$), LDL-cholesterol ($P<0.001$), apo B ($P<0.05$) and Lp(a) ($P<0.001$) levels were significantly decreased after L-T4 treatment but other parameters were not significantly changed after treatment. No correlation was found between Lp(a) and other parameters in patients before and after L-T4 therapy.

Discussion

Subclinical hypothyroidism is indicated by a high serum TSH level and a normal thyroid hormone concentration in the absence of overt symptoms [21]. The prevalence of subclinical hypothyroidism has been reported to be between 2.5% and 10.4%, depending on the sex and age of the population being screened [1]. The importance of subclinical hypothyroidism has been somewhat unclear, but in several studies it has been suggested to be a risk factor for CHD [1, 2]. An increase

Table 1. Thyroid function tests in patients with subclinical hypothyroidism (before and 3 months after levothyroxine treatment) and euthyroid controls

Thyroid tests	Subclinical hypothyroidism		Euthyroid control
	Before treatment	After treatment	
FT3 (pmol/L)	5.1 ± 0.25	5.3 ± 0.28	5.4 ± 0.25
FT4 (pmol/L)	15.1 ± 1.0	16.8 ± 0.8	16.0 ± 1.0
TSH (mU/L)	22.0 ± 3.9*	2.4 ± 0.29	1.1 ± 0.1

*$P<0.001$, *vs.* after treatment and *vs.* euthyroid control. FT3, free triiodothyronine; FT4, free thyroxine.

Table 2. Lp(a) and other lipoproteins in patients with subclinical hypothyroidism (before and 3 months after levothyroxine treatment) and euthyroid controls

Lipid parameters	Subclinical hypothyroidism		Euthyroid control
	Before treatment	After treatment	
Cholesterol (mmol/L)	5.5 ± 0.2	5.1 ± 0.2**	5.0 ± 0.2*
Triglyceride (mmol/L)	1.35 ± 0.12	1.28 ± 0.12	1.36 ± 0.11
HDL-cholesterol (mmol/L)	1.12 ± 0.07	1.22 ± 0.05	1.22 ± 0.07
LDL-cholesterol (mmol/L)	4.14 ± 0.18	3.63 ± 0.17**	3.45 ± 0.19*
Apo A-I (mg/dL)	143 ± 8	149 ± 5	153 ± 15
Apo B (mg/dL)	98 ± 5	86 ± 4*	84 ± 4*
Lp(a) (mg/L)	163 ± 15	126 ± 12**	131 ± 15

Values are the means ± SEM. HDL, high density lipoprotein; LDL, low density lipoprotein; Apo, apolipoprotein; Lp, lipoprotein. *$P<0.05$, *vs.* before treatment. **$P<0.001$, *vs.* before treatment.

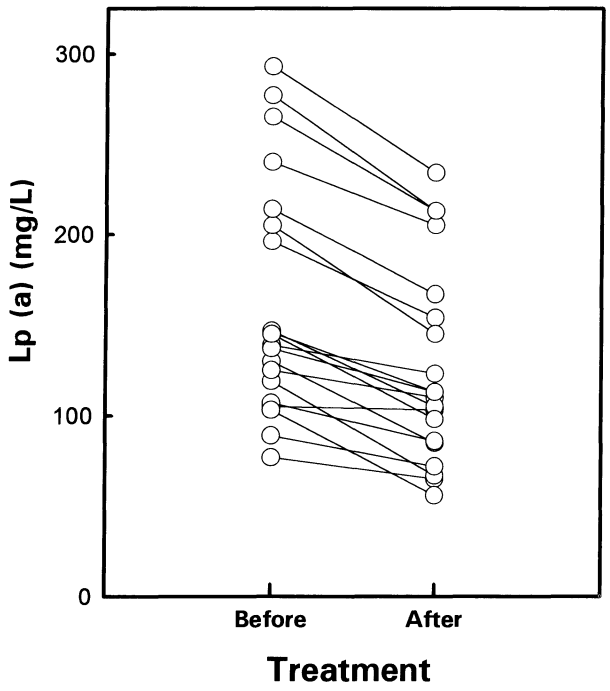

Fig. 1. Individual Lp(a) levels in patients with subclinical hypothyroidism before and after levo-thyroxine therapy.

in total cholesterol and LDL-cholesterol in this disorder has long been known [22], but changes in the HDL-cholesterol level are less apparent and less consistent [23]. Indeed, in our study it was observed that there was a 10% increase in total cholesterol, a 20% increase in LDL-cholesterol and no change in triglyceride and HDL-cholesterol levels, in untreated subclinical hypothyroidism when compared with the control group. We also found a 15% increase in the apo B concentration, which is the main apo of the LDL particle, but no significant change was observed in the apo A concentration, which is the main apo of the HDL particle. Our study also demonstrates that patients with subclinical hypothyroidism had slightly higher mean Lp(a) levels than in the control group, but almost all patients had initial Lp(a) values lower than the accepted cut off level for increased risk of atherosclerosis (300 mg/L). In agreement with our findings Kung *et al.* [24] and Arem *et al.* [19] have recently reported high Lp(a) levels in untreated subclinical hypothyroidism when compared to controls, but their patients had higher Lp(a) levels than the accepted cut off level of 300 mg/L.

Our findings suggest that L-T4 therapy in subclinical hypothyroidism causes a reduction in total cholesterol, LDL-cholesterol and apo B concentrations. These results are in accordance with those of the small number of published reports about the effect of L-T4 treatment on lipoprotein and apolipoprotein fractions in patients with subclinical hypothyroidism [25, 26]. But other studies have failed to demonstrate significant changes in total cholesterol following L-T4 treatment [27, 28]. These conflicting results may be explained by the difference in definition of subclinical hypothyroidism and/or the characteristics (sex, age, BMI) of patients and control groups. In our study, the patients with subclinical hypothyroidism were tested on at least three occasions 8 weeks apart before treatment and were matched for sex, age and BMI to controls. There is evidence that thyroid hormone enhances the removal of LDL particles by increasing the number and activity of LDL receptors [6]. The decrease in total and LDL-cholesterol levels observed after achievement of a euthyroid state probably results from the increase in LDL receptor activity caused by thyroid hormone. In the present study the observed decrease in apo B concentrations in the treated subclinical hypothyroid subjects had been caused by a reduction in serum total and LDL-cholesterol. This lipoprotein profile therefore suggests that patients with subclinical hypothyroidism are at increased risk for CHD.

We found a 25% decrease in the Lp(a) concentration after L-T4 therapy (25 μg/day) for 3 months. To our knowledge, only one study has evaluated the effect of L-T4 treatment on Lp(a) levels in subclinical hypothyroidism to date. In contrast to our study, Arem *et al.* found no change in Lp(a) levels after L-T4 treatment [19], but in that study, the daily dose of L-T4 was incremental and much higher at about 100–200 μg/day. The L-T4 dose and length of the treatment period may therefore be the reasons for this discrepancy between these two studies, and some previous studies, supporting this hypothesis, reported a dose-dependent effect of L-T4 therapy on Lp(a) levels in clinically overt hypothyroidism. De Bruin *et al.* [14] and Engler and Riesen [15] found that 25 μg L-T4 therapy decreased the Lp(a) concentration by 55%, but higher doses of L-T4 were associated with a secondary increase in Lp(a) in clinically overt hypothyroid patients. But, in other studies [17, 18, 29] no chang-

es in Lp(a) concentrations were observed.

The effects of thyroid hormones on possible routes of elimination of Lp(a) are unclear, but it is known that Lp(a) is eliminated at least in part via the LDL receptor [9]. Another mechanism leading to a decrease in Lp(a) is the synthesis of apo B. Plasma Lp(a) concentrations are reported to be genetically determined by the hepatic synthesis rate of the apo(a) moiety, the synthesis rate of apo B, and the assembly rate of the apo B and apo(a) moieties [9, 30]. Pharmacological doses of T3 administered to hypothyroid animals reduce plasma apo B levels and significantly suppress hepatic apo B synthesis [31, 32]. In rabbits, rats and humans, the synthesis and secretion of apo B are considered to be comparable processes [31, 32]. Since apo B and Lp(a) behaved in a parallel fashion in the hyperthyroid subjects [14], it is likely that thyroid hormones significantly suppressed apo B synthesis as well as the synthesis or secretion of apo(a).

In conclusion, increased Lp(a) and other atherogenic lipoprotein particles in subclinical hypothyroidism could be readily corrected with low dose thyroid hormone replacement therapy.

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