Effects of L-thyroxine treatment on early markers of atherosclerotic disease in children with subclinical hypothyroidism

Manuela Cerbone¹, Donatella Capalbo¹, Malgorzata Wasniewska², Sara Alfano¹, Giuseppina Mattace Raso³, Ugo Oliviero⁴, Antonio Cittadini⁴, Filippo De Luca² and Mariacarla Salerno¹

¹Pediatric Endocrinology Unit, Department of Translational Medical Sciences, University "Federico II" of Naples, Naples, Italy, ²Department of Pediatric, Gynecological, Microbiological and Biomedical Sciences, University of Messina, Messina, Italy, ³Department of Pharmacy and ⁴Internal Medicine Section, Department of Translational Medical Sciences, University "Federico II" of Naples, Naples, Italy

Correspondence should be addressed to M Salerno
Email
salerno@unina.it

Abstract

Objective: To investigate the effect of levothyroxine (L-T4) treatment on early markers of atherosclerotic disease in children with mild idiopathic subclinical hypothyroidism (SH).

Design: Two-year, open, case-control prospective study.

Methods: A total of 39 children, aged 9.18 ± 3.56 years, with SH and 39 healthy controls were enrolled in the study. Waist-to-height ratio (WHtR), blood pressure, triglycerides, total cholesterol (total-C), HDL-C, LDL-C, non-HDL-C, triglycerides/HDL-C, atherogenic index (AI), homocysteine (Hcy), asymmetric dimethylarginine (ADMA), flow-mediated dilation (FMD) and intima-media thickness (IMT) were evaluated at baseline and after 2 years of L-T4 treatment in SH children and after 2 years of follow-up in controls.

Results: At study entry WHtR was higher in SH subjects compared with controls (0.56 ± 0.08 vs 0.49 ± 0.07 , $P=0.04$) and significantly decreased after 2 years of treatment (0.50 ± 0.06 , $P<0.0001$). Mean HDL-C levels (50.47 ± 11.43 vs 61.06 ± 13.83 mg/dL, $P=0.002$) were lower, while triglycerides/HDL-C (1.63 ± 1.07 vs 1.19 ± 0.69 , $P=0.05$), AI (3.32 ± 0.90 vs 2.78 ± 0.68 , $P=0.005$), and Hcy (9.35 ± 2.61 vs 7.71 ± 1.94 μ mol/L, $P=0.01$) were higher in SH subjects compared with controls and improved after 2 years of treatment (HDL-C 56.26 ± 13.76 mg/dL, $P<0.0001$; triglycerides/HDL-C 1.23 ± 0.78 , $P=0.006$; AI 2.82 ± 0.68 , $P<0.0001$; and Hcy 8.25 ± 2.09 μ mol/L, $P=0.06$). ADMA concentrations at baseline were higher in SH subjects compared with controls (0.77 ± 0.21 vs 0.60 ± 0.16 μ mol/L, $P=0.001$) and decreased after therapy (0.58 ± 0.13 μ mol/L, $P<0.0001$). FMD, IMT and other metabolic parameters were not different among SH subjects and controls at baseline and after 2 years.

Conclusions: Children with SH may have subtle pro-atherogenic abnormalities. Although L-T4 treatment exerts some beneficial effects, the long-term impact of therapy on metabolic outcomes in SH children still remains unclear.

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Introduction

Subclinical hypothyroidism (SH) is a biochemical condition characterized by increased thyroid stimulating hormone (TSH) levels, with normal free T4 (FT4) concentrations (1). In adults SH is common with a prevalence of between 4 and 20% and a tendency to progress to overt

hypothyroidism (1). In children SH seems to be a benign and remitting condition (2, 3, 4) with a prevalence of less than 2% (5) and a low risk to progress to overt hypothyroidism, particularly in children with its mild and idiopathic form (2, 3, 4, 6).

Thyroid hormone plays a key role in the working of the cardiovascular (CV) system, with direct effects on cardiac function and vascular system and on several atherosclerotic factors (7). Indeed, SH in adults has been associated with dyslipidemia (8, 9), insulin resistance (10), and endothelial dysfunction (1, 11, 12). Coronary heart disease (CHD) and heart failure seem to occur more frequently in subjects with SH, particularly for TSH levels $>10\text{mU/L}$ (13, 14, 15) even though a recent systematic review suggests an increased stroke risk in SH subjects under the age of 50, with a higher risk of fatal stroke even for TSH levels between 7.0 and 9.9 mU/L (16).

Current recommendations from the American Thyroid Association suggest the start of levothyroxine (L-T4) treatment in adults with TSH levels between 4.5 and 10 mU/L in the presence of symptoms of hypothyroidism or positive thyroid antibodies or evidence of atherosclerotic CV disease (17). A recent systematic review on adults with mild to severe SH did not conclude for an improvement in survival or CV morbidity following L-T4 therapy; however, some beneficial effects of treatment on lipid profiles and left ventricular function were observed (18).

Health outcomes in children with untreated SH and the effects of L-T4 treatment have been less extensively evaluated; in particular, studies on the effects of mild SH on CV risk profile in children are still scanty. However, in a previous study, we documented slight alterations in visceral adiposity, lipid profile and homocysteine (Hcy) levels in a cohort of 49 prepubertal children with untreated and idiopathic SH (19). Whether this cluster of subtle pro-atherogenic abnormalities results in adverse CV outcomes in adulthood needs to be clarified. Understanding the clinical relevance of SH and L-T4 therapy on CV morbidity in SH children may help in driving the management of these subjects and in defining recommendations for their treatment. Indeed, current recommendations suggest L-T4 treatment only in children with TSH levels $>10\text{mU/L}$, whereas the management of mild idiopathic SH is still a matter of debate (1, 3, 20, 21, 22).

Only few studies have evaluated the effect of short-term trials with L-T4 in heterogeneous populations of children with mild to severe SH (5, 20, 23), and none of them have focused on the relationship between SH and metabolic aspects.

We designed this open, case-control prospective study in order to investigate the effects of 2 years of L-T4 treatment on clinical and biochemical CV risk factors in a selected cohort of children with mild idiopathic SH.

Subjects and methods

Subjects and controls

A total of 39 children (19 males), aged 9.18 ± 3.56 years, with untreated and idiopathic SH from two Italian pediatric endocrine units were enrolled in the study. These patients were a subset of the cohort of 49 SH children previously described (19).

Inclusion criteria were the following: (a) TSH values $>4.5\text{mU/L}$ and $<10\text{mU/L}$ with FT4 values within the reference range persisting for at least 24 months during the period preceding the study; (b) absence of anti-thyroglobulin (Tg-Ab) and anti-thyroperoxidase (TPO-Ab) antibodies and normal thyroid echogenicity on ultrasound; (c) absence of palpable goiter or symptoms related to hypothyroidism from the time of SH identification to the beginning of the study; and (d) adequate iodine urinary excretion.

Chronic diseases, chromosomal and genetic syndromes, previous or current thyroid diseases, use of drugs that may interfere with thyroid function, previous irradiation in the neck region, detection of SH at neonatal screening, and familial history of genetic lipid disorders or early CV diseases were considered exclusion criteria.

A total of 39 healthy euthyroid children, comparable for age, sex, height, and pubertal status, were enrolled in the study as controls.

All subjects were prepubertal at baseline; during the study 9 SH subjects and 9 controls attained puberty.

Informed consent was obtained from all parents, and the study was approved by the ethics committees of the institutions.

Study protocol

At study entry all SH subjects and controls underwent clinical evaluation through measurement of height, weight, waist circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP). The degree of overall adiposity was expressed as body mass index (BMI) and calculated as weight (kg)/height (m)². Height and BMI were normalized by age and sex in accordance with Italian standards and expressed as standard deviation score (SDS) (24).

Waist circumference was measured, by the same operator for each center, using a flexible steel tape while the subjects were standing, after gently exhaling, as the minimal circumference measurable on the horizontal plane between the lowest portion of the rib cage and the

iliac crest. Waist-to-height ratio (WHtR) was calculated and used as an indicator of abdominal adiposity (25).

SBP and DBP were measured at the right arm by a cuff manometer after 5 min of supine rest, according to the fourth report on National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (26).

Biochemical evaluation was performed at 0800 h after an overnight fast, by measurement of TSH, FT4, Tg-Ab and TPO-Ab, triglycerides, total cholesterol (total-C), HDL-C, glucose, insulin, and Hcy. LDL-C was calculated using the Friedewald equation. Moreover, non-HDL-C (total-C-HDL-C), atherogenic index (AI) (total-C/HDL-C ratio) and triglycerides/HDL-C ratio were calculated as predictors of CV disease (27, 28, 29).

The degree of insulin resistance was evaluated using the homeostasis model assessment (HOMA), by applying the formula: fasting serum insulin (mU/L) $\times$ fasting plasma glucose (mmol/L)/22.5 (30).

In addition to our previous evaluation (19), all subjects underwent evaluation of endothelial function by assessment of brachial flow-mediated dilation (FMD) and carotid intima-media thickness (IMT) and evaluation of serum levels of asymmetric dimethylarginine (ADMA) which represents a novel biomarker of endothelial dysfunction (31).

A 2-year L-T4 treatment was started in SH subjects at an initial dose of 50 μ g/day and then adjusted to keep both TSH and FT4 serum levels within the reference range.

All these measurements and evaluations were re-assessed in SH subjects after 2 years of L-T4 treatment and in controls after 2 years of follow-up.

Biochemical assays

TSH and FT4 dosages were measured by electrochemiluminescence immunoassay (ECLIA) using a commercial kit (Roche Diagnostics) (normal values: TSH: 0.3–4.2 mU/L, FT4: 0.8–1.7 pg/mL, Tg-Ab: 0–115 mU/L, TPO-Ab: 0–34 mU/L). Urine iodine levels were analyzed using an automated system (Autoanalyzer 3 system, Bran+Luebbe GmbH, Nordestedt, Germany).

Serum triglycerides, total-C, and HDL-C were determined by an enzymatic in vitro test using Roche automated clinical chemistry analyzers. Hcy levels were determined by a Fluorescence Polarization Immunoassay (Abbott); inter- and intra-assay coefficients of variance were 2 and 1%, respectively, and sensitivity was 0.8 μ mol/L.

ADMA was measured using ELISA (DLD, Diagnostika GMBH) (reference range 0.4–0.75 μ mol/L). The inter- and intra-assay coefficients of variations were 9.4 and 6.05%, respectively, and sensitivity was 0.05 μ mol/L.

Flow-mediated dilation and brachial artery distensibility

FMD was obtained by a single investigator for each center. All measurements were done after 8-h fasting

Table 1 Clinical details, thyroid hormones, and biochemical parameters in children with subclinical hypothyroidism (SH) before and after 2 years of L-T4 treatment compared with controls (ANCOVA after correction for BMI). Data are expressed as mean $\pm$ s.d.

	Baseline			2 years after L-T4 treatment		
	SH subjects	Controls	P	SH subjects	Controls	P
Chronological age (years)	9.18 $\pm$ 3.56	9.45 $\pm$ 3.62	NS	11.25 $\pm$ 3.57	11.42 $\pm$ 3.59	NS
TSH (mU/L)	6.30 $\pm$ 1.01	2.92 $\pm$ 0.68	0.003	2.82 $\pm$ 1.31	2.53 $\pm$ 0.67	NS
FT4 (pg/mL)	1.32 $\pm$ 0.18	1.26 $\pm$ 0.14	NS	1.37 $\pm$ 0.22	1.20 $\pm$ 0.15	<0.001
Height (SDS)	−0.65 $\pm$ 1.32	−0.47 $\pm$ 1.24	NS	−0.60 $\pm$ 1.31	−0.40 $\pm$ 1.47	NS
BMI (SDS)	0.31 $\pm$ 1.08	−0.10 $\pm$ 1.21	NS	0.49 $\pm$ 1.38	−0.09 $\pm$ 1.32	NS
Systolic BP (mmHg)	101.33 $\pm$ 10.82	102.36 $\pm$ 10.79	NS	102.36 $\pm$ 10.58	105.67 $\pm$ 11.33	NS
Diastolic BP (mmHg)	64.98 $\pm$ 9.09	66.50 $\pm$ 9.98	NS	64.54 $\pm$ 7.60	63.72 $\pm$ 8.02	NS
Waist to height ratio	0.56 $\pm$ 0.08	0.49 $\pm$ 0.07	0.04	0.50 $\pm$ 0.06	0.48 $\pm$ 0.05	NS
Triglycerides (mg/dL)	74.56 $\pm$ 39.35	65.32 $\pm$ 24.21	NS	63.18 $\pm$ 30.30	58.28 $\pm$ 19.92	NS
Total cholesterol (mg/dL)	159.72 $\pm$ 23.36	160.40 $\pm$ 21.20	NS	152.13 $\pm$ 26.59	154.40 $\pm$ 17.07	NS
HDL cholesterol (mg/dL)	50.47 $\pm$ 11.43	61.06 $\pm$ 13.83	0.002	56.26 $\pm$ 13.76	57.69 $\pm$ 11.20	NS
LDL cholesterol (mg/dL)	94.33 $\pm$ 22.25	87.19 $\pm$ 19.43	NS	83.23 $\pm$ 25.43	86.54 $\pm$ 18.01	NS
Non-HDL cholesterol (mg/dL)	109.24 $\pm$ 24.91	100.60 $\pm$ 20.95	NS	95.87 $\pm$ 27.30	98.34 $\pm$ 18.64	NS
Triglycerides/HDL cholesterol	1.63 $\pm$ 1.07	1.19 $\pm$ 0.69	0.05	1.23 $\pm$ 0.78	1.11 $\pm$ 0.52	NS
Atherogenic index	3.32 $\pm$ 0.90	2.78 $\pm$ 0.68	0.005	2.82 $\pm$ 0.68	2.77 $\pm$ 0.56	NS
Homocysteine (μ mol/L)	9.35 $\pm$ 2.61	7.71 $\pm$ 1.94	0.01	8.25 $\pm$ 2.09	8.37 $\pm$ 1.92	NS
Glucose (mg/dL)	77.35 $\pm$ 8.63	77.30 $\pm$ 8.62	NS	78.64 $\pm$ 8.31	77.97 $\pm$ 8.04	NS
Insulin (mU/L)	7.01 $\pm$ 4.25	5.68 $\pm$ 3.70	NS	6.92 $\pm$ 5.38	5.85 $\pm$ 2.94	NS
HOMA index	1.38 $\pm$ 0.90	1.09 $\pm$ 0.70	NS	1.40 $\pm$ 1.05	1.15 $\pm$ 0.60	NS
ADMA (μ mol/L)	0.77 $\pm$ 0.21	0.60 $\pm$ 0.16	0.001	0.58 $\pm$ 0.13	0.61 $\pm$ 0.15	NS

Figure 1

Waist-to-height ratio, HDL-C, triglycerides/HDL-C ratio, atherogenic index, homocysteine, and ADMA at baseline and after 2 years of L-T4 treatment in subjects with subclinical hypothyroidism (SH). * $P < 0.05$ comparing SH subjects at baseline with SH subjects after 2 years of therapy (see text for details on P values). The boxes show medians and 25th and 75th percentiles, and the whiskers represent the 5th and the 95th percentiles.

with subjects in supine position in a quiet, temperature-controlled room. Brachial artery reactivity was evaluated in each subject using validated protocol (32), with a 7.5 MHz multifrequency linear array probe (Aplio XG Imaging System, Toshiba). Electrocardiographic leads were connected and a sphygmomanometer cuff was placed on the right arm. The brachial artery was imaged 2–5 cm proximal to the antecubital crease in a longitudinal axis,

and the brachial artery diameter, from the intima–lumen interface on the near wall to the media–adventitia interface on the far wall, was measured at end-diastole cycle, on the electrocardiographic R-wave. Endothelium-dependent vasodilatation was assessed by measuring the maximum increase in brachial artery diameter during reactive hyperemia created by the inflation of the cuff (250 mmHg for 5 min) placed on the right arm. After sudden cuff deflation, flow velocity indexes were measured in the first 15 s; then brachial artery diameter was measured at least four times during the next 90 s. FMD resulted from the formula: ((post-hyperemia diameter – baseline diameter)/baseline diameter) $\times 100$.

The coefficient of distensibility (DC) of the brachial artery was assessed for the evaluation of arterial stiffness. The mean of three consecutive measurements was used in the analyses as recommended. Vessel diameters were measured in M-mode: the lowest end-diastolic arterial diameter (D_d) on the electrocardiographic R-wave, the highest end-systolic arterial diameter (D_s) on the electrocardiographic T-wave, and the diameter change during cardiac cycle (ΔD , defined as $D_s - D_d$). Brachial artery pulse pressure (ΔP), defined as “SBP minus DBP,” was measured by a sphygmomanometer and expressed in kPa. Finally, DC was calculated as $(2\Delta D/D_d)/\Delta P$ ($\text{kPa}^{-1} \times 10^{-3}$) (33).

Intima–media thickness

Carotid ultrasound examination was performed in each subject, by experienced vascular sonographers, with a 7.5 MHz multifrequency linear array probe. Briefly, ultrasound examination was made with the subject in a supine position, with a slight rotation of the neck. The probe was placed along the vessel axis, and carotid arteries were explored with longitudinal (anterior, lateral, and posterior) and transverse scans. Multiple long and short axis sections were employed, with special attention paid to defining the endothelial border from the origin of

Table 2 Ultrasound markers of endothelial dysfunction in children with subclinical hypothyroidism (SH) before and after L-T4 treatment compared with controls (ANCOVA after correction for BMI). Data are presented as mean $\pm$ s.d.

	Baseline			2 years after L-T4 treatment		
	SH subjects	Controls	P	SH subjects	Controls	P
IMT common carotid artery (mm)	0.44 ± 0.08	0.44 ± 0.06	NS	0.46 ± 0.07	0.46 ± 0.06	NS
IMT internal carotid artery (mm)	0.41 ± 0.09	0.41 ± 0.07	NS	0.39 ± 0.07	0.39 ± 0.07	NS
FMD (%)	12.64 ± 4.40	13.22 ± 3.96	NS	13.90 ± 4.92	11.85 ± 4.53	NS
CD	9.42 ± 3.89	8.80 ± 2.74	NS	8.89 ± 3.79	9.78 ± 4.59	NS

IMT, intima-media thickness; FMD, flow mediated dilation; CD, coefficient of distensibility.

common artery beyond the carotid artery bifurcation. On each side, IMT was measured at the bifurcation and at its proximal and distal segments, 1 cm before the bifurcation, on the echographic posterior wall of the common carotid artery, and 1 cm after the bifurcation, on the echographic posterior wall of the internal carotid artery always in the longitudinal scan.

Statistical analysis

Statistical analysis was performed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). Data are presented as mean $\pm$ S.D. or 95% CI. Differences between groups were evaluated by linear models after adjustment for BMI (ANCOVA). Changes after L-T4 therapy in SH patients were evaluated using *T*-test for paired samples. Differences in changes from baseline over the 2 years of the study in SH patients compared with healthy controls were evaluated through linear model entering BMI and basal values of each parameter as covariates (ANCOVA). Pearson's correlation coefficient was used to evaluate the relationship between variables. Significance was set at 5%.

Results

Clinical details, thyroid hormones and biochemical parameters in SH subjects and controls at baseline and after 2 years of treatment (in SH subjects) or follow-up

(in control group) are reported in Table 1. As expected, at study entry TSH levels were significantly higher in SH subjects and became comparable to controls after 2 years of L-T4 treatment. FT4 concentrations were similar in SH children and controls at baseline and became higher than controls in SH subjects after 2 years of treatment, still remaining within the normal range.

No significant differences were observed in height SDS between patients and controls, while BMI SDS was slightly higher in SH children even though this difference did not reach statistical significance at baseline or after 2 years.

SBP and DBP were comparable between the two groups at baseline and did not change during the study.

WHtR was significantly higher in SH subjects and significantly decreased after 2 years of treatment ($P < 0.0001$) (Fig. 1) becoming comparable to controls (Table 1).

Serum triglycerides, total-C, LDL-C, and non-HDL-C concentrations were comparable to controls at baseline and at the end of the study (Table 1). HDL-C levels were significantly lower whereas triglycerides/HDL-C ratio, AI, Hcy, and ADMA were significantly higher in SH subjects compared with controls at baseline. After 2 years of L-T4 therapy no more differences were observed between patients and controls in these parameters (Table 1). Indeed, a significant improvement was observed after L-T4 therapy with the exception of Hcy, which reduced without reaching statistical significance ($P < 0.0001$, $P = 0.006$, $P < 0.0001$, $P = 0.06$, $P < 0.0001$ respectively) (Fig. 1).

Table 3 Changes from baseline over 2 years of L-T4 therapy in patients with SH compared with controls (ANCOVA after correction for BMI and basal values). Data are presented as mean (95% CI).

	SH	Controls	P
WHtR	-0.05 (-0.06, -0.04)	-0.02 (-0.03, -0.01)	0.006
Triglycerides (mg/dL)	-9.1 (-16.3, -1.9)	-10.3 (-17.7, -2.9)	NS
Total cholesterol (mg/dL)	-7.7 (-13.7, -1.8)	-5.3 (-11.2, 0.6)	NS
HDL-C (mg/dL)	2.88 (-0.85, 6.62)	0.12 (-3.61, 3.86)	NS
LDL-C (mg/dL)	-9.3 (-15.5, -3.1)	-3.5 (-9.7, 2.7)	NS
Non-HDL-C (mg/dL)	-11.4 (-17.6, -5.1)	-4.7 (-11.0, 1.5)	NS
Triglycerides/HDL-C	-0.26 (-0.44, -0.07)	-0.21 (-0.41, -0.03)	NS
AI	-0.35 (-0.52, -0.17)	-0.15 (-0.33, 0.01)	NS
Hcy (μ mol/L)	-0.34 (-1.07, 0.38)	0.02 (-0.66, 0.72)	NS
Glucose (mg/dL)	2.1 (-0.5, 4.6)	0.6 (-1.8, 3.0)	NS
Insulin (mU/L)	0.47 (-0.81, 1.75)	-0.05 (-1.26, 1.15)	NS
HOMA index	0.118 (-0.134, 0.370)	-0.003 (-0.241, 0.236)	NS
ADMA (μ mol/L)	-0.144 (-0.186, -0.103)	-0.035 (-0.079, 0.004)	0.001
IMT common carotid artery (mm)	0.026 (0.004, 0.047)	0.014 (-0.008, 0.036)	NS
IMT internal carotid artery (mm)	-0.025 (-0.042, -0.008)	-0.015 (-0.032, 0.002)	NS
FMD (%)	1.04 (-0.41, 2.51)	-1.15 (-2.62, 0.33)	NS
CD	-0.22 (-1.65, 1.22)	0.73 (-0.75, 2.21)	NS

IMT, intima-media thickness; FMD, flow mediated dilation; CD, coefficient of distensibility.

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No differences were observed with regard to glucose, insulin, and HOMA levels between patients and controls throughout the course of the study (Table 1).

No significant differences were observed between SH subjects and controls in IMT, FMD and DC values at baseline and after 2 years of L-T4 treatment (Table 2).

Correlation studies revealed that changes in AI ($r=0.35$, $P=0.03$), ADMA ($r=0.40$, $P=0.02$) and HDL-C ($r=-0.38$, $P=0.02$) significantly correlated with changes in TSH levels during therapy, while changes in WHtR were significantly related with changes in FT4 levels ($r=-0.34$, $P=0.04$).

However, when changes from baseline between SH children and controls over the 2 years of study were compared through ANCOVA analysis (Table 3), only changes in WHtR and ADMA during therapy were deemed significant, while for other parameters the difference did not reach statistical significance.

None of our SH subjects developed adverse effects related to L-T4 treatment.

Discussion

To our knowledge, this is the first study evaluating the effect of 2 years of L-T4 treatment on early CV risk factors and vascular function in children with mild idiopathic SH. While there is general agreement in recommending L-T4 treatment for TSH levels >10 mU/L, the management of the mild form of SH is still debated. Although there are not sufficient data for a beneficial effect of L-T4 replacement on CV morbidity, data in SH adults suggest that L-T4 therapy improves lipid profile and left ventricular function (18).

The results of this case-control prospective study, in addition to confirming our previous findings that untreated SH in children is associated with a cluster of subtle pro-atherogenic abnormalities (19), documented an effect of L-T4 treatment on metabolic parameters.

Another novelty of our study is represented by the evaluation of precocious indices of vascular atherosclerosis in SH children before and after treatment. Even though no abnormalities were detected in vascular status, evaluated through FMD and IMT, ADMA concentrations, an early marker of endothelial dysfunction, were significantly increased at study entry in SH subjects and normalized after 2 years of L-T4 treatment, suggesting that mild thyroid abnormalities may be associated with early changes in pro-atherogenic profile.

ADMA is an amino acid derived from degradation of methylated proteins acting as an endogenous

competitive inhibitor of endothelial nitric oxide (NO) synthase (31). NO is an anti-atherosclerotic molecule involved in a wide variety of regulatory mechanisms of the CV system, including vascular tone and structure and platelet and monocyte adhesion (31, 34). Small modifications in ADMA concentrations may affect endothelial NO production, vascular tone, and systemic vascular resistance (35), and increased ADMA levels have been associated with endothelial dysfunction and increased IMT in adults (31).

Higher ADMA levels (36) as well as impaired FMD (11, 37, 38) and IMT (12) have been documented in adults with subclinical hypothyroidism with a beneficial effect of L-T4 therapy on endothelial function (37, 39, 40) and IMT in most (12, 41) but not all studies (42). Our finding of increased ADMA concentrations in SH children is in agreement with data on adults (36), although we did not observe atherosclerotic vascular wall abnormalities or endothelial dysfunction. The young age of our cohort of SH subjects could explain the absence of vascular abnormalities; indeed we could hypothesize that ADMA-mediated effects on NO availability might represent one of the mechanisms by which SH affects vascular function, and the increased levels of ADMA could represent the first step in the atherosclerotic process finally leading to structural abnormalities in adulthood.

The anthropometric evaluation did not reveal changes in height and BMI SDS after 2 years of L-T4 treatment in SH subjects, in agreement with previous data by Wasniewska *et al.* (20). However, after therapy we observed a significant reduction in WHtR values in SH children, suggesting that L-T4 treatment exerts a beneficial effect on visceral adiposity, as also suggested by correlation analysis which revealed a significant association between modifications in WHtR and FT4.

The relationship between lipid profile and thyroid function has been documented in childhood (43, 44, 45, 46) while so far there are no studies evaluating the effect of L-T4 therapy on lipid profile in SH children. A few randomized controlled trials in adults with SH found a beneficial effect of L-T4 treatment on lipid profile (40, 41, 47, 48), but this has not been confirmed by other studies (49).

Although the children in our study did not develop overt dyslipidemia, they showed at baseline a lipid profile prone to enhancement of atherosclerosis documented by subtle alterations in HDL-C, triglycerides/HDL-C, and AI. Indeed, HDL-C is inversely related to atherosclerosis, and triglycerides/HDL-C and AI are surrogate markers

of atherosclerosis and cardiometabolic risk even in childhood (28, 29, 50, 51). Furthermore, the risk profile of SH children was also documented by increased Hcy levels even though the role of Hcy as CV risk factor in adults and adolescents with mild SH remains controversial (9, 52).

During the 2 years of L-T4 therapy we observed an improvement in many lipid parameters and Hcy levels in SH children. However, the analysis of differences in changes over time between SH children and controls revealed a significant treatment effect only for WHtR and ADMA. Therefore, we cannot affirm that the mild improvement in lipid profile observed in SH subjects is effectively due to L-T4 treatment. However, we could assume that our results are affected by both the small sample size and the high physiological variability of lipid parameters, and thus no definitive conclusions on the effects of L-T4 therapy can be drawn.

In agreement with our previous results, glucose metabolism parameters in SH children were similar to controls at baseline and did not change on L-T4 therapy. Data about the association between insulin insensitivity and SH are inconsistent even in adult studies (10), leading to the conclusion that insulin insensitivity does not play a role in the increased CV risk of these subjects.

In summary, we confirmed our previous findings of subtle pro-atherogenic abnormalities in untreated SH children; in addition, we documented increased ADMA levels indicating an increased risk of early endothelial dysfunction. All these abnormalities were subtle and all the parameters were still within the normal range, thus not resulting in significant alterations in vascular status, assessed through FMD and IMT. L-T4 treatment was associated with an improvement in visceral adiposity and ADMA levels; for the other outcomes evaluated we were unable to document a significant effect of treatment in comparison with controls.

Further studies on larger cohorts of patients will help clarify the impact of L-T4 therapy on early markers of atherosclerotic disease and confirm if treatment might be beneficial for children with mild SH.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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