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Effect of Levothyroxine Replacement on Lipid Profile and Intima-Media Thickness in Subclinical Hypothyroidism: A Double-Blind, Placebo-Controlled Study

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Subclinical hypothyroidism (sHT) is associated with dyslipidemia and enhanced cardiovascular risk. We assessed carotid artery intima-media thickness (IMT, high-resolution ultrasonography) and lipoprotein profile in 45 sHT patients (aged 37 ± 11 yr) at baseline and after 6 months of randomized, placebo-controlled L-T_4 replacement. In comparison with 32 age- and sex-matched controls, sHT patients had elevated total and low-density lipoprotein (LDL) cholesterol and ApoB levels ($P = 0.002$, $P = 0.0007$, and $P = 0.01$, respectively) and higher mean-IMT values ($P < 0.0001$). In stepwise regression analysis, mean-IMT was positively related ($r^2 = 0.71$, $P < 0.0001$) to age, TSH, and LDL cholesterol. L-T_4 replacement significantly reduced both total and LDL cholesterol ($P < 0.0001$ for both) and mean-IMT (by 11%, $P < 0.0001$). The decrement in IMT was directly related to the decrements of both total cholesterol and TSH ($P = 0.02$ and $P = 0.0001$, respectively). We conclude that early carotid artery wall alterations are present in sHT patients. Whether such IMT increase is related to an early atherosclerotic involvement of the arterial wall cannot be clearly decided on the basis of the present results. However, the fact that L-T_4 replacement therapy was able to improve both the atherogenic lipoprotein profile and intima-media thickening suggests that lipid infiltration of arterial wall may represent a major mechanism underlying IMT increase in subclinical hypothyroidism. (*J Clin Endocrinol Metab* 89: 2099–2106, 2004)

OVERT HYPOTHYROIDISM, WITH its accompanying hypercholesterolemia, is widely recognized as a risk factor for atherosclerosis and cardiovascular disease (1). Subclinical hypothyroidism (sHT), characterized by elevated serum TSH levels despite normal free thyroid hormone (FT_3 and FT_4) values (2), has been detected with increasing frequency in recent years (3). Early clinical and autopsy studies have suggested an association between subclinical hypothyroidism and coronary heart disease (4–6). Although in a recent population-based survey sHT emerged as an independent risk factor for aortic atherosclerosis and myocardial infarction in elderly women (7), previous cohort studies had not substantiated this finding (8, 9).

Although the relationship between sHT and an atherogenic lipoprotein profile is still under debate (3, 8, 10, 11), a metaanalysis of 13 intervention studies showed that levothyroxine (L-T_4) therapy led to a significant reduction in both serum total and low-density lipoprotein (LDL) cholesterol levels (12). Moreover, in a randomized, placebo-controlled study, we recently demonstrated that in sHT patients serum LDL cholesterol levels are specifically and reversibly increased to an extent that may translate into a sizable cardiovascular risk (13). Also, one fourth of these patients showed elevated lipoprotein (a) [Lp(a)] values, which were unaffected by thyroid hormone replacement. Raised homocysteine levels have been described in overt hypothyroid patients and might contribute to their higher cardiovascular risk (14, 15). In contrast, in sHT serum homocysteine levels do not seem to be affected, although scant data are so far available (16, 17).

Whether L-T_4 replacement is able to improve the cardiovascular risk profile and slow down the progression of early atherosclerotic vascular wall lesions in sHT patients is still controversial. The assessment of subclinical atherosclerosis in the carotid arteries can be accomplished by means of B-mode ultrasonography (18). Intima-media thickness (IMT) is, at present, a close marker of early atherosclerotic changes and a widely accepted surrogate end point for cardiovascular events (19–21). The association of carotid artery IMT with major cardiovascular risk factors has been demonstrated in several studies (22–26), and both cross-sectional and prospective studies have clearly established its relationship with systemic atherosclerosis and coronary heart disease (27–29). Nagasaki *et al.* (30) recently described carotid artery intima-media thickening in patients with overt hypothyroidism, which was reversed by L-T_4 replacement therapy.

Aims of the present study were to assess IMT as well as

Abbreviations: $ApoA_1$, Apolipoprotein A_1; ApoB, apolipoprotein B; BMI, body mass index; CI, confidence interval; FT_3, free T_3; FT_4, free T_4; IMT, intima-media thickness; IRMA, immunoradiometric assay; LDL, low-density lipoprotein; LDLc, LDL cholesterol; Lp(a), lipoprotein(a); L-T_4, levothyroxine; sHT, subclinical hypothyroidism; TC, total cholesterol; TG, triglyceride; Tg-Ab, antithyroglobulin autoantibody; tHcy, total homocysteine; TPO-Ab, antithyroid peroxidase autoantibody; VSM, vascular smooth muscle.

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the lipoprotein profile and serum homocysteine values in a carefully selected group of sHT patients in comparison with matched euthyroid controls and test the effect of L-T_4 replacement therapy on these variables in a placebo-controlled fashion.

Patients and Methods

Patients

Forty-five sHT patients (eight men and 37 women) were recruited from the outpatient clinic of the Department of Internal Medicine of the University of Pisa. Thirty-six patients had Hashimoto's thyroiditis and were positive for both antithyroid peroxidase (TPO-Ab) and antithyroglobulin (Tg-Ab) autoantibodies, whereas nine developed sHT after radioiodine therapy for toxic adenoma or multinodular toxic goiter and had never shown positive autoantibody titers. To be enrolled, patients had to have documented sHT (TSH > 3.6 mIU/liter) for at least 6 months before the study. Thirty-two euthyroid subjects matched to the patients group for sex (27 women, 5 men), age, and body mass index (BMI) were recruited among staff and relatives of patients and served as the control group (Table 1). Thyroid autoimmunity was detected in none of the controls. All subjects were in good health, all women were premenopausal with regular menses, and none was pregnant. Individuals over age 55 yr; obese (BMI > 30 kg/m^2) subjects; smokers; and those with hypertension, diabetes mellitus, renal and hepatic failure, or other systemic diseases were excluded from the study. Routine laboratory chemistry was normal in all, and none was taking any drug. All subjects were on a free diet and were advised to maintain their dietary habits throughout the study. All study subjects gave their written informed consent to the study, which was approved by the Institutional Ethical Committee.

Biochemical measurements

At baseline, blood samples were drawn at 0800 h after an overnight fast for the determination of serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol, LDL cholesterol (LDLc), apolipoprotein A_1 ($ApoA_1$), apolipoprotein B (ApoB), Lp(a), total homocysteine (tHcy), folic acid, and vitamin B_{12} concentrations. For tHcy, vitamin B_{12}, and folate measurements, blood was drawn into tubes containing EDTA, placed on ice, and immediately centrifuged. Samples were stored at −70 C until analysis.

IMT assessment

Carotid arteries were investigated by high-resolution ultrasonography. The same investigator (C.P., blinded to the treatment status of the patients) performed all examinations using commercially available equipment (SONOS 2500, Hewlett-Packard, Andover, MA) and a 7.5-MHz linear transducer (Hewlett-Packard). Both image acquisition and IMT measurements were carried out according to the Atherosclerosis Risk in Communities study protocol (18, 31). The carotid images were obtained with the patient in the supine position with the neck mildly extended and the head rotated contralaterally to the side. The imaging protocol involved obtaining longitudinal B-mode image of the distal 10 mm of the right and left common carotid arteries; the carotid bifurcation; and the internal carotid artery from the anterior, lateral, and posterior angle. The whole imaging session was recorded on videotape. Good-quality images of internal carotid artery were obtained in 64% of subjects, and this segment was not included into IMT evaluation. Therefore, in the whole study population, the final analysis was performed with IMT images of both the near and far wall of the right ad left common carotid artery and carotid bifurcation. The recordings were evaluated by the same reader (M.K.) blinded to the clinical data, using a high-resolution video recorder (AG-MD830, Panasonic, Secaucus, NJ) coupled with the computer-driven image analysis system (Medical Image Processing; Institute of Clinical Physiology, National Research Council, Pisa, Italy) (32). Diastolic frames of each carotid segment were digitized. Lines were drawn along the lumen-intimal and medial-adventitial interfaces in all analyzed segments, and the IMT of each segment was automatically computed as an average of several measurements. For statistical analysis, IMT readings in all the eight carotid segments were averaged (mean IMT). In addition, the maximal thickness in any particular segment was also measured (maximal IMT). In our laboratory, the intra- and interobserver coefficients of variation for mean carotid IMT are 3.2 and 4.2%, respectively.

Study protocol

Patients were then randomly assigned to L-T_4 replacement (n = 23) or placebo (n = 22) in a blinded manner. Tablets containing L-T_4 or placebo were accurately counted and given to each patient. L-T_4 treatment always started with 25 μg, the dose being then gradually increased. All patients returned after 3 months for repeat thyroid function tests. One of us (N.C.) had access to the treatment code and increased the dose of L-T_4 by 25 μg if the TSH level was still higher than 3.6 mIU/liter. This process continued until euthyroidism was reached; the mean final re-

TABLE 1. Clinical features of euthyroid controls and subclinical hypothyroid patients

	Controls (n = 32)	Patients (n = 45)	*P*
Age (yr)	35 ± 10	37 ± 11	0.39
BMI (kg/m^2)	24.2 ± 3.7	24.7 ± 3.5	0.21
Gender (F/M)	27/5	37/8	0.8
SBP (mm Hg)	112 ± 13	115 ± 14	0.24
DBP (mm Hg)	70 ± 8	71 ± 10	0.15
TSH (mIU/liter)	1.19 (0.34–2.50)	6.31 (3.65–15.00)	<0.0001
FT_4 (pg/ml)	9.4 ± 1.4	8.6 ± 2.0	0.02
FT_3 (pg/ml)	3.5 ± 0.4	3.1 ± 0.6	0.0007
TC (mg/dl)	184.9 ± 22.9	213.6 ± 43.1	0.002
HDLc (mg/dl)	55.0 ± 14.3	56.6 ± 11.2	0.47
LDLc (mg/dl)	113.8 ± 21.1	138.0 ± 36.2	0.0007
Triglycerides (mg/dl)	80.5 ± 33.1	94.5 ± 44.7	0.13
$ApoA_1$ (mg/dl)	153 ± 29	166 ± 24	0.1
ApoB (mg/dl)	85 ± 20	111 ± 42	0.01
Lp(a) (mg/dl)	11.0 (9.0–69.0)	14.0 (9.0–162.0)	0.37
Homocysteine (μmol/liter)	9.5 ± 4.1	12.7 ± 14.0	0.47
Vit. B_{12} (pg/ml)	379.4 ± 180.2	403.8 ± 196.5	0.60
Folate (ng/ml)	5.3 ± 3.5	6.2 ± 9.0	0.91
Maximal IMT (mm)	0.74 ± 0.28	0.92 ± 0.28	0.003
Mean IMT (mm)	0.63 ± 0.07	0.75 ± 0.13	<0.0001

SBP, Systolic blood pressure; DBP, diastolic blood pressure; HDLc, HDL cholesterol. TSH and Lp(a) values are expressed as median and range, other data are expressed as mean ± SD. Conversion factors: total, HDL, and LDL cholesterol, 0.02586; triglycerides, 0.01129; FT_3, 1.536; FT_4, 1.286; folate, 2.27; Vit. B_{12}, 0.738.

placement dose of L-T_4 was 70 μg daily. Patients taking placebo completed an identical protocol, some of them being given additional placebo tablets to maintain the blindness of the study. Six months after the normalization of serum TSH level (median 10.5 months, range 9–15 from the beginning of the study in the L-T_4-treated group) or 6 months after the final dosage was assigned (median 10.5 months, range 9–12 in the placebo-treated group), the patients were readmitted for repeat evaluations of all parameters.

Analytical measurements

Serum FT_3 and FT_4 concentrations were measured by specific RIA (Techno-Genetics Recordati, Milan, Italy). Serum TSH levels were determined by an ultrasensitive immunoradiometric assay (IRMA) method (Cis Diagnostici, Tronzano Vercellese, Italy). Anti-Tg-Abs were measured by a specific IRMA assay (TG-Ab IRMA, Biocode, Sclessin, Belgium); anti-TPO-Abs were measured by a specific RIA (AB-TPO; Sorin Biomedica, Saluggia, Italy). TC and TG were assayed using enzymatic methods (Roche Diagnostics, Mannheim, Germany). high-density lipoprotein cholesterol was measured enzymatically after precipitation of LDL and very low-density lipoprotein with Mg^{2+}-dextran (Roche Diagnostics). LDLc was calculated by Friedewald's formula. $ApoA_1$ and ApoB were determined immunochemically (Nephelometer, Behring Diagnostics, Marburg, Germany). Lp(a) concentrations were determined in serum by nephelometry (N latex Lp(a) reagent, Behring Diagnostics). tHcy was measured by HPLC as previously described (33). Vitamin B_{12} as well as folic acid was measured by specific RIA (ICN Pharmaceuticals, Costa Mesa, CA). Normal ranges in our laboratory are as follows: FT_4 = 5.6–13 pg/ml (6.8–20 pmol/liter); FT_3 = 2.3–5.6 pg/ml (4.3–8.6 pmol/liter); TSH = 0.30–3.60 mIU/liter; Tg-Ab less than 50 IU/ml and TPO-Ab less than 10 IU/ml; $ApoA_1$ = 95–230 mg/dl; ApoB = 55–165 mg/dl; Lp(a) less than 30 mg/dl; tHcy = 5–15 μmol/liter; vitamin B_{12} = 261-1330 pg/ml (193–982 pmol/liter); folate = 1.3–7.5 ng/ml (3–17 nmol/liter).

Statistical analysis

Data were expressed as the mean ± SD unless otherwise stated. A Mann-Whitney *U* test was used for each comparison between two independent groups. Two-way ANOVA for repeated-measures (Friedman's rank test with Tukey multiple comparison) was used to compare data before and after therapy in L-T_4- and placebo-treated patients. Relationships among parameters were tested by Spearman correlation coefficient; in case of significant correlation between mean-IMT and the tested variable, a linear regression plot was also obtained. Stepwise regression analysis was performed with log-transformed mean-IMT as the dependent variable. The following continuous variables were selected for the stepwise regression model: age, TSH, FT_3, LDL, TG, Lp(a), ApoB, and systolic blood pressure. Correlation and regression analyses were always carried out on data from sHT patients. Significance was assumed for $P < 0.05$. SPSS for Windows XP (SPSS Inc., Chicago, IL) was used as statistical software.

Results

Clinical and biochemical characteristics of all study participants are summarized in Table 1. Euthyroid controls and sHT patients were well matched with respect to age, BMI, and gender. Except for serum TC, LDLc, and ApoB concentrations, there were no significant differences in clinical parameters between the two groups. Although serum Lp(a) values did not differ between patients and controls, elevated Lp(a) levels (>30 mg/dl) were significantly more frequent in sHT (33% of patients *vs.* 7% of controls, $P = 0.01$).

Baseline clinical and biochemical characteristics of sHT patients randomized to L-T_4 or placebo treatment were not significantly different (Table 2). After therapy, serum TSH levels had returned within the normal range in the L-T_4-treated group and were now significantly lower than in the placebo group ($P < 0.0001$). Serum FT_3 and FT_4 levels remained within the normal range during the entire treatment period both in placebo and L-T_4-treated patients; however, FT_4 levels rose significantly in the L-T_4-treated group (Table 2).

After 6 months of stable euthyroidism, L-T_4-treated patients showed a significant decrease in serum TC and LDLc concentrations; serum ApoB levels decreased slightly, and were no longer different from control values. The reduction

TABLE 2. Clinical features of sHT patients at baseline and after 6 months of stable euthyroidism or placebo

	Baseline		After therapy	
	sHT-LT_4 (n = 23)	sHT-placebo (n = 22)	sHT-LT_4 (n = 23)	sHT-placebo (n = 22)
BMI (kg/m^2)	24.3 ± 3.6	25.0 ± 3.5	23.7 ± 3.5	24.9 ± 3.8
SBP (mm Hg)	117 ± 15	112 ± 13	112 ± 15	114 ± 13
DBP (mm Hg)	72 ± 11	71 ± 9	69 ± 9	72 ± 8
TSH (mIU/liter)	6.03[a] (3.65–15.00)	5.68 (3.66–12.60)	1.32[c] (0.34–2.59)	6.01 (3.67–14.5)
FT_4 (pg/ml)	8.8 ± 1.9[a]	8.5 ± 2.0	10.8 ± 2.4[c]	8.4 ± 1.9
FT_3 (pg/ml)	3.1 ± 0.7	3.0 ± 0.5	3.2 ± 0.5	3.0 ± 0.6
TC (mg/dl)	214.2 ± 37.5[a]	213.0 ± 48.7	191.6 ± 32.5[d]	219.6 ± 48.9
HDLc (mg/dl)	56.5 ± 11.7	56.8 ± 10.6	54.7 ± 7.4	57.8 ± 11.6
LDLc (mg/dl)	138.9 ± 32.3[a]	137.2 ± 40.2	119.2 ± 27.8[d]	141.3 ± 38.6
Triglyceride (mg/dl)	94.0 ± 31.9	95.0 ± 57.6	88.1 ± 30.0	102.7 ± 53.1
$ApoA_1$ (mg/dl)	166 ± 24	165 ± 25	152 ± 38	169 ± 29
ApoB (mg/dl)	107 ± 32	117 ± 53	98 ± 24	118 ± 49
Lp(a) (mg/dl)	13.0 (9.0–83.0)	16.5 (9.0–162.0)	10.7 (9.0–65.0)	15.5 (9.0–160.0)
Homocysteine (μmol/liter)	15.2 ± 19.2	10.1 ± 3.2	13.6 ± 9.3	9.8 ± 3.1
Vit. B_{12} (pg/ml)	315 ± 164	280 ± 123	306 ± 122	290 ± 139
Folate (ng/ml)	11.8 ± 7.5	10.7 ± 4.8	11.1 ± 6.6	11.3 ± 5.2
Maximal IMT (mm)	0.95 ± 0.34[b]	0.89 ± 0.20	0.85 ± 0.32	0.91 ± 0.18
Mean IMT (mm)	0.76 ± 0.14[a]	0.74 ± 0.13	0.67 ± 0.13[d]	0.77 ± 0.14

SBP, Systolic blood pressure; DBP, diastolic blood pressure; HDLc, HDL cholesterol. TSH and Lp(a) values are expressed as median and range, other data are expressed as mean ± SD. Conversion factors: total, HDL, and LDL cholesterol, 0.02586; triglycerides, 0.01129; FT_3, 1.536; FT_4, 1.286; folate, 2.27; Vit. B_{12}, 0.738.

[a] $P < 0.0001$ *vs.* LT_4 therapy.
[b] $P = 0.001$ *vs.* LT_4 therapy.
[c] $P < 0.0001$ *vs.* placebo.
[d] $P = 0.03$ *vs.* placebo.

in serum TC averaged −22.6 ± 23.2 mg/dl (−0.58 ± 0.60 mmol/liter) (range −68.8–10.1, or −9.7%); for LDLc, the corresponding values were −19.7 ± 18.9 mg/dl (−0.50 ± 0.49 mmol/liter) (range −58.0–7.7, or −13.0%). Conversely, Lp(a) levels were unchanged both as a mean and in the nine patients with elevated baseline values (52 ± 14 *vs.* 48 ± 14 mg/dl, baseline, and after L-T_4 therapy, respectively) (Tables 1 and 2). Similarly, no significant changes were observed in serum tHcy, folic acid, and vitamin B_{12} levels (Table 2). However, the two patients with elevated baseline tHcy showed a reduction (62.6 *vs.* 20.0 and 83.4 *vs.* 46.9 μmol/liter, baseline, and after L-T_4 therapy, respectively), with a concomitant increment of plasma folate levels [0.6 *vs.* 2.6 and 1.6 *vs.* 4.7 ng/ml (1.4 *vs.* 5.9 and 3.6 *vs.* 10.7 nmol/liter), baseline, and after L-T_4 therapy, respectively]. In the placebo group, no significant changes in any of the biochemical parameters were seen.

IMT

The mean-IMT of sHT patients as a group was significantly higher than that of euthyroid controls (Table 1). Similar results were obtained when analyzing only the subgroup of sHT patients (n = 35) with serum TSH levels lower than 10 mIU/liter (median 5.23, range 3.65–9.32): mean-IMT 0.71 ± 0.10 mm, $P < 0.0001$ *vs.* euthyroid controls. Although we excluded subjects older than 55 yr as well as postmenopausal women, the mean-IMT was examined also in two age strata (older and younger than 35 yr) in view of the powerful effect of age on IMT. The number of sHT patients and euthyroid controls was comparable in both age strata (n = 24 and n = 15 in the older group; n = 21 and n = 17 in the younger group, respectively). The mean-IMT of sHT patients was significantly higher than that of controls in the older group (0.84 ± 0.10 *vs.* 0.64 ± 0.06 mm, $P < 0.0001$), whereas slightly, not significantly, increased in the younger group (0.66 ± 0.09 *vs.* 0.61 ± 0.07 mm, $P = 0.20$).

Six months of stable euthyroidism induced a significant decrease of mean-IMT, averaging 0.09 mm [95% confidence interval (CI) 0.06–0.11] or −10%. In particular, 19 patients (83%) showed a decrease in mean-IMT (Fig. 1A), whereas four (17%) had either no change (n = 3) or an increase (n = 1) (Fig. 1B). Although still slightly higher than in euthyroid controls ($P = 0.056$), mean-IMT of the L-T_4 treated group had become significantly lower ($P = 0.03$) than that of the placebo-treated group at the follow-up examination (Tables 1 and 2). L-T_4 replacement therapy was effective in also reducing mean-IMT in the subgroup of 18 sHT patients with serum TSH levels lower than 10 mIU/liter (mean reduction 0.08 mm, 95% CI 0.05–0.11) or −10% ($P < 0.0001$ *vs.* pretreatment). Furthermore, 6 months of stable euthyroidism induced a significant decrease of mean-IMT in both younger (n = 9) and older (n = 14) sHT patients, averaging 0.07 mm (95% CI 0.01–0.12) or −9% ($P = 0.03$ *vs.* pretreatment) in the former group and 0.09 mm (95% CI 0.05–0.13) or −11% ($P = 0.0003$ *vs.* pretreatment) in the latter group. Thus, in the younger group of sHT patients mean-IMT became equivalent to that of euthyroid controls (0.61 ± 0.08 *vs.* 0.61 ± 0.07 mm, respectively).

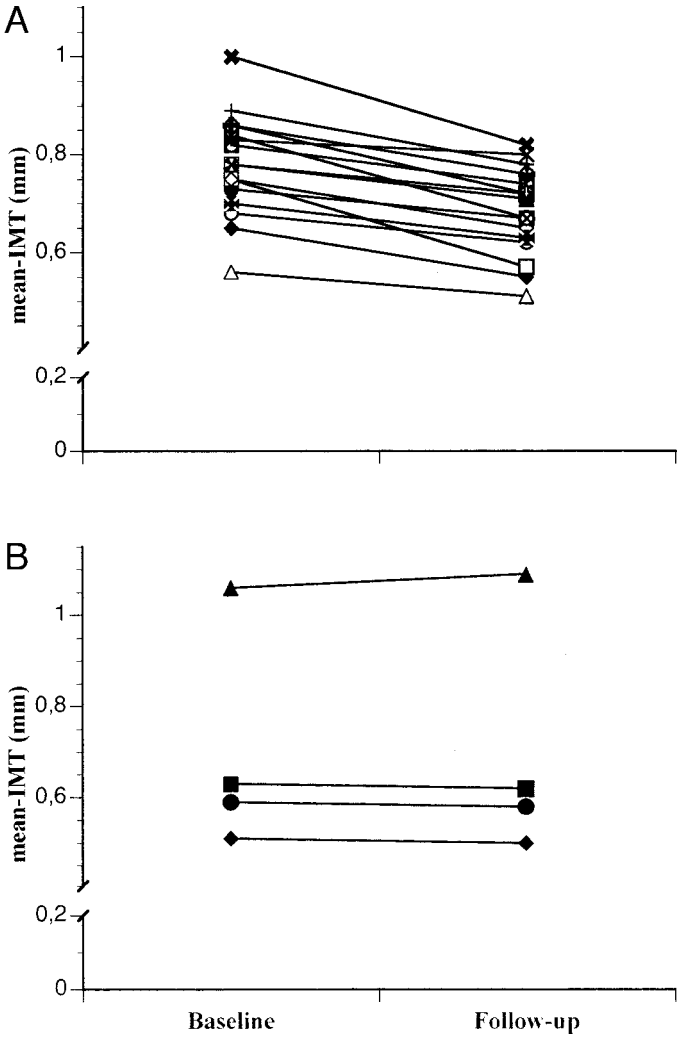

FIG. 1. Baseline and posttreatment mean-IMT values in individual patients with sHT undergoing L-T_4 replacement. A, Patients with a decrease in mean-IMT (n = 19). B, Patients with no change (n = 3) or an increase in mean-IMT (n = 1).

Correlations

Significant positive relationships were found between serum TSH and TC ($r = 0.39$, $P = 0.009$) and LDL levels ($r = 0.42$, $P = 0.005$). A positive relationship between the absolute decrements of both TC and LDL and the reduction of serum TSH was also observed ($r = 0.56$, $P = 0.0002$ and $r = 0.66$, $P < 0.0001$, respectively).

In addition to age ($r = 0.66$, $P < 0.0001$), mean-IMT correlated significantly with TC ($r = 0.55$, $P = 0.0003$) and LDLc ($r = 0.54$, $P = 0.0003$), TG ($r = 0.35$, $P = 0.02$), and ApoB ($r = 0.49$, $P = 0.003$). A strong positive relationship was also found between mean-IMT and serum TSH levels ($r = 0.51$, $P = 0.0008$) (Fig. 2). The absolute mean-IMT reduction was directly related to the absolute decrements of both serum TC and TSH values ($r = 0.35$, $P = 0.02$ and $r = 0.61$, $P = 0.0001$, respectively).

Stepwise regression analysis

In the final model that explained 71% of the variation in mean-IMT ($r^2 = 0.71$, $P < 0.0001$), age had the highest stan-

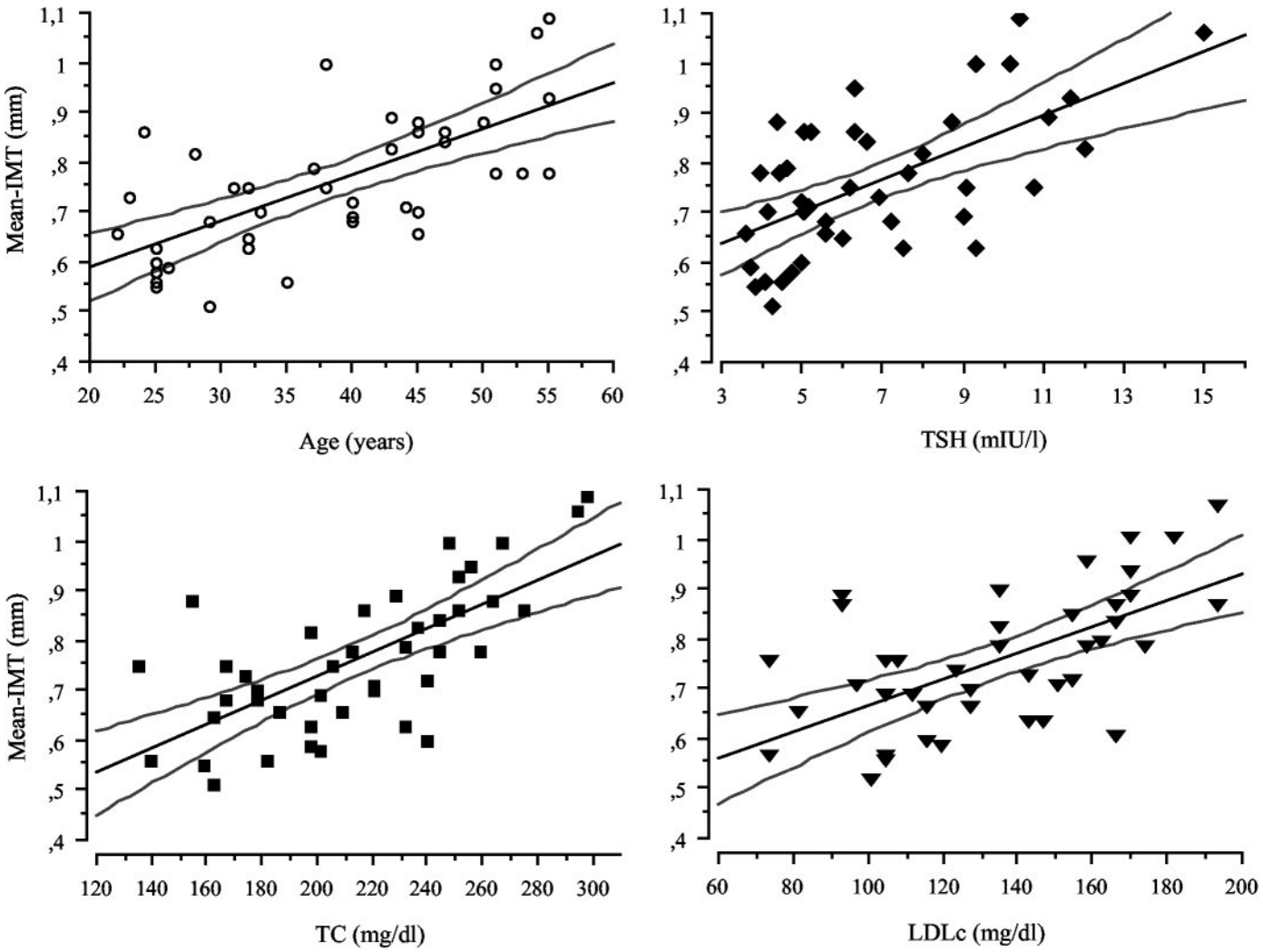

FIG. 2. Relationship between carotid artery mean-IMT and age, serum LDLc, TSH, and TC levels. The conversion factor for TC and LDLc was 0.02586.

dardized coefficient (0.46), followed by TSH (0.34) and LDLc (0.25), whereas the other parameters were excluded.

Discussion

To the best of our knowledge, this is the first study that examined the presence of early atherosclerotic vascular wall lesions by means of B-mode ultrasound measurement of IMT in sHT patients. To avoid possible confounding by factors independently associated with an increased carotid artery IMT, we focused on a selected series of patients with stable sHT who were younger than 55 yr and presented no other established risk factor for atherosclerosis.

Although consensus on the association between sHT and dyslipidemia is still lacking, most reports had documented a relationship between sHT and a reversible atherogenic lipoprotein profile (12). Two randomized, placebo-controlled studies recently demonstrated that in sHT serum LDL cholesterol levels are specifically and reversibly increased to an extent that may translate into a sizable cardiovascular risk (13, 34). The present study confirms this association and the efficacy of 6 months of stable euthyroidism in reducing both TC and LDLc (by 10 and 13%, respectively). It should be emphasized that the observed decrease in LDLc induced by L-T_4 replacement therapy is significant in terms of risk reduction for coronary heart disease (35). In fact, although comparable data are not available for premenopausal women, the Helsinki Heart Study has shown that in men a decrease by only 7% in serum LDLc levels is associated with a 15% reduction in the incidence of coronary heart disease (36). Because raised Lp(a) concentrations have been associated with the development of atherosclerosis and are viewed as a strong predictor of ischemic heart disease (37), attention has focused on serum Lp(a) levels in thyroid diseases. However, the relationship between hypothyroidism and Lp(a) levels and the effect of L-T_4 therapy are still under debate (38, 39). In agreement with the reported primary genetic determination of serum Lp(a) levels (40), we have recently shown that raised Lp(a) values are more frequent in sHT patients than controls, in association with a positive family history for coronary heart disease and/or diabetes mellitus (13). Accordingly, in the present study, elevated serum Lp(a)

concentrations were detected in 33% of sHT patients *vs.* 7% of euthyroid controls but were unaffected by L-T_4 replacement therapy. All in all, these and our previous findings suggest that altered Lp(a) values of sHT patients reflect a genetic influence rather than resulting from reduced thyroid hormone action; however, they may conspire with the atherogenic lipoprotein profile of untreated patients to enhance cardiovascular risk.

In recent years several novel risk factors for atherosclerotic cardiovascular disease have been identified, including hyperhomocystinemia. Several studies have demonstrated reversibly elevated homocysteine levels in overt hypothyroidism, with serum FT_4 being an independent determinant of homocysteine concentrations (14, 41). Furthermore, in adults with endemic goiter and raised TSH levels tHcy was shown to be significantly higher than in controls (42). Previous studies, however, failed to demonstrate an association between sHT and hyperhomocystinemia (1, 16). Accordingly, our results indicate that tHcy levels are not altered in sHT and are not changed by effective L-T_4 therapy. Besides renal function, plasma folic acid and vitamin B_{12} levels are known determinants of tHcy (16). Because vitamin B_{12} and folate deficiency are more frequently observed in overt than in subclinical hypothyroidism (43), it is possible that hyperhomocystinemia results from vitamin deficiency rather than thyroid hormone lack *per se*. Indeed, in the present study, the only two patients with elevated tHcy also had low plasma folate values and, after restoring euthyroidism the reduction of plasma homocysteine, was concomitant with an increment of folate levels.

The IMT measurement by carotid ultrasound represents a noninvasive, standardized, and validated approach for early atherosclerosis screening and provides a reliable surrogate end point to assess intervention (18–21, 44, 45). A thickening of carotid artery intima-media complex reflects not just regional alterations but also generalized atherosclerosis. In fact, a direct correlation between mean-IMT and the risk of myocardial infarction and stroke has been demonstrated (19). In the current study, the carotid artery mean-IMT was significantly higher in sHT patients than in euthyroid controls. As expected, mean-IMT correlated with most of lipid variables (TC, LDLc, ApoB, and TG) as well as with age. Interestingly, our data show a strong positive relationship between mean-IMT and serum TSH value, which emerged as one of the most important predictors of mean-IMT variation in stepwise regression analysis, preceded only by age and followed by LDLc. Furthermore, after 6 months of restored euthyroidism, carotid artery mean-IMT was reduced by almost 10%, and the absolute mean-IMT reduction was directly related to the absolute decrements of both serum TC and TSH values. Because no consensus exists on the issue of treatment of sHT when serum TSH level is slightly elevated (46), it is noteworthy that, in the current study, L-T_4 replacement therapy was effective in reducing mean-IMT also in the subgroup of sHT patients with serum TSH levels lower than 10 mIU/liter.

Although only a slight, not significant, difference in mean-IMT was observed, at baseline, between patients and controls younger than 35 yr, 6 months of stable euthyroidism induced a significant reduction of carotid artery mean-IMT in both the older (>35 yr) and younger group of sHT patients. Furthermore, mean-IMT became equivalent to that of euthyroid controls only in the latter group of patients. Strong evidence exists for the role of age as a major determinant of carotid IMT (21, 47). However, in addition to age, thickening of the arterial wall is also related to the cumulative exposure over time to various cardiovascular risk factors, such as hyperlipidemia and, possibly, hypothyroidism. Indeed, although the long-held association between hypothyroidism and hypercholesterolemia probably underlies much of the accelerated atherosclerotic disease described in patients with sHT (12, 13, 34), our data also suggest a direct effect of mild thyroid hormone deficiency. Accordingly, we recently demonstrated that in sHT, besides dyslipidemia, mild thyroid hormone deficiency *per se* is responsible for reversible endothelial dysfunction and reduced nitric oxide availability, which act as promoters of atherosclerosis (48).

The intimal and medial layers of the arterial wall cannot be distinguished from each other by ultrasound; thyroid hormones also exert a direct effect on the peripheral vasculature, which is best demonstrated by the reversible increase in systemic vascular resistance observed in humans and experimental animals with hypothyroidism (49). Accordingly, Mizuma *et al.* recently described the presence of an iodothyronine deiodinase in human vascular smooth muscle (VSM) cells (50). Although the target genes for T_3 action in VSM cells remain unknown, it could be speculated that, as described in cardiac myocytes, they are involved in the modulation of sarcoplasmic reticulum and sarcolemmal ion flux and VSM contractility (49). Indeed, the presence of an iodothyronine deiodinase suggests that VSM cells, which are in physiologic cross-talk with endothelium, may be a target for thyroid hormone action and lends support to the hypothesis of a direct involvement of thyroid hormone deficiency in IMT thickening. On the other hand, although significantly improved after restoration of euthyroidism, mean-IMT remained slightly higher in sHT patients than controls. This finding may be, at least in part, explained by the unchanged Lp(a) values; however, a possible additional role of autoimmunity and inflammation in the accelerated atherosclerotic process of sHT patients should not be overlooked and may account for the residual intima-media thickening (16).

Overall, our data indicate that early carotid artery wall lesions, as detected by B-mode ultrasound, are present in sHT patients mainly from the fourth decade of life onward. Whether such IMT increase is related to an early atherosclerotic involvement of the arterial wall cannot be clearly decided on the basis of the present results. However, the fact that L-T_4 replacement therapy was able to improve both the atherogenic lipoprotein profile and intima-media thickening suggests that lipid infiltration of arterial wall may represent a major mechanism underlying IMT increase in this selected subset of patients. Nonetheless, other pathologic processes can theoretically contribute to IMT elevation, namely VSM cells swelling and water accumulation in the arterial wall due to protein deposition in extracellular space as well as an increased capillary permeability (51). Advances in tissue characterization techniques by ultrasound may allow in the near future to get information, from quantitative acoustic

densitometry of images, on at least some of pathologic changes of the arterial wall.

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References

1. **Cappola AR, Ladenson PW** 2003 Hypothyroidism and atherosclerosis. J Clin Endocrinol Metab 88:2438–2444
2. **Surks MI, Ocampo E** 1996 Subclinical thyroid disease. Am J Med 100:217–223
3. **Canaris GJ, Manowitz NR, Mayor G, Ridgway EC** 2000 The Colorado thyroid disease prevalence study. Arch Intern Med 160:526–534
4. **Bastenie PA, Vanhaelst L, Neve P** 1967 Coronary artery disease in hypothyroidism: observation in preclinical myxoedema. Lancet 2:1221–1222
5. **Bastenie PA, Vanhaelst L, Bonnyns M, Neve P, Staquet M** 1971 Preclinical hypothyroidism: a risk factor for coronary heart disease. Lancet 1:203–204
6. **Fowler PBS, Swale J, Andrews H** 1970 Hypercholesterolaemia in borderline hypothyroidism: stage of premyxoedema. Lancet 2:488–491
7. **Hak AE, Pols HAP, Visser TJ, Drexhage HA, Hofman A, Witteman JCM** 2000 Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam study. Ann Intern Med 132:270–278
8. **Tunbridge WMG, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA** 1977 The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol (Oxf) 7:481–493
9. **Ladenson PW, Wilson MC, Gardin J, Kronmal R, Kuller L, Tracy R, Burke G, Fried LP** 1994 Relationship of subclinical hypothyroidism to cardiovascular risk factors and disease in an elderly population. Thyroid 4:S-18
10. **Geul KW, van Sluisveld IL, Grobbee DE, Docter R, de Bruyn AM, Hooykaas H, van der Merwe JP, van Hemert AM, Krenning EP, Hennemann G** 1993 The importance of thyroid microsomal antibodies in the development of elevated serum TSH in middle-aged women: association with serum lipids. Clin Endocrinol (Oxf) 39:275–280
11. **Parle JV, Franklin JA, Cross KW, Jones SR, Sheppard MC** 1992 Circulating lipids and minor abnormalities of thyroid function. Clin Endocrinol (Oxf) 37:411–414
12. **Danese MD, Ladenson PW, Meinert CL, Powe NR** 2000 Effect of thyroxine therapy on serum lipoproteins in patients with mild thyroid failure: a quantitative review of the literature. J Clin Endocrinol Metab 85:2993–3001
13. **Caraccio N, Ferrannini E, Monzani F** 2002 Lipoprotein profile in subclinical hypothyroidism: effect of levothyroxine replacement therapy. A randomized, placebo-controlled study. J Clin Endocrinol Metab 87:1533–1538
14. **Hussein WI, Green R, Jacobsen DW, Faiman C** 1999 Normalization of hyperhomocysteinemia with L-thyroxine in hypothyroidism. Ann Intern Med 131:348–351
15. **Lien EA, Nedrebo BG, Varhaug JE, Nygard O, Aakvaag A, Ueland PM** 2000 Plasma total homocysteine levels during short-term iatrogenic hypothyroidism. J Clin Endocrinol Metab 85:1049–1053
16. **Christ-Crain M, Meier C, Guglielmetti M, Huber PR, Riesen W, Staub JJ, Muller B** 2003 Elevated C-reactive protein and homocysteine values: cardiovascular risk factors in hypothyroidism? A cross-sectional and a double-blind, placebo-controlled trial. Atherosclerosis 166:379–386
17. **Luboshitzky R, Aviv A, Herer P, Lavie L** 2002 Risk factors for cardiovascular disease in women with subclinical hypothyroidism. Thyroid 12:421–425
18. **Howard G, Sharrett AR, Heiss G, Evans GW, Chambless LE, Riley WA, Burke GL** 1993 Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. Stroke 24:1297–1304
19. **O'Leary DH, Polak JF** 2002 Intima-media thickness: a tool for atherosclerosis imaging and event prediction. Am J Cardiol 90:18L–21L
20. **Allan PL, Mowbray PI, Lee AJ, Fowkes FG** 1997 Relationship between carotid intima-media thickness and symptomatic and asymptomatic peripheral arterial disease. The Edinburgh Artery Study. Stroke 28:348–353
21. **Salonen R, Salonen JT** 1991 Determinants of carotid intima-media thickness: a population-based ultrasonography study in eastern Finnish men. J Intern Med 229:225–231
22. **Gnasso A, Pujia A, Irace C, Mattioli PL** 1995 Increased carotid arterial wall thickness in common hyperlipidemia. Coron Artery Dis 6:57–63
23. **Gnasso A, Irace C, Mattioli PL, Pujia A** 1996 Carotid intima-media thickness and coronary heart disease risk factors. Atherosclerosis 119:7–15
24. **Wendelhag I, Wiklund O, Wikstrand J** 1992 Arterial wall thickness in familial hypercholesterolemia. Ultrasound measurement of intima-media thickness in the common carotid artery. Arterioscler Thromb 12:70–77
25. **Bots ML, Hofman A, de Bruyn AM, de Jong PT, Grobbee DE** 1993 Isolated systolic hypertension and vessel wall thickness of the carotid artery. The Rotterdam Elderly Study. Arterioscler Thromb 13:64–69
26. **Kawamori R, Yamasaki Y, Matsushima H, Nishizawa H, Nao K, Hougaku H, Maeda H, Handa N, Matsumoto M, Kamada T** 1992 Prevalence of carotid atherosclerosis in diabetic patients. Ultrasound high-resolution B-mode imaging on carotid arteries. Diabetes Care 15:1290–1294
27. **Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE** 1997 Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 96:1432–1437
28. **Mannami T, Konishi M, Baba S, Nishi N, Terao A** 1997 Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: the Suita study. Stroke 28:518–525
29. **Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G** 1995 Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke 26:386–391
30. **Nagasaki T, Inaba M, Henmi Y, Kumeda Y, Ueda M, Tahara H, Sugiguchi S, Fujiwara S, Emoto M, Ishimura E, Onoda N, Ishikawa T, Nishizawa Y** 2003 Decrease in carotid intima-media thickness in hypothyroid patients after normalization of thyroid function Clin Endocrinol (Oxf) 59:607–612
31. **Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Azola C** 1991 Carotid atherosclerosis measured by B-mode ultrasound in populations: association with cardiovascular risk factor in the ARIC study. Am J Epidemiol 134:250–256
32. **Mazzone AM, Urbani MP, Picano E, Paterni M, Borgatti E, De Fabritiis A, Landini L** 1995 *In vivo* ultrasound parametric imaging of carotid atherosclerotic plaque by videodensitometric technique. Angiology 46:663–672
33. **Ubbink JB, Vermaak WJH, Bissbort S** 1991 Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. J Chromatogr 565:441–446
34. **Meier C, Staub JJ, Roth CB, Guglielmetti M, Kunz M, Miserez AR, Drewe J, Huber P, Herzog R, Muller B** 2001 TSH-controlled L-thyroxine therapy reduces cholesterol levels and clinical symptoms in subclinical hypothyroidism: a double blind, placebo-controlled trial (Basel Thyroid Study). J Clin Endocrinol Metab 86:4860–4866
35. **Law MR, Wald NJ, Thompson SG** 1994 By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischemic heart disease? Br Med J 308:367–373
36. **Manninen V, Elo MO, Frick MH, Haapa K, Heinonen OP, Heinsalmi P, Helo P, Huttunen JK, Kaitaniemi P, Koskinen P, Maenpaa H, Malkonen M, Manttari M, Norola S, Pasternack A, Pikkarainen J, Romo M, Sjoblom T, Nikkila EA** 1988 Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study. JAMA 260:641–651
37. **Bostom AG, Cupples LA, Jenner JL, Ordovas JM, Seman LJ, Wilson PW, Schaefer EJ, Castelli WP** 1996 Elevated plasma lipoprotein (a) and premature coronary heart disease in Framingham men: a prospective study. JAMA 276:544–548
38. **Tzotzas T, Krassas GE, Konstantinidis T, Bougoulia M** 2000 Changes in lipoprotein (a) levels in overt and subclinical hypothyroidism before and during treatment. Thyroid 10:803–808
39. **Milionis HJ, Efstathiadou Z, Tselepis AD, Bairaktari E, Tsironis LD, Tsatsoulis A, Elisaf M** 2003 Lipoprotein (a) levels and apolipoprotein (a) isoform size in patients with subclinical hypothyroidism: effect of treatment with levothyroxine. Thyroid 13:365–369
40. **Boerwinkel E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH** 1992 Apolipoprotein (a) gene accounts for greater than 90% of the variation in plasma concentrations. J Clin Invest 90:52–60
41. **Diekman MJ, van der Put NM, Blom HJ, Tijssen JG, Wiersinga WM** 2001 Determinants of changes in plasma homocysteine in hyperthyroidism and hypothyroidism. Clin Endocrinol (Oxf) 54:197–204
42. **Ingenbleek Y, Barklay B, Dirren H** 1986 Nutritional significance of alterations in serum amino acid patterns in goitrous patients. Am J Clin Nutr 43:310–312
43. **Marqusee E, Mandel SJ** 2000 The blood in hypothyroidism. In: Braverman LE, Utiger RD, eds. The thyroid. 8th ed. Philadelphia: Lippincott Williams, Wilkins; 800–801
44. **Salonen RM, Nyyssonen K, Kaikkonen J, Porkkala-Sarataho E, Voutilainen S, Rissanen TH, Tuomainen TP, Valkonen VP, Ristonmaa U, Lakka HM, Vanharanta M, Salonen JT, Poulsen HE** 2003 Six-year effect of combined

vitamin C and E supplementation on atherosclerotic progression: the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study. Circulation 107:947–953
45. **Mithat E, Hakan I, Koray E** 2003 Effect of tibolone treatment on intima-media thickness and the resistive indices of the carotid arteries. Fertil Steril 79:268–273
46. **Vanderpump M** 2003 Subclinical hypothyroidism: the case against treatment. Trends Endocrinol Metab 14:262–266
47. **Zanchetti A, Bond MG, Hennig M, Neiss A, Mancia G, Dal Palu C, Hansson L, Magnani B, Rahn KH, Reid J, Rodicio J, Safar M, Eckes L, Ravinetto R** 1998 Risk factors associated with alterations in carotid intima-media thickness in hypertension: baseline data from the European Lacidipine Study on Atherosclerosis. J Hypertens 16:949–961
48. **Taddei S, Caraccio N, Virdis A, Dardano A, Versari D, Ghiadoni L, Salvetti A, Ferrannini E, Monzani F** 2003 Impaired endothelium-dependent vasodilation in subclinical hypothyroidism: beneficial effect of levothyroxine therapy. J Clin Endocrinol Metab 88:3731–3737
49. **Klein I, Ojamaa K** 2001 Thyroid hormone and the cardiovascular system. N Engl J Med 344:501–509
50. **Mizuma H, Murakami M, Mori M** 2001 Thyroid hormone activation in human vascular smooth muscle cells: expression of type II iodothyronine deiodinase. Circ Res 88:313–318
51. **Parving HH, Hansen JM, Neilsen SL, Rossing N, Munck O, Lassen NA** 1979 Mechanisms of oedema formation in myxedema: increased protein extravasation and relatively slow lymphatic drainage. N Engl J Med 301:460–466

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