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Aortic stiffness and left ventricular function in patients with differentiated thyroid cancer

--Manuscript Draft--

Manuscript Number:	JENI-D-14-00120R1
Full Title:	Aortic stiffness and left ventricular function in patients with differentiated thyroid cancer
Article Type:	Original Article
Abstract:	Objective: The aim of this study was to investigate aortic stiffness and left ventricular systolic and diastolic function in patients with differentiated thyroid cancer (DTC) on thyroxin (L-T4) therapy and after L-T4 withdrawal in order to assess the cardiovascular impact of long-term subclinical hyperthyroidism and short-term overt hypothyroidism. Design: Twenty four patients who had had total thyroidectomy and radioiodine ablation for differentiated thyroid cancer were studied on two occasions: on TSH suppressive L-T4 therapy (sTSH 0.24±0.11 mU/L), and four weeks after L-T4 withdrawal (sTSH 89.82±29.36 mU/L). Echocardiography was performed and thyroid function, serum thyroglobulin, lipid parameters, homocystine, C-reactive protein, fibrinogen and von Willebrandt factor activity (vWF) were measured. Twenty two healthy volunteers matched for age and sex served as euthyroid controls. Results: Aortic stiffness was increased both in hypothyroidism (6.04±2.88 cm2/dyn/103, p< 0.05) and subclinical hyperthyroidism (9.27±4.81 cm2/dyn/103, p<0.05) vs. controls (3.92±1.84 cm2/dyn/103). Subclinical hyperthyroidism had a more marked effect (p<0.05). LV dimensions and ejection fractions were similar before and after L-T4 withdrawal. The E'/A' was higher in euthyroid controls (1.34±1.02) as compared to both subclinical hyperthyroidism (1.0±0.14, p<0.05) and overt hypothyroidism (1.13±0.98, p<0.05). Change of aortic stiffness correlated with change of free-thyroxine (fT4), vWF and fibrinogen levels in a positive manner. Conclusion: Long-term thyrotropin-suppression therapy has continuous adverse effects on the arterial wall. The degree of TSH suppression in patients with DTC should be kept at the possible minimum, based on individually determined potential benefits and risks of treatment, especially in patients with cardiovascular comorbidities.
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Author Comments:	Luigi Bartalena, M.D. Editor-in-Chief Journal of Endocrinological Investigation

Dear Professor Bartalena,

We would like to thank you for the suggestions which have contributed to the improvement of our paper entitled „Aortic stiffness and left ventricular function in patients with differentiated thyroid cancer", JENI-D-14-00120 .

We have corrected the manuscript and we hope that you will find it worth publishing in the Journal of Endocrinological Investigation.

We provide a detailed point-by-point response to each of the referees' concerns, describing exactly how we responded to each point and where you can find the amendment in the revised manuscript.

Thank you very much for your patience and kind help.

Yours sincerely,

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Response to Reviewers:

Response to Reviewer I

We are highly thankful for the Reviewer's advice and remarks which have contributed to the improvement of the academic standards of our paper entitled „Aortic stiffness and left ventricular function in patients with differentiated thyroid cancer", manuscript JENI-D-14-00120.

We try to address your comments below.

Materials and Methods Section
•Did all the patients need LT4 suppressive therapy? According to current ATA and ETA guidelines this is not always necessary.

On the basis of the ATA guidelines (Cooper DS et al.: Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2009 Nov;19(11):1167-214) all patients in the study were classified as "high or intermediate risk" of DTC recurrence, hence they were on TSH-suppressive therapy (TSH 0.24±0.11 mU/L) continuously. We have inserted this information on page 4, line 42.

•How many time elapsed from thyroidectomy/RAI ablation and the current tests?

20±12,6 months elapsed before the start of this study.
This information has been added to page 4, line 47.

•What was the thyroglobulin serum level at the time of aortic examination? In other words, were all the patients without evidence of persistent/recurrent disease (also by the biochemical point of view)? / Did the Authors evaluate the level of serum anti-Tg antibodies?

The first off-T4 Tg measurrment was at least 6 months after RAI in parallel with anti-Tg antibody. Four of twenty four patients were Tg positive and 131I whole-body scan (WBS) was performed. WBS was positiv in 3 of the Tg positive patients due to small thyroid remnant. One Tg postive, WBS negative patient was followed, repeated Tg measurments were negative. Three patients were Tg negativ and anti-Tg positive. (Cooper DS et al. (2009): Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. American

Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. Nov;19(11):1167-214).
Page 4. line 51 has been supplemented with this.

•Why did the authors perform TSH-stimulated Tg evaluation after LT4 withdrawal? To date rhTSH stimulation is generally preferred due to the lack of clinical hypothyroidism.

The use of recombinant TSH (rhTSH) is covered only in the presence of severe cardiac comorbidities by the national health insurance plan in Hungary; these patients were excluded from the study..
This has been added to page 4. line 56.

•Most previous studies evaluated diastolic function also by measuring isovolumic relaxation time (see for example Monzani et al. J Clin Endocrinol Metab 2001; 86:110; Ann Intern Med. 2002 Dec 3; 137: 904-14. Review).

According to the suggestion, we inserted a supplementary information and two additional references in the Discussion section on Page 121, line 15.

Statistical analysis
Please specify the parameters included in the multivariate analysis and what of the parameters were log transformed for the skewed distribution

To improve the normality of the data distribution, triglyceride values were log-transformed for analysis.
The multivariate model consisted of the changes of stiffness index as dependent variable and independent variables were thos that had had significant correlation with changes of stiffness index in the simple linear regression analysis. These were vWF, fibrinogen, fT4 and LDL-C.
This is shown now at page 8, line 11.

Results Section
•Aortic stiffness was significantly increased both during short-term hypothyroidsm and subclinical hyperthyroidism as compared to healthy controls. Did the difference observed in the two former conditions reach the statistical significance?

Yes ($p<0,05$). This missing information was inserted to the text on page 9, line , and the Fig.1 was corrected.

•In order to better understand the significance of Hcys variation the level of folic acid should be reported.

Although we agree with the Reviewer, unfortunately, we have not measured folic acid in the patiens.

•Please, report the p values of the serum parameters described in the text.
P values have now been added to a new table, Table 1.

•By simple regression analysis, changes of aortic stiffness index during transition from subclinical hyperthyroidism to hypothyroidism correlated with changes of vWF, fT4 and fibrinogen in a positive manner, while with LDL-C in a negative manner. Did the Authors have an explanation for the lack of relationship with TSH value?

We speculate that the thyroid hormone concentration/action at tissue level changes more rapidly than TSH does. However, we do not feel that our data are strong enough to support this hypothesis.

Conclusions
•The last sentence of the conclusions "The degree of TSH suppression in patients with DTC should be kept at the possible minimum, based on individually determined potential benefits and risks of treatment, especially in patients with cardiovascular comorbidities" is not novel and already stated in the current DTC guidelines.

We agree with the Reviewer. We should have stated this. Now, this information has been added, and the section has been rewritten.

References
•Two early studies reporting the direct effect of NO and inflammation on endothelium dependent vasodilation in subclinical hypothyroid patients are not cited (Taddei et al. JCEM 2003 and JCEM 2006).

Taddei et al 2003 is cited now in Page 11, line 18 and 49., while Taddei et al 2006. is cited in Page 11, line 49.

English language
•Several minor mistakes are present in the text and should be checked. For example page 7: hormonal states were compared with data of the healthy euthyreoid control group.

We are sorry for this. We have now performed several spell-checks.

We would like to express our sincere gratitude for all the assistance given to us.

Response to Reviewer II.

We are highly thankful for the Reviewer's advice and remarks which have contributed to the improvement of the academic standards of our paper entitled „Aortic stiffness and left ventricular function in patients with differentiated thyroid cancer", manuscript JENI-D-14-00120.

We try to address your comments below.

Major comments:

•A table including all of the clinical characteristics of the patients and controls should be added.

According to the suggestion we inserted a new table containing clinical characteristics. This table was inserted to the manuscript as Table 1.

•The criteria for the selection of the patients and controls should be included.

According to your suggestion we detailed the selection criteria of our patients. A long extension containing this information has now been added to the "Patients" section on page 4.

•Some studies assessed arterial stiffness in patients with overt hyperthyroidism. These studies should be mentioned.

According to your suggestion, we inserted the appropriate citations into "Introduction" page 3, line 60.

•The authors should emphasize previous literature data on the cardiovascular alterations observed in short-term hypothyroidism.

According to your suggestion, we inserted supplementarry data and citations into "Introduction", page 3, line 40.

„In contrast, there is a few data on the effects of short term hypothyroidism induced by LT4-withdrawal on cardiovascular disease (Leonidas H Duntas and Bernadette Biondi (2007): Short-term hypothyroidism after Levothyroxine-withdrawal in of life consequences European Journal of Endocrinology 156 13–19). During short-term hypothyroidism night-time systolic, diastolic and mean blood pressure were increased, left ventricular diastolic function was impaired (Botella-Carretero JL et al. (2004): Chronic thyrotropin-suppressive therapy with levothyroxine and short-term overt hypothyroidism after thyroxine withdrawal are associated with undesirable cardiovascular effects in patientswith differentiated thyroid carcinoma. Endocrine Related Cancer. 11 345–356. ; Grossman G et al. (1994): Doppler echocardiographic evaluation of left ventricular diastolic function in acute hypothyroidism. ClinicalEndocrinology 40:227–233.), ejection fraction during effort was reduced documented by radionuclide ventriculography (Wieshammer S et al. (1989): Acute hypothyroidism slows the rate of left ventricular diastolic relaxation. Canadian Journal of Physiology and Pharmacology. 67:1007–1010.), afterload was increased (Fommei E & Iervasi G. (2002): The role of thyroid hormone in blood pressure homeostasis: evidence from short-term hypothyroidism in humans. Journal of Clinical Endocrinology and Metabolism. 87:1996–2000). Endothelial dysfunction in short-term hypothyroidism was reported in few study (Erbil et al. (2007): Effects of thyroxin replacement on lipid profile and endothelial function after thyreoidectomy. Br J Surg 94: 1485-90. ; Gazdag A. et al. (2010): Improved endothelial function and lipid profile compensate for impaired hemostatic and inflammatory status in iatrogenic chronic subclinical hyperthyroidism of thyroid cancer patients on L-t4 therapy. Exp Clin Endocrinol Diabetes 118:(6) 381-7).

•We reorganized the Discussion section and a more concise – at least we hoped - conclusion resulted.

•The authors should discuss the potential mechanism inducing increased arterial stiffness and the cardiovascular alterations during exogenous subclinical hyperthyroidism and short term hypothyroidism more clearly.

Now we described potential mechanisms for the vessel wall effects of both subclinical hyperthyroidism (page 11, line 31and short-term hypothyroidism (page 12, line 40).

In subclinical hyperthyroidism: „Sympathetic activation increases arterial wall stiffness [24]. Manifestations of hyperthyroidism resemble the effect of cathecholamine excess: the sensitivity of resistance vessels to the vasoconstrictive action of norepinephrine is enhanced [40]. β1-adrenergic blockade was associated with normalization of total arterial stiffness [28]. Our previous report of low-grade inflammation in subclinical hyperthyroidism has been confirmed by a recent study [41]. Vascular inflammation causes degradation of collagen and elastin, evokes changes in the proteoglycan composition and hydration status, and results in medial calcification [42] leading to increased arterial stiffness. Low-grade inflammation caused endothelial dysfunction and impaired NO availability in patients with subclinical hypothyroidism [43]. Thyroid hormone reduces systemic vascular resistance and causes activation the renin-angiotensin-aldosteron system. T3 directly stimulates the synthesis of renin substrate in the liver. Consequevent sodium reabsorption, increased blood volume and preload contribute to the characteristic increase in cardiac output [44]. Chronic hemodynamic overload causes increased myocardial contractility, cardiac hypertrophy, increased left-ventricular mass; contractile protein synthesis is increased. The faster heart rate in hyperthyroidism result an earlier return of the forward pressure wave in systole, resulting in a greater overlapping in the forward and reflected pressure waves [28]. vWf is reported to be a reliable marker of endothelial damage and subclinical atherosclerosis [45]. In the present study, vWF and fibrinogen as markers of endothelial dysfunction were more higher in subclinical hyperthyroidism than in overt hypothyroidism, and there was positive correlation between changes of aortic stiffness index, vWF and fibrinogen during transition from subclinical hyperthyroidism to hypothyroidism. These changes may be associated with relative hypercoagulability and increased thromboembolic risk [46].
Most previous studies used isovolumic relaxation time to evaulate diastolic dysfunction in subclinical hypo- and hyperthyroidism. Our results, albeit using another approach, are consonant with these studies Impaired diastolic function was detected in patients with subclinical hyperthyroidism[47, 8, 9, 48-50]. It has been suggested in earlier studies that diastolic dysfunction in subclinical hyperthyroidism resulted from increased LVM. However, no significant increases in LVM was found either by us or by other groups [8, 51]. Dörr et al. showed that decreased serum TSH levels were not associated with an elevated risk of left ventricular hypertrophy, but overt hyperthyrodism is an independent risk factor for left ventricular hypertrophy [52]. Thyroid hormones influence calcium regulation in myocytes, such as increase Ca++-ATPase activity and decrease phospholamban expression, and increase Ca-influx. [53]. Increase in intracellular calcium may be cause of mediated diastolic stiffness in

	hyperthyroid rats heart [54]." In short-term hypothyroidism: „We detected only slight impairerment in aortic stiffness and diastolic function in acute short-term hypothyroidism. Aortic stiffness is likely related to myxoedema of the arterial wall [15, 55]. However, our data do not support this notion and are consonant with the findings of other studies [56, 15, 30] that argued against the role of LDL-C in increased aortic stiffness. Impaired diastolic function in hypopthyroidism due to slow myocardial relaxation results from altered intracellular calcium handling, decreased activity of the sarcoplasmatic reticulum calcium ATPase and /or increased expression of phospholamban [2]. Myofibrill swelling, mucopolysaccharides accummlation can be detected in hypothyroid heart [53]." •Advice in order to avoid these negative cardiovascular findings should be given for patients receiving TSH suppressive therapy and in those performing L-T4 withdrawal. This information has now been added to the end of the Discussion section (page 13., line 11). Patients may benefit from the withespread use of rhTSH instead of thyroxin withdrawal to achieve high TSH during Tg measurement, as well as from beta-1 adrenergic blockade during iatrogenic subclinical hyperthyroidism. We would like to express our sincere gratitude for all the assistance given to us.
Suggested Reviewers:	

Aortic stiffness and left ventricular function in patients with differentiated thyroid cancer

Running Title: Cardiac effects of thyroid hormones

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Key words: cardiovascular risk, subclinical hyperthyroidism**,** aortic stiffness, differentiated thyroid cancer

Word Count: Text: 2686, Abstract: 247, Tables: 3, Figure: 4

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Abstract

Objective: The aim of this study was to investigate aortic stiffness and left ventricular (LV) systolic and diastolic function in patients with differentiated thyroid cancer (DTC) on thyroxin (L-T4) therapy and after L-T4 withdrawal in order to assess the cardiovascular impact of long-term subclinical hyperthyroidism and short-term overt hypothyroidism.

Methods: Twenty four patients who had had total thyroidectomy and radioiodine ablation for differentiated thyroid cancer were studied on two occasions: on TSH suppressive L-T4 therapy (sTSH 0.24±0.11 mU/L), and four weeks after L-T4 withdrawal (sTSH 89.82±29.36 mU/L). Echocardiography was performed and thyroid function, serum thyroglobulin, lipid parameters, homocystine, C-reactive protein, fibrinogen and von Willebrandt factor activity (vWF) were measured. Twenty two healthy volunteers matched for age and sex served as euthyroid controls.

Results: Aortic stiffness was increased both in hypothyroidism (6.04±2.88 $cm^2/dyn/10^3$, $p< 0.05$) and subclinical hyperthyroidism (9.27±4.81 $cm^2/dyn/10^3$, $p<0.05$) vs. controls (3.92±1.84 $cm^2/dyn/10^3$). Subclinical hyperthyroidism had a more marked effect ($p<0.05$). LV dimensions and ejection fractions were similar before and after L-T4 withdrawal. The E'/A' was higher in euthyroid controls (1.34±1.02) as compared to both subclinical hyperthyroidism (1.0±0.14, $p<0.05$) and overt hypothyroidism (1.13±0.98, $p<0.05$). Change of aortic stiffness correlated with change of free-thyroxine (fT4), vWF and fibrinogen levels in a positive manner.

Conclusion: Long-term thyrotropin-suppression therapy has continuous adverse effects on the arterial wall. The degree of TSH suppression in patients with DTC should be kept at the possible minimum, based on individually determined potential benefits and risks of treatment, especially in patients with cardiovascular comorbidities.

Introduction

Thyroid hormones have several profound effects on the cardiovascular system. [1-3]. Some of these effects can be studied in patients with differentiated thyroid cancer (DTC), who have been treated with total thyroidectomy and radioidine ablative therapy. These patients are kept lifelong on suppressive thyroxin (L-T4) therapy, which is interrupted on a yearly basis by short periods of hypothyroidism to detect thyroglobulin as a tumor marker.

Subclinical hyperthyroidism is associated with higher heart rate, frequent atrial premature beats, and increased prevalence of atrial fibrillation [4, 5]. Increased left ventricular mass (LVM) and diastolic dysfunction are also reported in subclinical hyperthyroidism [6, 4, 7-10]. However, the association between exogenous subclinical hyperthyroidism and cardiovascular morbidity and mortality is controversial [11].

Changes of the cardiovascular system are well characterized in long-standing hypothyroidism: bradycardia, prolongation and increased dispersion of the QT interval [12], increased blood pressure, particularly diastolic [13], increased periferial vascular resistance with a reduced cardiac output [14, 15], and left ventricular diastolic dysfunction [16]. Overt hypothyroidism is related to coronary artery disease because of atherogen lipid profile, hypertension, hyperhomocysteinemia, elevated C-reactive protein levels, coagulation factor abnormalities, and endothelial dysfunction [17]. In contrast, there are only limited data available on the effects of short term hypothyroidism induced by LT4-withdrawal on cardiovascular disease [18]. During short-term hypothyroidism night-time systolic, diastolic and mean blood pressures were increased and left ventricular diastolic function was impaired [10, 19], ejection fraction during effort was reduced [20] and afterload increased [13]. Endothelial dysfunction in short-term hypothyroidism has also been reported.[21, 22].

One of the recently described tools for determining cardiovascular risk is arterial wall stiffness which is an independent predictor of cardiac events [23] via several mechanisms. Increased cardiac afterload, impaired coronary blood flow, direct atherogenic action or microvascular damage may be contributing factors [23, 24]. Arterial stiffness can be calculated from the aortic diameter and blood pressure measured simultaneously [25] or can be determined by pulse wave analysis [26]. Central arterial stiffness is reduced in untreated hyperthyroidism based on analysis of the central arterial pressure waveform [27]. On the other hand, Palmieri et al. detected increased total arterial stiffness in overt hyperthyroidism using echocardiography.[28]. Antithyroid drug therapy significantly reduced the stiffness of the common carotid artery in patients with Graves' disease [29]. Overt and subclinical hypothyroid subjects have increased arterial stiffness and it is reversed by L-T4 replacement [15, 30]. Aortic stiffness has not been measured in subclinical hyperthyroidism.

The aim of this study was to investigate aortic stiffness and left ventricular systolic and diastolic functions in patients with DTC who are on TSH suppressive doses of L-T4, as well as after 4 weeks of L-T4 withdrawal, in order to assess the cardiovascular impact of both long-term subclinical hyperthyroidism and iatrogenic short-term hypothyroidism.

Patients and Methods

Patients

Twenty four women (mean age 42.4±8.07 years) who had had total or near-total thyroidectomy for DTC were included in the study. In 21/24 cases, ^{131}I ablation was also performed within 6 months. Three of the 24 patients declined the recommended ^{131}I treatment because they wanted to be pregnant within a year. On the basis of the ATA guidelines [31], all patients were classified as "high or intermediate risk" of DTC recurrence, hence they were on TSH-suppressive therapy (TSH 0.24±0.11 mU/L) continuously for at least 26 weeks and were taking no other medications. 20±12,6 months elapsed before the start of this study.

Their yearly follow up included TSH stimulated serum thyroglobulin (Tg) level, anti-Tg antibodies, neck ultrasonography and, if indicated, whole-body radioiodine scan. We performed TSH-stimulated Tg evaluation after L-T4 withdrawal and endogenous rise in TSH. The use of recombinant TSH (rhTSH) is covered only in the presence of severe cardiac comorbidities by the national health insurance plan in Hungary; these patients were excluded from the study. The first Tg measurment was at least 6 month after RAI in parallel with anti-Tg antobody (refence range: <1 IU/ml). Four of 24 patients were Tg positive (Tg > 2 ug/L); in these patients, whole-body scan (WBS) was performed. WBS was positive in 3 of them due to small thyroid remnant and RAI was repeated. One Tg postive, WBS negativ patient was followed and consequent Tg measurments were negative. Another 3 patients were Tg negativ, anti-Tg positive. Patients with known ischemic heart disease, stroke, cardiac failure, hypertension, diabetes mellitus, renal or liver failure, other systemic or malignant diseases (other than previous thyroid cancer) were excluded from the study. Twenty two healthy volunteers, matched for age, served as euthyroid controls (TSH: 1,64±1,05 mU/l). The same criteria were used in control subject selection, except that they had no history of thyroid disease. They were not taking any drug known to influence thyroid and/or cardiac function. The protocol was approved by the Institutional Ethics Committee. All study subjects gave written informed consent.

Study protocol

Patients were studied on the day before L-T4 withdrawal (subclinical hyperthyroidism) and four weeks later, before readministration of L-T4 (hypothyroidism). Controls were evaluated only once. Blood samples were collected between 08.00-09.00 am after an overnight fast for determination of TSH, free thyroxin (fT4), free triiodothyronin (fT3), thyroglobulin, cholesterol, triglyceride, low-densitiy lipoprotein cholesterol (LDL-C), high-densitiy lipoprotein cholesterol (HDL-C), total homocystine (Hcys), C-reactiv protein (CRP), fibrinogen and von Willebrand factor activity (vWF). Blood pressure was measured, body mass index (BMI) calculated (the individual's body mass divided by the square of their height) and echocardiography performed. Echocardiographic measurements were carried out by two independent investigators who were unaware of the patients' clinical data.

Aortic wall stiffness and left ventricular mass measurements

Transthoracic echocardiography was performed by using Philips HDI-5000 system (Philips Medical Systems, Bothell, USA) 2.5 Mhz- probe at the left lateral decubitus position in a standard manner. M mode tracings of the ascending aorta were obtained in the parasternal long axis views at a speed of 50 mm/s. Five consecutive cardiac cycles were averaged for every echocardiographic measurement. With M mode, aortic tracing was recorded at the level of approximately 3 cm above the aortic valve. From the M mode recordings, aortic systolic and diastolic diameters (Aos and Aod, respectively) were measured. Aos was determined at the time of the full opening of the aortic valve and Aod was determined at the peak of QRS. All parameters were measured in five consecutive cardiac cycles and averaged. Simultaneously, cuff brachial artery systolic (SBP) and diastolic (DBP) blood pressures were measured and recorded.

The aortic elasticity parameters, the aortic strain and aortic stiffness indexis were calculated using the following formulas [32]:

$$\text{Aortic Strain (\%)} = 100 \times (\text{Aos} - \text{Aod}) / \text{Aod}.$$

$$\text{Aortic Stiffness Index [beta]} = \ln(\text{SBP}/\text{DBP}) / \text{Aortic Strain}$$

M-mode measurements of LV internal dimension in diastolic (LVDD) and systolic (LVDS), and end-diastolic posterior wall (PW) and interventricular septum (IVS) thickness and left atrium anteroposterior diameter were obtained using the standard technique. LV fractional shortening , a measure of the percent change in LV dimensions with systole, was calculated as

$$\text{LV Fractional Shortening} = (\text{LVDD} - \text{LVDS}) / \text{LVDD}$$

LV mass (LVM) was calculated by using the formula:

$$0.8\ [1.04\ (\text{LVDD} + \text{ISV} + \text{PW})^3 - (\text{LVDD})^3 \rfloor + 0.6.$$

LVM was corrected for body surface area to obtain LVM index (LVMI). [33]. Two dimensional left ventricular ejection fraction (LVEF) was also aquired by the summation method. We used tissue doppler imaging (TDI) to determine LV diastolic function.

The peak early transmitral filling velocity during early diastole (E), peak transmitral atrial filling velocity during late diastole (A), and E/A ratio were used as left ventricular diastolic function parameters. Quantitative diastolic data were derived from TDI data. The sample volume (4 mm3) was placed in the LV basal portion of anterior, inferior, septal and lateral walls (using the 2- and 4-chamber images) The following parameters (mean values calculated from three consecutive beats) were derived: early diastlolic velocity (E'), and late diastolic velocitiy (A') and the E'/A' ratio. [34]. Parameters of the patient groups were compared to controls.

Biochemical measurements

TSH, serum fT4 and fT_3 and thyroglobulin levels were measured by chemiluminescence immunoassay (DiaSorin, Saluggia). Total serum cholesterol, triglyceride, LDL-C and HDL-C were assayed by enzimatic methods (Roche Diagnostics, Mannheim, Germany). Hcys levels were measured by fluorescence polarisation immunoassay (Abbott Laboratories, Abbott Park, Illinois, U.S.A.). Fibrinogen was assayed by the Clauss method (Diagnostico Stago, Asnieres-sur-Seine, France). vWF and CRP were assayed by latex sensitized immunturbidimetry (Diagnostico Stago, Asnieres-sur-Seine, France, and Roche Diagnostics, Mannheim, Germany, respectively).

Reference ranges are as follows: TSH: 0.4-4.2 mU/L, fT4: 9.0-23.2 pmol/L, fT_3: 3.5-6.2 pmol/L, thyroglobulin < 2 μg/L for thyroidectomized patients, cholesterol <5.2 mmol/L, triglyceride < 1.7 mmol/L, LDL-C < 3.4 mmol/l, Hcys < 12,5 μmol/L, fibrinogen: 1.5-4.0 g/L, vWF: 50-160 %, CRP < 4.6 mg/L.

Statistical analysis

All statistical analyses were performed by using the SAS for Windows (8.2 cary/nc SAS® Institute Inc. USA) statistical package. Continuous data were expressed as mean±standard deviation. Relationships between the continuous variables were evaluated by Pearson's or Spearman's correlation analysis. Comparisons between control, subclinical hyperthyroid and hypothyroid groups for continuous variable were made by one way ANOVA and post-hoc Tukey's test. To improve the normality of the data distribution, triglyceride values were log-transformed for analysis. Simple linear regression analyses was performed to asses the relationship between changes of aortic stiffness and other parameters. To investigate the independent effect of the different factors on changes of stiffness, a multiple stepwise linear regression model was used. The multivariate model consisted of the changes of stiffness index as dependent variable and independent variables that had had significant correlation with changes of stiffness index in the simple linear regression analysis, ie. vWF, fibrinogen, fT4 and LDL-C. $p<0.05$ was considered statistically significant.

Results

Clinical and laboratory parameters

In subclinical hyperthyroidism, the mean TSH level was 0.24 ± 0.11 mU /L while the fT3 and fT4 levels were within the normal range (4.79 ± 0.46 and 18.39 ± 2.33 pmol/L, respectively). After discontinuation of L-T4 for 4 weeks, all 24 subjects achieved a hypothyroid state, as evidenced by TSH levels (89.8 ± 29.36 mU/L) and low serum fT4 and fT3. Blood pressure and BMI were not significantly different in hypothyroidism compared to subclinical hyperthyroidism and euthyroid state. Cholesterol, triglyceride, LDL-C increased in hypothyroidism significantly compared to subclinical hyperthyroidism and were lower in subclinical hyperthyroidism than in hypothyroidism. Hcys was significantly higher in the hypothyroid state than in subclinical hyperthyroidism and was the lowest in euthyroid controls. Mean HDL-C levels were unchanged. Average CRP levels exceeded 1.0 mg/L in both hypothyroidism and subclinical hyperthyroidism (low cardiovascular risk: < 1.0 mg/L). However, CRP values were significantly higher in subclinical hyperthyroidism. The fibrinogen, vWF values were higher in subclinical hyperthyroidism, although the mean value of vWF remained within the reference range. The results of the two different hormonal states were compared with data of the healthy euthyreoid control group (Table 1.)

Aortic stiffness

Aortic stiffness increased significantly in both the hypo- ($p<0,05$) and subclinical hyperthyroid ($p<0,01$) groups compared to controls. However, in hypothyroidism, values falling between the subclinical hyperthyroid and control groups were observed. The difference in aortic stiffness was also significant between subclinical hyperthyroidism and overt hypothroidism ($p<0,05$) (Fig.1.).

As far as LV dimension and ejection fraction are concerned, no significant changes were observed in M-mode measurrments (LVEDD, LVESD, IVS, PW and fractional shortening) and in the two dimensional study (LVM, LVMI and LVEF) either during L-T4 withdrawal, or when compared with healthy controls (Table 2).

Diastolic function parameters, E-, A-, E'- waves were significantly lower in both subclinical hyperthyroidism and overt hypothyroidism compared to healthy controls. A'-wave was significantly higher in the two hormonal abnormalities compared to controls. The E/A and E'/A' were significantly lower in subclinical hyperthyroidism and in hypothyroidism than in controls, but this ratio was lower in subclinical hyperthyroidism than in hypothyroidism. The differences in E'/A' were significantly. Heart rate was lower in hypothyroidism and higher in subclinical hyperthyroidism compared with controls (Table 3.).

Correlation between changes in aortic stiffness and laboratory parameters

By simple regression analysis, changes of aortic stiffness index during transition from subclinical hyperthyroidism to hypothyroidism correlated with changes of vWF ($r=0.61$, $p=0.013$) (Fig. 2.), fT4 ($r=0.65$, $p=0.01$) (Fig. 3) and fibrinogen ($r=0.51$, $p=0.01$) (Fig. 4.) in a positive manner, while with LDL-C in a negative manner ($r= - 0.49$, $p=0.01$).

Stepwise multiple regression analysis to evaluate association between aortic wall stiffness index and other parameters

Table 4. presents the results of stepwise multiple regression analysis of changes in various clinical variables. In this model which included aortic stiffness index, vWF, fibrinogen, fT4 and LDL-C, only vWF and fT4 emerged as independent factors associated in a positive manner with aortic stiffness (Table 4.).

Discussion

In the present study we examined aortic stiffness as well as systolic and diastolic function in a cohort of patients in two subsequent measurements, first in subclinical hyperthyroidism while on L-T4 suppression therapy, and in overt hypothyroidism after L-T4 withdrawal. We found increased aortic stiffness and decreased diastolic function in both subclinical hyperthyroidism and overt hypothyroidism. These undesirable changes may represent lifelong cardiovascular risk in DTC patients. However, the effect the four weeks L-T4 withdrawal was less marked on both aortic stiffness and diastolic function. We speculate that four weeks are not enough to develop the entire effect of hypothyroidism on peripheral tissue. As subclinical hyperthyroidism is sustained for decades in these patients, the undesirable changes caused by this condition are more important in DTC patients. To the best of our knowledge, this is the first study which examines aortic stiffness and systolic and diastolic heart function simultaneously in subclinical hyperthyroidism.

The cardiovascular risk factors cause structural and functional vascular damage. One of the non-invasive methods used to study this functional damage is arterial stiffness measurement. Aortic stiffness can be assessed by pulse wave velocity or by ultrasonically measured pulsatile aortic dimension changes [24, 32]. We used the latter technique to evaluate the cardiovascular risk.

The relationship between cardiovascular morbidity and mortality in iatrogenic subclinical hyperthyroidism is controversial [11]. Only few data are available on aortic stiffness in hyperthyroidism. Obuobie et al. found lower central arterial stiffness in untreated thyreotoxic patients that may be cardioprotective [27]. In another study, systemic arterial stiffness was found increased parallel with decreased subendocardial perfusion in hyperthyroidism [35]. In hypothroidism, central arterial stiffness is increased [15], and it improves after restoration of euthyroidism [30].

In our study the aortic stiffness was significantly higher in long-term subclinical hyperthyroidism than in euthyroid controls and in short-term hypothyroidism. T4 was an independent factor associated in a positive manner with aortic stiffness. The mechanisms by which thyroid hormone excess affects vascular physiology are still unclear. Thyroid hormones have direct effects on the endothelium (endotehelium-dependent vasodilatation), that is modulated by T3 and the effector is nitrogen-monoxid [36, 37]. It has been also shown that thyroid hormones cause rapid relaxation of vascular smooth muscle (endothelium-independent vasodilatation)[38]. Recently, we found better endothelial function while slightly impaired vascular injury markers and inflammatory status in subclinical hyperthyroidism [39]. We concluded that these apparently opposing mechanisms may compensate for each other at the level of the vessel wall. Therefore, better endothelial function and decreased arterial stiffness can be expected in subclinical hyperthyroidism. Regarding aortic stiffness, our findings are in sharp contradiction with this assumption. Sympathetic activation increases arterial wall stiffness [24]. Manifestations of hyperthyroidism resemble the effect of cathecholamine excess: the sensitivity of resistance vessels to the vasoconstrictive action of norepinephrine is enhanced [40]. β1-adrenergic blockade was associated with normalization of total arterial stiffness [28]. Our previous report of low-grade inflammation in subclinical hyperthyroidism has been confirmed by a recent study [41]. Vascular inflammation causes degradation of collagen and elastin, evokes changes in the proteoglycan composition and hydration status, and results in medial calcification [42] leading to increased arterial stiffness. Low-grade inflammation caused endothelial dysfunction and impaired NO availability in patients with subclinical hypothyroidism [43]. Thyroid hormone reduces systemic vascular resistance and causes activation the renin-angiotensin-aldosteron system. T3 directly stimulates the synthesis of renin substrate in the liver. Consequevent sodium reabsorption, increased blood volume and preload contribute to the characteristic increase in cardiac output [44]. Chronic hemodynamic overload causes increased myocardial contractility, cardiac hypertrophy, increased left-ventricular mass; contractile protein synthesis is increased. The faster heart rate in hyperthyroidism result an earlier return of the forward pressure wave in systole, resulting in a greater overlapping in the forward and reflected pressure waves [28]. vWf is reported to be a reliable marker of endothelial damage and subclinical atherosclerosis [45]. In the present study, vWF and fibrinogen as markers of endothelial dysfunction were more higher in subclinical hyperthyroidism than in overt hypothyroidism, and there was positive correlation between changes of aortic stiffness index, vWF and fibrinogen during transition from subclinical hyperthyroidism to hypothyroidism. These changes may be associated with relative hypercoagulability and increased thromboembolic risk [46].

Most previous studies used isovolumic relaxation time to evaulate diastolic dysfunction in subclinical hypo- and hyperthyroidism. Our results, albeit using another approach, are consonant with these studies Impaired diastolic function was detected in patients with subclinical hyperthyroidism[47, 8, 9, 48-50];. It has been suggested in earlier studies that diastolic dysfunction in subclinical hyperthyroidism resulted from increased LVM. However, no significant increases in LVM was found either by us or by other groups [8, 51]. Dörr et al. showed that decreased serum TSH levels were not associated with an elevated risk of left ventricular hypertrophy, but overt hyperthyrodism is an independent risk factor for left ventricular hypertrophy [52]. Thyroid hormones influence calcium regulation in myocytes, such as increase Ca++-ATPase activity and decrease phospholamban expression, and increase Ca-influx. [53]. Increase in intracellular calcium may be cause of mediated diastolic stiffness in hyperthyroid rats heart [54].

We detected only slight impairerment in aortic stiffness and diastolic function in acute short-term hypothyroidism. Aortic stiffness is likely related to myxoedema of the arterial wall [15, 55]. However, our data do not support this notion and are consonant with the findings of other studies [56, 15, 30] that argued against the role of LDL-C in increased aortic stiffness. Impaired diastolic function in hypopthyroidism due to slow myocardial relaxation results from altered intracellular calcium handling, decreased activity of the sarcoplasmatic reticulum calcium ATPase and /or increased expression of phospholamban [2]. Myofibrill swelling, mucopolysaccharides accummlation can be detected in hypothyroid heart [53]

In conclusion, our results confirm that both long-term TSH suppressive L-T4 therapy with the consequent subclinical hyperthyroidism and thyroxin withdrawal have several adverse effects on the heart and vessel wall. Impaired diastolic function and increased aortic stiffness may increase the cardiovascular risk. The degree of TSH suppression in patients with DTC should be kept at the possible minimum, considering the potential benefits and risks of treatment, especially in patients with cardiovascular comorbidities, in agreement with the current international recommendations. Patients may benefit from the withespread use of rhTSH instead of thyroxin withdrawal to achieve high TSH during TG measurement, as well as from beta-1 adrenergic blockade during iatrogenic subclinical hyperthyroidism.

Declaration of interest

The authors declare that there is no conflict of interest.

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Figure 1.
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Fig. 1. Aortic stiffness in subclinical hyperthyroidism (ScH), hypothyroidism (H) and heathy controls.

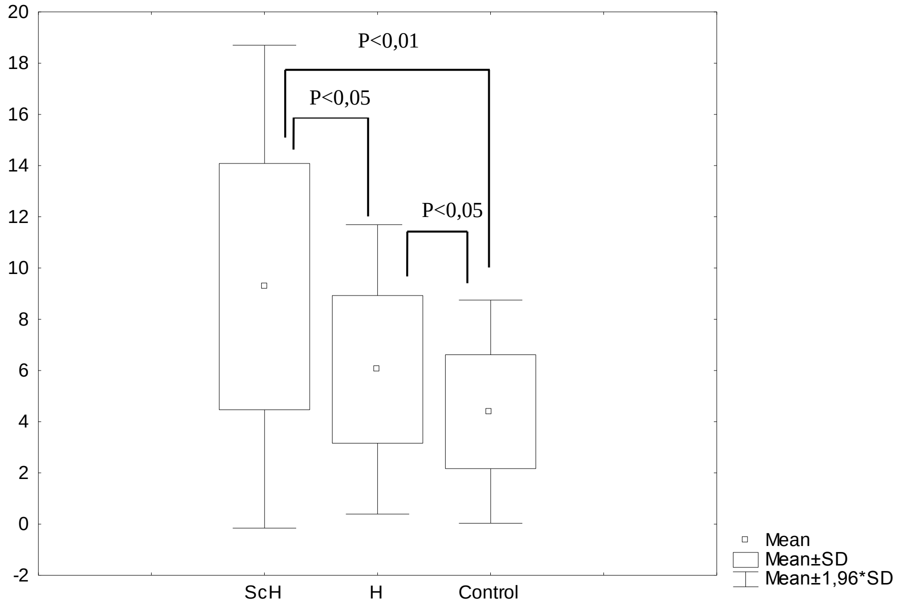

Figure 2.
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Fig. 2. Simple regression analysis to evaluate correlation of changes in vWF , with changes aortic stiffness during transition from subclinical hyperthyroidism to hypothyroidism.

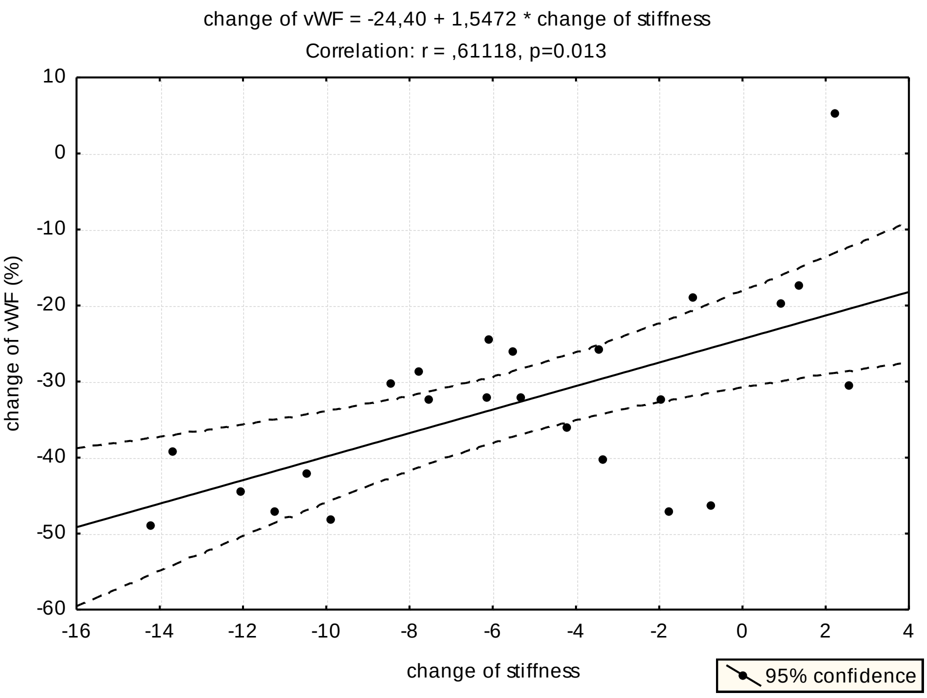

Figure 3.
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Fig 3. Simple regression analysis to evaluate correlation of changes in FT4 with changes in aortic stiffness during transition from subclinical hyperthyroidism to hypothyroidism.

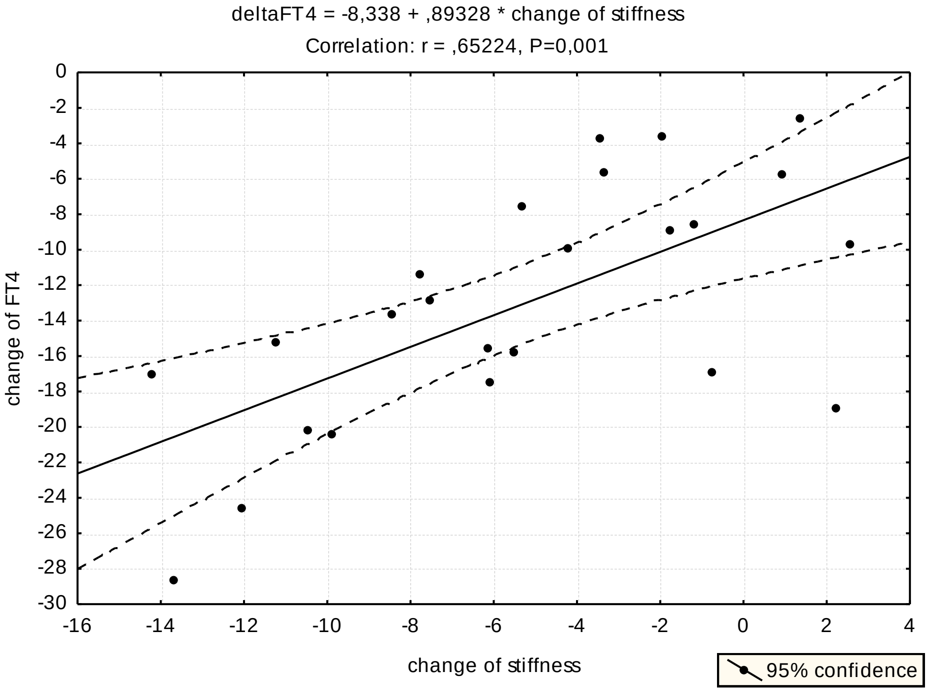

Figure 4.
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Fig 4. Simple regression analysis to evaluate correlation of changes in Fibrinogen with changes in aortic stiffness during transition from subclinical hyperthyroidism to hypo-thyroidism.

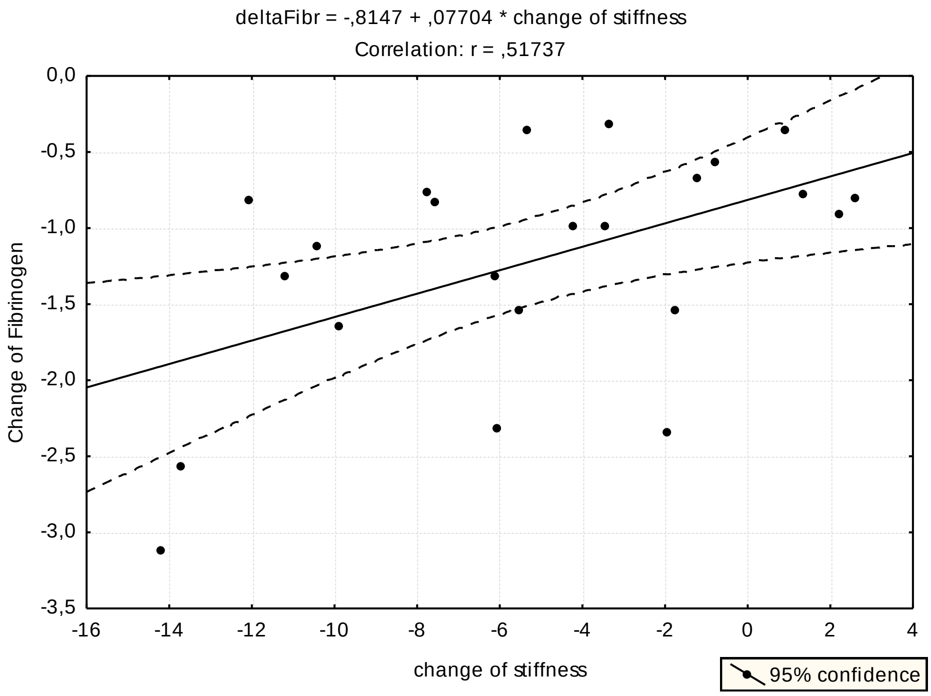

Table 1.
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Table 1. Clinical characteristics of the patient and control groups.

	Subclinical hyperthyroidism n=24	Hypothyroidism n=24	Control n=22
RR systolic (Hgmm)	125.82±7,02	128,63±7,17	126,3±5,67
RR diastolic (Hgmm)	85,18±5,82	82,29±3,98	84,21±4,56
BMI (Kg/m2)	26,98±4,56	27,34±4,87	26,54±3,45
sTSH (mU/L)	0,24±0,11#	89,82±29,36*#	1,64±1,05
fT4 (pmol/L)	18,39±2,33	2,11,24	12,16±3,11
fT3 (pmol/L)	4,79±0,46	2,0±0,82	3,12±0,54
Thyroglobuling (ug/L)	1,07±1,59	1,43±1,71	1,34±1,45
Cholesterol (mmol/l)	4,75±1,14#	7,43±1,23*#	5,12±1,21
Tryglyceride (mmol/L)	1,03±0,74#	1,79±1,12*#	1,32±0,87
LDL-C (mmol/L)	2,7±0,89#	4,55±1,1*#	2,83±1,02
HDL-C (mmol/L)	1,58±0,42	1,95±0,4	1,6±0,56
Hcys (umol/L)	9,62±2,29#	12,95±4,49*#	8,67±0,87
CRP (mg/L)	5,55±5,15#	4,39±5,16*#	2,11±0,21
VWF (%)	130,63±29,97#	90,09±25,92*#	92,36±20,6
Fibrinogen (g/L)	4,01±0,84#	3,23±0,5*#	3,05±1,2

RR: blood pressure; BMI: body-mass index; TSH: serum thyrotropin; fT4: free-thyroxine; fT3: free-triiodothyronine; LDL-C: low-densitiy lipoprotein cholesterol, HDL-c: high-densitiy lipoprotein cholesterol; Hcys: homocystein; CRP: C-reactive protein; vWF: von Willebrand factor activity

#p<0,05 compared to control

*p<0,01 compared to subclinical hyperthyrodism

Table 2. Effect of L-T4 withdrawal on LV dimensions and ejection fraction: no significant changes were observed either during L-T4 withdrawal, or when compared with healthy controls

	Subclinical hyperthyroidism (n=24)	Hypothyroidism (n=24)	Control (n=22)
LVDD (mm)	49.47±3.92	48.71±2.76	50.12±2.54
LVSD (mm)	33.23±3.47	33.11±2.98	34.98±2.89
LVEF (%)	69.50±4.93	68.92±3.12	69.12±3.87
Fractional shortening (%)	32.87±3.89	32.12±3.56	32.98±4.11
IVS (mm)	8.94±1.13	8.75±1.19	8.45±2.11
PW (mm)	8.88±1.13	8.92±1.18	8.32±1.23
LVM (g)	152.34±17.32	152.95±21	148±16.34
LVMI (gm^{-2})	87.9±12.18	87.98±11.21	85.34±6.78

LVDD: left ventricular end-diastolic diameter, LVDS: left ventricular end-systolic diameter, LVEF: left-ventricular ejection fraction, PW: end-diastolic posterior wall thickness, IVS: interventricular septum thickness, LVM: left-ventricular mass, LVMI: left ventricular mass index.

Table 3: Diastolic function in subclinical hyperthyroidism, hypothyroidism and euthyroid controls

	Subclinical hyperthyroidism (n=24)	Hypothyroidism (n=24)	Control (n=22)
E (cm/s)	60.30±10.53	62.98±9.76	72.12±7.23
A (cm/s)	43.75±9.37	42.71±7.78	45.23±4.67
E/A	1.37±4.16*	1.47±3.67*	1.59±3.67
E’	5.52±0.89*	5.78±1.02*	5.96±1.23
A’	5.48±0.9*	5.08±1.11*#	4.45±1.34
E’/A’	1.0±0.14*	1.13±0.98*#	1.34±1.02
Heart rate (beats/min)	78.35±7,23 *	70.6±6,78 *#	72.63±6.16

E: peak flow of early filling phase, A: peak flow in atrial filling phase, E’: peak flow of early filling phase measured by tissue Doppler imaging, A’: peak flow in atrial filling phase measured by tissue Doppler imaging * $p<0.05$ compared to controls, # $p< 0.05$ compared to subclinical hyperthyroidism

Table 4.
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Table 4. Stepwise multiple regression analysis to evaluate association of stiffness index with other characteristics. Standard regression coefficients (β) are given.

Dependent variable	Independent variable	β Coefficient	95% CI	P value	R^2
Changes of					
aortic stiffness	vWF	0,348	0.12 to 0.51	0.01	0.34
	fibrinogen	0.171	0.014 to 0.28	ns.	0.14
	fT4	0.47	0.33 to 0.53	0.01	0.38
	LDL-C	0.151	0.05 to 0.28	ns.	0.26

vWF: von Willebrandt factor activity, fT4: free thyroxin, LDL-C: low-densitiy lipoprotein choletesrol, ns.:non-significant