EFFECTS OF LONG-TERM L-THYROXINE TREATMENT ON ENDOTHELIAL FUNCTION AND ARTERIAL DISTENSIBILITY IN YOUNG ADULTS WITH CONGENITAL HYPOTHYROIDISM


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**Running title:** Endothelial function in CH

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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 arterial distensibility (BAD) with the measurement of the coefficient of distensibility (DC).

Methods: Thirty-two young adults with CH aged 18.9±0.2 years and thirty-two 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 under-treatment 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.
**Introduction**

Thyroid hormone has important effects on 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 been also 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 L-thyroxine (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 to 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 subclinical hypothyroidism. 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 intima-media thickness. 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 be also 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 brachial artery distensibility 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, 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/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 FT4 in the upper normal range. The etiological diagnosis of CH was made on the basis of 99mTc-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 six 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 less than 1.0 cm/yr during the preceding year) and females had regular menstrual cycles. (19). Serum TSH and thyroid hormones, and routine blood analysis were periodically assessed (every six months from the diagnosis of hypothyroidism). Three patients were mild smokers (less than 10 cigarettes per 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.

Thirty-two healthy young adults comparable for age, sex, body mass index and physical activity participated in the study as controls.

Informed consent was obtained from all patients or from their parents for the patients younger than 18 years. The study was approved by the Ethical Committee of the University of Naples, “Federico II”.

Study protocol

At study entry, all subjects underwent height, weight, BMI, heart rate, systolic (SBP) and diastolic blood pressure (DBP) measurements. TSH, thyroid hormones, total cholesterol and triglycerides were also evaluated.
Vascular function was measured in each patient at the time of the study by the measurements of FMD and DC.

In CH patients mean TSH and FT4 values were calculated from all the samples carried out during the study from the age of 1 year (mean total TSH and mean total FT4) 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 over-treatment (number of episodes with plasma TSH <0.5 mU/L) and an index of under-treatment (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 multifrequency linear-array probe (Aplio XG imaging system, Toshiba, 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 approximately 12.30 pm. 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 approximately 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-hyperaemia diameter – baseline diameter)/ baseline diameter] x 100. After subject had rested for at least 10 min, nitroglycerin spray (0.6 mg) was sublingually administered in order to assess
endothelium-independent vasodilatation (NMD). Peak nitroglycerin vasodilatation 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 = [(postnitroglycerin diameter – baseline diameter)/ baseline diameter] x 100.

Brachial artery distensibility

The distensibility coefficient of the brachial artery (DC) was assessed for the evaluation of arterial stiffness. All measurements were done after an 8-hour fasting. All the subjects rested in a supine position for 15 minutes in a quiet, temperature-controlled room before the measurements. DC was obtained by a single investigator using the same ultrasound system (AplioToshiba) equipped with a 7.5-MHz linear array probe under electrocardiographic monitoring. The mean of 3 consecutive measurements was used in the analyses as recommended. The brachial artery was imaged approximately 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 T-wave and the diameter change during cardiac cycle (ΔD, defined as Ds-Dd). Brachial artery pulse pressure (ΔP), defined as systolic minus diastolic blood pressure, was measured by a sphygmomanometer and expressed in kPa. Finally, DC was calculated as (2ΔD/Dd)/ ΔP (kPa⁻¹ x 10⁻³) (21).

Statistical analysis

Data are reported as mean±S.D., unless otherwise specified. The statistical analysis was performed using the U-Mann-Whitney rank-sum test. P value less than 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, 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\(^{-1}\)x10\(^{-3}\), 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), mean pubertal TSH (r=0.81 and r=0.87 respectively, p<0.0001) (Fig. 2, 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).

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=n.s.; DC 24.7±1.3 vs 24.5±1.8 kPa\(^{-1}\)x10\(^{-3}\), p=n.s.), not even when CH patients were separated into groups according to aetiological defect (FMD: ectopic gland 7.8±4.6, athyreosis 10.1±7.1, eutopic gland 10.0±6.9 %, p=n.s.; DC: ectopic gland 24.4±1.6, athyreosis 24.0±2.2, eutopic gland 24.8±1.0 kPa\(^{-1}\)x10\(^{-3}\), p=n.s.). 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 subclinical hypothyroidism episodes due to inadequate LT4 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 LT4 replacement therapy may occur during long-term treatment of CH patients. Indeed, our CH patients experienced periods of subclinical hypothyroidism, particularly during adolescence, when the compliance to the treatment becomes less regular, notwithstanding an accurate biochemical follow-up and frequent adjustments. 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 subclinical hyperthyroidism, 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 subclinical hypothyroidism (17). Other studies have shown a strong positive relationship between serum TSH values and endothelial dysfunction in adults with subclinical hypothyroidism (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 cell (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.

Disclosure

The study was supported by a grant of the Italian Ministry of Research and University (n. 2006069049, to M.S.). There is no conflict of interest.

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Legend to the figures

**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 whiskers represent the highest and lowest values.

**Figure 2** Linear regression analysis between flow mediated dilation (FMD) and total mean TSH ($r = 0.67$, $B = -1.09$) (right) and pubertal mean TSH ($r = 0.81$, $B = -1.25$) (left).

**Figure 3** Linear regression analysis between coefficient of distensibility of brachial artery (DC) and total mean TSH ($r = 0.79$, $B = -0.63$) (right) and pubertal mean TSH ($r = 0.87$, $B = -0.73$) (left).
### Table 1 Clinical and laboratory characteristics of CH patients and controls subjects at study entry.

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<th>Controls</th>
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<td>TSH (mIU/L)</td>
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<td>FT4 (pg/ml)</td>
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Data are expressed as mean±S.E.M; ns: not significant.
Table 2  Univariate predictors of impaired flow mediated dilation (FMD) and coefficient of distensibility of brachial artery (DC).

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<td>Number of pubertal episodes of hypothyroidism</td>
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<td></td>
<td>Mean total TSH</td>
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<td>DC</td>
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<td>Number of pubertal episodes of hypothyroidism</td>
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Figure 2
255x181mm (149 x 149 DPI)
Figure 3
258x182mm (148 x 148 DPI)