Effects of long-term L-thyroxine treatment on endothelial function and arterial distensibility in young adults with congenital hypothyroidism

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Abstract

Objective: Patients with congenital hypothyroidism (CH) display subclinical abnormalities of the cardiovascular system that are related to unphysiological fluctuations of TSH levels and occur despite careful replacement therapy.

Design: The aim of the present case–control study was to evaluate the effects of long-term levothyroxine (L-T4) replacement therapy on the vascular district in CH patients by assessing endothelial function with flow-mediated dilation (FMD) and brachial artery distensibility with the measurement of the coefficient of distensibility (DC).

Methods: Thirty-two young adults with CH aged 18.9 ± 0.2 years and 32 age- and sex-matched controls underwent brachial Doppler ultrasound examination to measure FMD and DC at the time of the study. Hypothyroidism was diagnosed by neonatal screening, and L-T4 treatment was initiated within the first month of life.

Results: Compared to healthy controls, CH patients had significantly reduced brachial artery reactivity with lower FMD values (8.9 ± 5.7 vs 14.1 ± 5.1%, P < 0.003) and decreased vascular distensibility (24.6 ± 1.6 vs 27.3 ± 3 kPa⁻¹ × 10⁻³, P < 0.0002). Linear regression analysis revealed that both total and pubertal mean TSH and number of episodes of undertreatment were independent determinants of FMD and DC. Pubertal mean TSH was the best predictor of both FMD and DC (r = 0.81 and r = 0.87 respectively, P < 0.001).

Conclusions: Young adults with CH treated with long-term L-T4 replacement therapy may have significant impairment of both FMD and DC. Our data suggest that high TSH levels, inadequately corrected by L-T4 replacement therapy in CH patients especially during puberty, can exert significant effects on the elastic and functional vessel properties.

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Introduction

Thyroid hormone has important effects on the cardiovascular system (1). Overt hypothyroidism represents a well-known risk factor for atherosclerotic disease. Specifically, it is characterized by endothelial dysfunction, increased peripheral vascular resistance, early development of structural atherosclerotic lesions, and the appearance of clinical vascular events (1–3). Moreover, cardiovascular abnormalities, such as left ventricular diastolic impairment, increase of carotid intima-media thickness (IMT), and endothelial dysfunction, have also been reported in patients with subclinical hypothyroidism (SH) (4–7). In this regard, two recent meta-analyses found that SH is associated with an increased risk of coronary heart disease (8, 9), while therapeutic trials with levothyroxine (L-T4) treatment in patients with SH showed a beneficial effect on both functional and structural atherosclerotic markers (10–14). All the determinants of cardiovascular risk in patients with thyroid function abnormalities are not fully understood, even if hypercholesterolemia and a direct effect of thyroid hormones on arterial wall are certainly involved (1).

Congenital hypothyroidism (CH) represents one of the most common endocrine diseases in newborns, with a worldwide incidence of 1:3000–1:4000 (15, 16). We recently provided the first evidence that patients with CH display early cardiac involvement as shown by an impairment of diastolic function and a reduction of exercise capacity and cardiopulmonary performance (17). Such abnormalities occurred despite careful
replacement therapy and were associated with fluctuations of TSH levels and episodes of SH. Episodes of subclinical hyperthyroidism were less frequently involved.

Endothelial dysfunction is an early event in atherogenesis and can precede the appearance of structural vascular changes such as the increase of carotid IMT. It can be examined by flow-mediated dilation (FMD) and can be used to predict clinical outcome in high-risk cohorts of patients (18). Vascular function, moreover, can also be evaluated in an early stage of atherogenesis by measuring brachial artery distensibility (BAD), a specific index of the vessel elastic properties inversely related to arterial stiffness.

The aim of the present study was to assess endothelial function and BAD in young adults with CH who were followed longitudinally starting from the first weeks of life, in comparison to matched euthyroid subjects and evaluate whether unphysiological fluctuation of TSH, associated with a long-term L-T4 replacement therapy, might place them at an increased risk of early atherosclerotic changes.

**Subjects and methods**

**Patient population**

Thirty-two young adults (21 females and 11 males) affected with CH, aged 18.9 ± 0.2 years, participated in the study. All patients were diagnosed by neonatal screening and were followed longitudinally from the time of diagnosis of CH to the time of the study. The diagnosis was confirmed by serum thyroid function tests. L-T4 replacement therapy was started immediately after the first evaluation at a mean age of 26 ± 0.9 days (range 12–30 days) and at a mean initial dose of 6.5 ± 0.1 μg/kg per day. Replacement therapy was modified during follow-up according to clinical and hormonal evaluation in order to maintain serum TSH in the normal range and serum free T4 (FT4) in the upper normal range. The etiological diagnosis of CH was made on the basis of 99m Tc-pertechnetate or iodine-123 thyroid scans at the time of diagnosis or at the age of 3 years, after the withdrawal of L-T4 therapy for 6 weeks. Cases were classified into three groups: athyreosis (n = 7), ectopic (n = 18), and eutopic gland (n = 7). At study entry, all subjects had completed their pubertal development and reached their final adult height (defined as a growth of <1.0 cm/year during the preceding year), and females had regular menstrual cycles (19). Serum TSH and thyroid hormones, and routine blood analysis were periodically assessed (every 6 months from the diagnosis of hypothyroidism). Three patients were mild smokers (<10 cigarettes/day) and three were moderate drinkers (beer or wine occasionally). Previous or current cardiovascular, respiratory, renal, or other chronic diseases as well as obesity were considered exclusion criteria.

In CH patients, mean TSH and FT4 values were calculated from all the samples collected during the study from the age of 1 year (mean total TSH and mean free T4) to the time of the study. Pubertal mean TSH was calculated on the samples collected from the age of the first sign of pubertal development (breast stage 2 for females and testicular volume of 4 ml for males) to the time of the study. Mean L-T4 dose taken in the same periods was also calculated.

Based on the normal TSH range in our laboratory (0.5–4.0 mU/l), we also calculated an index of overtreatment (number of episodes with plasma TSH <0.5 mU/l) and an index of undertreatment (number of episodes of plasma TSH >4.5 mU/l).

Measurements below 1 year of age were excluded since TSH plasma levels were still above the normal range in the vast majority of patients.

**Flow-mediated dilation**

Brachial artery reactivity was evaluated in each subject using validated protocol (20), with a 7.5 MHz multi-frequency linear array probe (Apio XG Imaging System, Toshiba, Tokyo, Japan). All subjects were evaluated in a quiet, temperature-controlled room and, from the day before the examination, had abstained from cigarette smoking. Measurements began at ~12 30 h. After resting for 10 min in a supine position, 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: FMD = ((post-hyperemia diameter − baseline diameter)/baseline diameter) × 100. After the subject had rested for at least 10 min, nitroglycerin spray (0.6 mg) was sublingually administered in order to assess endothelium-independent vasodilatation. Peak nitroglycerin mediated dilatation (NMD) occurs about 3 min after nitroglycerin administration. Both flow velocity measurements and brachial artery diameter were recorded four times during this period. NMD resulted from the formula: NMD = ((post-nitroglycerin diameter − baseline diameter)/baseline diameter) × 100.

**Brachial artery distensibility**

The DC of the brachial artery was assessed for the evaluation of arterial stiffness. All measurements were done after an 8-h fasting. All the subjects rested in a supine position for 15 min in a quiet, temperature-controlled room before the measurements. DC was obtained by a single investigator using the same ultrasound system (Apio Toshiba, Tokyo, Japan) equipped with a 7.5-MHz linear array probe under electrocardiographic monitoring. The mean of three consecutive measurements was used in the analyses as recommended. The brachial artery was imaged ∼2–5 cm proximal to the antecubital crease, in a longitudinal axis, and vessel diameters were measured in M-mode: the lowest end-diastolic arterial diameter (Dd) on the electrocardiographic R-wave, the highest end-systolic arterial diameter (Ds) on the electrocardiographic P-wave, and the diameter change during cardiac cycle (ΔD, defined as Ds−Dd). Brachial artery pulse pressure (ΔP), defined as systolic minus DBP, was measured by a sphygmomanometer and expressed in kPa. Finally, DC was calculated as (2ΔD/Dd)/ΔP (kPa−1 × 10−3) (21).

**Statistical analysis**

Data are reported as mean ± S.D., unless otherwise specified. The statistical analysis was performed using the Mann–Whitney U rank-sum test. P value < 0.05 was considered statistically significant.

Linear regression analysis was performed using FMD and DC as dependent variables and the following as independent variables: total and pubertal mean TSH, number of episodes of TSH > 4.5 or < 0.5 mU/l.

**Results**

At study entry, no differences were detected in clinical and laboratory findings such as BMI, heart rate, blood pressure, total cholesterol and triglycerides levels, and thyroid hormones between CH patients and controls (Table 1).

Compared to the control group, CH patients showed a significant reduction in both mean FMD value 8.9 ± 5.7 vs 14.1 ± 5.1%, P = 0.0003 and mean DC value (24.6 ± 1.6 vs 27.3 ± 3 kPa−1 × 10−3, P < 0.0002; Fig. 1). In contrast, no differences were detected in NMD values between CH patients and controls (20.2 ± 2.3 vs 22 ± 3.5%).

Linear regression analysis revealed that the mean total TSH (r = 0.67 and r = 0.79 respectively, P < 0.001), pubertal mean TSH (r = 0.81 and r = 0.87 respectively, P < 0.0001; Figs 2 and 3), total number of episodes of hypothyroidism (r = 0.58 and r = 0.62, P < 0.02 and P < 0.01 respectively), and pubertal episodes of hypothyroidism (r = 0.63 and r = 0.67, P < 0.002 and P < 0.001 respectively) were independent predictors of FMD and DC (Table 2).

**Table 1** Clinical and laboratory characteristics of congenital hypothyroidism (CH) patients and controls subjects at study entry.

<table>
<thead>
<tr>
<th></th>
<th>CH</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>32</td>
<td>32</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>11/21</td>
<td>11/21</td>
<td>NS</td>
</tr>
<tr>
<td>Age (year)</td>
<td>18.9±1.</td>
<td>19.5±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.4±0.5</td>
<td>21.9±0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>88±3</td>
<td>84±3</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113±2</td>
<td>110±2</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73±1</td>
<td>75±2</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>149±5</td>
<td>150±6</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>62±6</td>
<td>57±5</td>
<td>NS</td>
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<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>76±2</td>
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<td>NS</td>
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<td>HDL-Cholesterol (mg/dl)</td>
<td>57±2</td>
<td>64±2</td>
<td>NS</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>2.7±0.3</td>
<td>1.8±0.3</td>
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</tr>
<tr>
<td>FT4 (pg/ml)</td>
<td>12.3±0.3</td>
<td>11.4±0.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M.; NS, not significant.

**Figure 1** Flow-mediated dilation (FMD) (A) and coefficient of distensibility of brachial artery (DC) (B) in CH patients and controls. The boxes show medians, 25th and 75th percentiles, and the vertical bars represent the highest and lowest values.

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Both FMD and DC were more significantly associated with pubertal TSH (r=0.81 and r=0.87 respectively) compared to the other independent predictors (mean total TSH and number of episodes of hypothyroidism).

No significant correlation was detected between both FMD and DC and the number of episodes of subclinical hyperthyroidism in CH patients.

No significant differences were observed in mean FMD and DC between males and females with CH (FMD 7.6±5.2 vs 9.6±6.0%, P=NS; DC 24.7±1.3 vs 24.5±1.8 kPa⁻¹×10⁻³, P=NS), not even when CH patients were separated into groups according to etiological defect (FMD: ectopic gland 7.8±4.6, athyreosis 10.1±7.1, eutopic gland 10.0±6.9%, P=NS; DC: ectopic gland 24.4±1.6, athyreosis 24.0±2.2, eutopic gland 24.8±1.0 kPa⁻¹×10⁻³, P=NS). Moreover, no significant correlation was detected between the severity of CH at diagnosis, evaluated by the serum T₄ concentrations, and both FMD and DC.

**Discussion**

The results of the current case–control study indicate that young adults with CH treated with long-term L-T₄ replacement therapy present early vascular alterations, as demonstrated by the presence of endothelial dysfunction and arterial distensibility impairment. In fact, as compared to healthy controls, CH patients displayed a significant reduction of both FMD and DC values.

The impairment of functional and elastic vessel properties showed a strong correlation with higher mean values of TSH during the overall follow-up and in particular during the pubertal period. Moreover, the total number of SH episodes due to inadequate L-T₄ replacement therapy during the study, and the total number during puberty represent a predictive factor for the reduction of FMD and the impairment of the arterial distensibility.

Of course, episodes of inadequate L-T₄ replacement therapy may occur during long-term treatment of CH patients. Indeed, our CH patients experienced periods of SH, particularly during adolescence, when the compliance to the treatment becomes less regular, not withstanding an accurate biochemical follow-up and frequent adjustment. These episodes were strongly correlated with the impairment of both FMD and DC observed at the time of the study. On the contrary, no relationship was observed between episodes of SH, less frequently detected in CH patients, and vascular abnormalities at the time of the evaluation.

These results are in agreement with our previous study documenting an impairment of diastolic function and cardiopulmonary performance in young CH adults associated with episodes of SH (17). Other studies have shown a strong positive relationship between serum TSH values and endothelial dysfunction in adults with
SH (10). In some cases, however, the impairment of endothelial function was not explained by the presence of the usual cardiovascular risk factors (22), thus suggesting that TSH is itself endowed with atherogenic activity or it may regulate vascular homeostasis.

In agreement with this hypothesis, the presence of a functional TSH receptor was demonstrated in cardiomyocytes (23), in human coronary artery smooth muscle cells (24), and in human endothelial cells (25). Moreover, recombinant human TSH administration has been shown to acutely impair endothelium-dependent vasodilatation (26). Nevertheless, the intimate mechanisms of the interaction between TSH and vascular system have yet to be completely clarified.

In conclusion, our data indicate that young adults with CH treated with long-term L-T4 replacement therapy may have repeated episodes of TSH increase that can modify vascular reactivity and arterial distensibility by mechanisms not yet completely understood.

However, endothelial dysfunction and brachial artery distensibility are potentially reversible events, thus long-term studies are needed to clarify if these vascular abnormalities can be reversed after a sustained normalization of TSH concentration. In the meantime, we suggest careful follow-up with frequent dosage adjustment to avoid episodes of undertreatment, particularly frequent during adolescence, in order to prevent early atherosclerotic abnormalities. Moreover, the usefulness of systematic noninvasive cardiovascular screening in this population should be considered.

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.

Funding
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Table 2 Univariate predictors of impaired flow-mediated dilation (FMD) and coefficient of distensibility of brachial artery (DC).

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Independent predictors</th>
<th>r</th>
<th>P</th>
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<tr>
<td>FMD</td>
<td>Mean total TSH</td>
<td>0.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pubertal mean TSH</td>
<td>0.81</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Number of episodes</td>
<td>0.58</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>of hypothyroidism</td>
<td>0.63</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td></td>
<td>Number of pubertal</td>
<td>0.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>episodes of hypothyroidism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC</td>
<td>Mean total TSH</td>
<td>0.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pubertal mean TSH</td>
<td>0.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Number of episodes</td>
<td>0.62</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>of hypothyroidism</td>
<td>0.67</td>
<td>&lt;0.001</td>
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</table>

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