Elevated Arterial Stiffness and Diastolic Dysfunction in Subclinical Hypothyroidism

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Background: Thyroid hormone is associated with arterial stiffness and left ventricular diastolic function in hypothyroid disease. The relationship of thyroid hormone level to cardio-ankle vascular index (CAVI) and left ventricular diastolic function, however, remains unclear in subjects with subclinical hypothyroidism.

Methods and Results: We conducted a cross-sectional study of 83 patients with untreated subclinical hypothyroidism and compared them with 83 randomly selected controls from health check-ups. Log N-terminal prohormone of brain natriuretic peptide (NT-proBNP), C-reactive protein (CRP), and arterial stiffness were measured. In addition, we measured early diastolic mitral annular velocity (E') in 43 participants with subclinical hypothyroidism and in 40 controls. When compared with the control group, patients with subclinical hypothyroidism had higher logNT-proBNP (1.9±0.5 vs. 1.7±0.3 pg/ml, P<0.05), CRP (0.22±0.04 vs. 0.09±0.06 mg/dl, P<0.05), and CAVI (8.8±1.7 vs. 7.8±1.4, P<0.001) and lower E' (5.8±1.7 vs. 7.5±2.1 cm/s, P<0.001). CAVI was significantly associated with logNT-proBNP, CRP and E' in the subclinical hypothyroidism group.

Conclusions: High logNT-proBNP was associated with a raised CAVI in patients with subclinical hypothyroidism. Subclinical hypothyroidism may be a risk factor for cardiovascular events related to arterial stiffening and left ventricular diastolic dysfunction. (Circ J 2014; 78: 1494–1500)

Key Words: Atherosclerosis; Diastolic dysfunction; Stiffness; Thyroid
Blood Chemistry
We collected blood samples from the participants for the following: TSH, FT3, FT4, C-reactive protein (CRP), uric acid (UA), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and N-terminal prohormone of brain natriuretic peptide (NT-proBNP). All samples were measured with an autoanalyzer using the manufacturer’s standard kits (Cobas® 8000; Roche Diagnostics, Mannheim, Germany).

The control group consisted of patients with TSH 0.5–5.0 μIU/ml. The subclinical hypothyroidism group consisted of patients with TSH 5.1–20 μIU/ml. Both groups had FT3 2.3–4.0 pg/dl and FT4 0.9–1.7 ng/dl.

CAVI
To measure the vascular stiffness independently of the systemic blood pressure, CAVI was calculated using the vascular stiffness constant beta and the Bramwell-Hill equation.\(^{12,13}\) CAVI was measured using a VaSera-1500 (Fukuda Denshi, Tokyo, Japan). After a 10-min rest, the participants were asked to lie in the supine position on a bed with monitoring cuffs wrapped around each of their limbs. Limb lead electrocardiography and phonocardiography were performed using a microphone placed on the sternum at the level of the second intercostal space, and CAVI was automatically determined using the VaSera-1500.

Mean blood pressure was calculated from the formula: mean blood pressure = [(2×diastolic blood pressure)+systolic blood pressure]/3.

Echocardiography
Transthoracic echocardiography was recorded using an iE33 (Philips Medical Systems, Bothell, WA, USA). We conducted standard, comprehensive, M-mode (motion-mode), 2-D echocardiography and Doppler studies according to the American Society of Echocardiography guidelines.\(^{18}\) Left ventricular mass index and relative wall thickness were measured using the method described previously.\(^{19}\) Peak early diastolic phase (E) and late diastolic phase (A) mitral inflow velocities, and the E/A ratio were measured on pulsed-wave Doppler echocardiography with the sample volume between mitral leaflet tips. Mitral annulus velocity (E’) and the E/E’ ratio were measured at the septal annulus on tissue Doppler imaging.

Ethics
This study was conducted in accordance with the principles outlined in the Declaration of Helsinki after receiving approval from the institutional review board of Hyogo College of Medicine. All subjects provided written informed consent prior to participation.

Statistical Analysis
Continuous data are presented as mean±SD. We compared the control and subclinical hypothyroidism groups using unpaired t-test. The correlations between CAVI and TSH, FT3, FT4, NT-proBNP, CRP, and E’ were examined using linear regression analysis. CAVI was compared between the subclinical hypothyroidism group and control group using analysis of covariance, adjusting for mean blood pressure and heart rate as covariates. Multivariate analysis was done using stepwise regression analysis with forward elimination to identify independent factors associated with CAVI. Parameters with F>4.0 were entered into the regression analysis as independent variables.

P<0.05 was considered significant. Statistical analysis was performed using JMP version 10.0.1 (SAS Institute, Cary, NC, USA).

Results
Patient Characteristics
The baseline characteristics such as age, sex, body mass index (BMI), glycosylated hemoglobin (HbA1c), UA, Cre, HDL-C, LDL-C, and TG, were not different between the subclinical hypothyroidism and control groups. FT3 and FT4 were lower in the subclinical hypothyroidism group when compared with the control group, but FT3 and FT4 were within normal limits in both groups. Systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, CAVI, TSH, logNT-proBNP, and CRP were higher in the subclinical hypothyroidism group when compared with the control group. E’ and E/E’ were significantly different between the control and subclinical hypothyroidism groups (Tables 1,2). The adjusted CAVI for mean blood pressure and heart rate was significantly higher in the subclinical hypothyroidism group than in the control group (P<0.001).

CAVI, Thyroid Hormone, NT-proBNP, CRP, and E’
In the subclinical hypothyroidism group, CAVI was significantly associated with FT3 (r=-0.22, P<0.05), CRP (r=0.22,
CRP was negatively correlated with E' (r=−0.41, P<0.01) and positively correlated with logNT-proBNP (r=0.23, P<0.05) in the subclinical hypothyroidism group but not in the control group. In the subclinical hypothyroidism group, logNT-proBNP was solely and independently associated with CAVI after stepwise multiple regression analysis (regression coefficient, 0.827; standard error, 0.350; F=5.17). Non-accepted variables were SBP, DBP, mean blood pressure, heart rate, FT3, FT4, TSH, CRP, and the presence or absence of anti-hypertensive medication in the subclinical hypothyroidism group.

Discussion

The main findings in this study were: (1) CAVI increased in subclinical hypothyroidism; and (2) CAVI was correlated with CRP, E' and logNT-proBNP in subclinical hypothyroidism.

Subclinical Hypothyroidism and Arterial Stiffness

We determined that CAVI increased in patients with subclinical hypothyroidism. To our knowledge, this is the first study to identify a relationship between CAVI and subclinical hypothyroidism. Previous studies have reported that CAVI was associated with the arterial stiffness parameter beta, independently of blood pressure. P<0.05, logNT-proBNP (r=0.27, P<0.05), and E' (r=−0.42, P<0.01), and E/E' (r=0.38, P<0.05) but not with FT4 and TSH (Figures 1–3, Upper). In the control group, CAVI was significantly associated with logNT-proBNP (r=0.48, P<0.001) and E' (r=−0.47 P<0.01) but not with FT3, FT4, TSH, CRP, or E/E' (Figures 1–3, Lower). FT3 was inversely associated with logNT-proBNP in subclinical hypothyroidism (r=−0.27, P<0.05).

Table 2. Echocardiographic Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n=40)</th>
<th>Subclinical hypothyroidism (n=43)</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64±13</td>
<td>68±13</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>13/27</td>
<td>15/28</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>36±6</td>
<td>38±11</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>46±4</td>
<td>47±4</td>
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<tr>
<td>LVESD (mm)</td>
<td>29±3</td>
<td>29±4</td>
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<tr>
<td>RWT</td>
<td>0.35±0.06</td>
<td>0.39±0.04</td>
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<tr>
<td>LVEF (%)</td>
<td>68±6</td>
<td>67±6</td>
</tr>
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<td>LVMi (g/m²)</td>
<td>83±19</td>
<td>79±19</td>
</tr>
<tr>
<td>E (m/s)</td>
<td>65±12</td>
<td>60±14</td>
</tr>
<tr>
<td>A (m/s)</td>
<td>66±16</td>
<td>73±19</td>
</tr>
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<td>E/A</td>
<td>0.99±0.31</td>
<td>0.87±0.36</td>
</tr>
<tr>
<td>DT (ms)</td>
<td>207±48</td>
<td>220±78</td>
</tr>
<tr>
<td>E’ (cm/s)</td>
<td>7.5±2.1</td>
<td>5.8±1.6***</td>
</tr>
<tr>
<td>E/E'</td>
<td>9±3</td>
<td>11±3*</td>
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</table>

Data given as mean±SD. *P<0.05 vs. the control group, **P<0.01 vs. the control group, ***P<0.001 vs. the control group. DT, deceleration time; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; LVMi, left ventricular mass index; RWT, relative wall thickness.

Because CAVI is a useful tool for assessing the contraction of...
Figure 2. Correlation between CAVI and CRP or NT-proBNP in the (A) subclinical hypothyroidism (n=83) and (B) control (n=83) groups. (A) In the subclinical hypothyroidism group, CAVI was significantly associated with CRP \(r=0.22, P < 0.05\) and logNT-proBNP \(r=0.27, P < 0.05\). (B) In the control group, CAVI was associated with logNT-proBNP \(r=0.48, P < 0.001\) but not with CRP.

CAVI, cardio-ankle vascular index; CRP, C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

Figure 3. Correlation between the E’ and CAVI in the (A) subclinical hypothyroidism (n=43) and (B) control (n=40) groups. (A) In the subclinical hypothyroidism group, CAVI was significantly associated with E’ \(r=-0.42, P < 0.01\) and E/E’ \(r=0.38, P < 0.05\). (B) In the control group, CAVI was significantly associated with E’ \(r=-0.47, P < 0.01\) but not with E/E’. CAVI, cardio-ankle vascular index; E’, mitral annulus velocity; E, peak early diastolic phase; E/E’, ratio of E/E’.
smooth muscle cells and the degree of atherosclerosis, we anticipated its usefulness in assessing arterial stiffness in patients with subclinical hypothyroidism. To date, several studies have provided evidence that arterial stiffness is higher in subclinical hypothyroidism.\textsuperscript{3,8,9} In patients with subclinical hypothyroidism, increased blood pressure and arterial endothelial dysfunction resulting from a reduction in nitric oxide (NO) may be a major feature associated with increased arterial stiffness.\textsuperscript{3,4,2}

In the present study, FT4 was not associated with arterial stiffness as measured using CAVI. We speculate that this occurred because the molecular mechanism of T3-mediated vascular dilatation may be associated with a genomic pathway (ie, upregulation of the potassium channel through thyroid hormone receptor-\(\alpha\))\textsuperscript{33} or a non-genomic pathway (stimulating NO production).\textsuperscript{44} This would raise the possibility that FT3 may play a pivotal role in arterial stiffness because the local conversion from T4 to T3 induces aortic dilatation.

Previous reports found that subclinical hypothyroidism affects left ventricular diastolic dysfunction and arterial stiffness.\textsuperscript{3,8} Nagasaki et al suggested that elevated DBP might be associated with an increased pulse wave velocity (PWV) in subclinical hypothyroidism.\textsuperscript{3} Unfortunately, compared to PWV, CAVI is a less well recognized marker of arterial stiffness around the world because of the limited number of manufacturers. Shirai et al reported that CAVI measurement kit was more weakly correlated with SBP when compared with PWV in patients with atherosclerosis. Moreover, no relationship between CAVI and DBP existed.\textsuperscript{12} For the first time, we have found that CAVI was not correlated with DBP in the subclinical hypothyroidism group. Thus, CAVI appears to be a reliable vascular stiffness index that is less influenced by blood pressure than PWV.\textsuperscript{12,28} Therefore, CAVI was suitable for the assessment of arterial stiffness in subclinical hypothyroidism.

**Subclinical Hypothyroidism and Diastolic Dysfunction**

The present study has shown that the reduction in E’ and the increased E/E’ were associated with an elevation in CAVI. Previous studies have similarly confirmed a negative correlation between left ventricular diastolic dysfunction and CAVI.\textsuperscript{14,48} The thyroid gland primarily releases thyroid hormones as T4, with T3 typically comprising <5% of the thyroid’s output. T4, however, is converted to the biologically active T3 by the conversion of T4 with type I and type II 5’deiodinases. In particular, T3 affects the heart through both genomic effects (such as increased expression of sarcoplasmic reticulum calcium ATPase [SERCA] or cardiac \(\alpha\)-myosin heavy chain) and non-genomic effects (such as increased NO synthesis or NO release from endothelial cells).\textsuperscript{46,47} Subclinical hypothyroidism has been reported to impair left ventricular diastolic function by reducing calcium re-uptake into sarcoplasmic reticulum through decreased expression of SERCA and phospholamban activation.\textsuperscript{46,48–50} Moreover, Wassen et al identified type 3 deiodinase upregulation in cardiac tissue, which converts inactive T3 to its active form.\textsuperscript{51} Activity of type 3 deiodinase is stimulated by pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor-\(\alpha\)\textsuperscript{52} that are related to left ventricular diastolic dysfunction.\textsuperscript{53,54} These data suggest that subclinical hypothyroidism might have impaired both left ventricular relaxation and systemic vascular resistance in the present study.

In the subclinical hypothyroidism group, FT3 was inversely associated with logNT-proBNP. The association between BNP and FT3 is controversial. Schultz et al determined that BNP and FT3 were positively correlated in patients with subclinical hypothyroidism.\textsuperscript{55} This positive correlation may be attributable to the thyroid hormone increases in NT-proBNP gene transcription through genomic effects in cardiac myocytes.\textsuperscript{56} Conversely, similar to the present results, several studies have reported that T3 was negatively correlated with logBNP in patients with heart failure and preserved ejection fraction, idiopathic left ventricular dysfunction, and post-cardiac surgery status.\textsuperscript{57,59}

Therefore, in the present subjects, high logBNP would result from left ventricular diastolic dysfunction associated with hypothyroidism.

Takeda et al reported that endovascular aortic repair increased arterial stiffness and left ventricular diastolic dysfunction.\textsuperscript{60} Thus, the effect of ventriculo-arterial coupling may link left ventricular diastolic dysfunction with arterial stiffness. Masugata et al showed that arterial stiffness assessed using CAVI was positively correlated with plasma BNP in hypertensive patients, citing that plasma BNP reflects cardiac damage (including diastolic dysfunction or left ventricular hypertrophy).\textsuperscript{61} The present study suggests that increased CAVI is associated with high logNT-proBNP, which might be affected by left ventricular diastolic dysfunction and ventriculo-arterial uncoupling in subclinical hypothyroidism.

**Subclinical Hypothyroidism and CRP**

In the present study, subclinical hypothyroidism was associated with elevated CRP. Previous reports have found that subclinical hypothyroidism is associated with inflammation.\textsuperscript{62,63} Tian et al found that subclinical hypothyroidism was associated with increased carotid arterial stiffness caused by elevated CRP.\textsuperscript{64} Dardano et al reported that recombinant human TSH induced endothelial dysfunction and inflammation due to a reduction of vascular NO.\textsuperscript{65} Type 3 deiodinase upregulation, which converts inactive T3 to its active form, might possibly increase CRP in subclinical hypothyroidism. We found that CRP was positively correlated with CAVI and NT-proBNP, and negatively correlated with E’ in the subclinical hypothyroidism group, suggesting that elevated CRP may be associated with left ventricular diastolic dysfunction and increased arterial stiffness in subclinical hypothyroidism.

**Subclinical Hypothyroidism and Lipid Metabolism**

Although hypothyroidism is frequently associated with hyperlipidemia, the relationship between subclinical hypothyroidism and hyperlipidemia remains controversial.\textsuperscript{39} Some reports have found no differences in the lipid profile of subclinical hypothyroid patients compared with controls.\textsuperscript{86,67} Muller et al reported that the non-smokers with subclinical hypothyroidism had serum lipid levels that were not significantly different from those of controls. Moreover, smokers with subclinical hypothyroidism had higher serum lipid levels when compared with non-smokers with subclinical hypothyroidism. Further, Bakker et al reported that insulin resistance may play an important role in elevating serum lipids.\textsuperscript{68} The conflicting results in the present study might reflect differences in the subjects and the lesser impacts of smoking and insulin resistance compared with other studies.

Although hypothyroidism is usually associated with bradycardia, the present subclinical hypothyroidism patients had increased heart rate when compared with controls, but the heart rate in both groups tended to remain within the normal range. Similarly, the heart rate in the subclinical hypothyroidism and control subjects remained within the normal range in the previous studies.\textsuperscript{5,8} This conflicting result, although possibly due to chance, might reflect the differences in the variety of patients compared with other studies.

**Study Limitations**

There were several limitations to this study. First, this study was
conducted at a single center with a small sample size; therefore, a large multicenter study is needed to confirm the present results. Second, we did not measure anti-thyroid antibody titer. Nagasaki et al, however, reported that there was no significant correlation between arterial stiffness and autoimmune antibodies, such as anti-thyroglobulin antibody or anti-thyroid peroxidase antibody, in subclinical hypothyroidism. Therefore, such measurement may not have been necessary. Third, arterial stiffness is known to be greater in women than in men. Lambrinoudaki et al reported that serum TSH is a predictor of arterial stiffness in euthyroid postmenopausal women. When compared with lower levels, serum TSH over the upper reference range is typically associated with higher arterial stiffness. Further exploration of whether gender-related arterial stiffening is associated with subclinical hypothyroidism may be necessary. Fourth, PWV is recognized as the gold standard of arterial stiffness; thus, additional studies are needed to evaluate the relationship between CAVI and PWV in subclinical hypothyroidism. The present study could not definitively conclude that increased arterial stiffness was detectable using CAVI alone.

Perspective
We have shown that patients with subclinical hypothyroidism have increased arterial stiffening, as measured on CAVI, and that these abnormalities were associated with raised CRP and diastolic dysfunction. Thus, arterial stiffening may play a crucial role in the development of the cardiovascular mortality or morbidity in subclinical hypothyroidism. Further investigation is necessary to explore whether supplemental thyroxine therapy is a suitable preventative strategy for left ventricular diastolic dysfunction in patients with subclinical hypothyroidism. We have shown that patients with subclinical hypothyroidism have increased arterial stiffening, as measured on CAVI, and that these abnormalities were associated with raised CRP and diastolic dysfunction. Thus, arterial stiffening is associated with subclinical hypothyroidism may be necessary. Further investigation of whether gender-related arterial stiffening is associated with subclinical hypothyroidism may be necessary. Fourth, PWV is recognized as the gold standard of arterial stiffness; thus, additional studies are needed to evaluate the relationship between CAVI and PWV in subclinical hypothyroidism. The present study could not definitively conclude that increased arterial stiffness was detectable using CAVI alone.

Conclusions
High logNT-proBNP was associated with raised CAVI in subclinical hypothyroidism. Subclinical hypothyroidism may be a risk factor for cardiovascular events related to arterial stiffening and left ventricular diastolic dysfunction.

Acknowledgments
We thank Ms M. Tanaka, S. Makihara, C. Misumi, D. Maki, for their excellent technical assistance in the acquisition of echocardiographic tracings. This work was supported by Fukuda Densi, Tokyo, Japan and by a Grant-in-Aid for Researchers, Hyogo College of Medicine, 2011.

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