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# A Prospective Study of the Precision of Four Serum *Helicobacter pylori* Antibody Measurement Methods

Kazuhiko Inoue[1,2], Nobumi Hisamoto[2], Ken Haruma[1,2]

## Abstract

**Objective:** We prospectively examined the precision of four serum *Helicobacter pylori* (*H. pylori*) antibody measurement methods for diagnosing 'currently infected' vs. 'uninfected' and 'currently infected+previously infected' vs. 'uninfected.'

**Methods:** Subjects included 326 patients who underwent esophagogastroduodenoscopy (EGD) in a comprehensive health checkup system. Serum *H. pylori* antibodies were measured using three latex-based methods, namely, L-type Wako H. pylori antibody, J (Hp-W); Latex 'Seiken' H. pylori antibody (Hp-D); and LZ test 'Eiken' H. pylori antibody (Hp-L), and the ELISA-based method, E plate 'Eiken' H. pylori antibody II (Hp-E). *H. pylori* infection status was determined from $^{13}$C-urea breath test results and EGD according to the Kyoto classification of gastritis. The accuracy of each kit was examined according to set cut-off values. Further, receiver operating characteristic (ROC) curve analysis was performed to examine the precision of serum *H. pylori* antibody findings.

**Results:** Accuracy analysis using set cut-off values showed that Hp-W and Hp-D were equivalent to Hp-E, whereas Hp-L was inferior. In ROC analysis of 'currently infected' vs. 'uninfected' status, areas under the curve (AUCs) were 0.9996 for Hp-W, 0.9988 for Hp-D, 0.9893 for Hp-L, and 1.0000 for Hp-E. However, in ROC analysis for 'currently infected+previously infected' vs. 'uninfected,' AUCs decreased to 0.9251, 0.9037, 0.8886, and 0.9478 for Hp-W, Hp-D, Hp-L, and Hp-E, respectively.

**Conclusion:** Although Hp-W and Hp-D had good precision comparable to that of Hp-E, the cut-off value for Hp-L needs to be changed. The impact of 'previously infected' cases on precision should also be considered.

**Keywords** *Helicobacter pylori*, Serum antibody, Kyoto classification of gastritis, $^{13}$C-Urea breath test

Since *Helicobacter pylori* (*H. pylori*) was discovered by Warren and Marshall[1] in 1983, numerous studies have shown it to be strongly associated with the development of not only peptic ulcer but also gastric cancer. *H. pylori* infection has been positioned as a necessary condition for the development of gastric cancer[2,3], and determining *H. pylori* infection status during gastric cancer screening is considered important.

Methods for diagnosing *H. pylori* infection include the culture method, microscopic method, and rapid urease test (RUT), all of which require esophagogastroduodenoscopy (EGD) and biopsy, as well as a serum antibody test, urinary antibody test, fecal antigen test, and $^{13}$C-urea breath test (UBT), which do not require EGD. For screening, the method needs to be simple, non-invasive, inexpensive, and allow for testing of multiple patients at a time. In this context, serum antibodies offer the most suitable means and are widely used in comprehensive health checkup systems. Moreover, it is possible to evaluate the level of gastric health and to stratify the risk of gastric cancer by combining with the serum pepsinogen test, which has been used as a gastric cancer risk stratification test (ABC classification)[4,5]. In Japan, the E plate 'Eiken' H. pylori antibody II (Hp-E), an enzyme-linked immunosorbent assay (ELISA), has been widely used for serum *H. pylori* antibody measurement for many years.

Recently, due to the cumbersome nature of the ELISA-based method, several serum *H. pylori* measurement methods have been developed based on the latex test, which can be easily measured with a fully automated analyzer for general chemistry. Moreover, to assess the risk of gastric cancer, it is necessary to identify

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people with *H. pylori* infection, including those with a history of previous infection, as the risk remains in previously infected individuals. In the present study, we prospectively examined the diagnostic precision of the existing ELISA-based serum *H. pylori* antibody test and three new latex test-based serum *H. pylori* antibody measurement methods for 'currently infected' vs. 'uninfected' and 'currently infected+previously infected' vs. 'uninfected' cases, using the UBT and EGD as indicators.

## Methods

### Subjects

Subjects included 326 participants of a comprehensive health checkup system who underwent EGD and provided written consent from January 2017 to September 2018. The breakdown of the study cohort is as follows: 227 males, 99 females, age range 26–74 years, with a mean age of 50.5 years. Participants with a history of gastrectomy, taking oral antibiotics, proton pump inhibitors, or potassium competitive acid blocker, and those with advanced renal disorder were excluded.

### Serum *H. pylori* antibodies

Residual serum samples collected during the comprehensive health checkup system were frozen and stored at −20℃, and thawed for use at the time of measurement.

Measurements were performed using the following four methods: the ELISA method, E plate 'Eiken' H. pylori antibody II® (Eiken Chemical Co., Ltd, Tokyo, Japan) (Hp-E), and three new latex methods: L-type Wako H. pylori antibody, J® (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) (Hp-W); Latex 'Seiken' H. pylori antibody® (Denka company Limited, Tokyo, Japan) (Hp-D); and LZ test 'Eiken' H. pylori antibody® (Eiken Chemical Co., Ltd., Tokyo, Japan) (Hp-L).

The cut-off values set for each *H. pylori* antibody measurement method were 4 U/mL for Hp-W, 10 U/mL for Hp-D, 10 U/mL for Hp-L, and 10 U/mL for Hp-E.

### Esophagogastroduodenoscopy (EGD)

EGD was performed using a small-caliber endoscope manufactured by FUJIFILM Corporation (EG-L580NW, FUJIFILM Corporation, Tokyo, Japan). In addition to white light, we also linked color imaging (LCI) for special light observation to observe the condition of the gastric mucosa and to determine the status of *H. pylori* infection per the Kyoto classification of gastritis[6]. Specifically, cases with an atrophy pattern of C0 or C1 according to the Kimura and Takemoto classification (C0, C1, C2, C3, O1, O2, O3)[7], showing a regular arrangement of collecting venules (RAC)[8] in the gastric angle with no diffuse redness, were endoscopically determined as not having been infected with *H. pylori* (**Fig. 1**). Cases with an atrophy pattern of C2 or higher were excluded from the *H. pylori*-uninfected group and

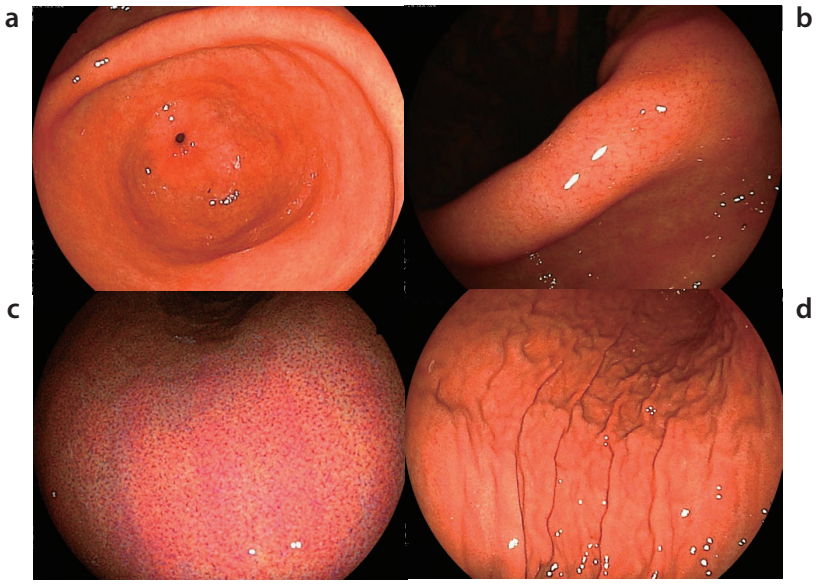


**Fig. 1. Endoscopic Image of an *H. pylori*-uninfected Case (58-year-old Male)**

a: No atrophy or diffuse redness is observed in the antrum. The atrophy pattern is C0. (WLI)
b: Regular arrangement of collecting venules (RAC) in the gastric angle is observed. (WLI)
c: Clear RAC in the lower body lesser curvature is observed. (LCI)
d: The folds of the greater curvature of the gastric body are narrow and uniform, and the entire gastric mucosa has a shine. (WLI)
$^{13}$C-urea breath test was negative at 0.7‰. Serum pepsinogen (PG) values were PG I: 39.1 ng/mL and PG II: 5.3 ng/mL, with a I/II ratio of 7.4.
LCI: linked color imaging, WLI: white light imaging.

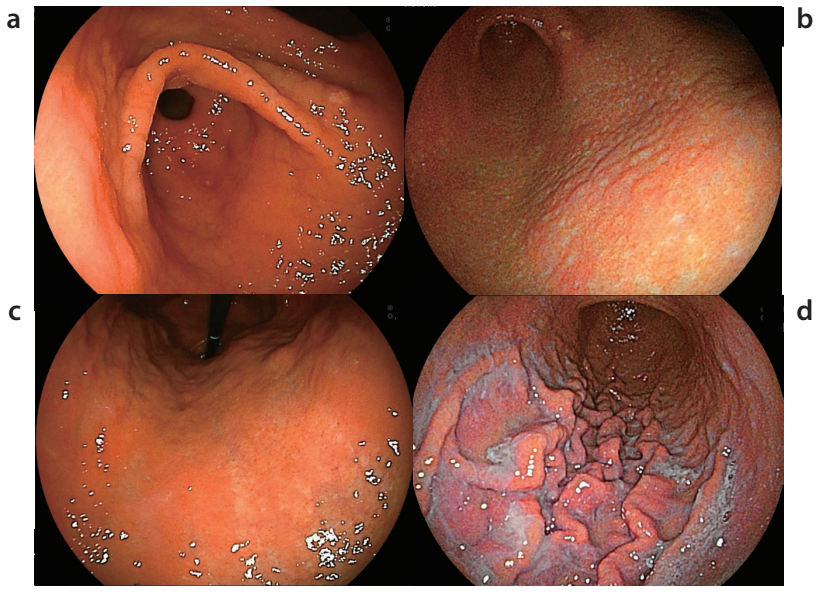


**Fig. 2. Endoscopic Image of a Current *H. pylori* Infection Case (58-year-old Female)**

a: Diffuse redness is observed in the antrum. (WLI)
b: Nodularity is observed in the antrum. (LCI)
c: Diffuse redness is observed in the gastric body, with C3 atrophy. (WLI)
d: Enlarged folds and sticky mucus attachment are observed in the greater curvature of the gastric body. (LCI)
Rapid urease test was positive, and $^{13}$C-urea breath test was also positive at 32.2‰. Serum pepsinogen (PG) values were PG I: 156.9 ng/mL and PG II: 94.4 ng/mL, with a I/II ratio of 1.7.
LCI: linked color imaging, WLI: white light imaging.

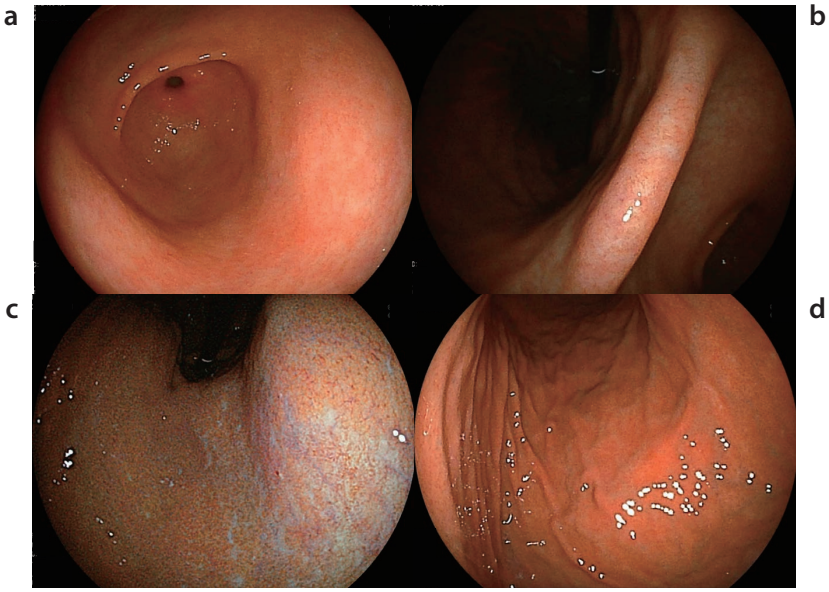


**Fig. 3. Endoscopic Image of a Previous *H. pylori* Infection Case (61-year-old Female, Underwent *H. pylori* Eradication Therapy 3 Years Prior)**

a: Atrophy is observed in the antrum, but no diffuse redness is observed. (WLI)
b: No regular arrangement of collecting venules (RAC) is observed in the gastric angle. (WLI)
c: The lesser curvature of the gastric body shows a patchy pattern of atrophic mucosa, which is assessed as C3. (LCI)
d: The greater curvature of the gastric body shows no diffuse redness. (WLI)
$^{13}$C-urea breath test was negative at 0.4‰. Serum pepsinogen (PG) values were PG I: 32.2 ng/mL and PG II: 6.3 ng/mL, with a I/II ratio of 7.4.
LCI: linked color imaging, WLI: white light imaging.

judged as being currently infected with *H. pylori* if diffuse redness was observed (**Fig. 2**), or as previously infected with *H. pylori* if no diffuse redness was observed (**Fig. 3**). Findings such as map-like redness, patchy redness, and a spotty pattern of atrophic mucosa in the lesser curvature of the gastric body suggested previous infection.

### $^{13}$C-Urea breath test (UBT)

Breath samples were collected before and 20 min after taking a UBIT® tablet 100 mg (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), and measurements were performed using POCone® (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), an infrared spectroscopy-based expiratory $^{13}CO_2$ analyzer. Samples with a $\Delta^{13}$C-$CO_2$ of 2.5‰ or higher were diagnosed as *H. pylori*-positive.

In cases in which the RUT using Helicocheck® (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) during EGD clearly indicated positive results, UBT was partly omitted and a diagnosis of current *H. pylori* infection was given. We never determined the *H. pylori*-negative status solely based on the RUT.

### Determination of *H. pylori* infection status

*H. pylori* infection status was determined based on UBT results and EGD findings. Specifically, cases in which UBT (RUT in some) results were positive, with EGD revealing C2 or higher atrophy and diffuse redness, were judged as 'currently infected.' In contrast, those with negative UBT results and EGD findings that included an atrophy pattern of C0 or C1 and RAC in the gastric angle were judged as 'uninfected with *H. pylori*.' However, those with negative UBT results and EGD showing atrophy of C2 or higher and no diffuse redness were judged as 'previously infected.' 'Previously infected' cases were further classified into those with and without a history of eradication, according to their medical history obtained by interview or medical records.

### Precision of each serum antibody measurement method

For *H. pylori* infection status ('currently infected' vs 'uninfected') determined based on UBT and EGD, the diagnostic accuracy was comparatively examined using cut-off values set for each serum *H. pylori* antibody measurement method. Statistical analyses were performed using JMP®14 (SAS Institute Inc., Cary, NC, USA). The overall agreement rate was assessed using κ coefficients, the disagreement rate was assessed using McNemar's test, and the diagnostic accuracy between kits was evaluated using a test for differences in population proportions, with the significance level set at 5%.

Furthermore, for each serum *H. pylori* antibody measurement method, the precision of diagnosing 'currently infected' vs. 'uninfected' cases, 'currently infected+previously infected (excluding post-eradication cases)' vs. 'uninfected' cases, and 'currently infected+previously infected (all cases)' vs. 'uninfected' cases was examined using receiver operating characteristic (ROC) curves. Then, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each optimal cut-off value were determined. ROC analysis was performed using JMP®14.

### Ethics

The Junpukai Health Maintenance Center ethics committee approved the protocol on October 8, 2016 (approval number: 20160002). Written informed consent was obtained from all subjects.

## Results

### *H. pylori* infection status according to UBT and EGD

**Table 1** shows the *H. pylori* infection status based on UBT results and EGD findings. There were 104 *H. pylori*-uninfected cases (75 males, 29 females; mean age, 46.1 years), 103 currently infected cases (66 males, 37 females; mean age, 49.9 years), and 119 previously infected cases (86 males, 33 females; mean age, 54.9 years). The 119 previously infected cases included 98 post-*H. pylori* eradication cases, with the remaining 21 having no history of eradication. The number of years elapsed after eradication varied, from six months to 20 years.

### Precision of serum *H. pylori* antibody measurement methods when using set cut-off values ('currently infected' vs. 'uninfected')

**Table 2-a** shows the precision of each serum *H. pylori* antibody measurement method when using the set cut-off values, with *H. pylori* infection status determined by UBT and EGD as an indicator. The accuracy rate was high at 99.0% (κ coefficient 0.9807) for the

**Table 1. Judgement of *H. pylori* Infection Status According to UBT and EGD Findings**

| *H. pylori* infection status | Number | Sex (M/F) | Age range (mean; years) |
|---|---|---|---|
| Uninfected | 104 | 75/29 | 26–67 (46.1) |
| Currently infected | 103 | 66/37 | 27–74 (49.9) |
| Previously infected | 119 | 86/33 | 33–73 (54.9) |
| [Post eradication] | [98] | [70/28] | [33–70 (54.5)] |
| [No history of eradication] | [21] | [16/ 5] | [38–71 (56.8)] |

UBT: $^{13}$C-Urea breath test, EGD: Esophagogastroduodenoscopy

**Table 2-a. Precision of Each Serum *H. pylori* Antibody Measurement Method by the Set Cut-off Values (Currently Infected vs. Uninfected)**

| | | UBT+EGD | | Total | Sensitivity | Specificity | Accuracy rate | False-negative rate | κ | *p* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Currently infected | Uninfected | | | | | | | |
| Hp-W | Positive | 102 | 1 | 103 | 0.990 | 0.990 | 0.990 | 0.010 | 0.9807 | 1.0000 |
| | Negative | 1 | 103 | 104 | | | | | | |
| | Total | 103 | 104 | 207 | | | | | | |
| Hp-D | Positive | 102 | 1 | 103 | 0.990 | 0.990 | 0.990 | 0.010 | 0.9807 | 1.0000 |
| | Negative | 1 | 103 | 104 | | | | | | |
| | Total | 103 | 104 | 207 | | | | | | |
| Hp-L | Positive | 84 | 2 | 86 | 0.816 | 0.981 | 0.899 | 0.184 | 0.7969 | 0.0002 |
| | Negative | 19 | 102 | 121 | | | | | | |
| | Total | 103 | 104 | 207 | | | | | | |
| Hp-E | Positive | 99 | 0 | 99 | 0.961 | 1.000 | 0.981 | 0.039 | 0.9613 | 0.0455 |
| | Negative | 4 | 104 | 108 | | | | | | |
| | Total | 103 | 104 | 207 | | | | | | |

Hp-W: L type Wako H. pylori antibody, J, cut-off value: 4 U/mL
Hp-D: Latex 'Seiken' H. pylori antibody, cut-off value: 10 U/mL
Hp-L: LZ test 'Eiken' H. pylori antibody, cut-off value: 10 U/mL
Hp-E: E plate 'Eiken' H. pylori antibody II, cut-off value: 10 U/mL
UBT: $^{13}$C-Urea breath test, EGD: Esophagogastroduodenoscopy

**Table 2-b. Comparison of Diagnostic Accuracy When Using Set Cut-off Values of Each Kit (Test for Differences in Population Proportions)**

| | Sensitivity | Specificity | Accuracy rate | False-negative rate |
|---|---|---|---|---|
| Hp-W vs. Hp-E | 0.0833 | — | 0.3173 | 0.0833 |
| Hp-D vs. Hp-E | 0.0833 | — | 0.3173 | 0.0833 |
| Hp-L vs. Hp-E | 0.0006 | — | 0.0002 | 0.0006 |
| Hp-W vs. Hp-D | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| Hp-W vs. Hp-L | <.0001 | 0.5637 | <.0001 | <.0001 |
| Hp-D vs. Hp-L | <.0001 | 0.5637 | <.0001 | <.0001 |

Hp-W: L type Wako H. pylori antibody, J, cut-off value: 4 U/mL
Hp-D: Latex 'Seiken' H. pylori antibody, cut-off value: 10 U/mL
Hp-L: LZ test 'Eiken' H. pylori antibody, cut-off value: 10 U/mL
Hp-E: E plate 'Eiken' H. pylori antibody II, cut-off value: 10 U/mL
UBT: $^{13}$C-Urea breath test, EGD: Esophagogastroduodenoscopy

latex methods Hp-W and Hp-D, and no significant difference was observed in the test for disagreement rates (McNemar's test) ($p$=1.0000). The accuracy rate was high at 98.1% (κ coefficient 0.9613) for Hp-E, but a significant difference was observed in the test for disagreement rates (McNemar's test) ($p$<0.05). In other words, the diagnostic accuracy of Hp-W and Hp-D was equivalent to or higher than that of the ELISA-based method Hp-E. In contrast, the accuracy rate was slightly low for Hp-L, at 89.9% (κ coefficient 0.7969), and a significant difference was observed in the test for disagreement rates (McNemar's test) ($p$<0.001). When comparing the diagnostic accuracy among kits (test for differences in population proportions), Hp-E, Hp-W, and Hp-D showed no significant between-kit differences for sensitivity, accuracy rate, or false-negative rate. In contrast, Hp-L showed significantly lower sensitivity, accuracy rate, and false-negative rate than each of Hp-E, Hp-W, and Hp-D kits ($p$<0.001) (**Table 2-b**).

**ROC analysis ('currently infected' vs. 'uninfected')**

For 103 currently infected cases and 104 uninfected cases determined by UBT and EGD findings, results of measurements using each serum *H. pylori* antibody test method were subjected to ROC analysis (**Fig. 4**). Areas under the curve (AUCs) were very good, at 0.9996 for Hp-W, 0.9988 for Hp-D, and 1.0000 for Hp-E. For Hp-L, the value was also sufficiently high at 0.9893. Optimal cut-off values were 4.4 U/mL for Hp-W, 12.2 U/mL for Hp-D, 5.2 U/mL for Hp-L, and 8.5 U/mL for Hp-E. While differences between these values and cut-off values set by each kit were small for Hp-W, Hp-D, and Hp-E, for Hp-L, the value was considerably lower than the set cut-off value. The sensitivity, specificity, PPV, and NPV determined using these optimal cut-off values were all very high for Hp-W, Hp-D, and Hp-E, and were satisfactory for Hp-L (**Table 3**).

**ROC analysis ('currently infected+previously infected (excluding post-eradication cases)' vs. 'uninfected')**

For a total of 124 cases, including 103 currently infected cases+21 previously infected cases (excluding post-eradication cases) and 104 uninfected cases deter-

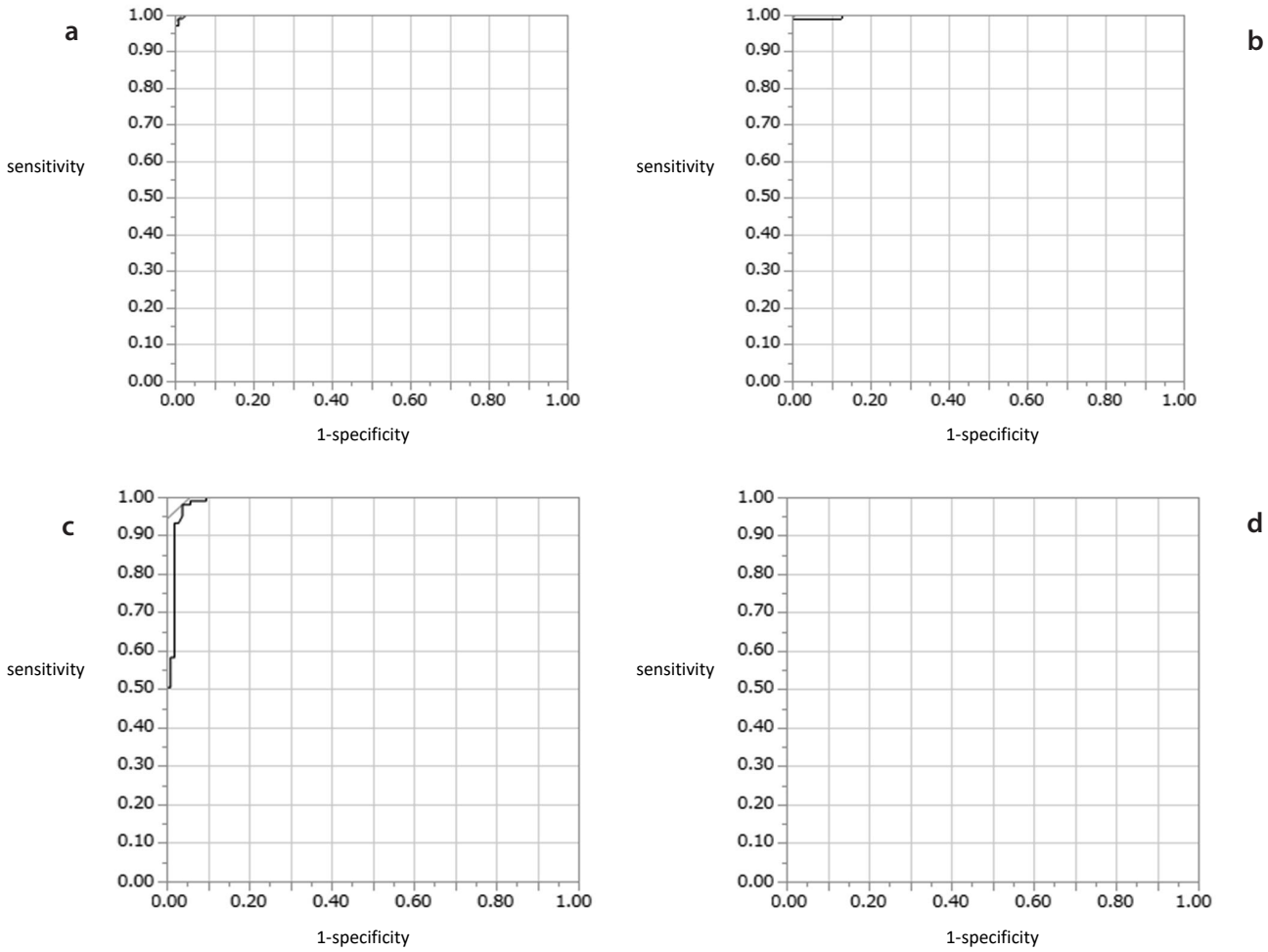


**Fig. 4. Receiver Operating Characteristic (ROC) Curve: Currently Infected vs. Uninfected**

a: Hp-W, b: Hp-D, c: Hp-L, d: Hp-E.
Hp-W: L type Wako H. pylori antibody, J, Hp-D: Latex 'Seiken' H. pylori antibody, Hp-L: LZ test 'Eiken' H. pylori antibody, Hp-E: E plate 'Eiken' H. pylori antibody II.

**Table 3. The Optimal Cut-off Value of Each Kit (ROC)**

| | Currently infected vs. Uninfected | | | | | |
|---|---|---|---|---|---|---|
| | AUC | Cut-off (U/mL) | Sensitivity | Specificity | PPV | NPV |
| Hp-W | 0.9996 | 4.4 | 0.990 | 0.990 | 0.990 | 0.990 |
| Hp-D | 0.9988 | 12.2 | 0.990 | 1.000 | 1.000 | 0.990 |
| Hp-L | 0.9893 | 5.2 | 0.981 | 0.962 | 0.962 | 0.980 |
| Hp-E | 1.0000 | 8.5 | 1.000 | 1.000 | 1.000 | 1.000 |

| | Currently infected+previously infected (excluding post eradication) vs. Uninfected | | | | | |
|---|---|---|---|---|---|---|
| | AUC | Cut-off (U/mL) | Sensitivity | Specificity | PPV | NPV |
| Hp-W | 0.9620 | 3.5 | 0.887 | 0.971 | 0.973 | 0.878 |
| Hp-D | 0.9470 | 12.2 | 0.831 | 1.000 | 1.000 | 0.832 |
| Hp-L | 0.9440 | 5.2 | 0.847 | 0.962 | 0.963 | 0.840 |
| Hp-E | 0.9740 | 3.6 | 0.879 | 0.981 | 0.982 | 0.872 |

| | Currently infected+previously infected (all cases) vs. Uninfected | | | | | |
|---|---|---|---|---|---|---|
| | AUC | Cut-off (U/mL) | Sensitivity | Specificity | PPV | NPV |
| Hp-W | 0.9251 | 2.8 | 0.784 | 0.942 | 0.967 | 0.671 |
| Hp-D | 0.9037 | 2.1 | 0.797 | 0.875 | 0.932 | 0.669 |
| Hp-L | 0.8886 | 5.2 | 0.842 | 0.827 | 0.912 | 0.711 |
| Hp-E | 0.9478 | 2.0 | 0.883 | 0.885 | 0.942 | 0.780 |

Hp-W: L type Wako H. pylori antibody, J, Hp-D: Latex 'Seiken' H. pylori antibody, Hp-L: LZ test 'Eiken' H. pylori antibody, Hp-E: E plate 'Eiken' H. pylori antibody II, ROC: receiver operating characteristic, AUC: area under the curve, PPV: positive predictive value, NPV: negative predictive value

mined by UBT and EGD findings, results of measurements using each serum *H. pylori* antibody test method were subjected to ROC analysis (**Fig. 5**). AUCs were 0.9620 for Hp-W, 0.9470 for Hp-D, 0.9440 for Hp-L, and 0.9740 for Hp-E. While AUCs were slightly lower when previously infected cases were included for all test methods, they were still all above 0.94. In addition, when we compared the optimal cut-off values with those obtained for 'currently infected' vs. 'uninfected,' the optimal cut-off values of Hp-D and Hp-L (12.2 U/mL and 5.2 U/mL, respectively) were the same as those obtained for 'currently infected' vs. 'uninfected.' In contrast, the optimal cut-off value of Hp-W (3.5 U/mL) differed slightly from that obtained for 'currently infected' vs. 'uninfected' (4.4 U/mL). On the other hand, the optimal cut-off value of Hp-E was 3.6 U/mL, which is considerably lower than the optimal cut-off value 8.5 U/mL obtained for 'currently infected' vs. 'uninfected' (**Table 3**).

**ROC analysis ('currently infected+all previously infected (including post-eradication cases)' vs. 'uninfected')**

For a total of 222 cases, including 103 currently infected cases+all 119 previously infected cases and 104 uninfected cases determined by UBT and EGD findings, results of measurements using each serum *H. pylori* antibody test method were subjected to ROC analysis (**Fig. 6**). AUCs for each test method were 0.9251 for Hp-W, 0.9037 for Hp-D, 0.8886 for Hp-L, and 0.9478 for Hp-E, which were lower than the AUCs obtained in the analyses of 'currently infected' vs. 'uninfected' and 'currently infected+previously infected (excluding post-eradication patients)' vs. 'uninfected' as a result of the inclusion of post-*H. pylori* eradication cases, which increased the number of previously infected cases. In addition, the optimal cut-off values were 2.8 U/mL for Hp-W, 2.1 U/mL for Hp-D, and 2.0 U/mL for Hp-E, which were lower than those of 'currently infected' vs.

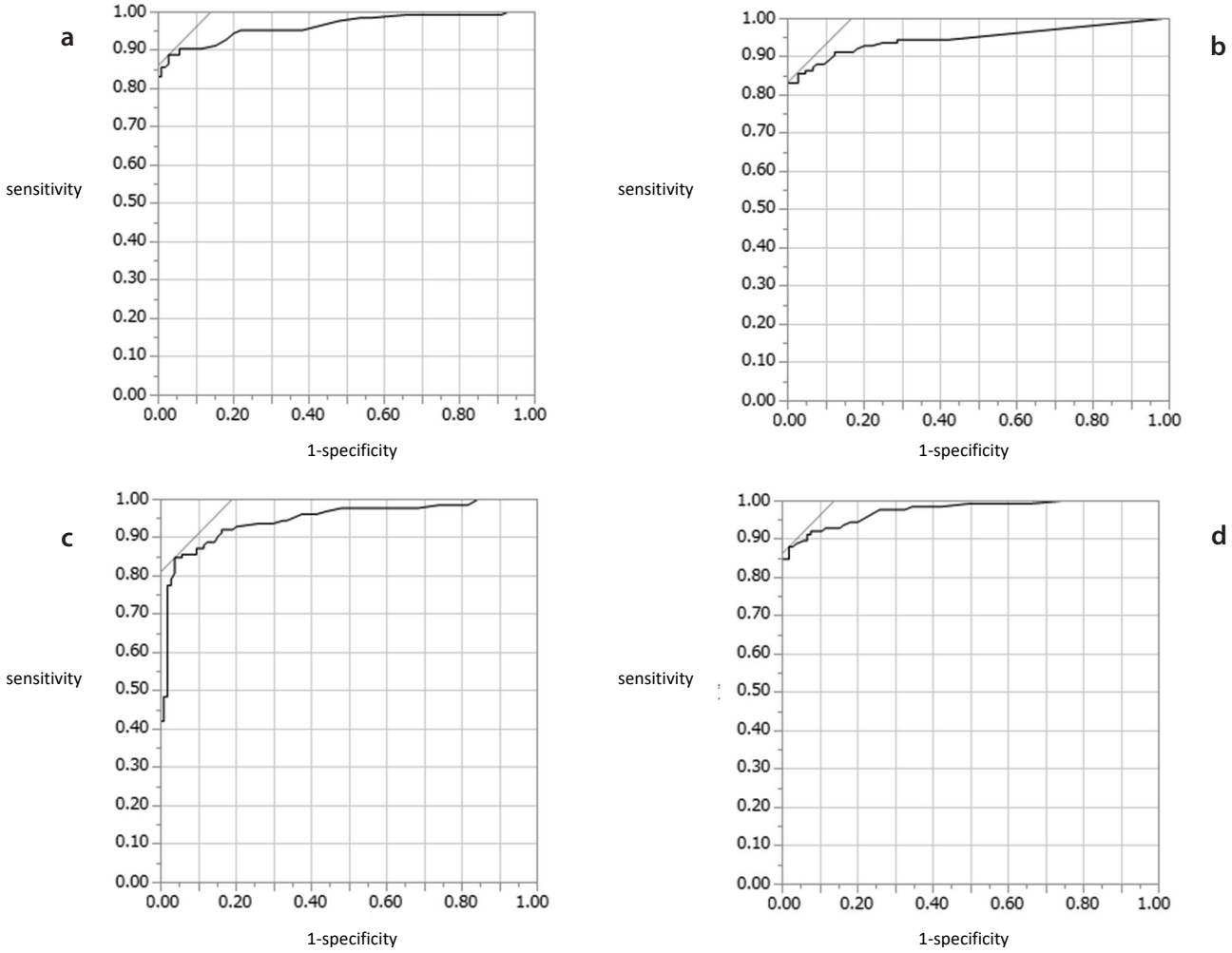


**Fig. 5. Receiver Operating Characteristic (ROC) Curve: Currently Infected+Previously Infected (Excluding Post-eradication Cases) vs. Uninfected**

a: Hp-W, b: Hp-D, c: Hp-L, d: Hp-E.
Hp-W: L type Wako H. pylori antibody, J, Hp-D: Latex 'Seiken' H. pylori antibody, Hp-L: LZ test 'Eiken' H. pylori antibody, Hp-E: E plate 'Eiken' H. pylori antibody II.

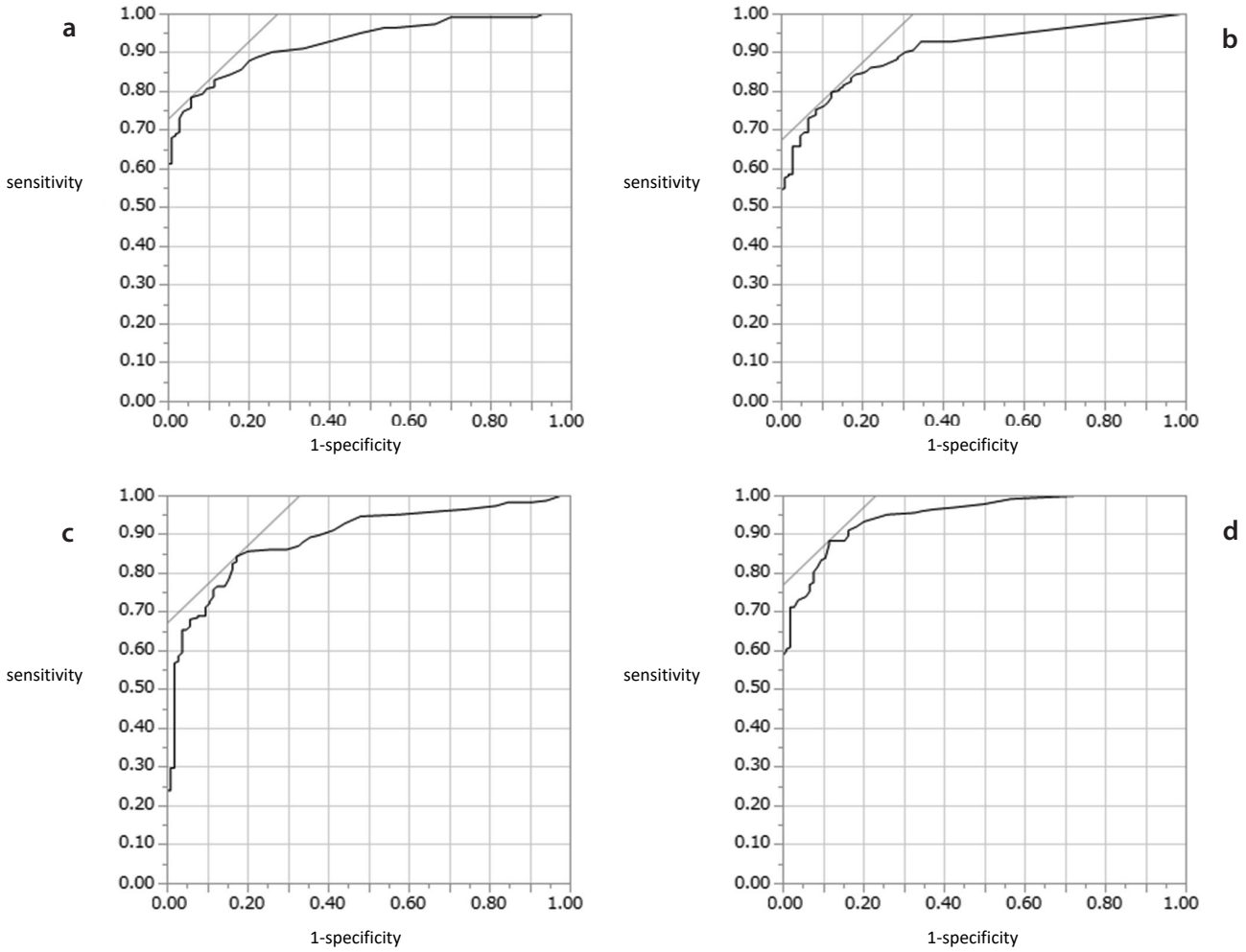


**Fig. 6. Receiver Operating Characteristic (ROC) Curve: Currently Infected+All Previously Infected vs. Uninfected**

a: Hp-W, b: Hp-D, c: Hp-L, d: Hp-E.
Hp-W: L type Wako H. pylori antibody, J, Hp-D: Latex 'Seiken' H. pylori antibody, Hp-L: LZ test 'Eiken' H. pylori antibody, Hp-E: E plate 'Eiken' H. pylori antibody II.

'uninfected' and 'currently infected+previously infected (excluding post-eradication cases)' vs. 'uninfected.' On the other hand, the optimal cut-off value of Hp-L was 5.2 U/mL, which was the same as that obtained for 'currently infected' vs. 'uninfected' and 'currently infected+previously infected (excluding post-eradication cases)' vs. 'uninfected' (**Table 3**).

## Discussion

Although serum *H. pylori* antibody measurement has mainly been performed using ELISA for many years in Japan, several latex-based serum *H. pylori* antibody measurement methods have been developed. The present study prospectively examined the precision of four measurement methods mainly by ROC analysis.

Serum *H. pylori* antibody measurement tools were designed and developed to identify individuals who are currently infected or uninfected with *H. pylori*. Precision assessment of each *H. pylori* antibody measurement method using their set cut-off values revealed that, among the new latex-based methods, the accuracy of Hp-W and Hp-D, as represented by accuracy rates, was equivalent to or higher than that of the ELISA-based method Hp-E. However, the number of false-negative cases for Hp-L was high. For Hp-L, the sensitivity and accuracy rate were also significantly lower and the false-negative rate was significantly higher than those of Hp-E, Hp-W, and Hp-D (**Table 2-a, b**). In a multicenter retrospective study, Ito *et al.*[9] found that for Hp-L compared to Hp-E, many subjects who were uninfected with *H. pylori* had values ≥3 U/mL, and some even had values higher than 10 U/mL, leading the authors to be concerned about false positives. In contrast, there were more false negatives in the present study. Importantly, many false-positive and false-negative cases identified using the set cut-off values for each measurement method had titer values close to the cut-off values (data not shown). This suggests that, in real-world clinical settings, patients whose antibody titers are close to cut-off values require careful judgment.

ROC analysis was performed to examine the precision of each serum *H. pylori* antibody measurement method for diagnosing 'currently infected' vs. 'uninfected' status. Satisfactory AUCs were obtained not only for Hp-W, Hp-D, and Hp-E, but also for Hp-L. While the differences between the obtained optimal cut-off values and set cut-off values for each test method were small for Hp-W, Hp-D, and Hp-E, for Hp-L, the cut-off value (5.2 U/mL) was considerably lower than the set cut-off of 10 U/mL (**Fig. 4**, **Table 3**). Aoyama *et al.*[10] reported similar results in a study targeting people who made clinic visits. While these findings warrant further examination with a larger number of cases, a correction of the cut-off value for Hp-L will likely be needed.

In Japan, public health insurance coverage for *H. pylori* eradication therapy was approved for gastric ulcer and duodenal ulcer in 2000. In 2013, the coverage was expanded to include chronic gastritis diagnosed by EGD. As a result, the number of people who undergo eradication therapy with the expectation of preventing the development of gastric cancer has increased considerably. In addition, in the comprehensive health checkup system and gastric cancer screening, or in clinical settings, we sometimes encounter people who have previously been infected with *H. pylori* but have no history of eradication. A multicenter, randomized controlled study in Japan demonstrated that the incidence of secondary cancer decreases in patients after endoscopic treatment for early gastric cancer, with a hazard ratio of 0.339 (95% confidence interval: 0.167–0.729)[11]. However, a meta-analysis by Ford *et al.*[12] showed that the preventive effect on the development of gastric cancer was somewhat limited, albeit significant, with an odds ratio of 0.66 (95% confidence interval: 0.46–0.95) for *H. pylori* gastritis alone, without gastric cancer or peptic ulcer. As the risk of gastric cancer development remains even after eradication, levels do not necessarily reach those of *H. pylori*-uninfected individuals. Hence, people with previous infection, including those who have had *H. pylori* eradicated, should not be classified as low-risk in the comprehensive health checkup system or gastric cancer screening.

Serum *H. pylori* antibody levels are not permanent, but rather gradually decrease after the disappearance of *H. pylori* due to eradication therapy or other processes; thus, many patients become *H. pylori*-negative. Thus, diagnostic precision is expected to decrease if an analysis included a high number of previously infected cases, including post-eradication cases. For this reason, in the present study, we also performed ROC analysis on the diagnostic precision for 'currently infected+previously infected' vs. 'uninfected' cases. Examination that excluded previously infected individuals with a history of *H. pylori* eradication demonstrated lower AUCs for all tests (Hp-W, Hp-D, Hp-L, and Hp-E) compared to the diagnostic precision for 'currently infected' vs. 'uninfected' cases. Nonetheless, all values were above 0.94 and were thus considered to be within an acceptable range (**Fig. 5**, **Table 3**). In addition, all three latex-based methods had the same or similar optimal cut-off values to those for 'currently infected' vs. 'uninfected' in the examination that excluded individuals with a history of *H. pylori* eradication from previously infected cases. Thus, it is feasible to use the same cut-off values if individuals with a history of *H. pylori* eradication are properly excluded. A multicenter retrospective study[9] conducted by Ito *et al.* that excluded people with a history of *H. pylori* eradication also showed that the cut-off values set by each test kit could be used for identifying the risk of gastric cancer in individuals, including those with previous infection, when using Hp-W and Hp-D.

However, AUCs further decreased when the proportion of individuals with previous *H. pylori* infection increased due to the inclusion of post-eradication cases. For the ELISA-based method Hp-E, the AUC was 0.948, which was considered to be within an acceptable range. In contrast, it is questionable as to whether the new latex-based methods Hp-W, Hp-D, and Hp-L can be used for screening purposes, given that their AUCs were 0.925, 0.904, and 0.889, respectively. Moreover, the optimal cut-off values for Hp-W and Hp-D were low at 2.8 U/mL and 2.1 U/mL, respectively, when individuals with a history of *H. pylori* eradication were included. In particular, for Hp-D, the optimal cut-off value for diagnosing 'currently infected' vs. 'uninfected,' and 'currently infected+previously infected (excluding post-eradication cases)' was 12.2 U/mL, whereas that for 'currently infected+previously infected (including post-eradication cases)' was very low at 2.1 U/mL (**Fig. 6**, **Table 3**). Thus, cut-off values can change when a large number of individuals with previous infection are included. Accordingly, it is impossible to definitely conclude that the same cut-off values used to distinguish currently infected people from uninfected people can be used to distinguish people with a risk of gastric cancer (people with current infection+people with previous infection) from people with a low risk of gastric cancer (i.e., uninfected people). Furthermore, the notion that people with a history of *H. pylori* eradication are unsuitable as subjects of serum *H. pylori* antibody titer determination should be promoted[13].

With respect to the ELISA-based method Hp-E, a multicenter study noted that persons with serum antibody titers ≥3 U/mL and <10 U/mL (i.e., high-titer negative cases) include many that were previously infected with *H. pylori*, including post-eradication cases. In fact, the cut-off value for assessing gastric cancer

risk has been changed to 3 U/mL[14,15]. Similarly, the present study, which was conducted prospectively, obtained a cut-off value of 8.5 U/mL for 'currently infected' vs. 'uninfected.' In comparison, cut-off values for 'currently infected+previously infected (excluding post-eradication cases)' vs. 'uninfected' and 'currently infected+previously infected (including post-eradication cases)' vs. 'uninfected' were 3.6 U/mL and 2.0 U/mL, respectively. These results suggest that the change to the above-mentioned cut-off value is reasonable.

The present prospective study used *H. pylori* infection status judged by EGD and UBT as an indicator. However, there are some limitations worth noting. First, the investigation was conducted at a single facility, and the number of subjects was not high. Moreover, selection bias is of concern, as our subjects were participants of a comprehensive health checkup system. A larger multicenter prospective study is needed. Further, it will also be necessary to confirm the applicability of our findings to participants receiving gastric X-ray examination and general medical care. Second, we could not perform histological examination of the gastric mucosa on all patients. It may be considered ethically problematic to perform biopsy and histological examination in all cases to diagnose *H. pylori* infection or histological gastritis, given that our subjects were participants of a comprehensive health checkup system. Furthermore, with regard to post-*H. pylori* eradication cases, the association between the number of years elapsed and *H. pylori* antibody titers has not been clarified. It should also be noted that serum antibody titers were measured at one time point; thus, we did not address the time course after eradication. These post-eradication changes in serum *H. pylori* antibody titer should be addressed in the future.

## Conclusion

Among the latex-based methods, the accuracy of Hp-W and Hp-D showed good precision, suggesting that they can be used as an alternative to ELISA. On the other hand, Hp-L was inferior to the other methods in the accuracy analysis when using the cut-off values set for each measurement method. However, ROC analyses clearly demonstrated that the three latex-based serum *H. pylori* antibody measurement methods have good precision, suggesting that they can be used as an alternative to ELISA. With an appropriate cutoff value, these methods may also be useful as diagnostic methods to identify at-risk groups for gastric cancer, including those with previous infection and excluding post-*H. pylori* eradication cases. However, the precision decreases with the inclusion of an increasing number of post-eradication cases.


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## Conflicts of interest

The authors have no conflicts of interest to declare in regard to this study.

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# Prevalence, Incidence, and Associated Factors of Retinal Vein Occlusion in a Japanese Population at a Medical Checkup Institution in Osaka, 2019–2020: A Cross-sectional Observational Study

Hiroshi Sonoo, Ryo Kobayashi, Keisuke Arai, Kiyotaka Fukumura

## Abstract

**Objective:** To describe the prevalence and one-year incidence of retinal vein occlusion (RVO) and associated risk factors in a Japanese population at a medical checkup institution in Osaka.

**Methods:** Fundus photography was performed on 28,781 participants aged 19–93 from April 2019 to March 2020 as part of the medical checkup. We determined the prevalence of RVO, including central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO) by assessing the photographs, and analyzed the associated risk factors for RVO using medical checkup data.

**Results:** Of the 28,781 participants, RVO, BRVO, and CRVO were found in 334, 311, and 23 participants with 1.2%, 1.1%, and 0.08% prevalence, respectively. New RVO, BRVO, and CRVO were found in 57, 52, and 5 participants with a one-year incidence of 0.2%, 0.18%, and 0.02%, respectively. According to multivariate logistic regression analyses of subjects with and without RVO, there was a significant difference in the age, with an odds ratio (OR) of 1.03 (95% confidence interval [CI]=1.01–1.04); hypertension (OR=2.56, 95%CI=1.90–3.44); and AV nicking (mild: [OR=9.73, 95%CI=2.3–41.1]; moderate: [OR=298, 95%CI=73.6–1,210]; and severe: [OR=1,210, 95%CI=289–5,080]); respectively.

**Conclusions:** The prevalence of RVO was 1.2% and the one-year incidence of RVO was 0.2%. The strong correlation between RVO and the risk factors of hypertension and AV nicking suggests that blood pressure control and the prevention of arteriosclerosis would be associated with a lower incidence and prevalence of RVO.

**Keywords** retinal vein occlusion, fundus photography, arteriovenous nicking, hypertension

Retinal vein occlusion (RVO) is the second most common sight-threatening retinal vascular disorder after diabetic retinopathy[1]. According to the site of occlusion, RVO can be broadly classified as branch RVO (BRVO) and central RVO (CRVO). BRVO occurs at an arteriovenous (AV) intersection, whereas CRVO occurs at or near the lamina cribrosa of the optic nerve[2,3]. Common complications of BRVO and CRVO include macular edema and macular ischemia, which are persistent and difficult to treat[2,3]. In AV nicking, thick-walled arteries compress and obstruct adjacent thin-walled veins, a process that is likely exacerbated by underlying retinal arterial disease, such as hypertension, or diabetes mellitus (DM), and dyslipidemia resulting in arteriosclerosis[4]. To our knowledge, to date there are few studies that have shown the relationship between RVO and the degree of arteriosclerosis at the AV intersection. We sought to investigate the prevalence and incidence of RVO and to identify AV nicking[5] and other risk factors for RVO using the data in a Japanese population at a medical checkup center, with fundus photography analysis.

## Methods

A single-institution, cross-sectional observational study in a Japanese population at a medical check-up institution in Sakai city, Osaka, was performed from April 2019 to March 2020. Of the 38,551 consecutive participants enrolled over a 12-month period in which medical checkup data were collected, fundus photography was performed on 28,781 participants. One nonmydriatic fundus photograph (CR-2; Canon) was taken

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of each eye: at 45° centered on the point between the macula and the optic disc.

The first grading of fundus photographs for the grading of AV nicking and the detection of RVO was carried out by three Japanese Society of Ningen Dock specialists. A Japanese Ophthalmological Society specialist judged the final grading.

In this study, new-onset RVO was defined as appearing within 12 months of the previous examination. New-onset CRVO was characterized by widespread scattered superficial or deep retinal hemorrhages with or without optic disc hyperemia or edema, venous dilatation, retinal edema, or occluded or sheathed veins in the four quadrants[6–8]. Chronic CRVO was detected by the presence of arteriovenous collaterals and neovascularization at the optic disc. Hemicentral RVO (HRVO), whose signs were present in the upper or lower retinal half, corresponding to the branch of the central vein in which the occlusion occurred, and that had been noted on at least one previous examination, was assigned to the CRVO group. New-onset BRVO involved a more localized area of the retina in the sector of the obstructed venule and was characterized by scattered superficial or deep retinal hemorrhages, venous dilatation, intraretinal microvascular abnormalities, and occluded and sheathed venules that had appeared since the previous examination[6–8]. Chronic BRVO was characterized by the presence of collateral vessels or intraretinal microvascular abnormalities in a retinal sector that had been noted on at least one previous examination (**Fig. 1**).

AV nicking was graded as present if there was a decrease in the diameter of the venule on both sides of the crossing of the arteriole, and in which there was at least 0.5 disc diameter from the optic disc. To grade AV nicking, we used a V2/V1 ratio (V1: normal venous caliber away from any from A/V crossing, V2: venous caliber at A/V crossing), none: absent/questionable; mild: 1/2 to 1; moderate: 1 to 1/2, severe: occlusion[5].

Face-to-face health interviews were conducted using standardized questionnaires, including taking present and past history, such as hypertension, DM, dyslipidemia, ischemic heart disease (IHD), previous stroke, and substance use, such as smoking and alcohol intake. Participants were categorized into 0 or 1 for either nonsmoker or current smoker, respectively. Participants were categorized into 0 or 1 for either nondrinker (including social drinker), or regular (everyday) drinker.

The health examination survey included anthropometric data, blood pressure, and biochemical data. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m$^2$). Blood pressure was measured two times from subjects in a sitting position after at least 5 minutes rest, and the mean of the two measurements was used for the analysis. Blood samples, including fasting glucose, glycated hemoglobin (HbA1c), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and creatinine, were collected after at least an 8-hour fasting period. The estimated glomerular filtration rate (eGFR) was calculated using the isotope dilution mass spectrometry-traceable creatinine-based four-variable modification of diet in renal disease (IDMS-MDRD) study equation in the Japanese population[9]. Hypertension was defined as a systolic

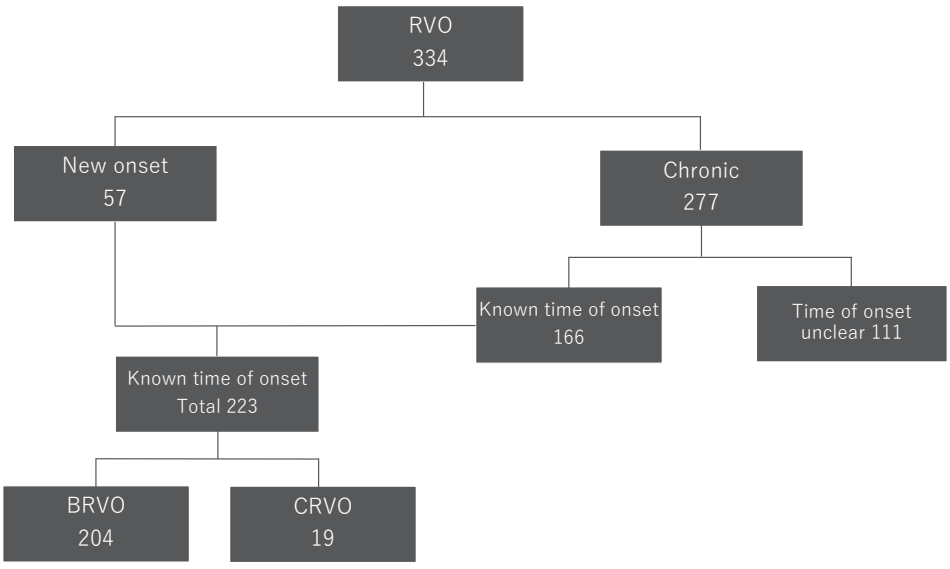


**Fig. 1. Flow Chart for Selection of Known Time of Onset RVO**

In addition to the recent RVO data, the old RVO data where onset of occlusions were confirmed, were defined as known time of onset RVO.
BRVO: branch retinal vein occlusion, CRVO: central retinal vein occlusion, RVO: retinal vein occlusion

blood pressure (SBP) ≧140 mmHg, a diastolic blood pressure (DBP) ≧90 mmHg, or a current prescription for antihypertensive medication. Diabetes was defined as fasting glucose ≧126 mg/dL, HbA1c≧6.5%, or a current prescription for antihyperglycemic medication. Dyslipidemia was defined as an LDL-C concentration ≧140 mg/dL, HDL-C concentration <40 mg/dL, or a current prescription for lipid-lowering medication. Cases of chronic kidney disease (CKD) were defined as eGFR <60 mL/min/1.73 $m^2$ [10]. Obesity was defined as BMI≧25 kg/$m^2$ according to Japanese criteria[11].

This study was conducted in accordance with the Helsinki Declaration, with all participants providing written informed consent, and this study design was approved by the Institutional Review Board (IRB) of the Bell Clinic.

Prevalence is given as the percentage of subjects with either new-onset or chronic RVO, among all subjects whose retinas were photographed. One-year incidence is given as the percentage of new-onset RVO cases identified from April 2019 to March 2020. The relationships between risk factors and the prevalence of RVO were examined. Age, BMI, SBP, DBP, fasting glucose, HbA1c, HDL-C, LDL-C, and eGFR were treated as continuous variables and sex, obesity, smoking and drinking habits, CKD, medical history, DM, dyslipidemia, hypertension, and AV nicking as categorical variables. Differences in the baseline continuous variables between the subjects with and without RVO were evaluated using either Student's *t*-test or the Mann–Whitney *U*-test, and those of categorical variables were evaluated using Fisher's exact test. Risk factors for RVO, including age, sex, obesity, smoking and drinking habits, CKD, DM, dyslipidemia, hypertension, and AV nicking were entered into multivariate logistic regression analyses. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated after adjusting for age and all other confounders, and $p<0.05$ was considered statistically significant.

Statistical analyses were performed with open-source software (EZR, Saitama Medical Center, Jichi Medical University, Saitama, Japan)[12], which is a graphical user interface for R (The R foundation for Statistical Computing, Vienna, Austria).

## Results

Of the 28,781 participants aged 19–93 (mean age, 52.1±8.5 years) with fundus photography, RVO was found in 334 participants, for a prevalence of 1.2% (**Fig. 2**). BRVO was found in 311 participants and CRVO was found in 23 participants, including 7 HRVOs, for a prevalence of 1.1% and 0.08%, respectively. New-onset RVO, BRVO, and CRVO were found in 57, 52, and 5 participants for an incidence of 0.2%, 0.18%, and 0.02%, respectively.

The locations of occlusions are shown in **Table 1**. The proportion of BRVO in the right eye was similar to that

**Table 1. The Location of Occlusions for All RVO**

| Factor | Group | BRVO | CRVO |
|---|---|---|---|
| *n* | | 311 | 23 |
| side (%) | right | 144 (46.3) | 16 (69.6) |
| | left | 153 (49.2) | 4 (17.4) |
| | both | 14 (4.5) | 3 (13.0) |
| location (%) | superotemporal | 195 (63.1) | 6 (66.7) |
| | inferotemporal | 105 (34.0) | 1 (11.1) |
| | both | 9 (2.9) | 2 (22.2) |

BRVO: branch retinal vein occlusion, CRVO: central retinal vein occlusion, DM: diabetes mellitus, RVO: retinal vein occlusion

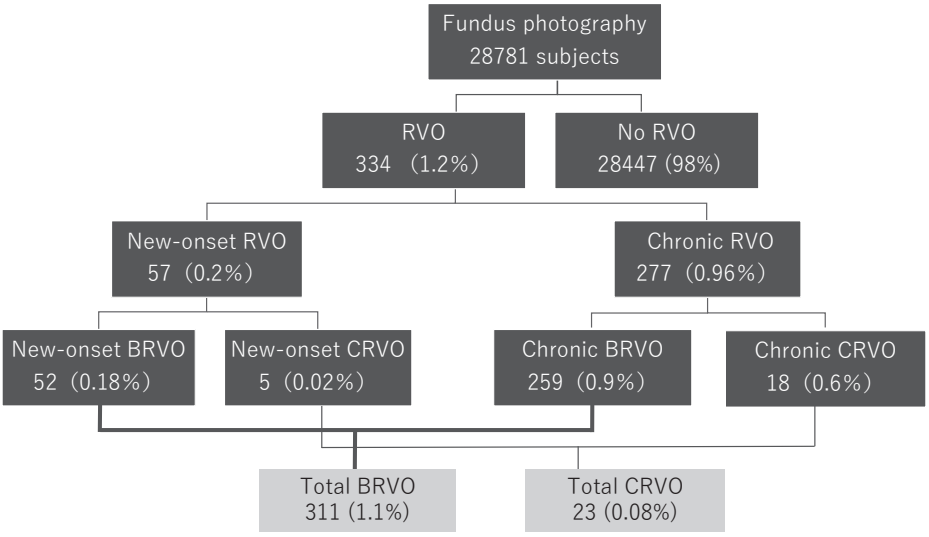


**Fig. 2. Any Prevalence of RVO, CRVO, and BRVO**

Prevalence is given as relative frequencies for the number of RVO, including recent and old RVO, with all the participants having fundus photographs taken.
BRVO: branch retinal vein occlusion, CRVO: central retinal vein occlusion, RVO: retinal vein occlusion

in the left eye (144/311 46.3% vs. 153/311 49.2%), and 14 persons had bilateral BRVO (4.5%). The site of BRVO involved the superotemporal quadrant in 63.1% of eyes (195/311), and the inferotemporal quadrant in 34.0% of eyes (105/311).

Of all 334 RVO cases, 223 were detected as having new-onset disease (**Fig. 1**).

Comparisons of characteristics between BRVO and CRVO are shown in **Table 2**. BRVO was found in 204 participants and CRVO was found in 19 participants, including 5 HRVOs. The mean ages were similar in both groups (58.4±8.6 years for BRVO and 56.5± 10.0 years for CRVO). According to univariate logistic regression analysis, there was no significant difference between the two groups except AV nicking and HDL-C. No significant differences were found between the two groups in multivariate logistic regression analysis.

Comparisons of characteristics between non-RVO and RVO are shown in **Table 3**. According to univariate logistic regression analysis, some risk factors were significantly associated with RVO, but there were no significant differences in BMI, LDL-C, alcohol intake, smoking, antihyperglycemic medication, DM, or IHD history between the two groups. There were significant differences in antihypertensive medication and lipid-lowering medication between the two groups. Previous stroke was associated with RVO. Logistic regression multivariate analysis of associations between potential risk factors and RVO are shown in **Table 4**. In consideration of the multicollinearity, risk factors for RVO, including age, sex, obesity, smoking and drinking habits, CKD, DM, dyslipidemia, hypertension, and AV nicking

**Table 2. Comparisons of Characteristics Between BRVO and CRVO**

| Factor | Group | BRVO | CRVO | *p*-value |
|---|---|---|---|---|
| *n* | | 204 | 19 | |
| RVO age (year) | | 58.4±8.58 | 56.5±9.97 | 0.363 |
| Sex (%) | Female | 84 (41.2) | 11 (57.9) | 0.225 |
| | Male | 120 (58.8) | 8 (42.1) | |
| BMI (kg/m$^2$) | | 24.40±3.43 | 23.89±3.50 | 0.538 |
| SBP (mmHg) | | 133.50±16.40 | 132.37±19.66 | 0.779 |
| DBP (mmHg) | | 86.26±10.96 | 85.00±9.95 | 0.628 |
| HDL (mg/dL) | | 64.25±17.63 | 73.79±24.90 | <0.05 |
| LDL (mg/dL) | | 127.29±30.59 | 113.63±36.17 | 0.068 |
| eGFR (mL/min/1.73 m$^2$) | | 71.86 (12.41) | 66.71 (21.91) | 0.12 |
| FBS (mg/dL) | | 100.00 [81.00, 189.00] | 103.00 [80.00, 149.00] | 0.229 |
| HbA1c (%) | | 5.60 [4.30, 7.70] | 5.60 [5.10, 7.70] | 0.886 |
| AV nicking (%) | absent/questionable | 0 (0.0) | 2 (10.5) | <0.01 |
| | mild | 21 (10.3) | 4 (21.1) | |
| | moderate | 145 (71.1) | 12 (63.2) | |
| | severe | 38 (18.6) | 1 (5.3) | |
| Alcohol intake (%) | Non-drinker, social drinker | 138 (67.6) | 14 (73.7) | 0.798 |
| | Regular drinker | 66 (32.4) | 5 (26.3) | |
| Smoking (%) | Non-smoker | 167 (81.9) | 18 (94.7) | 0.21 |
| | Current smoker | 37 (18.1) | 1 (5.3) | |
| Obesity (%) | BMI (kg/m$^2$)<25 | 124(60.8) | 11(57.9) | 0.81 |
| | BMI (kg/m$^2$) ≧ 25 | 80(39.2) | 8(42.1) | |
| CKD (%) | eGFR ≧ 60 | 168 (82.4) | 13 (72.2) | 0.339 |
| | eGFR<60 | 36 (17.6) | 5 (27.8) | |
| Antihyperglycemic medication (%) | No treatment | 196 (96.1) | 16 (84.3) | 0.056 |
| | treatment | 8 (3.9) | 3 (15.8) | |
| Lipid-lowering medication (%) | No treatment | 167 (81.9) | 9 (47.4) | 0.068 |
| | treatment | 37 (18.1) | 12 (63.2) | |
| Antihypertensive medication (%) | No treatment | 144 (70.6) | 13 (68.4) | 0.799 |
| | treatment | 60 (29.4) | 6 (31.6) | |
| Ischemic heart disease history (%) | No history | 203 (99.5) | 18 (94.7) | 0.163 |
| | Current or Past history | 1 (0.5) | 1 (5.3) | |
| Stroke history (%) | No history | 196 (96.1) | 19 (100.0) | 1 |
| | Current or Past history | 8 (3.9) | 0 (0.0) | |
| DM (%) | absent | 186 (91.2) | 15 (78.9) | 0.102 |
| | present | 18 (8.8) | 4 (21.1) | |
| Dyslipidemia (%) | absent | 108 (52.9) | 9 (47.4) | 0.811 |
| | present | 96 (47.1) | 10 (52.6) | |
| Hypertension (%) | absent | 85 (41.7) | 8 (42.1) | 1 |
| | present | 119 (58.3) | 11 (57.9) | |

AV: arteriovenous, BRVO: branch retinal vein occlusion, CRVO: central retinal vein occlusion, DM: diabetes mellitus, RVO: retinal vein occlusion

**Table 3. Comparisons of Characteristics Between Normal and RVO**

| Factor | Group | non RVO | RVO | *p*-value |
|---|---|---|---|---|
| *n* | | 28447 | 223 | |
| RVO age (year) | | 51.94±10.30 | 58.22±8.77 | <0.001 |
| Sex (%) | Female | 14931 (52.5) | 95 (42.6) | <0.01 |
| | Male | 13516 (47.5) | 128 (57.4) | |
| BMI (kg/m$^2$) | | 23.08±3.64 | 24.36±3.44 | 0.982 |
| SBP (mmHg) | | 119.14±15.25 | 133.40±16.65 | <0.001 |
| DBP (mmHg) | | 76.19±11.23 | 86.16±10.86 | <0.001 |
| HDL-C (mg/dL) | | 70.14±18.26 | 65.07±18.48 | <0.001 |
| LDL-C (mg/dL) | | 123.93±29.84 | 126.13±31.25 | 0.274 |
| eGFR (mL/min/1.73 m$^2$) | | 74.26 (13.54) | 71.44 (13.43) | <0.001 |
| FBS (mg/dL) | | 96.00 [53.00, 399.00] | 100.00 [80.00, 189.00] | <0.001 |
| HbA 1 c (%) | | 5.60 [2.90, 13.50] | 5.60 [4.30, 7.70] | <0.05 |
| AV nicking (%) | absent/questionable | 11836 (41.6) | 2 (0.9) | <0.001 |
| | mild | 13868 (48.8) | 25 (11.2) | |
| | moderate | 2577 (9.1) | 157 (70.4) | |
| | severe | 157 (0.6) | 39 (17.5) | |
| Alcohol intake (%) | Non-drinker, Social drinker | 20638 (72.5) | 152 (68.2) | 0.152 |
| | Regular drinker | 7809 (27.5) | 71 (31.8) | |
| Smoking (%) | Non-smoker | 23714 (83.4) | 185 (83.0) | 0.857 |
| | Current smoker | 4733 (16.6) | 38 (17.0) | |
| Obesity (%) | BMI (kg/m$^2$)<25 | 21137 (74.3) | 135 (60.5) | <0.001 |
| | BMI (kg/m$^2$) ≧ 25 | 7309 (25.7) | 88 (39.5) | |
| CKD (%) | eGFR ≧ 60 | 24437 (87.2) | 181 (81.5) | <0.05 |
| | eGFR<60 | 3574 (12.8) | 41 (18.5) | |
| Antihyperglycemic medication (%) | No treatment | 27263 (95.8) | 212 (95.1) | 0.502 |
| | Current treatment | 1184 (4.2) | 11 (4.9) | |
| Lipid-lowering medication (%) | No treatment | 25086 (88.2) | 179 (80.3) | <0.001 |
| | Current treatment | 3361 (11.8) | 44 (19.7) | |
| Antihypertensive medication (%) | No treatment | 24190 (85.0) | 157 (70.4) | <0.001 |
| | Current treatment | 4257 (15.0) | 66 (29.6) | |
| DM (%) | absent | 26429 (92.9) | 201 (90.1) | 0.115 |
| | presence | 2018 (7.1) | 22 (9.9) | |
| Dyslipidemia (%) | absent | 17039 (59.9) | 117 (52.5) | <0.05 |
| | presence | 11408 (40.1) | 106 (47.5) | |
| Hypertension (%) | Non presence | 21271 (74.8) | 93 (41.7) | <0.001 |
| | presence | 7176 (25.2) | 130 (58.3) | |
| Ischemic heart disease history(%) | No history | 28103 (98.8) | 221 (99.1) | 1 |
| | previous history | 344 (1.2) | 2 (0.9) | |
| Stroke history (%) | No history | 28143 (98.9) | 215 (96.4) | <0.01 |
| | Previous history | 304 (1.1) | 8 (3.6) | |

AV: arteriovenous, BRVO: branch retinal vein occlusion, CRVO: central retinal vein occlusion, DM: diabetes mellitus, RVO: retinal vein occlusion

**Table 4. Logistic Regression Multivariate Analysis of Associations Between Potential Risk Factors and RVO**

| Factor | Odds ratios | 95% Confidence Intervals | *p*-value |
|---|---|---|---|
| RVO age (year) | 1.03 | 1.01–1.04 | <0.05 |
| Sex (%) | 1.04 | 0.77–1.39 | 0.79 |
| Alcohol use (%) | 0.89 | 0.65–1.21 | 0.51 |
| Smoking (%) | 1.04 | 0.72–1.50 | 0.84 |
| Obesity (%) | 1.30 | 0.97–1.74 | 0.1 |
| CKD | 0.89 | 0.62–1.27 | 0.53 |
| DM (%) | 0.68 | 0.43–1.07 | 0.13 |
| Dyslipidemia (%) | 0.81 | 0.61–1.06 | 0.14 |
| HT (%) | 2.56 | 1.90–3.44 | <0.001 |
| AV nicking (%) | | | |
| mild | 9.73 | 2.3–41.1 | <0.01 |
| moderate | 298 | 73.6–1210 | <0.001 |
| severe | 1210 | 289–5080 | <0.001 |

DM: diabetes mellitus, RVO: retinal vein occlusion

were entered into multivariate logistic regression analyses. There was a significant difference in age between persons with RVO (58.22±8.77 years) and without RVO (51.9±10.3 years) (OR 1.03; 95%CI=1.01–1.04) ($p$<0.05). Hypertension was strongly associated with RVO (OR 2.56; 95%CI=1.90–3.44) ($p$<0.001). AV nicking was highly associated with RVO ($p$<0.001), mild; (OR 9.73; 95%CI=2.3–41.1), moderate; (OR 298; 95%CI=73.6–1,210), severe; (OR 1,210; 95%CI=289–5,080), respectively.

## Discussion

RVO is a degenerative vascular disease of the retina. An increasing trend of prevalence associated with advanced age has been widely observed in previous epide-

miological studies[1,13–15].

Laouri *et al.*[16] compared the data of one pooled analysis and seven population-based studies to assess the prevalence of RVO in 2011. According to this systematic review, the prevalence of RVO is relatively constant across all countries; in populations older than 40 years, it ranges from 0.3% to 2.1%, with highest values in Japan and Australia and lowest values in the United States (US), Europe, and Singapore[17]. In all studies, the prevalence of BRVO was higher than that of CRVO ranging from three (Singapore[18]) to 10 (China[19]) times higher[15]. More recently, a systematic review and meta-analysis in 2019 from the United Kingdom (UK), which analyzed 17 studies and provided data on RVO prevalence, was published, reporting that in 2015, the global prevalence of RVO, BRVO, and CRVO in people aged 30–89 years was 0.77%, 0.64%, and 0.13%, respectively[13]. In our study, the prevalence of RVO, BRVO, and CRVO were 1.2%, 1.1%, and 0.08%, respectively. Our RVO prevalence estimates are moderate compared to the results of the previous study. However, our prevalence of BRVO was eleven times higher than that of CRVO, partly because old occlusions were diagnosed comparing the most recent fundus photographs with those from the past. Of all 334 RVO cases, we read the past fundus photographs comparatively for 330 RVO subjects. Therefore, we might have detected even smaller occlusions in asymptomatic cases.

A systematic review and meta-analysis in 2019 from the UK, which analyzed six individual studies with data on RVO incidence, reported that the pooled five-year and ten-year cumulative incidence of RVO was 0.86% and 1.63%, respectively[13]. Additionally, the Hisayama study reported a nine-year cumulative incidence of any RVO as 3.0% in Japan[20], and the Beaver Dam Eye study reported a 15-year cumulative incidence of any RVO as 2.3% in the US[21]. In our study, one-year incidences of any RVO, BRVO, and CRVO were 0.2%, 0.18%, and 0.02%, respectively. Our RVO incidence estimates are moderate compared to earlier studies.

Findings from most studies[6,7,22,23] have suggested that BRVO more frequently involves the superotemporal quadrant. Given that a greater number of AV crossings are present in the superotemporal region, approximately two-thirds of major BRVO occur in the superotemporal quadrant, followed by the inferotemporal quadrant, and less commonly, in the nasal quadrants[4,7,21,24]. Two-thirds of BRVO foci occurred in the superotemporal region in our cases.

Although the exact mechanism of BRVO has not been completely elucidated, BRVO is thought to follow the principle of Virchow's triad for intravascular thrombus, such that there is endothelial damage, hemodynamic changes in blood flow, and hypercoagulability[25]. Sclerotic arteriolar walls compress underlying venules at the AV crossing, leading to reduced blood flow, which in turn may facilitate the development of a thrombus and downstream venular occlusion[26]. Findings that retinal arteriosclerosis signs such as AV nicking are independent predictors of BRVO risk, are consistent with earlier data[7–9]. The Beaver Dam Eye study[7] in the US in 2000 reported that retinal arterioles were found anterior to venules nearest the occlusion in 87.1% of eyes, and retinal vein occlusions were more common in eyes with AV nicking.

Some reports mentioned that CRVO and BRVO have different risk factors[27,28]. However, in our study, according to multivariate logistic regression analysis, there was no significant differences between the two groups. Therefore, we analyzed the data of CRVO and BRVO as RVO.

The majority of patients with RVO had underlying systemic arterial disease, such as hypertension, diabetes mellitus, and hyperlipidemia, resulting in arteriosclerosis, a process that is characterized by thickening and hardening of the arterial wall with a loss of elasticity[4]. In our study, RVO was highly associated with a hypertension OR of 2.56 (95%CI=1.90–3.44). The associations of RVO with hypertension were in line with those found in other studies[27,29–33]. A systematic review and meta-analysis in 2019 from the UK, which analyzed 12 individual studies that reported the prevalence of RVO additionally examined potential risk factors for RVO using a multivariate design, reported that hypertension was revealed as the strongest risk factor for any RVO (meta-OR: 2.82 [95%CI=2.12–3.75]), and advanced age was found to be a significant risk factor for any RVO with a meta-OR of 1.60 (95%CI=1.38–1.84)[1,13–15]. In our study, AV nicking positively correlated with RVO; mild with an OR=9.73, moderate with an OR=298, severe with an OR=1,210, suggesting that anti-arteriosclerosis therapy beginning at a younger age, including medications, and lifestyle improvements such as healthy eating habits and regular exercise, may be important for the prevention of RVO. In addition, the strong and consistent link between hypertension and RVO suggests the benefits of blood pressure management in the prevention of RVO[34], especially, for persons with AV nicking.

Our study has several strengths, including large sample size, and investigating all fundus photos, which had been taken in previous medical checkups in our institution. However, several limitations should be considered when interpreting our data. The nature of its cross-sectional observational design means selection bias might not be excluded. Therefore, we used major risk factors for RVO, which have been reported by many previous studies, and categorized the data in the traditional way

to reduce the selection bias. Second, new-onset RVO was defined as onset within 12 months according to the interval of medical examination because we use annual medical checkup data, and it may have been subject to overestimation. Third, we did not divide RVO into BRVO and CRVO for identifying potential risk factors because sample sizes for CRVO were too small to analyze properly and robustly. Although our study had a large sample size for one year, we didn't have enough cases to analyze due to the low prevalence of CRVO. Further investigation is needed.

## Conclusions

In conclusion, the prevalences of RVO, BRVO, and CRVO were 1.2%, 1.1%, and 0.08% and the one-year incidences of RVO, BRVO, and CRVO were 0.2%, 0.18%, and 0.02%, respectively, in a Japanese population at a medical checkup institution in Osaka. RVO was associated with advanced age, hypertension, and AV nicking, which suggests that optimal blood pressure control and anti-arteriosclerosis therapy, including medications and lifestyle improvements, may prevent RVO.


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## Conflict of Interest

The authors have no conflict of interest to declare.

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Original Article

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# Analysis of the Relationship Between Plasma and Urinary Glucose and the Significance of Urinary Glucose Levels in Patients Started on SGLT-2 Inhibitors

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## Abstract

**Objective:** Urinary glucose testing is simple, and has been used as a screening test. However, SGLT-2 inhibitors, drugs for diabetes, which promote massive urinary glucose excretion, are now commercially available, and the number of patients strongly positive for urinary glucose (4+) on health checkups has increased. In this study, we investigated the relationship between the urinary and fasting plasma glucose levels and their changes in persons who had undergone Ningen Dock during 5-year periods before and after SGLT-2 inhibitors became commercially available (2009–2013)(2014–2018).

**Methods:** The subjects were persons who had undergone health checkups. The fasting blood and urinary glucose levels were measured, and their relationship was investigated.

**Results:** In the diabetes treatment group, the mean rates of males and females with urinary glucose 4+ among those with a fasting plasma glucose level of <126 mg/dL during the 5 years before SGLT-2 inhibitors became commercially available (launching) were 0.8 and 0%, respectively. However, the percentages 5 years after launching were 25.7 and 16.1%, respectively, showing rapid increases. The specificity, false positive rate, and positive predictive value during the 5 years before launching were 99.4, 0.6, and 53.6%, respectively. The percentages 5 years after launching were 98.5, 1.5, and 21.2%, respectively.

**Conclusions:** Urinary glucose testing has been used for screening on health checkups. The launching of SGLT-2 inhibitors, which promote massive urinary glucose excretion, markedly influenced the specificity, false positive rate, and positive predictive value of urinary glucose testing.

**Keywords** urinary glucose, health checkup, SGLT-2 inhibitors

The renal glucose threshold is considered to be a plasma glucose level of 160 to 180 mg/dL[1]. Therefore, persons with positive (≥1+) reactions on urinary glucose testing may have hyperglycemia (plasma glucose level: ≥160 to 180 mg/dL). Urinary glucose testing facilitates diabetes mellitus screening, excluding renal glycosuria, when a blood test is not conducted, as well as severity assessment to some degree based on several grades of qualitative results related to the urinary glucose concentration. This testing is incorporated in urine test papers, and this paper is commercially available at pharmacies. It is a highly useful diagnostic method that can be easily used.

Diabetes mellitus screening on health checkups primarily consists of urinary glucose, plasma glucose, and HbA1c.

Recently, a large number of new oral drugs for diabetes mellitus have been approved. In April 2014, sodium-glucose cotransporter 2 (SGLT-2) inhibitors[2] became commercially available, and were covered by health insurance. SGLT-2 inhibitors have a unique mechanism: they act on the proximal renal tubule, promoting urinary glucose/Na excretion. Their use leads to massive urinary glucose excretion. As a result, the urinary glucose level becomes abnormally high during the administration of SGLT-2 inhibitors even when the plasma glucose level is slightly increased.

On health checkups, urinary glucose testing has been useful for evaluating the presence or absence of diabetes mellitus or its severity. However, with the appearance

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of SGLT-2 inhibitors, which promote massive urinary glucose excretion, the urinary-glucose-positive rate has increased in persons undergoing health checkups/Ningen Dock. Even in those with a plasma glucose level of <160 mg/dL, which is the renal glucose threshold for diabetes, the number of those strongly positive for urinary glucose (4+) has recently and markedly increased.

In this study, we investigated the relationship between the blood and urinary levels of glucose during 5-year periods before (2009–2013) and after (2014–2018) SGLT-2 inhibitors became commercially available. We primarily analyzed the relationship between the fasting plasma glucose level and rate of strongly urinary-glucose-positive (4+) persons in the diabetes treatment and non-treated groups. In addition, we reviewed the significance of urinary glucose testing on health checkups/Ningen Dock involving simultaneous blood and urinary glucose tests based on the specificity and positive predictive value of urinary glucose testing.

## Methods

The subjects were 97,128 persons who had undergone Ningen Dock in the Health Care Center at the Jikei University School of Medicine between 2009 and 2018 (10 years) (65,205 males, 31,923 females). To clarify changes in the urinary-glucose-positive rate related to the appearance of SGLT-2 inhibitors, the survey periods were established as 5-year periods before and after these inhibitors became commercially available.

Prior to Ningen Dock, a self-reporting questionnaire prepared in accordance with the questionnaire for specific health checkups was delivered. Questions regarding diabetes treatment consisted of the presence or absence of blood-glucose-lowering drugs or insulin injection. On a medical interview, a public health nurse reconfirmed the status of treatment based on a drug notebook.

After ≥10-hour fasting, a blood test was conducted. The fasting plasma glucose (FPG) level was measured using the glucose oxidase (GOD) method with an automatic glucose analyzer (GA, A&T Corporation, Kanagawa Prefecture, Japan) between 2009 and 2010 and GA08III (A&T Corporation, Kanagawa Prefecture, Japan) between 2011 and 2017. In 2018, it was measured by amperometry with GOD-immobilized enzyme membrane and hydrogen peroxide electrodes (ADAMS Glucose GA-1172, ARKRAY, Inc., Kyoto, Japan). The HbA1c level was measured using reverse-phase partition cation exchange chromatography (DS120, Tosoh Corporation, Tokyo, Japan) between 2009 to 2010 and HLC-723G9 (TOSOH CORPORATION, Tokyo, Japan) from 2011 until 2017. In 2018, it was measured by amperometry with GOD-immobilized enzyme membrane and hydrogen peroxide electrodes using an ADAMS A1C HA-8190V (ARKRAY, Inc., Kyoto, Japan). For urinary glucose measurement, the reflectance method was adopted using an automatic urine analyzer AX-4030 (ARKRAY, Inc., Kyoto, Japan) from 2009 until 2014 and AX-4060 (ARKRAY, Inc., Kyoto, Japan) from 2015 until 2018. As a reagent, Uriflet S 7UA was used. Measurements were conducted at the Central Laboratory, Jikei University Hospital. FPG and urinary glucose were analyzed in the diabetes treatment and non-treatment groups each year.

All data are expressed as mean±standard deviation (SD). All statistical analyses were performed using commercial software (IBM SPSS Statistics version 27.0, IBM Corporation, Armonk NY, USA).

This study was conducted according to the Helsinki Declaration. Its protocol was approved by the Ethics Review Board of the Jikei University School of Medicine (17-015).

## Results

The subjects' profiles are shown in **Table 1**. Using April 2014, when SGLT-2 inhibitors became commercially available (launching), as a starting date, the survey period was divided into a 5-year period before launching (2009 to 2013) and a 5-year period after launching (2014 to 2018).

The mean rates of males and females with urinary glucose (4+) during the 5 years before launching were 0.36 and 0.07%, respectively. The percentage was higher in males, because the rates of males and females receiving diabetes treatment were 5.29 and 1.67%, respectively.

The mean rates of males and females with urinary glucose (4+) during the 5 years after launching were 0.97 and 0.33%, respectively. In males, the percentage in 2018, that is, 5 years after launching, was 2.08%, being 5.78 times higher than the mean rate during the 5 years before launching (0.36%). In females, the percentage in 2018, that is, 5 years after launching, was 0.49%, being 7 times higher than the mean rate during the 5 years before launching (0.07%). The mean rate of males receiving diabetes treatment during the 5 years before launching was 5.29%. It increased to 6.01% (+13.6%) in 2018, that is, 5 years after launching. In females, there was an increase from 1.67 to 1.88% (+12.6%).

Next, we analyzed the subjects by dividing them into two groups: diabetes treatment and non-treated groups (**Table 2**). In the diabetes treatment group, the mean rates of urinary glucose 4+ males and females in those with an FPG level of <126 mg/dL during the 5 years before launching were 1.0 and 0%, respectively. The rate of males 5 years after launching was 25.7%, showing a 32.1-fold increase. That of such females 5 years

**Table 1. Subjects' Profile in the Present Study**

| | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | SGLT2 inhibitor was launched in April, 2014 | | | | |
| number | 10235 | 9972 | 10053 | 10332 | 9971 | 9501 | 9327 | 9465 | 9404 | 8868 |
| age (mean±SD) | 49.2±11.0 | 49.8±11.0 | 50.2±10.9 | 50.8±10.7 | 51.1±10.8 | 51.6±10.9 | 52.0±10.7 | 52.2±10.8 | 52.4±10.8 | 53.2±10.9 |
| BMI (mean±SD) | 22.8±3.3 | 22.9±3.3 | 23.0±3.4 | 23.1±3.4 | 23.0±3.4 | 23.0±3.4 | 23.0±3.4 | 23.0±3.4 | 23.0±3.4 | 23.1±3.5 |
| FPG (mean±SD) | 98.7±18.4 | 99.3±19.2 | 99.3±17.5 | 100.2±18.2 | 100.7±17.6 | 101.1±17.7 | 100.9±17.2 | 100.9±17.8 | 100.5±16.4 | 94.1±16.6 |
| FPG<126 | 9759 | 9449 | 9533 | 9758 | 9404 | 8928 | 8787 | 8937 | 8985 | 8504 |
| 126≤FPG<140 | 194 | 216 | 222 | 250 | 273 | 255 | 240 | 230 | 211 | 169 |
| 140≤FPG<180 | 188 | 205 | 218 | 235 | 211 | 238 | 238 | 215 | 207 | 147 |
| 180≤FPG | 93 | 99 | 73 | 86 | 78 | 75 | 60 | 73 | 59 | 44 |
| UG (−) | 10046 | 9782 | 9874 | 9788 | 9800 | 9339 | 9164 | 9287 | 9216 | 8672 |
| UG (±) | 65 | 64 | 67 | 28 | 65 | 65 | 51 | 41 | 43 | 23 |
| UG (1+) | 33 | 37 | 35 | 16 | 32 | 27 | 74 | 21 | 20 | 14 |
| UG (2+) | 18 | 19 | 15 | 4 | 14 | 12 | 16 | 14 | 17 | 16 |
| UG (3+) | 20 | 29 | 24 | 7 | 25 | 24 | 13 | 21 | 12 | 10 |
| UG (4+) | 43 | 30 | 24 | 7 | 20 | 20 | 33 | 66 | 84 | 126 |
| ratio of UG (4+) | 0.40% | 0.30% | 0.30% | 0.30% | 0.20% | 0.20% | 0.40% | 0.70% | 0.90% | 1.40% |
| under treatment ratio | 3.50% | 4.03% | 4.10% | 4.19% | 4.41% | 4.60% | 4.44% | 4.00% | 4.24% | 4.60% |

BMI: body mass index, FPG: fasting plasama glucose, UG: urinary glucose
No urine test for those on dialysis or menstruating

**Table 2. Correlation Between Urinary Glucose and Fasting Plasma Glucose in Diabetes Treatment and Non-treated Groups**

| | | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | SGLT2 inhibitor was launched in April 2014 | | | | |
| under medication | | | | | | | | | | | |
| UG (−, ±) | FPG<126 | 97.3% | 97.4% | 97.0% | 97.6% | 97.60% | 97.6% | 96.9% | 89.3% | 79.6% | 74.2% |
| | 126≤FPG<140 | 94.0% | 97.8% | 97.5% | 95.2% | 97.50% | 97.5% | 90.3% | 86.7% | 75.6% | 74.1% |
| | 140≤FPG<180 | 88.2% | 84.9% | 82.9% | 90.5% | 88.40% | 88.4% | 80.7% | 79.1% | 77.2% | 67.0% |
| | 180≤FPG | 24.6% | 25.7% | 26.0% | 39.7% | 35.70% | 35.7% | 32.6% | 28.6% | 31.0% | 25.0% |
| UG (4+) | FPG<126 | 0.9% | 0.9% | 0.7% | 0.8% | 0.8% | 0.8% | 2.3% | 9.2% | 18.3% | 24.2% |
| | 126≤FPG<140 | 0.0% | 1.1% | nil | nil | nil | nil | 4.3% | 12.0% | 24.4% | 24.7% |
| | 140≤FPG<180 | nil | 4.0% | 0.7% | 1.4% | 0.7% | 0.7% | 7.3% | 14.0% | 14.0% | 29.0% |
| | 180≤FPG | nil | 17.1% | 28.0% | 24.1% | 17.9% | 17.9% | 30.2% | 31.0% | 33.3% | 37.5% |
| without medication | | | | | | | | | | | |
| UG (−, ±) | FPG<126 | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% | 99.9% |
| | 126≤FPG<140 | 99.2% | 98.4% | 98.6% | 98.6% | 98.7% | 98.7% | 97.3% | 98.1% | 99.2% | 97.6% |
| | 140≤FPG<180 | 83.8% | 88.6% | 91.7% | 87.5% | 91.7% | 91.7% | 89.8% | 89.4% | 90.1% | 76.6% |
| | 180≤FPG | 15.7% | 27.6% | 21.7% | 21.4% | 13.6% | 13.6% | 35.3% | 38.7% | 35.3% | 15.0% |
| UG (4+) | FPG<126 | nil | 0.1% | nil | nil | nil | nil | 0.0% | 0.0% | 0.0% | 0.0% |
| | 126≤FPG<140 | nil | 0.5% | nil | nil | nil | nil | nil | 0.6% | nil | 1.2% |
| | 140≤FPG<180 | 2.9% | 2.9% | nil | nil | nil | nil | nil | 1.2% | 2.8% | 6.4% |
| | 180≤FPG | 53.1% | 17.2% | 47.8% | 46.4% | 36.4% | 36.4% | 41.2% | 32.3% | 17.6% | 60.0% |

FPG: fasting plasma glucose, UG: urinary glucose
nil: no case

after launching was 16.1%. In the non-treated group, the mean rates of urinary glucose 4+ males and females in those with an FPG level of <126 mg/dL during the 5 years before launching were 0%. The rates of such males and females 5 years after launching were also 0%. Before launching, "urinary glucose 4+" was noted only in males and females with an FPG level of ≥180 mg/dL regardless of the presence or absence of diabetes treatment before launching.

To verify the significance of urinary glucose testing as a health checkup item, we calculated the sensitivity, specificity, false negative rate, false positive rate, positive predictive value, and negative predictive value of this test from the data on an FPG level of ≥180 mg/dL and urinary glucose 4+ (**Fig. 1**). In the diabetes treatment group (left row of **Fig. 1**), the specificity, false positive rate, and positive predictive value during the 5 years before launching were 93.1, 6.92, and 62.9%, respectively. The percentages 5 years after launching were 72.3, 27.7, and 14.5%, respectively, showing marked changes. Overall (right row of **Fig. 1**), the specificity, false positive rate, and positive predictive value during the 5 years before launching were 99.4, 0.6, and 53.6%, respectively. The percentages 5 years after launching were 98.5, 1.5, and 21.2%, respectively; there were marked changes in the false positive rate and positive predictive value.

## Discussion

After 2014, when SGLT-2 inhibitors, which potently

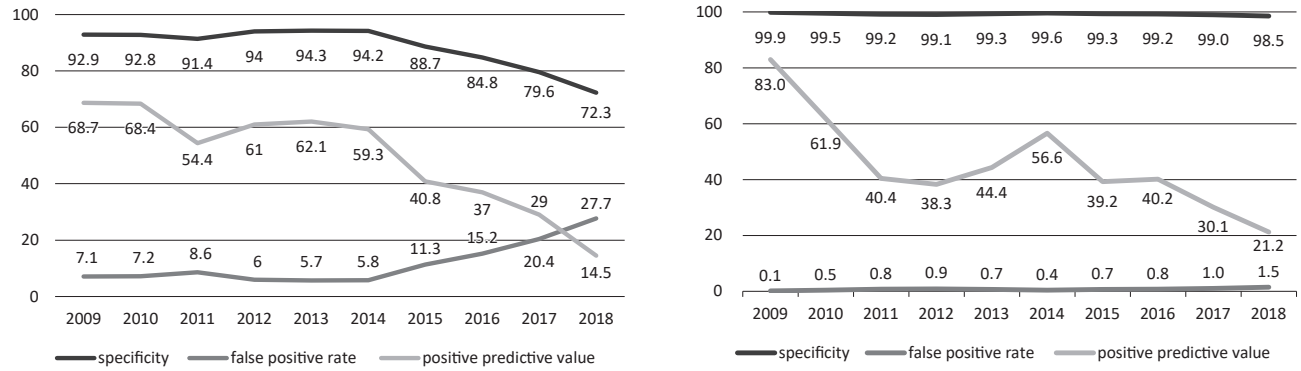


**Fig. 1. Specificity, False Positive Rate, and Positive Predictive Value of Urinary Glucose 4+ in Subjects with a Fasting Plasma Glucose Level of ≥ 180 mg/dL among Those Receiving Diabetes Treatment (left) and All Subjects (right)**

promote urinary glucose excretion, became commercially available, the number of males and females receiving diabetes treatment who were strongly positive for urinary glucose (4+) despite an FPG level of <126 mg/dL, gradually increased. On the other hand, none of the non-treated patients showed urinary glucose (4+) with an FPG level of <126 mg/dL during the survey periods before and after launching.

Urinary glucose has been used for health checkups, such as those for school children, over many years. Iseki *et al.*[3] investigated the morbidity rate and outcome of glucosuria in subjects with and without diabetes mellitus (DM) among 209,060 participants in a national survey involving 6 areas of Japan in 2008, regarding urine test paper 1+ or higher subjects as having glucosuria. They reported the hazard ratio (HR) of the mortality rate, with a median follow-up of 3.57 years. The rough mortality rates in glucosuria-free subjects and those with glucosuria were 1.2 and 3.4%, respectively. After correcting various factors, the HR for urine test paper glucosuria was 1.475. In subjects with DM, the HR was higher than in glucosuria-free subjects with DM. In non-DM subjects with glucosuria, the HR was higher than in glucosuria-free non-DM subjects. They indicated the significance of urinary glucose testing.

Renal glucosuria refers to a condition in which urinary-glucose-positive reactions are noted although the blood glucose level is not elevated. It is related to a low renal glucose threshold as a glucose transporter abnormality in the kidney[4,5]. In the non-treated group, there was no urinary glucose 4+ subject with an FPG level of <180 mg/dL during the 5 years (2009 to 2013) before SGLT-2 inhibitors became commercially available. In the diabetes treatment group, urinary glucose 4+ males and females with an FPG level of <180 mg/dL accounted for <1 and 0%, respectively. This suggests a lack of influence of subjects with renal glucosuria on the data after SGLT-2 inhibitor launching.

After SGLT-2 inhibitors for diabetes became commercially available, there were marked changes in the results of urinary glucose testing. For screening, a high specificity is initially important. In the diabetes treatment group, the mean specificity during the 5 years before launching was 93.1%, but it decreased to 72.3% five years after launching. Overall, that before launching was 99.4%, decreasing to 98.5%. In the diabetes treatment group, the mean positive predictive value during the 5 years before launching was 69.2%, but it rapidly decreased to 14.5% five years after launching. Overall, that before launching was 53.6%, rapidly decreasing to 21.2%. SGLT-2 inhibitors have been approved as drugs for diabetes mellitus. In November 2020, they were approved as drugs for chronic heart failure. In August 2021, they were approved as drugs for chronic kidney disease. In particular, there is less drug treated chronic kidney disease directly until now. Thus, SGLT-2 inhibitors may be widely used in patients without diabetes. In the future, blood sugar can no longer be inferred from urine sugar results in diabetics under treatment. Also, urine sugar results make it difficult to pick up diabetes. An increase in the false positive rate may lead to time/cost wastes related to unnecessary tests, mental/physical stress, and the risk of complications. Actually, urinary-glucose-positive persons are evaluated as requiring additional or detailed examinations even in the absence of abnormalities in the blood glucose level at many medical examination centers.

As a limitation of this study, the presence or absence of SGLT-2 inhibitor administration was not confirmed, although the presence or absence of anti-diabetic drug use was investigated. The data are based on simply calculated statistics in each year, and duplicated subjects were not counted. Furthermore, the diagnostic criteria for renal glucosuria were not adopted, and the data were simply evaluated based on the results of urinary glucose testing at the laboratory alone. With respect to the relationship with the estimated glomerular filtration rate (eGFR), which alters the renal glucose threshold,

the number of subjects with a reduction in the eGFR was small, and they were not included for analysis. In the non-treated group before launching, the rates of urinary glucose 4+ males and females in those with an FPG level of <140 mg/dL were 0%. However, after launching, there were a small number of urinary glucose 4+ subjects among those with an FPG level of <140 mg/dL. Although information on the presence or absence of diabetes treatment was obtained using a questionnaire for inquiry or through a medical interview, it was insufficient in subjects who did not bring a drug notebook on the day of Ningen Dock. Actually, it could not be accurately evaluated which of two factors: the use of SGLT-2 inhibitors or the presence of diseases that influence the appearance of urinary glucose, such as pheochromocytoma and hyperthyroidism, was involved.

As a screening test, urinary glucose testing is significant especially when blood glucose testing is not conducted. However, in adults, blood glucose testing is performed on Ningen Dock or specific health checkups, reducing the significance of urinary glucose testing. In addition, urinary glucose is not included in diagnostic criteria for diabetes mellitus. Therefore, according to the judgment criteria of Japan Society of Ningen Dock, urine sugar (±) or greater is judged as a mild abnormality[6]. Based on the results of this survey, the rate of urinary glucose 4+ subjects rapidly increased after the launching of SGLT-2 inhibitors (April 2014) in comparison with that before launching. In particular, an increase in the number of subjects, strongly positive for urinary glucose (4+), evaluated as healthy based on an FPG level of <126 mg/dL raised the necessity of reviewing the contents of a health checkup. In addition, SGLT-2 inhibitors will be increasingly administered to patients with heart failure or chronic kidney disease in the future; therefore, the number of urinary-glucose-positive persons other than diabetics will increase. On health checkups/Ningen Dock, the value of urinary glucose testing in the presence of simultaneous blood glucose testing may reduce. On health checkups involving blood glucose and HbA1c tests, urinary glucose testing may not always be necessary.

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## Conflict of Interest

The authors have no conflict of interest to declare.

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Original Article

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# Obesity and Metabolic Syndrome in Urban Vietnam

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## Abstract

**Objective:** Obesity, in particular abdominal obesity, often leads to a metabolic syndrome. Abdominal obesity is defined as a visceral fat area (VFA) >100 $cm^2$ using abdominal CT. Waist circumference (WC) is an alternative indirect and simple value for abdominal obesity. Among 191 countries, Vietnam has the lowest obesity rate (2.10%) as defined by body mass index (BMI) ≥30. The prevalence of obesity and metabolic syndrome has not been well studied in developing urban Vietnam, which is experiencing rapid changes in dietary patterns.

**Methods:** A total of 659 adult Vietnamese (373 males, ages 22 to 79, mean 48.9±0.5; and 286 females, ages 22 to 87, mean 49.9±0.7) underwent abdominal CT to measure VFA and physical examinations, including measurement of WC and BMI, at the HECI Center, Ho Chi Minh, Vietnam in 2019. The prevalence of obesity and metabolic syndrome, and the relationship among the three parameters were analyzed.

**Results:** The overall rate of obesity was 2.6% (males, 3.5%; females 1.4%), and overweight (BMI≥25 kg/$m^2$) was 30.8% (males 41.0%; females 17.4%). Abdominal obesity (VFA≥100 $cm^2$) was found in 44.3% (males 63.8%; females 18.9%) and metabolic syndrome using VFA≥100 $cm^2$ was found in 34.3% (males 49.9%; females 14.3%). VFA, WC and BMI values were positively correlated in both males and females.

**Conclusion:** In urban Vietnam, the prevalence of obesity (BMI≥30) is low, but being overweight and having metabolic syndrome with abdominal obesity were found in up to 40% of participants.

**Keywords** visceral fat area, BMI, waist circumference, obesity, metabolic syndrome

More than half of adults (people aged 18 years or more) in the world exceeded normal body mass index (BMI) in 2016[1]. In developed and developing countries, changes in dietary and physical activity patterns often underly obesity[2]. An elevated BMI is a major risk factor for noncommunicable diseases such as cardiovascular diseases, diabetes, musculoskeletal disorders and some cancers (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon)[1].

Vietnam has an average BMI of 21.6 kg/$m^2$ and an obesity rate of 2.1% (obesity defined as BMI≥30), which is the lowest in the world according to the 2021 World Population Review[3]. Over the past 20 years, however, Vietnam has been among the fastest economically developing countries, which has resulted in rapid changes in dietary patterns and increases in the population's BMI[4]. Ho Chi Minh City is the country's largest city and a major economic hub, with a population of 8.8 million in 2021[5].

Obesity, in particular abdominal (or visceral) obesity, often leads to metabolic syndrome. It is now accepted as a global health concern with a worldwide prevalence ranging from 10% to 84% depending on ethnicity, age and gender[6,7]. Metabolic syndrome represents various complex health conditions, consisting of a combination of adiposity, hypertension, hypertriglycemia, low high-density lipoprotein cholesterol, glucose intolerance, and insulin resistance[6,7]. The presence of metabolic syndrome leads to an approximately 2-fold increase in risk for coronary heart disease and a 1.5-fold increase in mortality rate[8,9]. In addition, metabolic syndrome and type 2 diabetes are strongly associated, yet it is still debatable whether diabetes is a component or an outcome of metabolic syndrome[10].

Abdominal obesity is defined as visceral fat area (VFA) >100 $cm^2$, as measured from images obtained by abdominal computed tomography (CT)[11]. Without

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requiring an elaborate technique, measuring waist circumference (WC) is a simple way to assess abdominal obesity[12]. Cut-off lines for WC and/or components of metabolic syndrome are slightly different in various guidelines, including those published by the International Diabetes Federation (IDF), National Cholesterol Education Program, and World Health Organization[13,14]. As the prevalence of metabolic syndrome is influenced by ethnicity, age and gender, the IDF defines ethnicity- and gender-specific cut-off lines of WC for abdominal obesity. For instance, the cut-off value for obesity in South Asians is defined ≥90 cm in males, ≥80 cm in females; in those of European descent it is ≥94 cm for males, 80 cm for females[13,14].

The prevalence of obesity and metabolic syndrome, and correlations among obesity indices including VFA, WC and BMI, have not been well studied in the Vietnamese. We examined these issues in economically developing, urban Vietnam, which is undergoing rapid changes in diet and physical activities.

## Methods

### Study design and participants

The study was conducted in 2019 at the Health Evaluation and Promotion Center Cho Ray Hospital International University Health and Welfare (HECI Center), which is a bilateral corporation unit between Vietnam and Japan, located in Ho Chi Minh city, Vietnam. Six hundred and fifty-nine adult Vietnamese who underwent a comprehensive physical check-up were analyzed for VFA, WC, BMI, as well as the presence of metabolic syndrome as evaluated by levels of blood pressure, triglyceride, HDL-cholesterol, and fasting blood glucose, or medical treatment for hypertension, dyslipidemia, or diabetes mellitus. More details are presented in the Results. The study conformed to the guidelines of the Declaration of Helsinki, 1964. All protocols were approved by the Institutional Review Board of the Cho Ray Hospital, where de-identified clinical data has been approved for use in research. De-identified data were analyzed at the International University of Health and Welfare Narita Hospital.

### Measurements and data collection

Abdominal CTs were taken using the Aquilion One 640-slice (Toshiba Corp., Tokyo, Japan), and images were analyzed by the Vitrea™ Advanced Visualization CT Fat Measurement (Canon Inc., Tokyo, Japan). WC was measured around the abdomen at the level of the umbilicus.

### Statistical analysis

Data are expressed as mean values plus or minus the standard deviation. For analyses we used SPSS (version 27 or 28) to conduct chi-square test, linear regression, independent T-tests and ANOVA. Levene's test was used for equality of variance, and $p$ values were calculated depending on the assurance of equality. $p$ values less than 0.05 were considered significant.

## Results

### Participant population and obesity indices

Data from a total of 659 adult Vietnamese were analyzed: 373 males (ages 22 to 79, mean 48.9±0.5) and 286 females (ages 22 to 87, mean 49.9±0.7, $p$=0.247), who underwent a complete physical check-up including an abdominal CT measurement for VFA at the HECI Center, Ho Chi Minh, Vietnam in 2019.

As shown in **Table 1**, the overall average BMI was 23.8±3.1 kg/m$^2$, 24.6±3.0 kg/m$^2$ in males, and 22.7 ±2.8 in females. These values are within the normal BMI<25. The average WC was 87.7±8.2 cm in males and 77.5±8.1 cm in females. In Vietnam, the IDF criterion for abdominal obesity ethnicity-specific for South Asians has been used in most studies[15], which is ≥90 cm in males and ≥80 cm in females. Thus, the average WCs were also below the cut-off values in both sexes. However, the average VFA in males was 116.1±49.3 cm$^2$, which exceeded the normal level (<100 cm$^2$). VFA in females was 66.0±37.3 cm$^2$, which is within the normal value.

In summary, the average obesity indices BMI, WC and VFA were below the cut-off level for obesity, with the exception of male VFA. Males showed significantly higher values than females in all three indices **(Table 1)**.

### Prevalence of obesity in urban Vietnam

**Table 2** summarizes the prevalence of obesity. First, overweight participants (BMI≥25 kg/m$^2$) accounted for 30.8% of the total (males 41.0%; females 17.4%); the prevalence of obesity (BMI≥30) was 2.6% (males 3.5%; females 1.4%). Second, regarding abdominal obesity, when VFA≥100 cm$^2$ is applied, 44.3% of the total study population (males 63.8%; females 18.9%) were considered to have abdominal obesity. Third, when the IDF definition of WC≥90 cm in males and ≥80 cm in females was applied, 39.3% of the total (males 38.1%; females 40.9%) were considered to have abdominal obesity. Of note, in Japan, different cut-off values for WC are used, namely ≥85 cm for males and ≥90 cm for females[16,17]. Applying these values to the Vietnamese data, 40.1% of all participants (males 64.8%; females 7.7%) were considered abdominally obese. All three obesity values (VFA, WC, and BMI) were significantly higher in males than females, with the exception of the obesity rate (BMI≥30), which showed no gender difference.

### Obesity indices in three different age groups

Participant ages ranged from 22 to 79 years in males and 22 to 87 years in females. We categorized them into three groups: group 1 (21–40 years old), group 2 (41–

**Table 1. Obesity Indices in the Total Population, Males and Females**

| Obesity index | Total (*n*=659) | Male (*n*=373) | Female (*n*=286) | Male vs. Female *p* value |
|---|---|---|---|---|
| BMI (kg/m$^2$) | 23.8±3.1 | 24.6±3.0 | 22.7±2.8 | 0.000* |
| Waist circumference (cm)# | 83.3±9.6 | 87.7±8.2 | 77.5±8.1 | 0.000* |
| Visceral fat area (cm$^2$) | 94.4±50.9 | 116.1±49.3 | 66.0±37.3 | 0.000* |

*$p \leq 0.05$. #Waist circumference for obesity is ethnicity specific: Europid males ≥94 cm, females ≥80 cm; South Asians males ≥90 cm, females ≥80 cm[13,29]. Mean±S.D.

**Table 2. Prevalence of Obesity in the Total Population, Males and Females**

| Obesity index | Total (*n*=659) | Male (*n*=373) | Female (*n*=286) | Male vs. Female *p* value | Male vs. Female $\chi^2$ |
|---|---|---|---|---|---|
| BMI ≥ 25 kg/m$^2$ | 203 (30.8%) | 153 (41.0%) | 50 (17.4%) | 0.000* | 132.4 |
| BMI ≥ 30 kg/m$^2$ | 17 (2.6%) | 13 (3.5%) | 4 (1.4%) | 0.094 | 2.805 |
| Waist circumference (cm)[a] | 259 (39.3%) | 142 (38.1%) | 117 (40.9%) | 0.000* | 134.1 |
| Waist circumference (cm)[b] | 264 (40.1%) | 242 (64.8%) | 22 (7.7%) | 0.000* | 220.9 |
| Visceral fat area ≥ 100 cm$^2$ | 292 (44.3%) | 238 (63.8%) | 54 (18.9%) | 0.000* | 42.1 |

*$p \leq 0.05$. [a]Following the IDF definition: males ≥90 cm, females ≥80 cm[13,29]. [b]Following the Japanese obesity society's definition: waist circumference ≥85 cm for male, ≥90 cm for female. Mean±S.D.

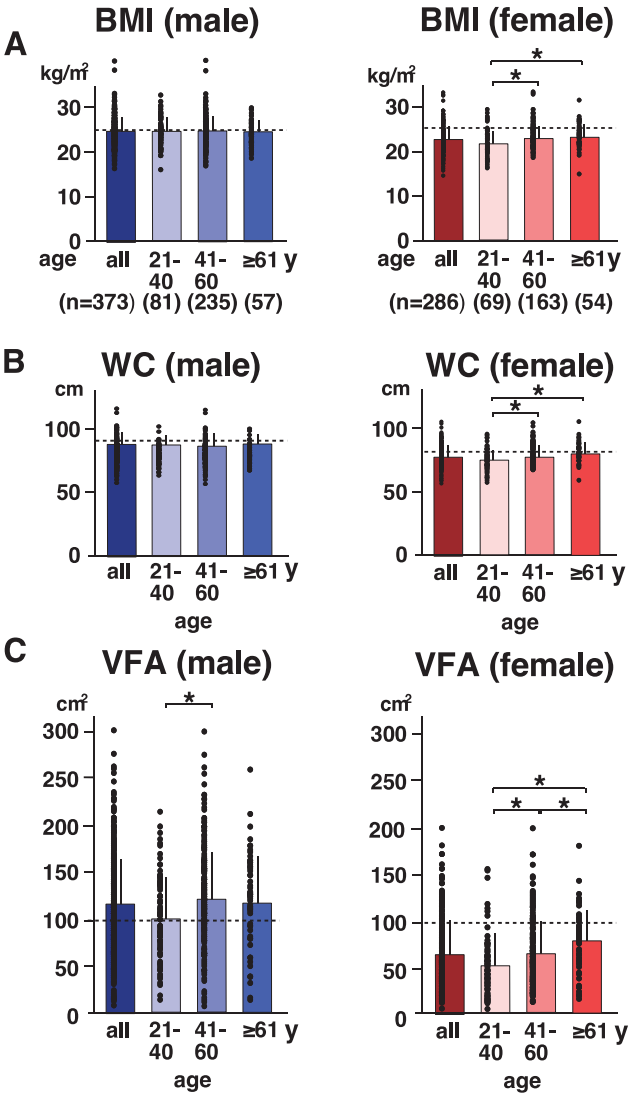


**Fig. 1. Obesity Indices in Three Different Age Groups**

(A) BMI, (B) WC and (C) VFA in total and three different age groups: group 1 (21–40 years old), group 2 (41–60 years old), and group 3 (≥61 years old) in males and females. Data are expressed as mean±S.D.; individual data are represented as circles. *$p<0.05$.

60 years old), and group 3 (≥61 years old). **Fig. 1** shows the obesity indices (Mean±S.D. with individual values) overall and in the three groups of males and females.

In males, only VFA in group 1 (21–40 years old) versus group 2 (41–60 years old) showed a significant difference. In contrast, in females, most of the obesity indices showed significant age-dependent increases, but not in groups 2 (41–60 years old) and 3 (≥61 years old) in BMI and WC. In summary, obesity indices were mostly unchanged among the three age groups in males, whereas they generally increased in an age-dependent manner in females.

## Correlations among VFA, WC, and BMI

We tested for correlations among the three obesity indices (VFA, WC, and BMI) in males and females separately using liner regression analysis (**Fig. 2**). The coefficient determination, R-squared ($R^2$), for VFA and WC, was 0.404 for males and 0.533 for females; $R^2$ for VFA and BMI was 0.326 for males and 0.433 for females; and $R^2$ for BMI and WC was 0.745 for males and 0.626 for females. The strongest correlation was between BMI and WC, followed in order by that between VFA and WC, and VFA and BMI, in both males and females.

The cut-off level for normal VFA is 100 cm$^2$ [11]. Following the regression equation, this value corresponds to an estimated WC of 85.98 cm for males and 83.12 cm for females (**Fig. 2**). It also corresponds to estimated values of BMI 24.10 kg/m$^2$ for males and 24.42 kg/m$^2$ for females. The cut-off level for normal BMI is 25 kg/m$^2$, which corresponds to estimated values of WC 88.65 cm for males and 83.0 cm for females.

## Prevalence of metabolic syndrome in urban Vietnam

We evaluated the presence of metabolic syndrome following the IDF definition (**Fig. 3**): having central obesity plus any two of the following four components;

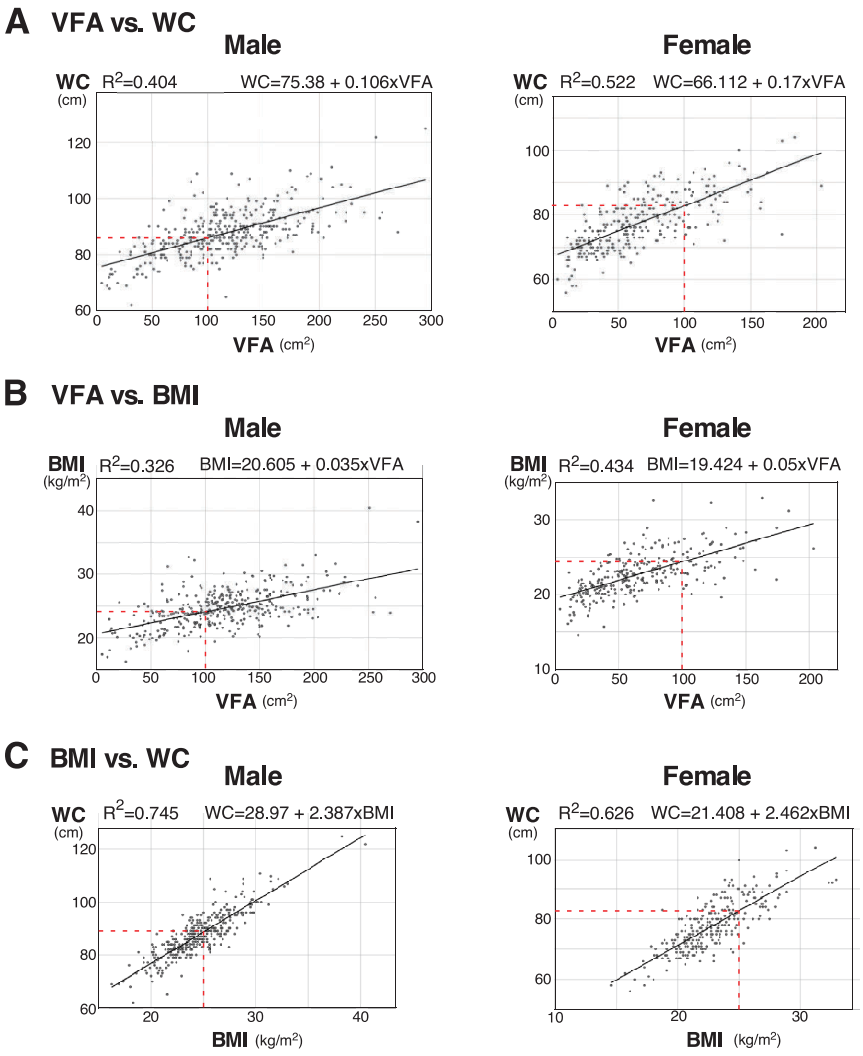


**Fig. 2. Linear Regression Analysis among Three Obesity Indices Demonstrating Individual Data, Coefficient of Determinations R-squared and Regression Equations in Males and Females**

(A) VFA and waist circumference, (B) VFA and BMI, (C) BMI and waist circumference. VFA (100 $cm^2$) or BMI (25 $kg/m^2$) was used as the normal cut-off value indicated by dotted lines
VFA, visceral fat area; WC, waist circumference; BMI, body mass index.

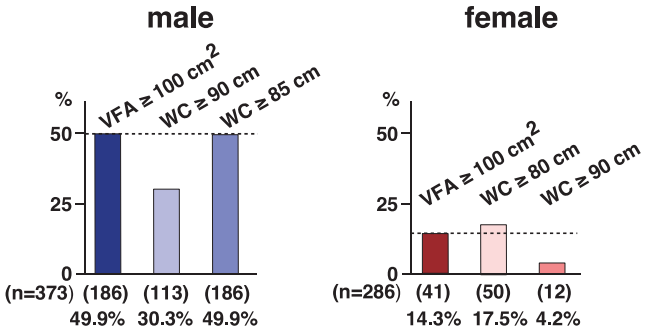


**Fig. 3. Prevalence of Metabolic Syndrome in Urban Vietnam**

The prevalence of metabolic syndrome using direct measurement of VFA≥100 $cm^2$, WC for IDF's South Asian populations (≥90 cm for males and ≥80 cm for females) or Japanese criteria (≥85 cm for males and ≥90 cm for females) for central obesity in males and females. Data are expressed as percentage and number (*n*).

1) hyper-triglyceridemia (≥150 mg/dL), 2) low high-density lipoprotein cholesterol (<40 mg/dL in males and <50 mg/dL in females), 3) high blood pressure (systolic BP≥130 mmHg or diastolic BP≥85 mmHg), and 4) high fasting glucose (≥100 mg/dL). Central obesity was defined by VFA≥100 $cm^2$, WC for IDF's South Asian populations (≥90 cm for males and ≥80 cm for females), and the Japanese criteria (≥85 cm for males and ≥90 cm for females).

The prevalence of metabolic syndrome using central obesity VFA≥100 $cm^2$ was 49.9% in males and 14.3% in females. In males, when WC≥90 cm was applied, the prevalence of metabolic syndrome decreased from 49.9% to 30.3%, whereas when WC≥85 cm was applied, it remained the same. In females, when WC≥80 cm was applied, prevalence increased from 14.3% to

17.5%, whereas when WC≥90 cm was applied, it decreased to 4.2%.

## Discussion

Obesity is an emerging disease in developed and developing countries worldwide. In 2016, more than half of adults, i.e., people aged >18 years, exceeded normal BMI across the globe[1]. In this study, we examined the prevalence of obesity (BMI≥30) in urban Vietnam, the country with the lowest obesity rate (2.10%) according to the WHO[3]. In agreement, the overall obesity rate in our study was 2.6%, despite the fact that the participants underwent a comprehensive health check-up in an urban city hospital and may not represent Vietnamese people in general. However, we found that nearly one-third of the participants exceeded the normal BMI of 25 kg/m$^2$, and more than one-third of the participants met the criteria of abdominal obesity defined as VFA≥100 cm$^2$ and WC≥90 cm in males or ≥80 cm in females. Furthermore, metabolic syndrome was found in nearly 50% of males and in 14.3% of females, following the IDF definition[13,14], except that central obesity was evaluated by direct measurement of VFA≥100 cm$^2$.

Being overweight and obese are key risk factors for many health problems including diabetes, hypertension and osteoarthritis. In addition, many studies have shown that abdominal obesity, i.e., the accumulation of visceral fat, is a key risk factor for developing metabolic syndrome[6–10]. Adipose tissue was once considered a mere energy storage site; however, it is now attributed with additional important roles, including regulating insulin sensitivity in adipocytes and secreting numerous proteins, including hormones (adipokines), lipids and nucleic acid factors that act on other nearby or distant tissues in a paracrine and endocrine manner[14,18,19]. In developed countries, such as the United States, 70.2% of adults are considered to be either overweight (BMI 25–30, 32.5%) or obese (BMI≥30, 37.7%). By 2012, more than one-third of all American adults met the criteria for metabolic syndrome[20–23]. In Europe, there has been a sharp increase in the prevalence of metabolic syndrome among older adults[24].

VFA 100 cm$^2$ is the cut-off value for abdominal obesity[11]. Following the regression equations, estimated values of WC corresponding to VFA 100 cm$^2$ were approximately 85.98 cm in males and 83.12 cm in females, and estimated values of BMI were 24.10 kg/m$^2$ and 24.42 kg/m$^2$ respectively. BMI 25 kg/m$^2$ corresponds to a WC of 88.65 cm in males and 82.96 cm in females. In Vietnam, the IDF definition of ethnicity-specific central obesity[15] is widely used, in which abnormal WC is over or equal to 90 cm in males and 80 cm in females for South Asians[13]. Our results show that these values appeared slightly higher (less stringent criteria) in males, in contrast to the lower values (more stringent criteria) for females.

Other cut-off values for WC for abdominal obesity are ≥85 cm in males and ≥90 cm in females, as used by the Japanese Internal Medicine Committee[16,17]; ≥102 cm for males, ≥88 cm for females, used by the National Cholesterol Education Program, and ≥94 cm for males and ≥88 cm for females, used by the European Group for the Study of Insulin Resistance[13]. The use of different definitions affects the prevalence of metabolic syndrome[25,26], which ranges between 17.7% according to the WHO definition and 41.5% according to the IDF definition in Finland[26].

The prevalence of obesity and/or metabolic syndrome in Vietnamese has been reported, but in limited studies[4,15,27,28]. The most recent review[15] stated that the overall prevalence of metabolic syndrome in adults was 16.1% (range, 6.7%–28.9%), 14.76% in males (range, 9.3%–36%), and 17.3% in females (range, 6.8%–76.1%), based on a total of 35,421 participants in 18 studies published between 2004 and 2019. Potential risk factors for metabolic syndrome are gender (higher in females than males), living area (higher in urban areas than in rural areas) and smoking, but not alcohol intake.

In this study, metabolic syndrome was found in nearly 50% of males and 14.3% of females according to the IDF definition, but with direct measurement of VFA≥100 cm$^2$. This prevalence in males is higher than those in the studies in Vietnam mentioned above, as well as in the United States, where one-third of all American adults meet the criteria for metabolic syndrome[20–23]. One reason for the difference is that the definition of central obesity using VFA≥100 cm$^2$ is more stringent than the IDF's WC≥90 cm for males, resulting in higher prevalence. In addition, this study was conducted in urban Vietnam, which is expected to show a higher prevalence of metabolic syndrome[15]. However, such a high prevalence was not found in female participants, who were tested in the same facility. Furthermore, the outcomes of this study may have been affected by additional factors such as socioeconomic status and lifestyle behavior, as well as ethnicity, gender, and living area, resulting in a higher prevalence of obesity and metabolic syndrome in males. Among children (11–14 years old) in Ho Chi Minh City, being overweight was more prevalent in boys living in wealthier districts, compared to girls and children living in less wealthy districts[27]. These relationships need to be examined in future studies.

In summary, we report the prevalence of obesity and metabolic syndrome and correlations among VFA, WC, and BMI measures in urban Vietnam.

## Disclosures

None.


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## Author Contributions

Data collection procedures were designed and performed by NL, TL, TN, TY, TA and HK. The manuscript was prepared by HK. All authors read and approved the final manuscript.

## Competing Financial Interests

The authors declare no potential conflicts of interest.

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# Association Between Low-normal Range Free Thyroxine Concentration and Increase in Carotid Intima–Media Thickness with Age among Euthyroid Japanese Men

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## Abstract

**Objective:** This study aimed to verify whether low-normal range serum thyroid hormone concentration is associated with carotid arteriosclerosis in men as they age.

**Methods:** This cross-sectional study included 1,087 men with a euthyroid state who had not been treated for or diagnosed with cardiovascular diseases and/or metabolic disorders in a comprehensive medical examination at Hidaka Hospital in Japan. Participants' weight, blood pressure, plasma glucose, serum lipid profile, free thyroxine ($FT_4$), and thyrotropin were measured and they were interviewed about their smoking habit. Carotid maximum intima–media thickness (max IMT) was determined by ultrasonography.

**Results:** Max IMT increased with age and began to exceed 1.1 mm, an indicator of the presence of carotid artery plaque, at ≥50 years of age. Serum $FT_4$ concentrations were significantly lower in those with carotid plaque ($p$=0.001) and, in multivariate logistic regression analysis were independently associated with carotid plaque only in men aged ≥50 years (β=−1.800, $p$=0.002). Moreover, max IMT was significantly higher in men with serum $FT_4$ concentration values of <0.97 ng/dL than in those with ≥0.97 ng/dL. These differences were not observed in men aged <50 years.

**Conclusion:** Serum $FT_4$ concentration was independently associated with age-related increases in carotid max IMT in euthyroid Japanese men. A serum $FT_4$ concentration of <0.97 ng/dL in men aged ≥50 years may help determine their risk for early carotid arteriosclerosis.

**Keywords** thyroid hormone, free thyroxine, atherosclerosis, carotid maximum intima–media thickness

Cardiovascular diseases (CVD) have become the leading cause of disability and premature mortality globally[1]. Therefore, early identification of atherosclerosis must prevent CVD. Carotid intima–media thickness (CIMT) is one of the best indices for detecting early-stage atherosclerosis[2]. CIMT, carotid plaque, and carotid stenosis develop with age[1]. In addition, men have a higher risk of developing carotid arteriosclerosis than women[1]. The association between aging in men and risk factors of atherosclerosis has gained attention over recent years[3].

Thyroid hormones, which promote systemic metabolism, also play an important role in the cardiovascular system[4]. Overt hypothyroidism increases the risk for atherosclerotic CVD by increasing the circulating levels of highly atherogenic low-density lipoprotein cholesterol (LDL-C) particles, inducing diastolic hypertension, altering coagulability, and directly affecting vascular smooth muscles[5]. Subclinical hypothyroidism (SCH), which is defined by elevated levels of thyrotropin (TSH) despite concentrations of free 3,5,3'-triiodothyronine ($FT_3$) and free thyroxine ($FT_4$) being within their reference ranges, is also associated with dyslipidemia, hypertension, impaired renal function, accelerated atherosclerosis, and coronary artery disease[6]. Levothyroxine ($LT_4$) replacement therapy, which is used to normalize thyroid function in patients with SCH, decreases carotid mean IMT[7]. After adjustment for age and sex, carotid IMT inversely correlates with $FT_4$ and positively correlates with TSH in euthyroid patients[8]. Although the carotid maximum (max) IMT is more predictive of coronary artery stenosis than mean IMT[9], the associa-

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tion between carotid max IMT and thyroid function in euthyroid cases, especially in men, remains poorly understood.

Thyroid hormones should also be within their reference ranges during pregnancy, as a TSH level of ≥2.5 μIU/mL is associated with a significant increase in the pregnancy loss rate[10]. Therefore, detailed management of thyroid function is needed during pregnancy for fetal development[11]. Recently, we reported a significantly higher carotid max IMT without hypercholesterolemia in postmenopausal women with serum TSH concentrations of ≥2.5 μIU/mL[12]. In contrast, no association was observed between the thyroid function parameter and carotid max IMT in pre- and perimenopausal women. However, compared with women, the association between thyroid function and arteriosclerosis development with age in men is unclear. Elucidation of a relationship could suggest the usefulness thyroid function tests at comprehensive medical examinations for preventing arteriosclerosis in men according to age.

In this study, we aimed to verify whether a low-normal range serum thyroid hormone concentration is associated with carotid arteriosclerosis with age in men, as we did in our previous report in women.

## Methods

### Study population

**Fig. 1** shows the process of enrollment of the study participants. From 2015 to 2019 (5 years), 1,969 Japanese men from the general population underwent carotid ultrasonography, and were interviewed about their smoking habit during a comprehensive medical examination at Hidaka Hospital in Takasaki, Japan. Our study only included men with a euthyroid state without CVD and/or metabolic diseases associated with atherosclerosis risk. Thus, we excluded 19 participants taking current medication for thyroid disorders, 202 with diabetes mellitus, 272 with dyslipidemia, and 280 with hypertension. We also excluded 7 participants with a history of cerebral infarction or hemorrhage, 46 taking current medication for and/or with a past history of CVD, and 2 undergoing hemodialysis. The data examined in this study did not include the results of repeated examinations of the same participants. Thyrotoxicosis was defined by serum $FT_4$ and TSH concentrations of >1.48 ng/dL and <0.35 μIU/mL, respectively, and subclinical thyrotoxicosis as a serum $FT_4$ concentration in the range 0.70–1.48 ng/dL and serum TSH concentration <0.35 μIU/mL. Subclinical hypothyroidism was defined by a serum $FT_4$ concentration in the range 0.70–1.48 ng/dL and serum TSH concentration >4.94 μIU/mL. Among the 1,141 men who had their serum $FT_4$ and TSH concentrations measured, 21 men with subclinical hypothyroidism, 30 with subclinical thyrotoxicosis, and 3 with thyrotoxicosis were excluded. Ultimately, 1,087 men were included for further analysis. The ethics committee of Hidaka Hospital approved this study (approval no. 186).

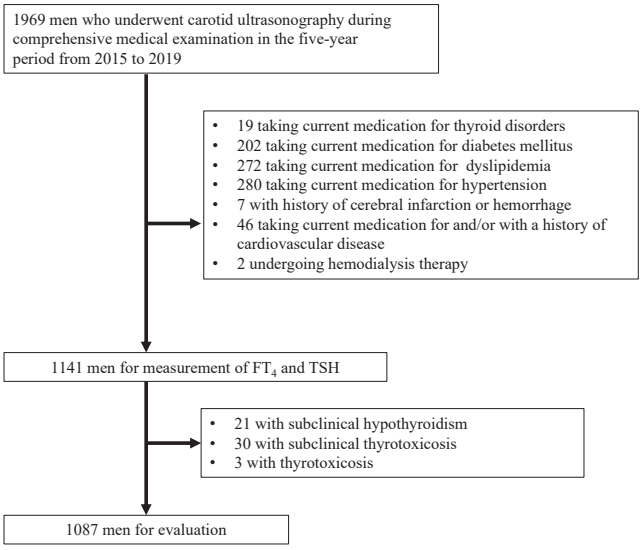


**Fig. 1. Enrollment of the Study Participants**
$FT_4$, free thyroxine; TSH, thyrotropin

### Physical examination and laboratory analyses

Physical examination and laboratory analyses were performed as previously described[12]. In detail, height and weight were measured, and body-mass index (BMI) was calculated as weight divided by height squared (kg/$m^2$). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also measured and blood samples were obtained from an antecubital vein while the participant was sitting after an overnight fast. Serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), and LDL-C were measured by enzymatic assays using an automatic analyzer (TBA-c8000; Canon Medical Systems Corporation). Furthermore, plasma glucose (PG) concentrations were measured using the glucose oxidase method, and hemoglobin A1c (HbA1c) levels were measured by high-performance liquid chromatography using automatic analyzers (GA 08 II; A&T and HLC-723G9; Tosoh). Additionally, serum $FT_4$ and TSH concentrations were measured by chemiluminescence immunoassay using an automatic analyzer (ARCHITECTi2000SR; Abbott). In this study, a euthyroid state was defined by serum $FT_4$ concentrations of 0.70–1.48 ng/dL and serum TSH concentrations of 0.35–4.94 μIU/mL, as previously described[12].

### Measurement of carotid artery max IMT

Measurement of carotid artery max IMT was performed as previously described[12]. In detail, max IMT of the left or right carotid arteries was measured by ultrasonography (Aplio 300; Canon Medical Systems

Corporation) using a 7.5 MHz linear array transducer. The greatest thickness measured on both sides of the common carotid artery, bulbus, and internal carotid artery, excluding the external carotid artery, was indicative of the max IMT. The presence of carotid plaque was defined by a max IMT≥1.1 mm. All scans were conducted by three well-trained and experienced medical technologists without any knowledge of the participants' clinical details. The intra-observer coefficient of variation for IMT measurement was 5.2%±0.8%, as previously described[12].

### Statistical analysis

Data are expressed as median and 25th–75th percentiles. Kruskal–Wallis tests were performed to compare men allocated to five age groups binned by decade or four groups according to quartiles of serum $FT_4$ concentration. Statistically significant differences between two groups were identified using the Mann–Whitney *U* test or chi-squared test, as appropriate. Independent factors contributing to carotid plaque development were identified by multivariate logistic regression analysis. As serum TG and TSH concentrations were not normally distributed, logarithmic transformation was performed on these parameters for multivariate logistic regression analysis. Differences and correlations were considered significant at *p*-values <0.05. All statistical data were analyzed using StatFlex software version 7 (Artech, Osaka, Japan).

## Results

### Comparison of max IMT between five and two age groups

**Fig. 2A** shows the results of the comparison of max IMT between male participants divided into the following age groups: <40, 40–49, 50–59, 60–69, and ≥70. Max IMT was significantly different between the five groups by Kruskal–Wallis test ($p<0.001$). Max IMT increased with age and began to exceed 1.1 mm, an indicator the presence of carotid artery plaque, from the age of 50 years. **Fig. 2B** shows the results of the comparison of max IMT between men aged <50 and ≥50 years. Max IMT was significantly higher in men aged ≥50 years than in those aged <50 years by Mann–Whitney *U* test ($p<0.001$).

### Comparison of clinical variables between men with and without plaque aged <50 and ≥50 years

**Table 1** shows the results of the comparison of clinical variables between men aged <50 years ($n=430$) and ≥50 years ($n=657$), who were each further divided according to the absence or presence of plaque (max IMT≥1.1 mm). Those with plaque (plaque group) ($n=56$) were significantly older ($p<0.001$) than those without plaque (non-plaque group) ($n=374$) among men aged <50 years. The plaque group also had significantly higher SBP ($p=0.047$), DBP ($p=0.009$), LDL-C ($p=0.028$), and max IMT ($p<0.001$) than the non-plaque group. Among men aged ≥50 years, the plaque group ($n=284$) were significantly older ($p<0.001$) than the non-plaque group ($n=373$) and had significantly higher SBP ($p<0.001$), DBP ($p=0.031$), and max IMT ($p<0.001$). Moreover, the plaque group had significantly lower $FT_4$ ($p=0.001$) than the non-plaque group.

### Multivariate logistic regression analysis of factors contributing to carotid artery plaque

**Table 2** shows the potential independent risk factors of carotid artery plaque identified by multivariate logistic regression analysis based on the results of comparison of plaque and non-plaque groups among men aged

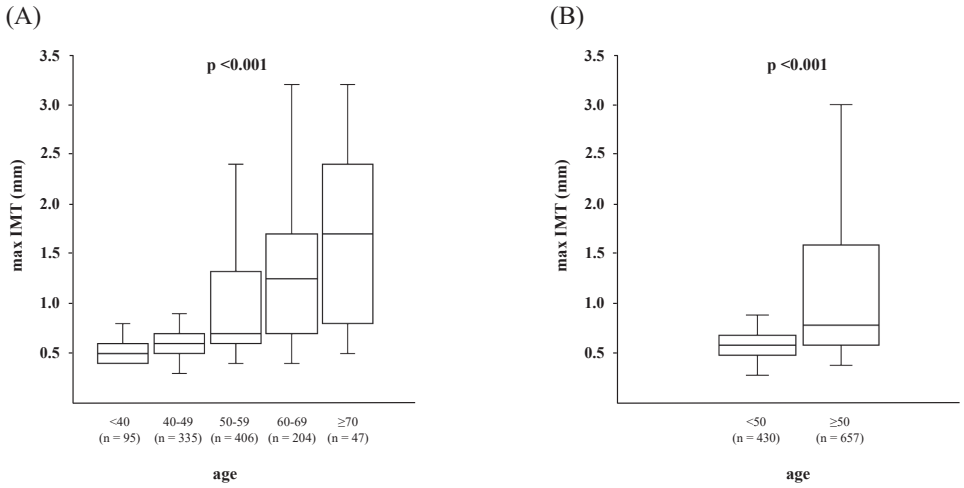


**Fig. 2. Comparison of Max IMT Between Five or Two Age Groups**

Comparison of max IMT between five age groups binned by decade as follows: <40, 40–49, 50–59, 60–69, and ≥70 (A). Comparison of max IMT between men aged <50 and ≥50 years (B). Kruskal–Wallis test and Mann–Whitney *U* tests were used to compare max IMT.
max IMT, maximum intima–media thickness

**Table 1. Comparison of Clinical Variables Between Men with and Without Plaque Aged Under 50 Years and 50 Years and Over**

| | Aged under 50 years | | | Aged 50 years and over | | |
|---|---|---|---|---|---|---|
| | Non-plaque group (*n*=374) | Plaque group (*n*=56) | *p*-value | Non-plaque group (*n*=373) | Plaque group (*n*=284) | *p*-value |
| Age (years) | 44 (40–47) | 46 (44–48) | <0.001 | 56 (53–60) | 60 (55–65) | <0.001 |
| Smoking (%) | 32.9 | 26.8 | 0.362 | 24.9 | 22.9 | 0.543 |
| BMI (kg/m$^2$) | 23.4 (21.8–25.6) | 23.6 (21.9–26.0) | 0.683 | 23.0 (21.3–24.8) | 23.3 (21.4–25.1) | 0.524 |
| SBP (mmHg) | 115 (107–123) | 119 (111–130) | 0.047 | 119 (108–128) | 123 (115–133) | <0.001 |
| DBP (mmHg) | 75 (67–82) | 79 (72–89) | 0.009 | 79 (71–87) | 81 (73–89) | 0.031 |
| TC (mg/dL) | 204 (183–231) | 217 (196–237) | 0.051 | 211 (189–235) | 213 (195–233) | 0.388 |
| HDL-C (mg/dL) | 54 (47–65) | 55 (46–65) | 0.799 | 57 (48–68) | 57 (47–66) | 0.378 |
| TG (mg/dL) | 105 (71–157) | 112 (77–138) | 0.908 | 101 (76–140) | 110 (79–149) | 0.207 |
| LDL-C (mg/dL) | 125 (104–149) | 134 (117–157) | 0.028 | 126 (107–148) | 131 (113–151) | 0.126 |
| PG (mg/dL) | 100 (95–105) | 100 (98–106) | 0.218 | 102 (97–107) | 102 (97–109) | 0.131 |
| HbA1c (%) | 5.6 (5.4–5.8) | 5.6 (5.5–5.8) | 0.407 | 5.6 (5.5–5.9) | 5.7 (5.5–5.9) | 0.096 |
| Max IMT (mm) | 0.5 (0.5–0.6) | 1.4 (1.3–1.8) | <0.001 | 0.6 (0.6–0.7) | 1.7 (1.4–2.1) | <0.001 |
| $FT_4$ (ng/dL) | 1.09 (0.98–1.20) | 1.04 (0.95–1.16) | 0.087 | 1.08 (0.99–1.20) | 1.04 (0.95–1.16) | 0.001 |
| TSH (μIU/mL) | 1.30 (0.89–1.76) | 1.29 (0.89–1.80) | 0.843 | 1.34 (0.95–1.91) | 1.31 (0.97–2.05) | 0.453 |

Data are expressed as median (25th–75th percentile). The presence of carotid plaque was defined by a max IMT ≥1.1 mm. The plaque group was compared with the non-plaque group using the chi-squared test or Mann–Whitney *U* test, as appropriate.
BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; PG, plasma glucose; HbA1c, hemoglobin A1c; max IMT, maximum intima–media thickness; $FT_4$, free thyroxine; TSH, thyrotropin

**Table 2. Multivariate Logistic Regression Analysis of Factors Contributing to Carotid Artery Plaque**

| Factor | Aged under 50 years | | | Aged 50 years and over | | |
|---|---|---|---|---|---|---|
| | β | *p*-value | OR (95%CI) | β | *p*-value | OR (95%CI) |
| Age (years) | 0.123 | 0.002 | 1.130 (1.040–1.220) | 0.080 | <0.001 | 1.080 (1.050–1.110) |
| SBP (mmHg) | −0.004 | 0.854 | 1.000 (0.950–1.040) | 0.031 | 0.005 | 1.030 (1.010–1.050) |
| DBP (mmHg) | 0.037 | 0.185 | 1.040 (0.980–1.100) | −0.016 | 0.239 | 0.980 (0.960–1.010) |
| TC (mg/dL) | −0.001 | 0.945 | 1.000 (0.980–1.020) | −0.005 | 0.252 | 0.990 (0.990–1.000) |
| logTG (mg/dL) | −0.656 | 0.057 | 0.520 (0.260–1.020) | 0.036 | 0.856 | 1.040 (0.710–1.520) |
| LDL-C (mg/dL) | 0.012 | 0.230 | 1.010 (0.990–1.030) | 0.008 | 0.097 | 1.010 (1.000–1.020) |
| PG (mg/dL) | −0.020 | 0.333 | 0.980 (0.940–1.020) | 0.006 | 0.591 | 1.010 (0.980–1.030) |
| HbA1c (%) | 0.277 | 0.642 | 1.320 (0.410–4.240) | −0.055 | 0.869 | 0.950 (0.500–1.810) |
| $FT_4$ (ng/dL) | −0.980 | 0.337 | 0.380 (0.050–2.770) | −1.800 | 0.002 | 0.170 (0.050–0.520) |
| logTSH (μIU/mL) | −0.056 | 0.849 | 0.950 (0.530–1.680) | −0.099 | 0.549 | 0.910 (0.660–1.250) |

OR, odds ratio; CI, confidence interval; BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; PG, plasma glucose; HbA1c, hemoglobin A1c; $FT_4$, free thyroxine

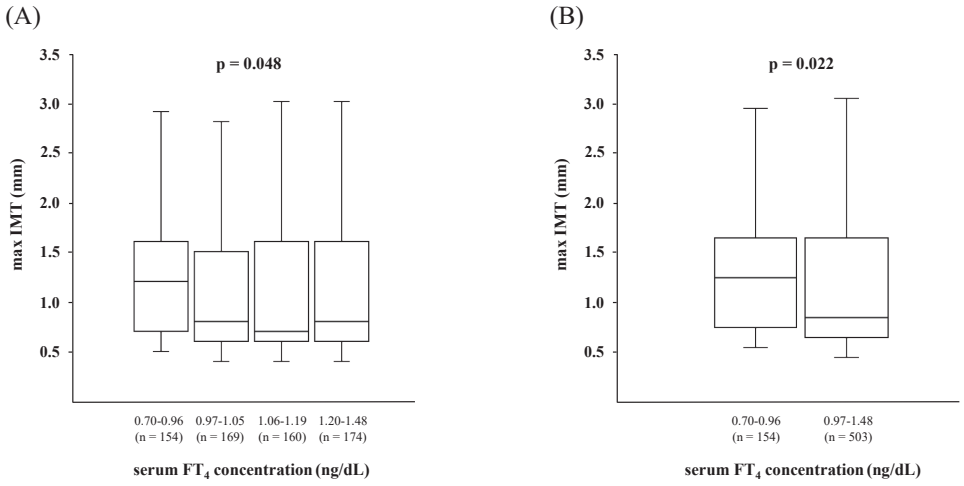


**Fig. 3. Comparison of Max IMT by Serum $FT_4$ Concentration Within the Normal Range in Men Aged ≥50 Years**

Comparison of max IMT according to quartiles of serum $FT_4$ concentration in men aged ≥50 years (A). Comparisons of max IMT based on a serum $FT_4$ concentration cutoff value of 0.97 in men aged ≥50 years (B). Kruskal–Wallis and Mann–Whitney *U* tests were used to compare max IMT.
max IMT, maximum intima–media thickness; $FT_4$, free thyroxine

**Table 3. Comparison of Max IMT and Other Clinical Variables in Men Aged 50 Years and Over Divided by a $FT_4$ Cutoff of 0.97**

| | <0.97 (*n*=154) | ≥0.97 (*n*=503) | *p*-value |
|---|---|---|---|
| Age (years) | 57 (53–63) | 58 (54–62) | 0.890 |
| Smoking (%) | 20.8 | 25.0 | 0.278 |
| BMI (kg/m$^2$) | 23.3 (21.2–24.8) | 23.1 (21.4–25.0) | 0.988 |
| SBP (mmHg) | 122 (112–130) | 121 (112–129) | 0.235 |
| DBP (mmHg) | 80 (74–90) | 80 (71–87) | 0.073 |
| TC (mg/dL) | 214 (189–233) | 211 (191–234) | 0.904 |
| HDL-C (mg/dL) | 58 (49–71) | 57 (48–66) | 0.079 |
| TG (mg/dL) | 101 (80–142) | 106 (76–144) | 0.862 |
| LDL-C (mg/dL) | 128 (107–146) | 129 (109–151) | 0.231 |
| PG (mg/dL) | 102 (96–108) | 102 (97–108) | 0.998 |
| HbA1c (%) | 5.7 (5.5–5.9) | 5.7 (5.5–5.9) | 0.771 |
| max IMT (mm) | 1.2 (0.7–1.6) | 0.8 (0.6–1.6) | 0.022 |
| $FT_4$ (ng/dL) | 0.90 (0.86–0.94) | 1.10 (1.03–1.20) | <0.001 |
| TSH (μIU/mL) | 1.52 (1.12–2.27) | 1.30 (0.93–1.86) | 0.001 |

Data are expressed as median (25th–75th quartile). $p<0.05$, comparison of variables between two groups divided based on a $FT_4$ cutoff concentration of 0.97 using the chi-squared test or Mann–Whitney *U* test.
BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; PG, plasma glucose; HbA1c, hemoglobin A1c; max IMT, maximum intima–media thickness; $FT_4$, free thyroxine; TSH, thyrotropin.

<50 or ≥50 years. Age (β=0.123, $p$=0.002) was independently associated with carotid artery plaque in men aged <50 years. Conversely, SBP, DBP, TC, logTG, LDL-C, PG, HbA1c, $FT_4$, and logTSH were not independently associated with carotid artery plaque. In men aged ≥50 years, age (β=0.080, $p$<0.001), SBP (β=0.031, $p$=0.005), and $FT_4$ (β=−1.800, $p$=0.002) were independently associated with carotid artery plaque.

### Comparison of max IMT by serum $FT_4$ concentration among men with low-normal range values aged ≥50 years

**Fig. 3A** shows the results of the comparison of max IMT conducted between four groups of men aged ≥50 years divided according to quartiles of serum $FT_4$ concentration. The ranges of the quartiles were 0.70–0.96, 0.97–1.05, 1.06–1.19, and 1.20–1.48 ng/dL. Max IMT was significantly different among the four groups of men aged ≥50 years ($p$=0.048). Given that the median max IMT was highest in the 25% quartile ($FT_4$ concentration 0.70–0.96), we further divided the men aged ≥50 years into two groups based on a cutoff value of 0.97 ng/dL. Max IMT was significantly higher in men with serum $FT_4$ concentrations of 0.70–0.96 ng/dL than in those with 0.97–1.48 ng/dL ($p$=0.022) (**Fig. 3B**). TSH was also significantly higher in men with serum $FT_4$ concentrations of <0.97 ng/dL than in those with ≥0.97 ng/dL ($p$=0.001) (**Table 3**).

## Discussion

The present study demonstrated an association between carotid atherosclerosis risk with age and thyroid function in euthyroid Japanese men. Max IMT increased with age and exceeded the value used to define the presence of carotid artery plaque (max IMT ≥1.1 mm) in men aged ≥50 years. Serum $FT_4$ concentrations were significantly lower in the plaque group and independently associated with carotid plaque in men aged ≥50 years based on logistic regression analysis. Moreover, when using a cutoff value of 0.97 ng/dL for serum $FT_4$ concentration in men aged ≥50 years, max IMT was significantly higher in men with concentrations <0.97 ng/dL than in those with values ≥0.97 ng/dL.

Although reports on the relationship between thyroid function and factors such as blood pressure, lipid profile, glucose metabolism, and atherosclerosis in euthyroid women are numerous, those in euthyroid men remain limited. According to age-adjusted multivariate linear regression analysis, $FT_4$, and log TSH are significantly correlated with CIMT in women, but not in men[8]. Using multiple linear regression analysis, a previous study demonstrated a significant inverse relationship between $FT_4$ and IMT in predominantly middle-aged euthyroid men and women after adjustment for obesity, pulse pressure, and lipid levels[13]. We recently reported that serum TSH concentrations were independently associated with carotid plaque by multivariate logistic regression analysis in euthyroid women. The significant positive relationship between the max IMT of the carotid artery and serum TSH concentrations of ≥2.5 μIU/mL was only observed in postmenopausal women, but not in pre- or perimenopausal women[12]. In the present study, we focused on subjects aged between 50–59 years, when the max IMT begins to exceed 1.1 mm, as the turning point of early age-related atherosclerosis, compared to those aged ≥60 years with completed carotid atherosclerosis. Therefore, we used age 50 years as the cutoff. In support of our selection of this value, a previous study demonstrated the significance of a cutoff age of 50 years in assessing future cardiovascular events[14]. In our study, multiple logistic regression analysis showed an inverse relationship between $FT_4$ and max IMT independently in euthyroid men aged ≥50 years, although carotid artery plaque in men aged <50 years was only independently associated with age. This report is the first to focus on the relationship between the early age-related progression of carotid arteriosclerosis and mild hypothyroidism in euthyroid men. In a previous study, $LT_4$ treatment did not significantly improve endothelial function or reduce the carotid IMT in patients with mild subclinical hypothyroidism[15]. However, in a different study, $LT_4$ significantly reduced both LDL-C and the mean IMT; thus, lipid infiltration of the arterial wall may represent the main mechanism underlying the increase in IMT in patients with subclinical hypothyroidism[7]. Previously, low normal $FT_4$

was reported to be associated with insulin resistance, which is related to higher TG, lower HDL-C, and abdominal obesity[16]. After adjusting for potential cardiovascular risk factors, mean DBP, homeostatic model assessment-estimated insulin resistance (HOMA-IR), and branchial–ankle pulse wave velocity (baPWV) levels decreased across increasing quartiles of $FT_4$ in both male and female euthyroid participants. DBP, HOMA-IR, and baPWV were reported to be significantly higher in euthyroid individuals when $FT_4$ concentrations were <0.97[17]. The present study revealed a relationship between the carotid max IMT, which increased with age, and a serum $FT_4$ concentration cutoff value of 0.97 ng/dL, but not other risk factors. However, the significance of a serum $FT_4$ cutoff value of 0.97 ng/dL in CVD risk needs to be clarified. As we enrolled participants without CVD risk factors such as hypertension and dyslipidemia and without overt thyroid dysfunction, mild differences in serum $FT_4$ levels within the reference range might be less likely to be associated with differences in other risk factors, including lipid and blood pressure. In contrast, based on the evidence from molecular studies of the direct effects of thyroid hormones on blood vessel cells[18–22], there may be a relationship between a serum $FT_4$ concentration of <0.97 ng/dL and an increase in max IMT in men aged ≥50 years in this study.

However, the present study has some limitations. First, $FT_3$ and anti-thyroid antibodies, including anti-thyroglobulin and anti-thyroid peroxidase antibodies, were not measured. Thyroid autoimmunity is reportedly associated with increased carotid IMT independent of thyroid function[23]. The effect of serum $FT_4$ cutoff values on carotid atherosclerosis with or without anti-thyroid antibodies should be evaluated in all participants in future studies. Second, this study was cross-sectional in design, and excluded participants with general risk factors for CVDs. Therefore, it is not be possible to assess the effect of a decrease in serum $FT_4$ concentration on the development of atherosclerosis based on the available data. Further prospective studies are needed to assess the actual occurrence of CVDs for each aging man according to the serum $FT_4$ cutoff value. Third, the sample size was relatively small, owing to the exclusive enrollment of male participants who were not taking current medication for CVD-associated metabolic diseases. We expect that our hypothesis will be validated by future studies with a larger sample size and adjustment for age and blood pressure to assess the association between carotid IMT and detailed thyroid function parameters with age.

In conclusion, serum $FT_4$ concentration was independently associated with age-related increases in carotid max IMT in euthyroid Japanese men. Especially at age ≥50 years, men with serum $FT_4$ levels <0.97 ng/dL, even within the reference range, and without metabolic dysfunctions, including hypercholesterolemia, had a significantly higher carotid max IMT. Therefore, detailed laboratory assessment of thyroid function parameters within the normal range and measurement of other risk factors may be useful for preventing atherosclerosis in men aged ≥50 years.

## Conflict of Interest

The authors declare that they have no competing interests.

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# Relationship Between Sleep and Obesity: A Cross-sectional and Longitudinal Observational Study of Healthy Adults in Japan

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## Abstract

**Objectives:** The aim of this study was to elucidate the effects of sleep habits on obesity in Japan.

**Methods:** We analyzed data from healthy people who underwent the "Ningen Dock" examination between 2012 and 2020. A total of 5,518 subjects (age: 57.5±13.1 years) were included in a cross-sectional analysis, and 1,515 (age: 58.2±11.9 years) were followed for 6 years in a longitudinal analysis.

**Results:** The mean sleep duration was 6.4±1.0 hours and was significantly longer in young and elderly people than in middle-aged people. Obesity prevalence was 25.6%, and 11.5% had obesity onset during the follow-up period. In both univariate and cross-sectional multivariate analyses, sleep was significantly associated with obesity. Other significant factors were age, current smoking in males, and habitual drinking and exercise in females. Compared to the 6-hour group, the odds ratio for obesity was significantly higher in males in the <6-hour sleep group and lower in the 7-hour and ≥8-hour groups. In females, there were tendencies towards higher obesity rates in the <6-hour group, and lower rates in the 7-hour group. In the longitudinal multivariate analysis, the odds ratio for sleep in relation to obesity onset tended to be higher in the <6-hour group and lower in the 7-hour group.

**Conclusions:** Short sleep duration was significantly correlated with obesity in males, and a similar tendency was found in females. Therefore, a sleep duration of approximately 7 hours may be advantageous in terms of obesity prevention in adults.

**Keywords** sleep, obesity, body mass index, multivariate analysis, longitudinal analysis

In recent years, the sleep deficit in the "working generation" has become a healthcare problem. The mean daily sleep duration in Japan is reported to be 7.4 hours[1]; however, nearly half of individuals in their 40s and 50s sleep for less than 6 hours a night[2]. Inversely to the sleep duration decrease, the proportion of obese males has increased from 21.8% to 33.0% over the past 30 years, with males in their 40s and 50s accounting for nearly 40% of these, which is a larger proportion than in other age groups[3]. By contrast, the proportion of females with obesity has remained relatively stable, changing from 20.3% to 22.3% in this time-period[3].

Since the 1990s, extensive epidemiological evidence, mainly from Europe and the USA, has been highlighting the relationship of sleep with mortality rate[4] and various pathological conditions (obesity[5–7], diabetes[8], hypertension[9], cardiovascular disease[10], cerebrovascular disease[11], gastroesophageal reflux disease[12], etc). Furthermore, interventional studies have shed light on the mechanism of onset of these diseases[13,14]. Currently, the recommended sleep duration for adults in Europe and the USA is at least 7 hours per night[15], or 7–9 hours for young people and adults and 7–8 hours for older individuals[16], to prevent lifestyle-related diseases. Lifelong sleep education is commonly provided to this effect. By contrast, research in the field of sleep is scarce in East Asia, including Japan. Therefore, public awareness of the importance of sleep is poor; compared with diet- and physical activity-related guidance, sleep health

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guidance is not commonly provided in the clinical setting.

According to previous epidemiological studies in Europe and the USA[5,6], a U-shaped or inverse linear relationship was observed between insufficient or excessive sleep duration and obesity. A short sleep duration was associated with obesity, obesity onset, increased body mass index (BMI), and weight gain. However, the relationship weakened or disappeared depending on the sex and/or age of the subjects, study design (cross-sectional, longitudinal, classification of sleep duration, and data measurement methods), confounding factors (demographic factors, socioeconomic factors, and lifestyle), and intermediate factors (related metabolic diseases). Concerning results restricted to the Japanese population[17–21], the findings were similar to those from other countries; that is, short sleep duration (<5–6 hours) significantly correlates with obesity in males, although findings in females are divergent. The difference between the sexes may be attributed to the effects of single-institution cohorts, the predominance of studies including only males, and/or the smaller proportion of participants. These factors have contributed to the limited generalizability of previous findings.

Japanese people's sleep duration is shorter than that of people in any other member country of the Organisation for Economic Co-operation and Development. However, ensuring appropriate sleep duration is important for the health of Japanese people. Nevertheless, as explained above, there has been a delay in obtaining evidence for supporting better sleep-related policies in Japan and the rest of East Asia. Here, we aimed to evaluate the most recent data from healthy East Asian (Japanese) people to elucidate the relationship between sleep status and obesity, using cross-sectional and longitudinal analyses.

## Methods

### Study subjects

The cross-sectional analysis included 6,711 subjects from among 22,371 individuals who underwent the "Ningen Dock" (Comprehensive Health Checkup System) at the Center for Epidemiology and Preventive Medicine, the University of Tokyo Hospital, between October 1, 2012, and December 28, 2020. If an individual underwent examination more than once, only the data on the first examination were used in this study. Overall, 5,518 subjects were included in the analysis, after excluding 1,193 people who had missing data on laboratory test items or lifestyle-related questions (**Fig. 1A**).

For the longitudinal analysis, the enrollment period ran from October 1, 2012 until December 26, 2014; each individual's data were subsequently reviewed at each annual visit, from January 5, 2015 to December 28, 2020. The study population consisted of 3,560 of the 5,578 people who underwent the "Ningen Dock" evaluations during the enrollment period. If someone underwent the "Ningen Dock" twice or more, only data from the first evaluation were used. After excluding 714 subjects with missing data on laboratory test items or lifestyle-related questions, 2,011 subjects who had repeated examinations during the follow-up period were included in the baseline examination. Additionally, 1,515 subjects were included in the analysis, excluding 496 subjects who were already obese at baseline. These subjects were followed for 6 years (median: 2,187 days) (**Fig. 1B**).

### Data collection

Self-administered questionnaires related to medical history and lifestyle (sleeping, smoking, drinking, exercise, etc.) were mailed to the participants in advance. Measurements taken included blood pressure, using a fully automated sphygmomanometer (UDEX-i; Elquest Corporation, Chiba, Japan), and height and weight, using a fully automated height and weight meter (DC270A; Tanita Corporation, Tokyo, Japan). Blood samples were collected in accordance with standardized procedures. Subsequently, laboratory tests were run at the Department of Clinical Laboratory, University of Tokyo Hospital (ISO 15189-accredited).

### Observation items

The dependent variables included the presence or absence of obesity in the cross-sectional analysis, and presence or absence of obesity onset in the longitudinal analysis. The independent variables were general laboratory test results, lifestyle factors, and age at screening as follows: 1) Systolic blood pressure (SBP); 2) Diastolic blood pressure (DBP); 3) Fasting blood glucose (FPG); 4) Hemoglobin A1c (HbA1c); 5) Low-density lipoprotein cholesterol (LDL-C); 6) High-density lipoprotein cholesterol (HDL-C); 7) Triglycerides (TG); 8) Uric acid (UA); 9) Answers to a question about smoking, i.e., "Do you have a smoking habit?", which were classified as (a) current smoker; (b) previous smoker; or (c) lifetime non-smoker; 10) Answers to a question about alcohol consumption, i.e., "How many days a week do you consume alcohol?", which were used to classify a habitual drinker as someone who reported drinking 3 or more days per week; 11) Answers to a question about exercise, i.e., "Do you engage in light exercise sufficient to break out in sweat, for at least 30 minutes, at least 3 times a week?", which were used to classify habitual exercisers when the response was "yes" rather than "no"; 12) Age; 13) Answers to a sleep-related question, i.e., "How many hours of sleep do you have on average per day?", with the responses given as integers, which were used to classify participants into the following four

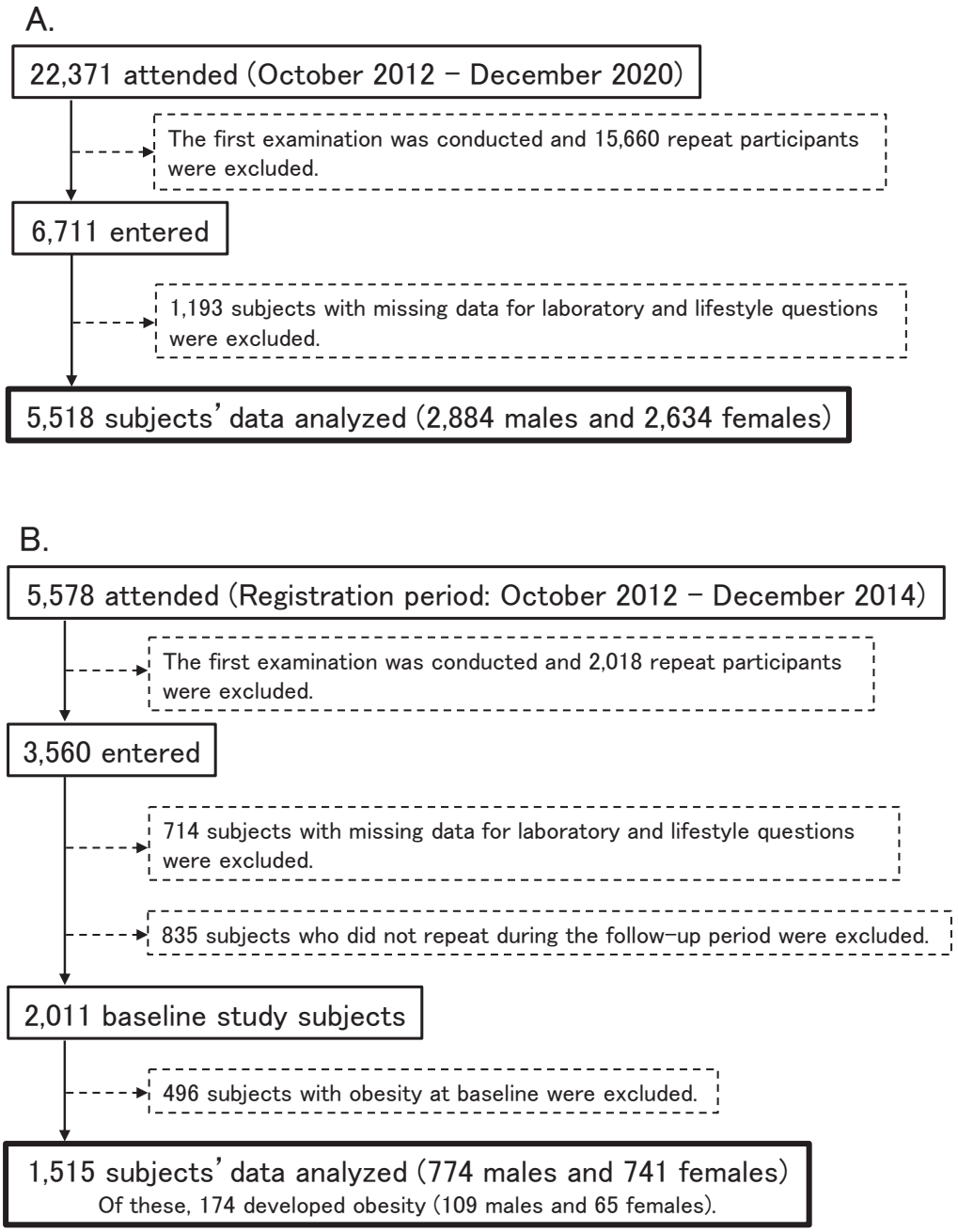


**Fig. 1. Study Recruitment Flowchart**

groups: <6 hours; 6 hours; 7 hours; and ≥8 hours. Responses stating that the sleep duration was 0 hours, or >24 hours, were excluded. Sleep duration class intervals were decided upon by evaluating frequency distributions on a histogram.

**Judgment criteria**

Regarding the laboratory tests performed, the following results were considered abnormal, in accordance with the criteria of the indicated societies: (a) Japan Society of Hypertension: SBP ≥140 mmHg and DBP ≥90 mmHg; (b) Japan Diabetes Society: FPG ≥126 mg/dL and HbA1c ≥ 6.5%; (c) Japanese Atherosclerosis Society: TG ≥150 mg/dL, LDL-C ≥140 mg/dL, and HDL-C <40 mg/dL; and (d) Japanese Society for Gout and Nucleic Acid Metabolism: UA >7 mg/dL, UA ≥8 mg/dL, and UA ≥9 mg/dL. BMI was calculated as weight (kg)/height $(m)^2$. In accordance with Japan Society for the Study of Obesity criteria, BMI ≥25 $kg/m^2$ indicated obesity, BMI 18.5–24.9 $kg/m^2$ was considered normal, and BMI <18.5 $kg/m^2$ indicated underweight. In the cross-sectional analysis, obesity was defined as BMI ≥25 $kg/m^2$. In the longitudinal analysis, obesity was defined as an increase in BMI from <25 $kg/m^2$ to ≥25 $kg/m^2$.

**Sleep duration analysis**

The 5,518 subjects in the cross-sectional analysis

were classified into six age groups (<30, 30s, 40s, 50s, 60s, and ≥70s), and the distributions of summary statistics (mean and median) were confirmed to assess data quality. The mean was used to present the sleep duration, as there was no marked difference between the mean and median, and because the mean was reported in the National Health and Nutrition Survey (Ministry of Health, Labor, and Welfare), the Survey on Time Use and Leisure Activities (Statistics Bureau, Ministry of Internal Affairs and Communications), and the Japanese Time Use Survey (NHK Broadcasting Culture Research Institute), which are the principal Japanese resources for sleep statistics.

One-way analysis of variance was used to test differences in mean sleep duration between age groups, and Tukey's HSD test was used for post-hoc comparisons when significant differences were found. The median values were included in the tables for reference, and differences were compared using the Kruskal–Wallis and Steel–Dwass tests.

### Identification of background factors associated with obesity (cross-sectional analysis) and obesity onset (longitudinal analysis)

Univariate analyses were performed to determine whether there were differences in the test values and lifestyle items (the above items 1 to 13), depending on the presence or absence of obesity. The nature of the quantitative data (normal distribution and equal variance) was confirmed beforehand by the Anderson-Darling test, and, as most of the test values in the two groups (with and without obesity and obesity onset), were not normally distributed, the median was used as the appropriate descriptive statistic (data omitted). Wilcoxon's rank-sum test was used for age, BMI, SBP, DBP, FPG, HbA1c, LDL-C, HDL-C, TG, and UA comparisons, and the $\chi^2$ test was used for categorical data, i.e., smoking, drinking, and exercise (Fisher's exact test was used when the expected value was less than 5 in at least 20% of the cells in the contingency table). For quantitative data, the bias in the number of individuals in the contingency table was also included in the table for reference.

### Relationship between sleep duration and obesity and obesity onset, considering the effect of multiple background factors

As preliminary preparation, data were checked for multicollinearity and sample size, and 0-1 type dummy variables were prepared for nominal-scale variables (smoking, habitual drinking, habitual exercise, and sleep duration groups). Multivariate analyses (multiple logistic regression analysis for cross-sectional analyses, and Cox proportional hazards analysis for longitudinal analyses) were then performed using the lifetime nonsmoking, no habitual drinking, no habitual exercise, and 6-hour sleep duration groups as references, followed by evaluation with likelihood ratio tests and 95% confidence intervals (CIs) for odds ratios (ORs) or hazard ratios (HRs). To select explanatory variables, adjustment was made for five background factors (age, weight, smoking, habitual drinking, and habitual exercise at entry), with reference to the results of previous studies on background factors in obesity[20,22], as well as the results of univariate analysis.

All statistical analyses were performed using JMP® 16 software (SAS Institute Inc., Cary, NC, USA), with a significance level of 5%. In addition, all analyses were performed separately for males and females, as the influence of genetic sex on obesity onset could not be neglected.

### Ethical considerations

This study was approved by the Ethics Committee of the University of Tokyo School of Medicine and the Clinical Research Review Committee of the University of Tokyo (review no.: 2498) and was performed in compliance with the Declaration of Helsinki.

## Results

### Cross-sectional analysis

The cross-sectional analysis was conducted on data from 5,518 subjects (mean age: 57.5±13.1 years; mean sleep duration: 6.4±1.0 hours), including 2,884 males (52.3%) and 2,634 females (47.7%), with a mean age of 57.4±13.1 years for males (median: 59 years; range: 21–98 years) and 57.6±13.1 years for females (median: 58 years; range: 19–91 years). The mean sleep duration was 6.4±1.0 hours (median: 6 hours; 3–12 hours) in males, and 6.4±1.1 hours (median: 6 hours; 1–13 hours) in females.

### Current state of sleep duration

Sleep duration was used to categorize the following four groups: 968 subjects (17.5%) in the <6-hour group, 2,074 subjects (37.6%) in the 6-hour group, 1,761 (31.9%) in the 7-hour group, and 715 (13.0%) in the ≥8-hour group. Most subjects belonged to the 6-hour group, which accounted for 36.6% of males and 38.6% of females. Another 16.5% of males and 18.7% of females slept <6 hours per night. This percentage was highest in males in their 30s and 40s, and in females in their 40s and 50s (exceeding 20%) (**Fig. 2**).

The comparison of mean sleep duration across all age groups showed that both males and females slept significantly longer at younger and older ages than did males in their 30s to 50s and females in their 40s and 50s (**Table 1**).

### Identification of background factors associated with obesity

The baseline characteristics of the 5,518 subjects included in the cross-sectional analysis, including sleep

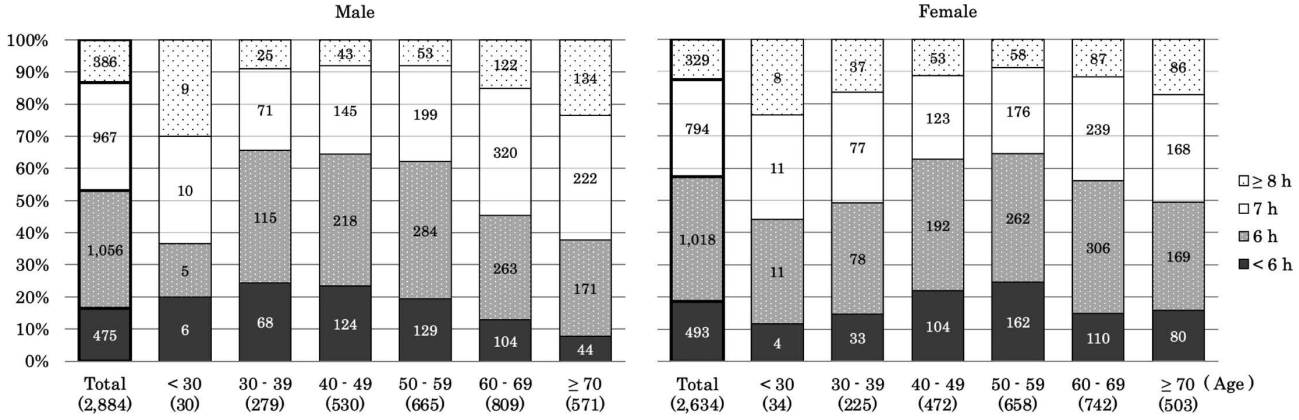


**Fig. 2. Distribution of Sleep Duration by Age Group**

**Table 1. Sleep Duration by Age Group**

| Age (years) | Sleep duration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | All participants | | | Male | | | Female | | |
| | *n* | Mean±SD | Median (IQR) | *n* | Mean±SD | Median (IQR) | *n* | Mean±SD | Median (IQR) |
| <30 | 64 | 6.7±1.1 | 7 (6–8) | 30 | 6.7±1.2 | 7 (6–8) | 34 | 6.7±1.0 | 7 (6–7.3) |
| 30–39 | 504 | 6.3±1.1 | 6 (6–7) | 279 | 6.2±1.1 | 6 (6–7) | 225 | 6.6±1.2 | 7 (6–7) |
| 40–49 | 1002 | 6.2±1.0 | 6 (6–7) | 530 | 6.2±1.0 | 6 (6–7) | 472 | 6.2±1.1 | 6 (6–7) |
| 50–59 | 1323 | 6.2±1.0 | 6 (6–7) | 665 | 6.2±1.0 | 6 (6–7) | 658 | 6.2±1.0 | 6 (6–7) |
| 60–69 | 1551 | 6.5±1.0 | 6 (6–7) | 809 | 6.6±1.0 | 7 (6–7) | 742 | 6.4±1.0 | 6 (6–7) |
| ≥70 | 1074 | 6.7±1.1 | 7 (6–7) | 571 | 6.8±1.0 | 7 (6–7) | 503 | 6.5±1.1 | 7 (6–7) |
| Total | 5518 | 6.4±1.0 | 6 (6–7) | 2884 | 6.4±1.0 | 6 (6–7) | 2634 | 6.4±1.1 | 6 (6–7) |
| *p*-value | | <0.0001*** | <0.0001*** | | <0.0001*** | <0.0001*** | | <0.0001*** | <0.0001*** |

One-way ANOVA was used for mean values, and Kruskal-Wallis test was used for median values to test for differences.

duration in relation to obesity, are shown in **Table 2**. The obesity prevalence was 25.6% (34.4% in males; 16.0% in females), and the median BMI was 22.7 kg/m$^2$ (23.8 [15.4–45.6] kg/m$^2$ in males; 21.3 [12.8–41.8] kg/m$^2$ in females). The SBP, DBP, FPG, HbA1c, LDL-C, TG, and UA levels were significantly higher, while the HDL-C level was significantly lower in the obese group in both sexes. Additionally, significant differences were found in exercise habits in both sexes, in smoking and sleep duration in males, and in age and drinking habits in females.

### Relationship between sleep duration and obesity, considering effects of multiple background factors

Model 1 was adjusted for age, and Model 2 was adjusted for age, smoking, habitual drinking, and habitual exercise (**Table 3**).

Sleep duration correlated significantly with obesity in males, but not in females. In Model 1, in males, the OR for obesity was significantly higher in the <6-hour group (OR: 1.39; 95%CI: 1.11–1.74) and significantly lower in the 7-hour (OR: 0.81; 95%CI: 0.67–0.98) and ≥8-hour groups (OR: 0.73; 95%CI: 0.56–0.96) compared to the 6-hour group. This relationship remained significant in Model 2, after further adjustments. In Model 2, in females, the OR tended to be higher in the <6-hour group (OR: 1.06; 95%CI: 0.79–1.41) and lower in the 7-hour (OR: 0.83; 95%CI: 0.64–1.08) and ≥8-hour groups (OR: 0.95; 95%CI: 0.67–1.33) groups compared to the 6-hour group. Thus, obesity and sleep showed an inverse linear relationship, with significantly higher ORs for shorter sleep duration in males. By contrast, the ORs in females were highest with shorter (<7 hours) or longer (≥8 hours) sleep duration, with the lowest point in the 7-hour group, showing a U-shaped profile (**Fig. 3**).

Besides sleep, age was significantly correlated with obesity in both sexes (OR: 1.01; 95%CI: 1.00–1.02). Current smoking (OR: 1.35; 95%CI: 1.08–1.69) correlated significantly with obesity only in males, whereas habitual drinking (OR: 0.48; 95%CI: 0.36–0.63) and habitual exercise (OR: 0.77; 95%CI: 0.60–0.98) correlated significantly with obesity only in females.

### Longitudinal analysis

Of the 1,515 subjects included in the longitudinal analysis (mean age: 58.2±11.9 years; mean sleep duration: 6.4±1.0 hours), 774 were male (51.1%) and 741 were female (48.9%), with a mean age of 58.4±12.0 years (median: 60 years; range: 25–86 years) in males and 58.0±11.7 years (median: 59; range: 22–89 years) in females. The mean sleep duration was 6.5±1.0 hours (median: 7 hours; 3–10 hours) in males and 6.4±1.0 hours (median: 6 hours; 1–10 hours) in females. Of the 1,515 non-obese subjects at baseline, 174 (11.5%) developed obesity during the follow-up period.

**Table 2. Characteristics of Background Factors in 5,518 Participants by Presence or Absence of Obesity[‡]**

| Variables | Male (*n*=2884) | | | Female (*n*=2634) | | |
|---|---|---|---|---|---|---|
| | Obesity (*n*=992) | Non-obesity (*n*=1892) | *p*-value | Obesity (*n*=422) | Non-obesity (*n*=2212) | *p*-value |
| BMI, kg/m$^2$ | 27.0 (25.8–28.6) | 22.6 (21.2–23.7) | <0.0001*** | 27.0 (25.9–29.1) | 20.7 (19.2–22.4) | <0.0001*** |
| <18.5 | 0 | 66 | | 0 | 343 | |
| 18.5–<25 | 0 | 1,826 | <0.0001*** | 0 | 1869 | <0.0001*** |
| ≥25 | 992 | 0 | | 422 | 0 | |
| Age, years | 58 (48.3–66) | 59 (47–68) | 0.4736 | 60 (51–69) | 58 (48–67) | 0.0027** |
| <30 | 4 | 26 | | 3 | 31 | |
| 30–39 | 75 | 204 | | 20 | 205 | |
| 40–49 | 197 | 333 | <0.0001*** | 65 | 407 | 0.0028** |
| 50–59 | 266 | 399 | | 117 | 541 | |
| 60–69 | 273 | 536 | | 118 | 624 | |
| ≥70 | 177 | 394 | | 99 | 404 | |
| SBP, mmHg | 124 (115–133) | 119 (110–129) | <0.0001*** | 124 (115–133) | 114 (104–126) | <0.0001*** |
| SBP ≥140 | 135 (13.6) | 157 (8.3) | <0.0001*** | 60 (14.2) | 162 (7.3) | <0.0001*** |
| DBP, mmHg | 79 (72–86) | 76 (69–82) | <0.0001*** | 78 (72–84) | 72 (66–79) | <0.0001*** |
| DBP ≥90 | 149 (15.0) | 152 (8.0) | <0.0001*** | 49 (11.6) | 118 (5.3) | <0.0001*** |
| FPG, mg/dL | 102 (94–113) | 96 (90.3–104) | <0.0001*** | 96 (92–106) | 91 (87–97) | <0.0001*** |
| FPG ≥126 | 130 (13.1) | 110 (5.8) | <0.0001*** | 39 (9.2) | 34 (1.5) | <0.0001*** |
| HbA1c, % | 5.7 (5.5–6.1) | 5.6 (5.4–5.9) | <0.0001*** | 5.8 (5.5–6.1) | 5.6 (5.4–5.8) | <0.0001*** |
| HbA1c ≥6.5 | 161 (16.2) | 157 (8.3) | <0.0001*** | 58 (13.7) | 56 (2.5) | <0.0001*** |
| LDL-C, mg/dL | 126 (105–147) | 122 (102–142) | 0.0002*** | 128 (106.8–151) | 122 (103–143) | 0.0002*** |
| LDL-C ≥140 | 333 (33.6) | 528 (27.9) | 0.0016** | 149 (35.3) | 640 (28.9) | 0.0088** |
| HDL-C, mg/dL | 52.6 (45.3–61.2) | 61.7 (52.6–72.3) | <0.0001*** | 62.5 (54.2–72.7) | 75.4 (65–87.8) | <0.0001*** |
| HDL-C <40 | 95 (9.6) | 61 (3.2) | <0.0001*** | 11 (2.6) | 12 (0.5) | 0.0003***,† |
| TG, mg/dL | 124 (88–176) | 89 (65–125) | <0.0001*** | 100 (72–135.3) | 70 (52–96.8) | <0.0001*** |
| TG ≥150 | 358 (36.1) | 298 (15.8) | <0.0001*** | 86 (20.4) | 129 (5.8) | <0.0001*** |
| UA, mg/dL | 6.5 (5.7–7.2) | 6.0 (5.3–6.7) | <0.0001*** | 5.2 (4.6–6) | 4.6 (4–5.2) | <0.0001*** |
| UA >7.0 | 310 (31.3) | 341 (18.0) | <0.0001*** | 20 (4.7) | 36 (1.6) | <0.0001*** |
| UA ≥8.0 | 98 (9.9) | 90 (4.8) | <0.0001*** | 2 (0.5) | 9 (0.4) | 0.6924† |
| UA ≥9.0 | 19 (1.9) | 14 (0.7) | 0.0048** | 0 (0.0) | 2 (0.1) | 1.0000† |
| Smoking | | | | | | |
| Current smoker | 185 | 288 | | 25 | 119 | |
| Past smoker | 414 | 765 | 0.0155* | 55 | 292 | 0.9022 |
| Never smoker | 393 | 839 | | 342 | 1801 | |
| Habitual drinking | 532 (53.6) | 1022 (54.0) | 0.8427 | 68 (16.1) | 641 (29.0) | <0.0001*** |
| Habitual exercise | 284 (28.6) | 623 (32.9) | 0.0182* | 96 (22.7) | 609 (27.5) | 0.0420* |
| Sleep duration, hours | | | | | | |
| Mean values | 6.3 | 6.5 | <0.0001*** | 6.3 | 6.4 | 0.8009 |
| Median values | 6 (6–7) | 6 (6–7) | <0.0001*** | 6 (6–7) | 6 (6–7) | 0.2836 |
| <6 | 205 | 270 | | 86 | 407 | |
| 6 | 375 | 681 | <0.0001*** | 169 | 849 | 0.4520 |
| 7 | 300 | 667 | | 114 | 680 | |
| ≥8 | 112 | 274 | | 53 | 276 | |

[‡] Obesity: BMI ≥25 kg/m$^2$

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; UA, uric acid.

Data show median (IQR) or headcount of each variable.

Continuous scale was based on the Wilcoxon rank-sum test, and nominal scale was the $\chi^2$ test.

[†] Fisher's exact test was applied.

*p*-value less than 0.05 was considered statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

## Identification of background factors associated with obesity onset

**Table 4** shows the characteristics of the 1,515 non-obese subjects at baseline, including sleep duration in relation to obesity onset during the follow-up period. Of those who developed obesity during the follow-up period, 109/774 (14.1%) were males and 65/741 (8.8%) were females. The BMI at baseline in the obesity-onset group was 24.2 kg/m$^2$ (24.3 [22.3–24.9] kg/m$^2$ in males, and 24.0 [18.6–24.9] kg/m$^2$ in females). The HDL-C level was significantly lower in the male obesity-onset group, and a significant difference was observed in sleep duration. Additionally, the SBP, FPG, TG, and UA levels were significantly higher in the female obesity-onset group.

## Inference of causal relationship between sleep duration and obesity onset

Model 1 was adjusted for age, and Model 2 was adjusted for age, smoking, habitual drinking, habitual exercise, and weight at baseline (**Table 5**).

**Table 3. Multivariate Analysis of the Association Between Sleep Duration and Obesity‡ ; Cross-sectional Analysis**

| Variables | | Male | | | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Unadjusted | | | Model 1¶ | | Model 2§ | | | Unadjusted | | | Model 1¶ | | Model 2§ | | |
| | | OR | 95%CI | *p*-value | OR | 95%CI | *p*-value | OR | 95%CI | *p*-value | OR | 95%CI | *p*-value | OR | 95%CI | *p*-value |
| Sleep duration group | <6 | 1.38 | [1.11, 1.72] | 0.0045** | 1.39 | [1.11, 1.74] | 0.0037** | 1.36 | [1.09, 1.70] | 0.0074** | 1.06 | [0.80, 1.41] | 0.6823 | 1.07 | [0.81, 1.43] | 0.6349 | 1.06 | [0.79, 1.41] | 0.7026 |
| | 6 | | Reference | | | Reference | | | Reference | | | Reference | | | Reference | | | Reference | |
| | 7 | 0.82 | [0.68, 0.98] | 0.0324* | 0.81 | [0.67, 0.98] | 0.0265* | 0.81 | [0.67, 0.98] | 0.0304* | 0.84 | [0.65, 1.09] | 0.1906 | 0.83 | [0.64, 1.07] | 0.1489 | 0.83 | [0.64, 1.08] | 0.1643 |
| | ≥8 | 0.74 | [0.58, 0.96] | 0.0199* | 0.73 | [0.56, 0.94] | 0.0150* | 0.72 | [0.56, 0.93] | 0.0117* | 0.96 | [0.69, 1.35] | 0.8341 | 0.94 | [0.67, 1.31] | 0.7050 | 0.95 | [0.67, 1.33] | 0.7513 |
| Age, years | | | | | 1.00 | [1.00, 1.01] | 0.3954 | 1.00 | [1.00, 1.01] | 0.2262 | | | | 1.01 | [1.01, 1.02] | 0.0006*** | 1.01 | [1.00, 1.02] | 0.0018** |
| Smoking | Current | | | | | | | 1.35 | [1.08, 1.69] | 0.0088** | | | | | | | 1.43 | [0.90, 2.27] | 0.1395 |
| | Past | | | | | | | 1.16 | [0.97, 1.37] | 0.1034 | | | | | | | 1.16 | [0.84, 1.60] | 0.3643 |
| | Never | | | | | | | | Reference | | | | | | | | | Reference | |
| Habitual drinking | Yes | | | | | | | 0.97 | [0.83, 1.14] | 0.7342 | | | | | | | 0.48 | [0.36, 0.63] | <0.0001*** |
| | No | | | | | | | | Reference | | | | | | | | | Reference | |
| Habitual exercise | Yes | | | | | | | 0.85 | [0.71, 1.01] | 0.0593 | | | | | | | 0.77 | [0.60, 0.98] | 0.0328* |
| | No | | | | | | | | Reference | | | | | | | | | Reference | |
| Model $\chi^2$ test | | | *p*<0.0001*** | | | *p*<0.0001*** | | | *p*<0.0001*** | | | *p*=0.4470 | | | *p*=0.0063** | | | *p*<0.0001*** | |

‡ Obesity: BMI ≥25 kg/m$^2$
OR, odds ratio; CI, confidence interval; *p*-value, likelihood ratio test.
¶ Model 1: Adjusted for age.
§ Model 2: Adjusted for the variables in model 1 plus smoking, habitual drinking, habitual exercise.
*p*-value less than 0.05 was considered statistically significant (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).




**Fig. 3. Adjusted Odds Ratios for each Sleep Duration Group Associated with Obesity**

Sleep duration showed no significant correlation with obesity onset in either males or females. However, in Model 2, compared to the 6-hour group, the HRs tended to be higher in the <6-hour group (HR: 1.38; 95%CI: 0.81–2.31 for males; and HR: 1.12; 95%CI: 0.56–2.15 for females), and lower in the 7-hour group (HR: 0.69; 95%CI: 0.43–1.11 for males; and HR: 0.63; 95%CI: 0.33–1.15 for females) and the ≥8 hours group (HR: 0.99; 95%CI: 0.52–1.79 for males; and HR: 0.91; 95%CI: 0.38–1.95 for females). In summary, obesity and sleep duration showed a U-shaped relationship in both males and females, with the lowest level of obesity at 7 hours of sleep and the HR increasing with shorter (<7 hours) and longer (≥8 hours) sleep duration (**Fig. 4**).

In addition to sleep, weight at baseline showed a significant association with obesity development for both genders (HR: 1.13; 95%CI: 1.10–1.16 for males; and HR: 1.21; 95%CI: 1.16–1.27 for females); age was also significantly associated with obesity, but only in females (HR: 1.03; 95%CI: 1.00–1.06).

## Discussion

We evaluated current sleep status, and the relationship between sleep and obesity using epidemiological data from Japanese individuals who underwent the "Ningen Dock" examination. The most frequently reported sleep duration was 6 hours. Sleep duration was shortest in people in their 40s and 50s, and significantly longer in younger and older groups. Additionally, in both univariate analysis and confounding factor-adjusted cross-sectional multivariate analysis, sleep duration was identified as a background factor for obesity, with shorter sleep time correlating significantly with obesity in males, and a 17% reduction in obesity risk in females sleeping 7 rather than 6 hours. Furthermore, results of the longitudinal multivariate analysis suggested that short sleep duration (<7 hours) in males is a long-term risk factor for obesity onset, and a similar tendency was

**Table 4. Characteristics of Background Factors at Baseline in 1,515 Participants by Presence or Absence of Obesity Onset[‡]**

| Variables | Male (*n*=774) | | | Female (*n*=741) | | |
|---|---|---|---|---|---|---|
| | Onset of obesity (*n*=109) | Non-obesity (*n*=665) | *p*-value | Onset of obesity (*n*=65) | Non-obesity (*n*=676) | *p*-value |
| BMI, kg/m$^2$ | 24.3 (23.7–24.7) | 22.2 (21–23.3) | <0.0001*** | 24.0 (23.3–24.5) | 20.6 (19.2–22) | <0.0001*** |
| <18.5 | 0 | 25 | 0.0373*,† | 0 | 111 | <0.0001***,† |
| 18.5–<25 | 109 | 640 | | 65 | 565 | |
| Age, years | 58 (45–66) | 60 (50–67) | 0.1275 | 58 (51–65) | 59 (50–66) | 0.6872 |
| <30 | 0 | 2 | | 1 | 6 | |
| 30–39 | 13 | 45 | | 2 | 42 | |
| 40–49 | 17 | 113 | 0.1257 | 10 | 108 | 0.7881 |
| 50–59 | 27 | 159 | | 23 | 196 | |
| 60–69 | 40 | 212 | | 21 | 221 | |
| ≥70 | 12 | 134 | | 8 | 103 | |
| SBP, mmHg | 118 (112–126.5) | 119 (109–129) | 0.8965 | 118 (108–129) | 113 (103–125) | 0.0229* |
| SBP ≥140 | 5 (4.6) | 47 (7.1) | 0.3376 | 4 (6.2) | 43 (6.4) | 1.0000† |
| DBP, mmHg | 75 (68.5–82) | 75 (69–82) | 0.8014 | 73 (67–78) | 72 (65–78) | 0.2185 |
| DBP ≥90 | 4 (3.7) | 50 (7.5) | 0.1437 | 4 (6.2) | 30 (4.4) | 0.5293† |
| FPG, mg/dL | 96 (91–103) | 96 (91–104) | 0.9120 | 94 (89–101) | 91 (86–96) | 0.0007*** |
| FPG ≥126 | 2 (1.8) | 34 (5.1) | 0.1320 | 1 (1.5) | 9 (1.3) | 0.6031† |
| HbA1c, % | 5.5 (5.4–5.8) | 5.5 (5.3–5.8) | 0.5623 | 5.7 (5.3–5.9) | 5.5 (5.3–5.7) | 0.0599 |
| HbA1c ≥6.5 | 2 (1.8) | 47 (7.1) | 0.0376* | 2 (3.1) | 13 (1.9) | 0.3847† |
| LDL-C, mg/dL | 127 (104–149.5) | 122 (103–144) | 0.2413 | 124 (106–149.5) | 124 (104–144) | 0.4859 |
| LDL-C ≥140 | 37 (33.9) | 192 (28.9) | 0.2821 | 23 (35.4) | 206 (30.5) | 0.4131 |
| HDL-C, mg/dL | 59.7 (48.6–67.6) | 62.6 (52.9–74.7) | 0.0041** | 71.2 (59.4–85.8) | 75.7 (66–88.5) | 0.0348* |
| HDL-C <40 | 5 (4.6) | 16 (2.4) | 0.2004† | 0 (0.0) | 0 (0.0) | — |
| TG, mg/dL | 98 (72–149.5) | 93 (66–126.5) | 0.1029 | 93 (62–114.5) | 71 (53–96) | 0.0021** |
| TG ≥150 | 27 (24.8) | 97 (14.6) | 0.0072** | 8 (12.3) | 31 (4.6) | 0.0155*,† |
| UA, mg/dL | 6.3 (5.5–7.1) | 6.1 (5.3–6.7) | 0.0771 | 5.2 (4.3–5.7) | 4.6 (3.9–5.2) | 0.0013** |
| UA >7.0 | 27 (24.8) | 115 (17.3) | 0.0615 | 2 (3.1) | 15 (2.2) | 0.6547† |
| UA ≥8.0 | 5 (4.6) | 31 (4.7) | 0.9727 | 0 (0.0) | 4 (0.6) | 1.0000† |
| UA ≥9.0 | 1 (0.9) | 5 (0.8) | 0.5990† | 0 (0.0) | 0 (0.0) | — |
| Smoking | | | | | | |
| Current smoker | 20 | 99 | | 4 | 41 | |
| Past smoker | 43 | 292 | 0.5535 | 7 | 81 | 0.9592 |
| Never smoker | 46 | 274 | | 54 | 554 | |
| Habitual drinking | 57 (52.3) | 387 (58.2) | 0.2481 | 24 (36.9) | 192 (28.4) | 0.1488 |
| Habitual exercise | 37 (33.9) | 227 (34.1) | 0.9690 | 18 (27.7) | 208 (30.8) | 0.6068 |
| Sleep duration, hours | | | | | | |
| Mean values | 6.3 | 6.5 | 0.0370* | 6.2 | 6.4 | 0.1675 |
| Median values | 6 (6–7) | 7 (6–7) | 0.0365* | 6 (6–7) | 6 (6–7) | 0.2569 |
| <6 | 22 | 84 | | 14 | 107 | |
| 6 | 42 | 233 | 0.0637 | 26 | 265 | 0.4715 |
| 7 | 30 | 256 | | 17 | 232 | |
| ≥8 | 15 | 92 | | 8 | 72 | |

[‡] Onset of obesity: BMI ≥25 kg/m$^2$

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; UA, uric acid.

Data show median (IQR) or headcount of each variable.

Continuous scale was based on the Wilcoxon rank-sum test, and nominal scale was the $\chi^2$ test.

† Fisher's exact test was applied.

*p*-value less than 0.05 was considered statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

found in females. Therefore, the adverse effects of short sleep duration on obesity have also been observed in Japanese individuals. Importantly, our study was based on current Japanese data from a broad group of healthy individuals of both sexes. Furthermore, the results of this study were considered reliable, as the findings of cross-sectional analysis were consistent with the results of longitudinal analysis.

First, the sleep duration status was compared with data from the 2019 National Health and Nutrition Survey (Ministry of Health, Labor, and Welfare)[3], the 2016 Survey on Time Use and Leisure Activities (Statistics Bureau, Ministry of Internal Affairs and Communications)[1], and the JACC Study[23]. The sleep distribution and trend by age were generally consistent, and the present study population had a similar composition to that of the general Japanese population.

Second, our cross-sectional analysis showed a significant inverse linear relationship between sleep duration and obesity in males, and a U-shaped profile, with 7 hours as the lowest point, in females. In addition, our longitudinal analysis showed a U-shaped relationship between sleep duration and obesity onset, with 7 hours as the lowest point in both sexes. These findings indicate that short sleep duration is associated with increased risks of obesity and obesity onset,

**Table 5. Multivariate Analysis of the Association Between Sleep Duration and Onset of Obesity‡; Longitudinal Analysis**

| Variables | | Male | | | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Unadjusted | | Model 1¶ | | Model 2§ | | Unadjusted | | Model 1¶ | | Model 2§ | | | |
| | | HR | 95%CI | *p*-value | HR | 95%CI | *p*-value | HR | 95%CI | *p*-value | HR | 95%CI | *p*-value | HR | 95%CI | *p*-value |
| Sleep duration group | <6 | 1.49 | [0.87, 2.47] | 0.1401 | 1.43 | [0.83, 2.38] | 0.1903 | 1.38 | [0.81, 2.31] | 0.2330 | 1.34 | [0.68, 2.53] | 0.3836 | 1.34 | [0.70, 2.57] | 0.3847 | 1.12 | [0.56, 2.15] | 0.7301 |
| | 6 | | Reference | | | Reference | | | Reference | | | Reference | | | Reference | | | Reference | |
| | 7 | 0.69 | [0.43, 1.10] | 0.1184 | 0.72 | [0.44, 1.15] | 0.1665 | 0.69 | [0.43, 1.11] | 0.1266 | 0.78 | [0.41, 1.42] | 0.4145 | 0.78 | [0.42, 1.43] | 0.4145 | 0.63 | [0.33, 1.15] | 0.1336 |
| | ≥8 | 1.01 | [0.54, 1.78] | 0.9725 | 1.13 | [0.59, 2.04] | 0.6997 | 0.99 | [0.52, 1.79] | 0.9848 | 1.21 | [0.51, 2.55] | 0.6469 | 1.21 | [0.55, 2.67] | 0.6470 | 0.91 | [0.38, 1.95] | 0.8158 |
| Age, years | | | | | 0.99 | [0.97, 1.01] | 0.1773 | 1.00 | [0.99, 1.02] | 0.7625 | | | | 1.00 | [0.98, 1.02] | 0.9982 | 1.03 | [1.00, 1.06] | 0.0220* |
| Smoking | Current | | | | | | | 1.16 | [0.67, 1.95] | 0.5897 | | | | | | | 1.52 | [0.45, 3.85] | 0.4546 |
| | Past | | | | | | | 0.97 | [0.63, 1.49] | 0.8835 | | | | | | | 0.87 | [0.35, 1.81] | 0.7222 |
| | Never | | | | | | | | Reference | | | | | | | | | Reference | |
| Habitual drinking | Yes | | | | | | | 0.78 | [0.53, 1.14] | 0.1999 | | | | | | | 1.18 | [0.68, 2.01] | 0.5388 |
| | No | | | | | | | | Reference | | | | | | | | | Reference | |
| Habitual exercise | Yes | | | | | | | 1.22 | [0.80, 1.85] | 0.3444 | | | | | | | 0.81 | [0.45, 1.39] | 0.4512 |
| | No | | | | | | | | Reference | | | | | | | | | Reference | |
| Weight at baseline | | | | | | | | 1.13 | [1.10, 1.16] | <0.0001*** | | | | | | | 1.21 | [1.16, 1.27] | <0.0001*** |
| Model $\chi^2$ test | | | $p$=0.0599 | | | $p$=0.0556 | | | $p$<0.0001*** | | | $p$=0.4671 | | | $p$=0.6365 | | | $p$<0.0001*** | |

‡ Onset of obesity: BMI ≥ 25 kg/m$^2$

HR, hazard ratio; CI, confidence interval; *p*-value, likelihood ratio test.

¶ Model 1: Adjusted for age.

§ Model 2: Adjusted for the variables in model 1 plus smoking, habitual drinking, habitual exercise, weight at baseline.

*p*-value less than 0.05 was considered statistically significant (*$p$<0.05, ***$p$<0.001).




**Fig. 4. Adjusted Hazard Ratios for Each Sleep Duration Group Associated with Obesity**

irrespective of sex and study design. This association can be explained physiologically, as sleep restriction has been shown to impact obesity via changes in appetite-regulating hormones (increased caloric intake), and decreased physical activity (and energy expenditure), due to fatigue[6,14,24]. Our findings are also in agreement with those of other epidemiological studies: a cross-sectional study[18] (inverse linear association between sleep duration and obesity) of Japanese males who underwent the "Ningen Dock" evaluation; an ultra-large-scale study[25] (in the cross-sectional analysis part, inverse linear relationship for males, and U-shaped with 7 hours as the lowest point for females) of over 1.1 million individuals in the USA; a longitudinal study of a Japanese corporate employee cohort[19] (in males, higher risk at <6-hours sleep duration than at 7-hour sleep duration); and two large-scale longitudinal studies in the USA[26,27] (higher risk at <5 hours of sleep in males, and <7 hours in females, than with 7 hours).

In this study, smoking, alcohol consumption, exercise, and age were identified as background factors other than sleep that were associated with obesity in the cross-sectional univariate and multivariate analyses. Current smoking in men was associated with a 35% increased risk compared with lifetime non-smoking; habitual drinking in women was associated with a 52% decreased risk compared with a no-drinking habit; and exercise in women was associated with a 23% decreased risk compared with a no-exercise habit. The association between current smoking and obesity in men can be attributed to the fact that smoking stimulates the expression of male hormones, causing visceral fatty obesity, and lowers the levels of adiponectin, a substance that inhibits obesity[28]. Our study has further established the fact that heavy smokers are more likely to be obese[29].

The association between alcohol consumption and obesity in women may reflect the "desire to be thin" among Japanese women. According to the 2022 National Health and Nutrition Survey (Ministry of Health, Labor and Welfare)[3], 3.9% of men and 11.5% of wom-

en were thin (BMI <18.5 kg/m$^2$) were; furthermore, the age range of thin people is wider among women than among men (not only among young people but also among those in their 40s, which has increased in recent years). In addition, the common impression is that a higher proportion of men drink alcohol compared to women; the difference between men and women reflects the fact that women have a much stronger desire to remain thin, which can explain the gender difference among heavy alcohol drinkers.

Finally, the two analytical methods, cross-sectional and longitudinal, also yielded some different results. The relationship of sleep duration with obesity and obesity onset, as shown by the multivariate analysis, was the same (U-shaped) in both cross-sectional and longitudinal analyses for females, whereas it was different for males, being inverse linear in the cross-sectional analysis, and U-shaped in the longitudinal analysis. The data do not clarify whether long sleep is a protective factor or a risk factor in males. There is no national or international consensus on the effects of long sleep duration[5,30], making the interpretation challenging. This was also a limitation of the present study, along with the restricted numbers of subjects (obese persons and obesity-onset subjects) with long sleep duration or in whom events occurred. The validity of the adjustment model was limited to a certain level of assurance obtained from the longitudinal multivariate analysis. Additionally, there may have been time-dependent covariates (sleep duration, smoking status, alcohol consumption, exercise status, oral medication use, etc.), and unmeasured variables that were confounding factors. The non-exclusion of obstructive sleep apnea as a cause of sleep disturbance (the number of patients with moderate or severe sleep apnea in Japan is 9 million, and the prevalence rate is 14.0%[31]), and the absence of lifestyle data of individual participants were also additional study limitations.

The results of our cross-sectional and longitudinal analyses showed that, in general, short sleep duration (<7 hours) is associated with obesity in adults of both sexes, but the direction of the causal relationship remains unclear. Moreover, our results alone cannot be used to clarify guidelines for healthier sleep. Future epidemiological studies should clarify the nature of the relationship, preferably using large-scale, multicenter cohorts, with approaches including analysis using classification by sleep duration of 8 hours and ≥9 hours; subgroup analyses of young adults, adults, and older individuals; combined analysis of parameters other than BMI; and exclusion of diseases with bidirectional effects on sleep. Further steps should include the development of tools for sleep health guidance, and interventional studies to verify their effectiveness, covering, for example, whether sleep health guidelines actually improve sleeping habits, and whether increased sleep duration prevents obesity onset or leads to weight loss in obese people. We believe that our findings highlight the importance of sleep for Japanese preventive medicine, and will lay the groundwork for promoting sleep counseling as a new pillar of lifestyle disease prevention measures (i.e., health counseling).

## Conclusions

Shorter sleep duration correlated statistically significantly with obesity in males. A similar tendency was found in females. Furthermore, in both males and females, sleeping <7 hours per night may be associated with increased risk of future obesity onset, suggesting that sleep duration of approximately 7 hours is preferable for obesity prevention.

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## Conflicts Of Interests

The authors have no conflicts of interest regarding this study.

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Original Article

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# Ningen Dock Database-based Criteria for Assessing the Significance of Changes in Test Values of Individuals

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## Abstract

**Objective:** The Japan Society of Ningen Dock does not provide any criteria for determining whether changes from previous test values are within the range of natural biological variation or indicate clinical improvement or deterioration. Thus, when test values are classified into the same category as previously, deciding the significance of changes in the values has been left to the discretion of examiners. This study utilized data collected from participants to investigate new indices that would allow us to objectively determine whether changes in test values were significant within the respective criteria categories.

**Methods:** Participants comprised individuals who had undergone comprehensive health checkups (Ningen Dock) at the Jikei University School of Medicine for 25 years. Their data were used to calculate the level-specific, within-individual coefficient of variation ($CV_I$), and the reference change value (RCV) based on this $CV_I$. We then investigated significant changes in test values according to the criteria categories. Differences due to sex and age were also evaluated.

**Results:** Level-specific $CV_I$ was estimated from measurements of 20,262 individuals with stable health status over time. We calculated RCV based on $CV_I$ estimated without considering sex and age, and then classified RCV according to the criteria categories.

**Conclusions:** RCV estimation allows an objective evaluation of the significance of changes in test values, which helps determine whether the values have improved or deteriorated based on scientific evidence. RCV is expected to be widely used for Ningen Dock and in future clinical practice.

**Keywords** within-individual variation, reference change value, health checkup, reference interval

The role of a comprehensive health checkup (Ningen Dock) is to classify individuals into those who require treatment, those who require lifestyle guidance, and those who do not require either, by assessing their history and conducting basic tests. When evaluation is made based on test values, comparing them to previous values is extremely important, especially in cases of individuals who have undergone several Ningen Dock sessions. The Japan Society of Ningen Dock provides a criteria category scheme for the basic test items[1], but does not provide any criteria for determining whether changes from the previous values are within the range of natural biological variation or indicate clinical improvement or deterioration. For example, when a low-density lipoprotein cholesterol (LDL-C) level changed from 140 mg/dL in the previous year to 170 mg/dL in the following year, both levels are classified as category C (Requires follow-up). The criteria category for these levels does not change. The decision on whether an increase of 30 mg/dL is a "significant" deterioration has been left to the discretion of examiners.

For objective evaluation of the significance of changes in test values, the use of the following indices is internationally recommended: the within-individual coefficient of variation ($CV_I$) estimated from serial measurements in individuals, and the reference change value (RCV)[2] generally calculated based on the biological coefficient of variation (CV)[3–8]. $CV_I$ indicates the average variability in test values, usually estimated based on prospective serial measurements in healthy individuals whose lifestyles are controlled to remain unchanged[9–12]. Studies on estimation of $CV_I$ for test values around the median value of the reference range

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are often limited to sample sizes of several tens up to approximately 100 because of cost issues. Moreover, the intervals between serial measurements vary among the studies. Thus, the low accuracy of estimation is a concern. As our previous study revealed that $CV_I$ for certain test items is dependent on the levels of test values[13], it is not always appropriate to directly apply $CV_I$ for test values around the median value of the reference range to the abnormal range. For these reasons, RCV is rarely used in clinical practice at present.

Therefore, in this study, we used a database that contained serial measurements taken from many individuals undergoing Ningen Dock for 25 years to calculate level-specific $CV_I$ and RCV based on the obtained $CV_I$. We then investigated significant changes in test values according to the criteria categories. In addition, we evaluated the extent to which estimation of $CV_I$ was affected by sex and age, which are important factors for fluctuations in test values. Based on examination of these data, we aimed to develop new indices that would allow us to objectively determine whether changes in test values were significant.

## Methods

The data analyzed in the present study were obtained from individuals who underwent Ningen Dock at the Health-Care Center, Jikei University School of Medicine, during a 25-year period from fiscal year 1995 to fiscal year 2019 and who consented to participate (in total, 80,665 individuals: 55,569 men and 25,096 women). Estimation of $CV_I$ requires selecting individuals whose health status is stable over time. For this reason, the following inclusion criteria were set: received Ningen Dock twice or more during the study period; standard deviation of body mass index (BMI) less than 0.5 kg/m$^2$; not receiving drug therapies for dyslipidemia, hyperglycemia, hyperuricemia, hepatic disease, or cardiovascular disease; and received Ningen Dock sessions at intervals of <5 years. A BMI standard deviation of less than 0.5 kg/m$^2$ corresponds to a 95% confidence interval of −2.9 to 2.9 kg for body weight of an individual with a height of 1.7 m. The final analyses included 20,262 participants (13,941 men and 6,321 women) selected under these criteria. The following items were analyzed: Systolic blood pressure (SBP), diastolic blood pressure (DBP), total protein (TP), albumin (Alb), creatinine (Cre), estimated Glomerular Filtration Rate (eGFR), uric acid (UA), high density lipoprotein cholesterol (HDL-C), LDL-C, triglyceride (TG), ALT, AST, γ-GT (γ-GTP), fasting plasma glucose (FPG), HbA1c, white blood cell count (WBC), hemoglobin (Hb) and platelet count (PLT). Demographic information and summary statistics of laboratory results for the enrolled individuals are presented in **Table 1**.

Level-specific $CV_I$ was estimated according to the method developed by Kawano *et al.*[13]. For each test item, the participant's mean value and CV were calculated, and the median CV, in other words $CV_I$, was estimated according to the mean value. For this study we assumed that a larger number of Ningen Dock sessions was associated with greater contribution to the estimation. Therefore, estimation accuracy was improved by weighing the estimation according to the number of Ningen Dock sessions. To examine the validity of the estimated level-specific $CV_I$, $CV_I$ for test values around the median value of the reference range was compared to $CV_I$ reported by Westgard *et al.*[5]. For each test item, Westgard *et al.* reviewed multiple articles and integrated the data to calculate $CV_I$; the information is publicly available in a database and is the most reliable reference value.

RCV was calculated based on the estimated $CV_I$. RCV is conventionally computed as the 95% confidence limit (CL) of the difference between any two measurements. There are two types of RCV: $RCV_{\%}$ is a ratio (%) expressing the relatively significant changes to level T of a test value of interest, while $RCV_X$ expresses significant changes in actual test values. Each type is calculated using the following respective equation.

$$RCV_{\%} = \sqrt{2} Z_\alpha \times CV_I \quad (1)$$

$$RCV_X = \sqrt{2} Z_\alpha \left( T \times CV_I \div 100 \right) \quad (2)$$

where $Z_\alpha$ denotes the z-score of the standard Gaussian distribution corresponding to two-tail probability of $\alpha = (1 - p/100)$. The values of $Z_\alpha$ for $p$=80, 85, 90, 95% are 1.28, 1.44, 1.64 and 1.96, respectively. Conventionally, $Z_\alpha$=1.96 is used. Fraser, who proposed RCV, valued CL ($p$%) as follows because interpretation of the significance of RCV varies depending on CL. According to Fraser, an 80% CL is likely, a 90% CL is more likely, and a 95% CL is very likely[2]. In screening contexts such as Ningen Dock, changes in metabolic syndrome are considered to be most effectively detected with $p$=80%[13]. Thus, $RCV_X$ was calculated from the equation using $Z_\alpha$=1.28 in the present study.

$CV_I$ for test values around the median value of the reference range was estimated separately for men and women, and compared using the standardized difference[14]. The standardized difference is an index used to evaluate the balance between groups and is frequently used in propensity score matching. A standard difference of 0.1 or higher is a indicator of the presence of imbalance. Similarly, to evaluate impacts age-related effects, $CV_I$ was estimated for participants were stratified according to the age at the first Ningen Dock session: <40 years, 40–65 years, and ≥65 years. Differences be-

**Table 1. Summary Statistics of the Source Data**

| Test item | Levels | *n* | Median (Percent) | Mid 95% |
|---|---|---|---|---|
| Sex, *n* (%) | Male | 13941 | 68.8 | |
| | Female | 6321 | 31.2 | |
| Age, year | | 20262 | 46 | 30–68 |
| Age group, *n* (%) | –39 | 6163 | 30.4 | |
| | 40–64 | 13134 | 64.8 | |
| | 65– | 965 | 4.8 | |
| Number of times underwent | –4 | 15064 | 74.3 | |
| health checkups, *n* (%) | 5– | 5198 | 25.7 | |
| BMI, kg/m$^2$ | | 20250 | 22.2 | 17.2–28.8 |
| body fat, % | | 20065 | 21.6 | 12.6–33.5 |
| Abdominal circumference, cm | | 14454 | 80.0 | 64.0–98.5 |
| SBP, mmHg | | 20253 | 117 | 91–149 |
| DBP, mmHg | | 20253 | 73 | 55–95 |
| TP, g/dL | | 19840 | 7.2 | 6.5–8.0 |
| Alb, g/dL | | 19659 | 4.5 | 3.9–5.0 |
| Cre, mg/dL | | 20254 | 0.8 | 0.5–1.1 |
| eGFR, mL/min/1.73 m$^2$ | | 20254 | 80.3 | 56.2–116.4 |
| UA, mg/dL | | 20254 | 5.6 | 3.1–8.4 |
| HDL-C, mg/dL | | 20254 | 61 | 36–100 |
| LDL-C, mg/dL | | 13579 | 118 | 65–183 |
| TG, mg/dL | | 20254 | 87 | 36–305 |
| AST, U/L | | 20254 | 20 | 13–43 |
| ALT, U/L | | 20254 | 19 | 8–65 |
| γ-GT, U/L | | 20254 | 28 | 11–174 |
| FPG, mg/dL | | 20254 | 95 | 80–130 |
| HbA1c (NGSP), % | | 19578 | 5.5 | 4.8–6.8 |
| WBC, 10$^3$/μL | | 20254 | 5.4 | 3.3–9.5 |
| Hb, g/dL | | 20254 | 14.6 | 11.5–16.9 |
| PLT, 10$^4$/μL | | 20237 | 2.3 | 1.5–3.5 |

Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cre, creatinine; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; γ-GT, γ-glutamyl transferase; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; PLT, platelet count; SBP, systolic blood pressure; TG, triglyceride; TP, total protein; UA, uric acid/urate; WBC, while blood cell count

tween these groups were evaluated using the standardized difference.

SAS (version 9.4) was used for all analyses. The study was conducted in compliance with the Declaration of Helsinki and approved by the Ethics Committees of the Jikei University School of Medicine (31-156) and Hiroshima University Hospital (E-1640).

## Results

Concerning sex differences in $CV_I$, **Table 2** shows the separately estimated test values around the median value of the reference range in men and women, as well as the standardized difference. For each test item, the standardized differences, which indicate the degree of imbalance between groups, were below 0.1, which is a indicator of the presence of imbalance. **Table 2** also shows the results of comparison between participants <40 years and ≥65 years, who were expected to show the largest differences among the age groups. The standardized differences were less than 0.1 for all test items except SBP (0.12), TP (0.11), and Alb (0.10). The results of comparisons between participants aged <40 years and 40–65 years, and between those aged 40–65 years and ≥65 years are shown in the **Supplemental Table**. The standardized differences were less than 0.1 for all test items. These results indicate that the impacts of sex and age on estimation of $CV_I$ were negligible. $CV_I$ was estimated without consideration of sex and age.

**Table 3** summarizes the $CV_I$ estimated for test values around the median value of the reference range in the present and the values reported by Westgard *et al.* No substantial differences were observed for the test items except for SBP, DBP, eGFR, and WBC, which are not available in the database created by Westgard *et al.*

**Fig. 1** shows plots of the profiles of within-individual variations in HDL-C and γ-GT that allow us to visually evaluate the dependence of $CV_I$ on the test value levels. The corresponding plots for other test items examined are shown in **Supplemental Figures**. These plots are scattergrams with the x-axis representing the mean value and the y-axis representing CV for each participant. Each point represents a participant who underwent Ningen Dock. The solid line represents the estimated level-specific $CV_I$, and the dash line represents $RCV_{\%}$

**Table 2. Comparison of Sex and Age Differences in $CV_I$**

| Test item | Typical level | Sex | | | Age (–39 vs. 65–) | | |
|---|---|---|---|---|---|---|---|
| | | Category | $CV_I$ | Std Diff | Category | $CV_I$ | Std Diff |
| SBP, mmHg | 110 | Male | 5.6 | 0.01 | –39 | 5.2 | 0.12 |
| | | Female | 5.7 | | 65– | 6.4 | |
| DBP, mmHg | 70 | Male | 5.9 | 0.00 | –39 | 5.6 | 0.09 |
| | | Female | 6.1 | | 65– | 6.8 | |
| TP, g/dL | 7.0 | Male | 2.5 | 0.06 | –39 | 2.7 | 0.11 |
| | | Female | 2.7 | | 65– | 2.2 | |
| Alb, g/dL | 4.5 | Male | 3.3 | 0.01 | –39 | 3.3 | 0.10 |
| | | Female | 3.4 | | 65– | 3.0 | |
| Cre, mg/dL | 0.8 | Male | 6.2 | 0.06 | –39 | 6.3 | 0.02 |
| | | Female | 6.3 | | 65– | 5.8 | |
| eGFR, mL/min/1.73 $m^2$ | 78 | Male | 6.9 | 0.02 | –39 | 7.0 | 0.09 |
| | | Female | 7.2 | | 65– | 6.5 | |
| UA, mg/dL | 4.8 | Male | 6.9 | 0.01 | –39 | 7.1 | 0.01 |
| | | Female | 7.8 | | 65– | 7.0 | |
| HDL-C, mg/dL | 68 | Male | 7.4 | 0.03 | –39 | 7.5 | 0.03 |
| | | Female | 7.1 | | 65– | 6.7 | |
| LDL-C, mg/dL | 115 | Male | 8.2 | 0.00 | –39 | 8.4 | 0.02 |
| | | Female | 8.4 | | 65– | 7.5 | |
| TG, mg/dL | 72 | Male | 19.2 | 0.07 | –39 | 19.6 | 0.00 |
| | | Female | 19.3 | | 65– | 17.9 | |
| AST, U/L | 19 | Male | 11.1 | 0.06 | –39 | 10.8 | 0.01 |
| | | Female | 10.3 | | 65– | 9.8 | |
| ALT, U/L | 16 | Male | 16.8 | 0.09 | –39 | 17.7 | 0.06 |
| | | Female | 17.3 | | 65– | 15.4 | |
| γ-GT, U/L | 20 | Male | 11.7 | 0.05 | –39 | 11.9 | 0.00 |
| | | Female | 12.3 | | 65– | 10.4 | |
| FPG, mg/dL | 93 | Male | 4.1 | 0.04 | –39 | 4.0 | 0.01 |
| | | Female | 4.0 | | 65– | 3.7 | |
| HbA1c (NGSP), % | 5.4 | Male | 2.5 | 0.02 | –39 | 2.6 | 0.09 |
| | | Female | 2.4 | | 65– | 2.0 | |
| WBC, $10^3/\mu L$ | 5 | Male | 9.9 | 0.05 | –39 | 11.2 | 0.06 |
| | | Female | 11.7 | | 65– | 9.8 | |
| Hb, g/dL | 15.0 | Male | 2.3 | 0.00 | –39 | 2.4 | 0.02 |
| | | Female | 2.6 | | 65– | 2.3 | |
| PLT, $10^4/\mu L$ | 2.2 | Male | 6.0 | 0.00 | –39 | 6.4 | 0.01 |
| | | Female | 6.7 | | 65– | 5.6 | |

Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cre, creatinine; CV, coefficient of variation; $CV_I$, within-individual CV; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; γ-GT, γ-glutamyl transferase; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; PLT, platelet count; SBP, systolic blood pressure; Std Diff, standardized difference; TG, triglyceride; TP, total protein; UA, uric acid/urate; WBC, while blood cell count

calculated based on this $CV_I$. For HDL-C, which is a typical test item with normally distributed values, $CV_I$ was found to be constant regardless of the test value levels. By contrast, for γ-GT, which is a typical test item without normally distributed values, $CV_I$ was found to increase gradually with higher test value levels. In addition, $CV_I$ was found to be dependent on the levels of TG, AST, and ALT measurements, which are not normally distributed.

With reference to the table of the criteria categories presented by the Japan Society of Ningen Dock, we calculated level T of test values around the median value in category A (Normal), category B (Slightly abnormal), category C (Requires follow-up [life improvement, re-examination]), and category D (Medical care needed). $RCV_X$ for the test values was calculated from equation (2). **Table 4** summarizes these values. RCV is presented in the upper half of each cell, and level T is presented in the lower half. For example, changes in LDL-C levels are considered statistically significant when $RCV_X$ exceeds 17.4 mg/dL in category A, 18.3 mg/dL in category B, 20.0 mg/dL in category C, and 21.0 mg/dL in category D.

## Discussion

In general, $CV_I$ is estimated from serial measurements taken over time in healthy individuals whose health status is carefully controlled to remain stable. However, studies on $CV_I$ are often conducted with limited sample sizes of several tens of participants because of complicated of conventional study designs. Such

**Table 3. Comparison of $CV_I$ Derived in This Study with Those Listed in Westgard Web-site**

| Test item | Typical level | $CV_I$ | |
|---|---|---|---|
| | | This study | Westgard database |
| SBP, mmHg | 110 | 5.6 | **NA** |
| DBP, mmHg | 70 | 6.0 | **NA** |
| TP, g/dL | 7.0 | 2.5 | 2.8 |
| Alb, g/dL | 4.5 | 3.4 | 3.2 |
| Cre, mg/dL | 0.8 | 6.2 | 6.0 |
| eGFR, mL/min/1.73 $m^2$ | 78 | 7.0 | **NA** |
| UA, mg/dL | 4.8 | 7.2 | 8.6 |
| HDL-C, mg/dL | 68 | 7.3 | 7.3 |
| LDL-C, mg/dL | 115 | 8.3 | 7.8 |
| TG, mg/dL | 72 | 19.2 | 19.9 |
| AST, U/L | 19 | 10.8 | 12.3 |
| ALT, U/L | 16 | 17.0 | 19.4 |
| γ-GT, U/L | 20 | 11.9 | 13.4 |
| FPG, mg/dL | 93 | 4.1 | 5.6 |
| HbA1c (NGSP), % | 5.4 | 2.5 | 1.9 |
| WBC, $10^3/\mu L$ | 5 | 10.4 | **NA** |
| Hb, g/dL | 15.0 | 2.4 | 2.9 |
| PLT, $10^4/\mu L$ | 2.2 | 6.2 | 9.1 |

Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cre, creatinine; CV, coefficient of variation; $CV_I$, within-individual CV; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; γ-GT, γ-glutamyl transferase; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; PLT, platelet count; SBP, systolic blood pressure; TG, triglyceride; TP, total protein; UA, uric acid/urate; WBC, while blood cell count

sample sizes may be insufficient. Furthermore, problems with study designs make it impossible to estimate $CV_I$ for levels of test values in abnormal ranges, which are important for health checkups. In the present study, we used data based on the Ningen Dock health checkup system to acquire a large sample size, and attempted to estimate $CV_I$ for abnormal ranges by including not only healthy individuals, but also individuals with a "stable" health status. To select individuals who were stable over time, we excluded not only individuals treated for certain diseases but also those with extreme changes in BMI, which were regarded as evidence of substantial changes in lifestyle. Individuals with an extremely long interval between Ningen Dock sessions were also excluded because any changes that had occurred were unclear. The validity of these extraction criteria was verified by comparing the database used in the present study with one developed using data from multiple studies and made publicly available by Westgard *et al.*[5]. As there were no substantial differences in any test items, we regard our extraction criteria as valid.

Although the method for estimating level-specific $CV_I$ using a Ningen Dock database was based on the previous study[13], in the present study we attempted to improve the accuracy of estimation of $CV_I$ by weighing for the number of Ningen Dock sessions. In addition, the study period was extended by approximately 10 years more than is typical of previous studies, allowing us to acquire a sample that was 1.5 times larger. These efforts allowed us to consistently estimate $CV_I$ for a wide range of abnormal test values that could not have been estimated in other studies.

Many test values are clearly distributed differently according to sex and age. However, sex- and age-related differences in $CV_I$ have not been fully investigated because issues with study design, including insufficient sample sizes. In the present study, we separately estimated $CV_I$ for men and women, and different age groups, and compared them using the standardized difference. The results showed no substantial differences for either factor, quantitatively demonstrating that estimation of $CV_I$ did not require consideration of either sex or age. To our knowledge, there are no other published quantitative evaluations of impacts of sex and age. The present study provides important evidence for studies on biological CV of test values.

RCV, which we estimated based on level-specific $CV_I$, is an index for evaluating the statistical significance of changes in test values. We developed profile plots of within-individual variations to facilitate visual inspection and intuitive understanding of the profiles (**Fig. 1** and **Supplemental Figure**). We also summarized the criteria for the significance of changes in test values in tabular form (**Table 3**), similar to the table of the criteria categories developed by the Japan Society of Ningen Dock[1] for ease of use for Ningen Dock. These plots and criteria allowed us to evaluate the significance of improvement or deterioration of test values based on scientific evidence, rather than subjective judgment based on experience. Whereas changes from before to after interventions have been evaluated only in groups, our results allowed us to evaluate improvement or deterioration in individuals with not only test values within the reference ranges but also those well beyond the reference ranges. These results appear to be applicable to not only health checkups but also outpatient care and other settings.

The application of the criteria for the significance of changes in test values is simple. First, the previous year's value is classified according to the criteria categories, and $RCV_X$ corresponding to the identified category is determined. When the value improves from the previous year by more than the $RCV_X$ determined, the improvement is significant. In contrast, when the value deteriorates by more than the $RCV_X$, deterioration is significant. In the example described above in which LDL-C levels increase from 140 mg/dL in the preceding year to 170 mg/dL, the increase of 30 mg/dL is evaluated as follows. The previous year's value is classified into category C so that $RCV_X$ is 20.0 mg/dL. As the observed change exceeds $RCV_X$, the LDL-C level has

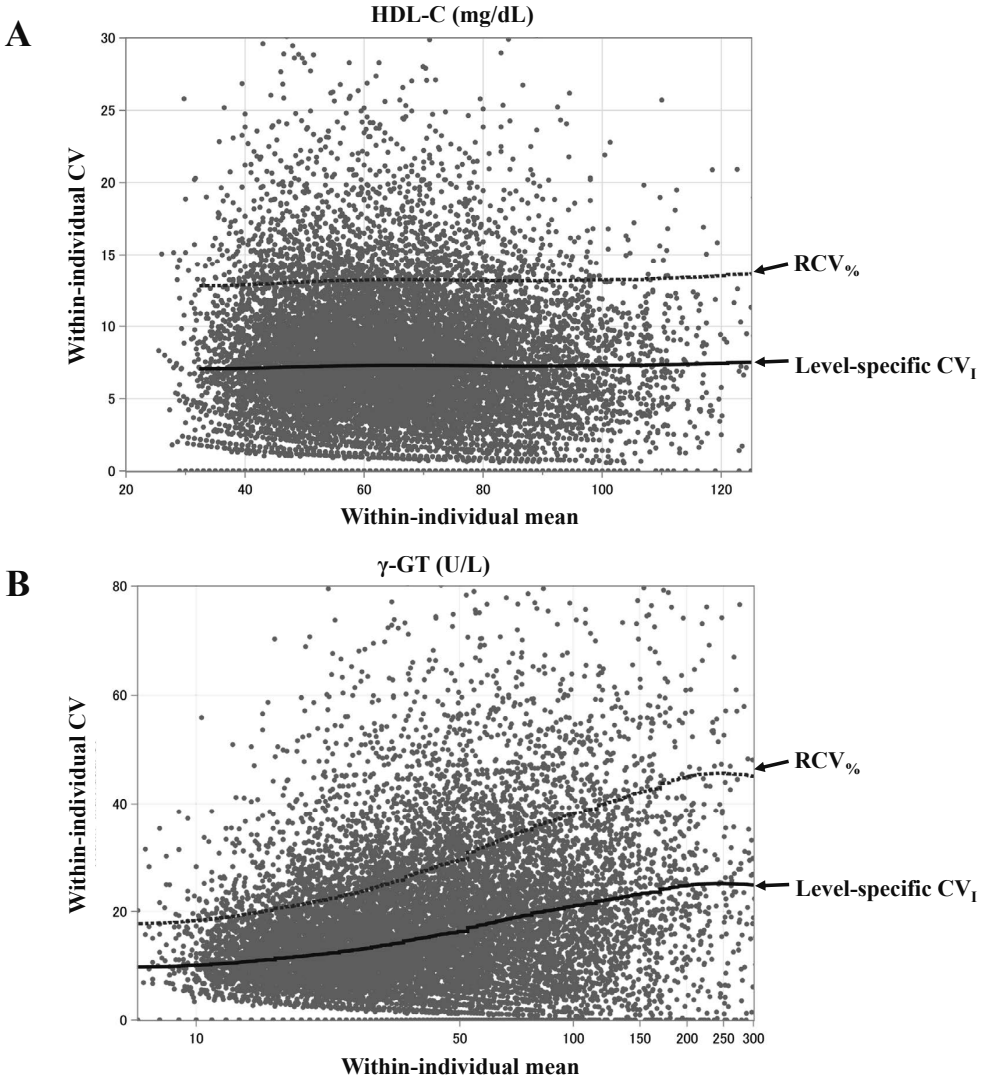


**Fig. 1. The Profile of $RCV_X$ Based on Level-specific $CV_I$ at Confidence Levels (80%) on the Scattergram of Mean vs. CV Plot for All Subjects Examined**

Plotted on the scattergram were mean (on x-axis) and CV (on y-axis) computed for each individual from the serial results over a period of 2–25 years. Typical scattergrams are shown for HDL-C (A) and γ-GT (B). The solid line in the center represents level-specific $CV_I$, while the dash line represents $RCV_{\%}$ determined using the confidence level of α=80%.

CV, coefficient of variation; $CV_I$, within-individual CV; HDL-C, high-density lipoprotein-cholesterol; $RCV_{\%}$, RCV expressed as CV or a relative change ratio (%); $RCV_X$, RCV expressed as an absolute change in a given test value

deteriorated significantly. However, it should be noted that RCV is not a decision limit for the diagnosis of a specific disease, but a reference value such as a reference interval. In the present study, CL ($p$%) was set at 80% for the calculation of RCV in consideration of the outcomes of a previous study[13] and the roles of screening by Ningen Dock. However, when it is preferable to calculate RCV with more significant CL of 85%, 90%, and 95%, RCV can be calculated from equation (1) or (2) using $Z_\alpha$=1.44, 1.64, 1.96, respectively. In fact, the values presented in **Table 3** match $RCV_X$ calculated from equation (2) using $CV_I$ determined for level T of test values corresponding to each criteria category in the profile plot and $Z_\alpha$=1.28. Thus, when $RCV_X$ for level T of a given test value is calculated, it can be calculated using $CV_I$ determined for level T in the plot of the profile of within-individual variation.

In the present study, we estimated level-specific $CV_I$ and RCV by optimizing the benefits of the Ningen Dock database. However, because fewer individuals with more abnormal test values undergo Ningen Dock, studies may be needed to develop mathematical models for extrapolation and validation. Moreover, because we were unable to estimate $CV_I$ for BMI, which was used as a criterion for identifying individuals with stable health status, and items highly associated with BMI, other approaches to identifying such individuals may be advantageous.

**Table 4. Criteria for Assessing the Significance of Changes in Test Values of Each Individual**

| Test item | | | A: Normal | B: Slightly abnormal | C: Require follow-up (life improvement, re-examination) | D: Medical care needed |
|---|---|---|---|---|---|---|
| SBP, mmHg | | $RCV_X$ | **11.5** | **12.3** | **12.8** | **14.4** |
| | | T | (110) | (130) | (140) | (160) |
| DBP, mmHg | | $RCV_X$ | **7.7** | **8.2** | **8.6** | **9.3** |
| | | T | (70) | (85) | (90) | (100) |
| TP, g/dL | | $RCV_X$ | **0.3** | **0.4** | **0.3** | **0.3** |
| | | T | (7.0) | (8.0) | (6.3) | (6.0) |
| Alb, g/dL | | $RCV_X$ | **0.3** | | **0.3** | **0.3** |
| | | T | (4.5) | | (3.8) | (3.6) |
| Cre, mg/dL | Male | $RCV_X$ | **0.1** | **0.1** | **0.1** | **0.1** |
| | | T | (0.8) | (1.0) | (1.1) | (1.3) |
| | Female | $RCV_X$ | **0.1** | **0.1** | **0.1** | **0.1** |
| | | T | (0.6) | (0.7) | (0.8) | (1.0) |
| eGFR, mL/min/1.73 $m^2$ | | $RCV_X$ | **9.5** | | **8.4** | **7.6** |
| | | T | (78) | | (60) | (45) |
| UA, mg/dL | | $RCV_X$ | **0.6** | **0.8** | **0.9** | **1.0** |
| | | T | (4.8) | (7.0) | (8.0) | (9.0) |
| HDL-C, mg/dL | | $RCV_X$ | **8.5** | | **6.0** | **5.9** |
| | | T | (68) | | (37) | (34) |
| LDL-C, mg/dL | | $RCV_X$ | **17.4** | **18.3** | **20.0** | **21.0** |
| | | T | (115) | (130) | (160) | (180) |
| TG, mg/dL | | $RCV_X$ | **25.9** | **66.6** | **169.3** | **277.4** |
| | | T | (72) | (200) | (400) | (500) |
| AST, U/L | | $RCV_X$ | **3.8** | **7.0** | **9.6** | **17.9** |
| | | T | (19) | (33) | (40) | (55) |
| ALT, U/L | | $RCV_X$ | **5.1** | **9.7** | **12.1** | **16.8** |
| | | T | (16) | (35) | (45) | (55) |
| γ-GT, U/L | | $RCV_X$ | **4.5** | **20.0** | **26.6** | **33.3** |
| | | T | (20) | (70) | (90) | (110) |
| FPG, mg/dL | | $RCV_X$ | **6.9** | **7.1** | **7.5** | **8.0** |
| | | T | (93) | (105) | (120) | (130) |
| HbA1c (NGSP), % | | $RCV_X$ | **0.2** | **0.2** | **0.2** | **0.3** |
| | | T | (5.4) | (5.7) | (6.0) | (6.5) |
| WBC, $10^3/\mu L$ | | $RCV_X$ | **1.0** | **1.3** | **1.4** | **1.6** |
| | | T | (5.0) | (8.5) | (9.0) | (10.0) |
| Hb, g/dL | Male | $RCV_X$ | **0.6** | **0.6** | **0.6** | **0.8** |
| | | T | (15) | (17) | (13) | (11) |
| | Female | $RCV_X$ | **0.6** | **0.6** | **0.7** | **1.0** |
| | | T | (13) | (15) | (12) | (10) |
| PLT, $10^4/\mu L$ | | $RCV_X$ | **2.5** | **2.2** | **2.1** | **2.1** |
| | | T | (22) | (13) | (11) | (9) |

Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cre, creatinine; CV, coefficient of variation; $CV_I$, within-individual CV; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; γ-GT, γ-glutamyl transferase; Hb, hemoglobin; HbA1c, hemoglobin $A_{1c}$; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; PLT, platelet count; RCV, reference change value; $RCV_X$, RCV expressed as an absolute change in a given test value; SBP, systolic blood pressure; TG, triglyceride; TP, total protein; UA, uric acid/urate; WBC, while blood cell count

## Conclusion

Evaluation of the significance of changes in test values of Ningen Dock has conventionally been left to the subjective discretion of examiners. RCV, which was calculated based on level-specific $CV_I$ in the present study, is a new index for objectively evaluating the significance of changes in test values of individuals. In other words, it allows us to determine whether test values have improved or deteriorated based on scientific evidence. RCV is expected to be widely used not only for Ningen Dock but also in clinical practice in the future.


## Acknowledgement

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## Conflicts of Interest

The authors declared no conflict of interest.

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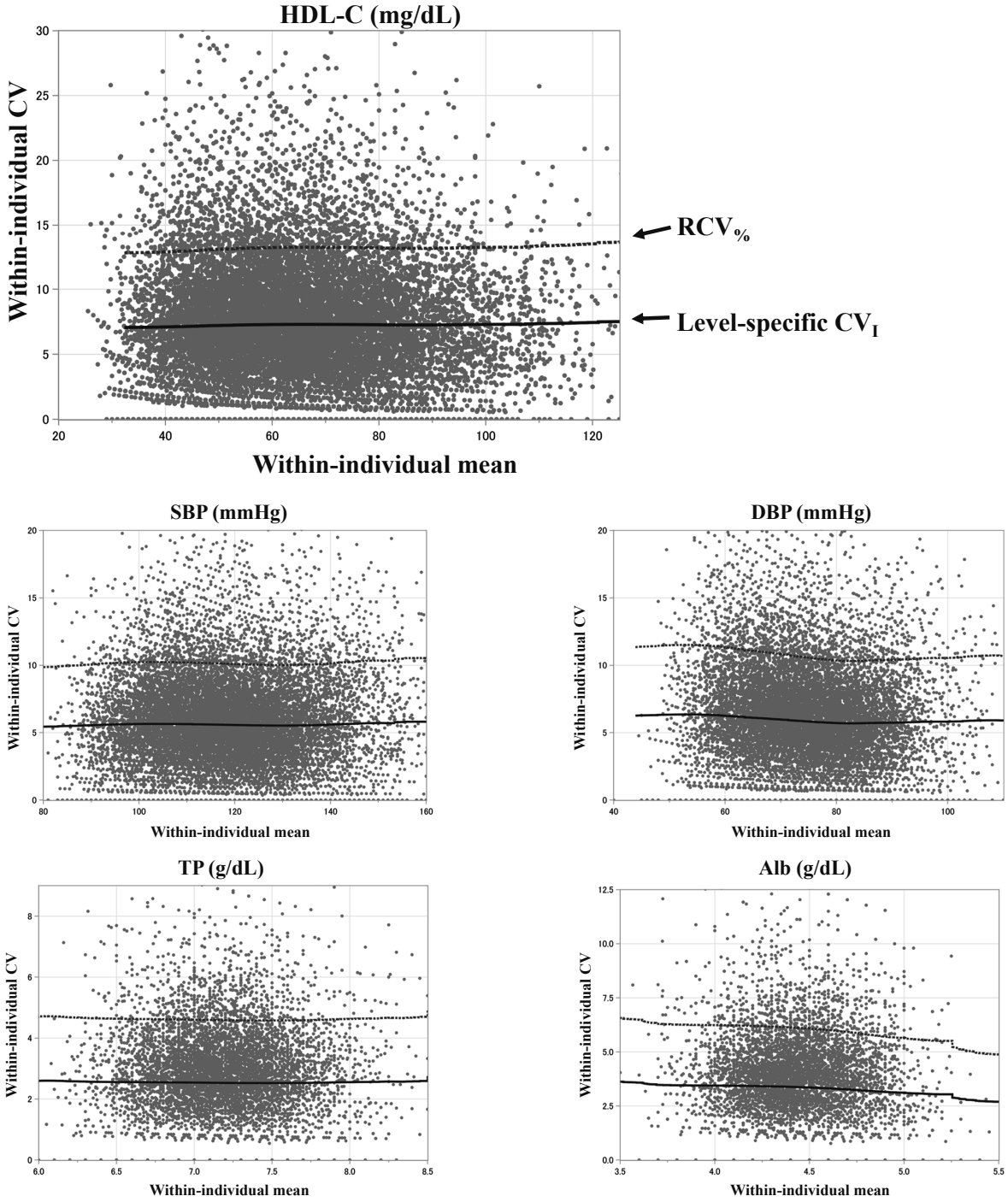
**Supplemental Table. Comparison of Age Differences in $CV_I$**

| Test item | Typical level | Age (−39 vs. 40−64) | | | Age (40−64 vs. 65−) | | |
|---|---|---|---|---|---|---|---|
| | | Category | $CV_I$ | Std Diff | Category | $CV_I$ | Std Diff |
| SBP, mmHg | 110 | −39 | 5.2 | 0.06 | 40−64 | 5.8 | 0.06 |
| | | 40−64 | 5.8 | | 65− | 6.4 | |
| DBP, mmHg | 70 | −39 | 5.6 | 0.04 | 40−64 | 6.1 | 0.06 |
| | | 40−64 | 6.1 | | 65− | 6.8 | |
| TP, g/dL | 7.0 | −39 | 2.7 | 0.03 | 40−64 | 2.5 | 0.08 |
| | | 40−64 | 2.5 | | 65− | 2.2 | |
| Alb, g/dL | 4.5 | −39 | 3.3 | 0.00 | 40−64 | 3.4 | 0.09 |
| | | 40−64 | 3.4 | | 65− | 3.0 | |
| Cre, mg/dL | 0.8 | −39 | 6.3 | 0.00 | 40−64 | 6.2 | 0.02 |
| | | 40−64 | 6.2 | | 65− | 5.8 | |
| eGFR, mL/min/1.73 $m^2$ | 78 | −39 | 7.0 | 0.03 | 40−64 | 7.0 | 0.06 |
| | | 40−64 | 7.0 | | 65− | 6.5 | |
| UA, mg/dL | 4.8 | −39 | 7.1 | 0.01 | 40−64 | 7.3 | 0.01 |
| | | 40−64 | 7.3 | | 65− | 7.0 | |
| HDL-C, mg/dL | 68 | −39 | 7.5 | 0.01 | 40−64 | 7.3 | 0.02 |
| | | 40−64 | 7.3 | | 65− | 6.7 | |
| LDL-C, mg/dL | 115 | −39 | 8.4 | 0.02 | 40−64 | 8.3 | 0.03 |
| | | 40−64 | 8.3 | | 65− | 7.5 | |
| TG, mg/dL | 72 | −39 | 19.6 | 0.03 | 40−64 | 19.2 | 0.03 |
| | | 40−64 | 19.2 | | 65− | 17.9 | |
| AST, U/L | 19 | −39 | 10.8 | 0.02 | 40−64 | 10.9 | 0.03 |
| | | 40−64 | 10.9 | | 65− | 9.8 | |
| ALT, U/L | 16 | −39 | 17.7 | 0.01 | 40−64 | 16.8 | 0.07 |
| | | 40−64 | 16.8 | | 65− | 15.4 | |
| γ-GT, U/L | 20 | −39 | 11.9 | 0.02 | 40−64 | 12.1 | 0.03 |
| | | 40−64 | 12.1 | | 65− | 10.4 | |
| FPG, mg/dL | 93 | −39 | 4.0 | 0.05 | 40−64 | 4.1 | 0.05 |
| | | 40−64 | 4.1 | | 65− | 3.7 | |
| HbA1c (NGSP), % | 5.4 | −39 | 2.6 | 0.02 | 40−64 | 2.4 | 0.07 |
| | | 40−64 | 2.4 | | 65− | 2.0 | |
| WBC, $10^3/\mu L$ | 5 | −39 | 11.2 | 0.05 | 40−64 | 10.2 | 0.01 |
| | | 40−64 | 10.2 | | 65− | 9.8 | |
| Hb, g/dL | 15.0 | −39 | 2.4 | 0.00 | 40−64 | 2.4 | 0.02 |
| | | 40−64 | 2.4 | | 65− | 2.3 | |
| PLT, $10^4/\mu L$ | 2 | −39 | 6.4 | 0.00 | 40−64 | 6.2 | 0.00 |
| | | 40−64 | 6.2 | | 65− | 5.6 | |

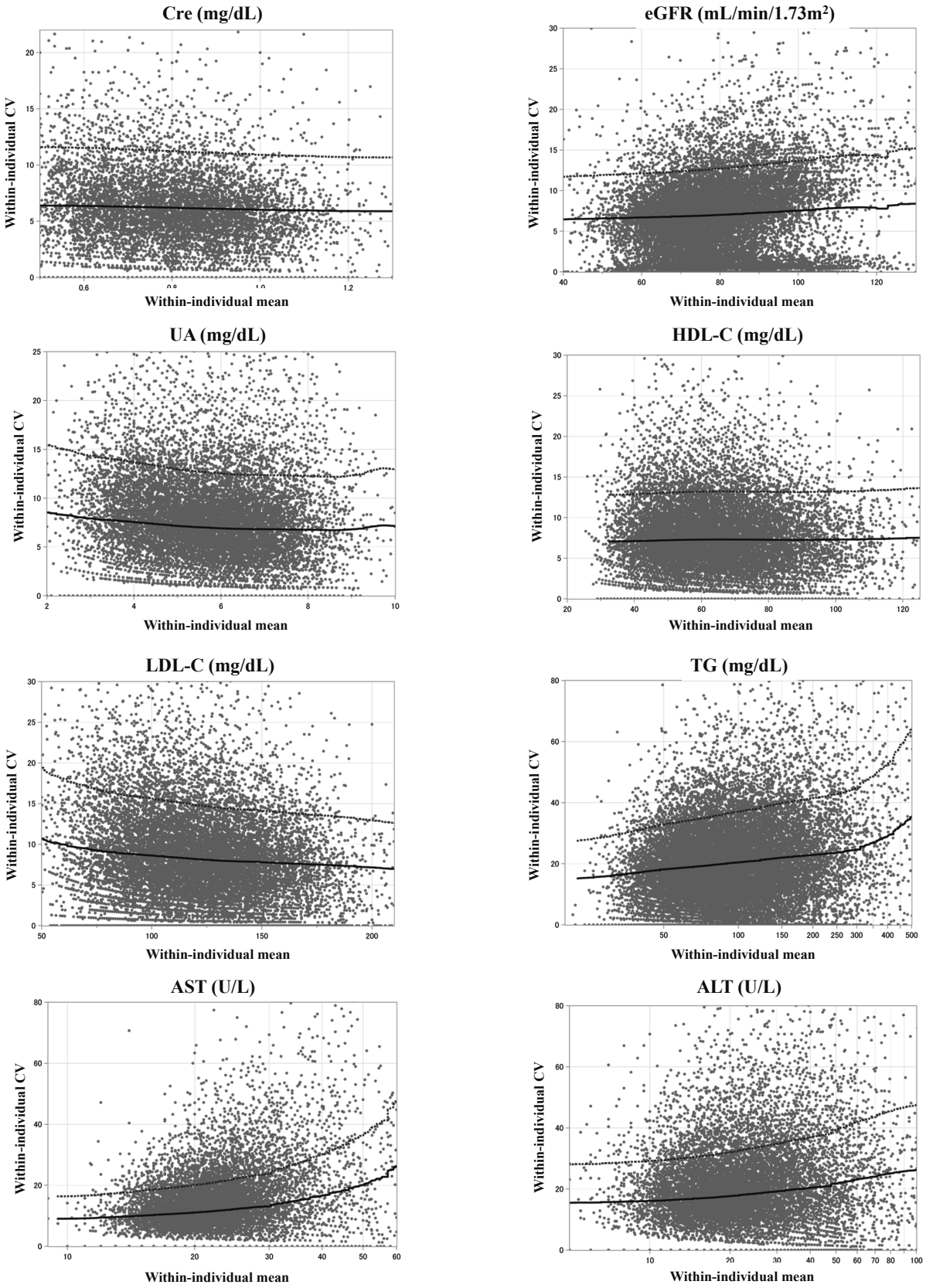
Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cre, creatinine; CV, coefficient of variation; $CV_I$, within-individual CV; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; γ-GT, γ-glutamyl transferase; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; PLT, platelet count; SBP, systolic blood pressure; Std Diff, standardized difference; TG, triglyceride; TP, total protein; UA, uric acid/urate; WBC, while blood cell count



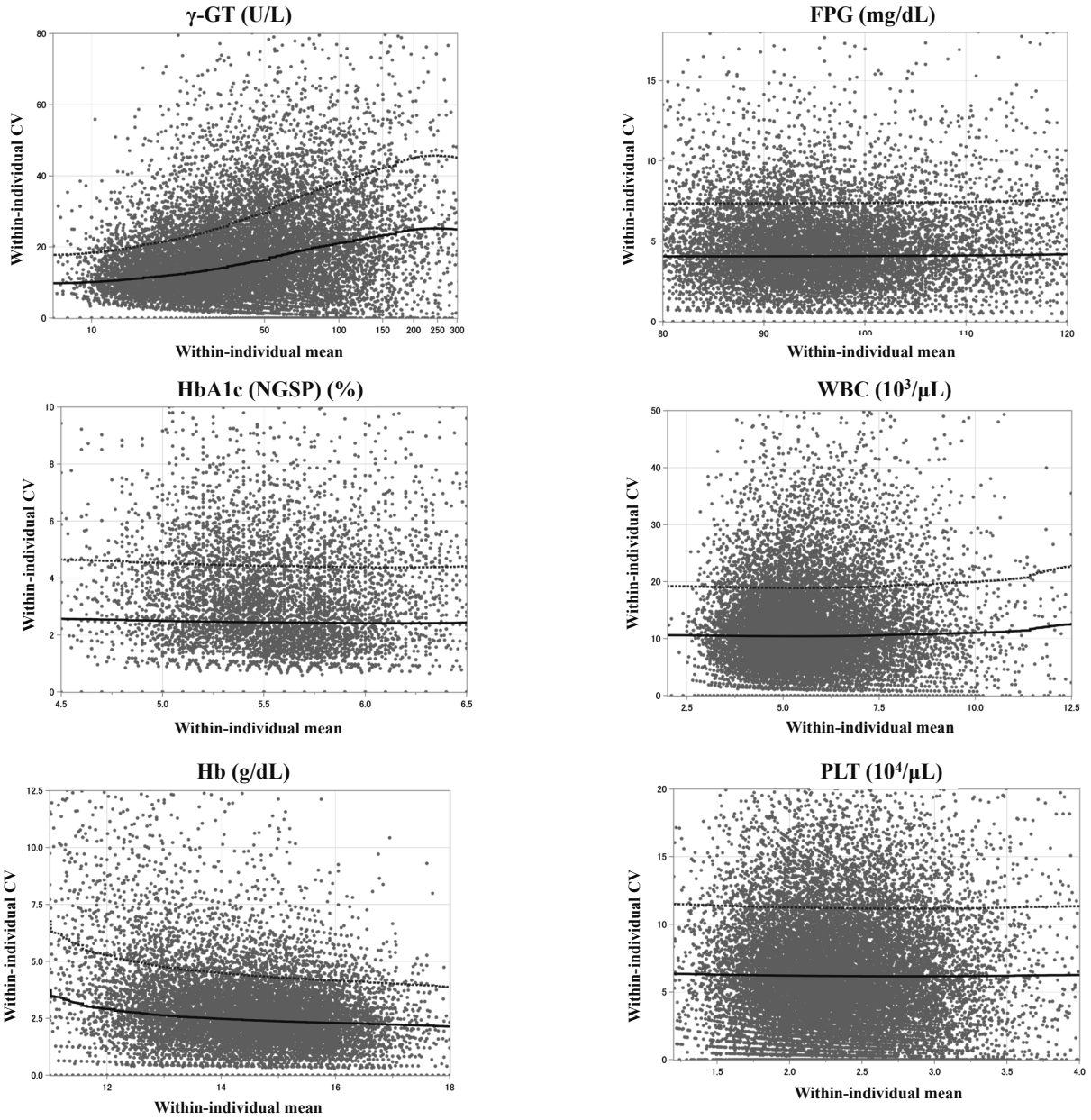

**Supplemental Figures. The Profile of $RCV_X$ Based on Level-specific $CV_I$ at Confidence Levels (80%) on the Scattergram of Mean vs. CV Plot for All Subjects Examined**

Plotted on the scattergram were mean (on x-axis) and CV (on y-axis) computed for each individual from the serial results over a period of 2–25 years. The solid line in the center represents level-specific $CV_I$, while the dash line represents $RCV_{\%}$ determined using the confidence level of $\alpha = 80\%$. The graphs were drawn for all test items.

Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cre, creatinine; CV, coefficient of variation; $CV_I$, within-individual CV; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; γ-GT, γ-glutamyl transferase; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; PLT, platelet count; RCV, reference change value; $RCV_{\%}$, RCV expressed as CV or a relative change ratio (%); $RCV_X$, RCV expressed as an absolute change in a given test value; SBP, systolic blood pressure; TG, triglyceride; TP, total protein; UA, uric acid/urate; WBC, while blood cell count




Supplemental Figures. The Profile of $RCV_X$ Based on Level-specific $CV_I$ at Confidence Levels (80%) on the Scattergram of Mean vs. CV Plot for All Subjects Examined (continue)




Supplemental Figures. The Profile of $RCV_X$ Based on Level-specific $CV_I$ at Confidence Levels (80%) on the Scattergram of Mean vs. CV Plot for All Subjects Examined (continue)

Original Article

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# Carotid Mean Intima-media Thickness is Related to Moderate-to-severe Calcified Coronary Artery

Takashi Kikuchi[1,2], Yuki Ohmoto-Sekine[1], Yumiko Ohike[1], Mariko Inoue[2], Maki Higuchi[2], Tomomi Matsushita[2], Satomi Yamashita[2], Makiko Yamaguchi[2], Aya Omura[2], Yuki Jinnai[1], Rieko Niitsu-Ishimura[1], Akiko Toda[1], Takatoshi Kasai[3], Ritsuko Yamamoto-Honda[1], Yasuji Arase[1], Yukako Koike[2]

## Abstract

**Objective:** This study investigated the relationship between a coronary artery calcium score (CACS) ≥100 and mean intima-media thickness (IMT), and whether the discrimination ability of mean IMT for CACS≥100 differs from that of entire carotid artery (C-max) IMT and max IMT.

**Methods:** A total of 878 consecutive subjects without any history of cardiovascular disease who had undergone atherosclerosis dock were examined by logistic regression analysis to determine whether there is a significant association between mean IMT and CACS≥100. The analysis was adjusted for risk factors with quartile values that showed an IMT-positive likelihood ratio ≥2.0 for CACS≥100. The discrimination ability of each IMT parameter for CACS≥100 was compared using the area under the curve (AUC) as the index.

**Results:** The average age of the study subjects was 61.4±11.0 years, and the prevalence of CACS≥100 was 19.7%. Mean IMT values in the 75th percentile or higher (≥0.95 mm) showed a significant association with CACS≥100 (odds ratio: 2.28). The AUC of mean IMT in relation to CACS≥100 was 0.725, which was not significantly different from that of C-max IMT (0.729) or max IMT (0.728; $p>0.05$ for both).

**Conclusions:** The discrimination ability of mean IMT for CACS≥100 was not significantly different from that of C-max IMT or max IMT in Japanese subjects. Therefore, mean IMT may be as useful as max IMT for CAD risk stratification in this cohort.

**Keywords** mean IMT, calcium score, Japanese, coronary artery disease

Among the carotid intima-media thickness (IMT) parameters, maximum IMT of the entire carotid artery (max IMT)[1,2] and maximum IMT of the common carotid artery (C-max IMT)[3,4] are useful for stratification of coronary artery disease (CAD) risk. In contrast, as atherosclerosis-related lifestyle modification[5] and medical treatment[6] can reduce atherosclerosis progression, some experts have questioned the usefulness of mean IMT for stratifying CAD risk in clinical practice[7–10]. The MESA study[11] demonstrated that IMT reference values differ between ethnicities. Previous studies[7–9] with multiethnic data have included few Japanese participants and only a limited number of Japanese-specific cohort studies have focused on mean IMT[12,13]. Moreover, only one Japanese-specific cohort study has evaluated the usefulness of mean IMT as an indicator of high-risk populations for CAD among asymptomatic individuals[14]. Given that mean IMT has high reproducibility and can be estimated in short test times[15,16], it would be advantageous to examinees if it were a suitable index for screening populations at high risk for CAD, similar to max IMT and C-max IMT.

In this study, moderate or higher coronary calcification[17–20], a CAD high-risk marker, was used as a surrogate outcome to examine the relationship between CAD and mean IMT. In addition, we investigated the predictive value of mean IMT, and whether there are differences in discrimination ability between mean IMT and other measurements obtained by carotid ultrasonography.

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## Subjects and Methods

### Subjects

Of the 1,208 subjects who underwent atherosclerosis dock at Toranomon Hospital Health Management Center between October 2013 and July 2020, 878 subjects who underwent computed tomography (cardiac CT) and carotid ultrasonography (carotid US) at the time of human dock and did not meet the exclusion criteria (**Fig. 1**) were included in the study. Individuals with duplicate data (i.e., individuals examined twice or more during the enrollment period), with an unmeasurable coronary artery calcium score (CACS), for whom carotid intervention had been performed, or with a history of cardiovascular events, arteriosclerosis obliterans (ankle-brachial index below 0.9) were excluded. All subjects underwent the examination voluntarily and had no symptoms.

Various conditions were defined as meeting the following criteria at the time of the dock: (i) hypertension: systolic blood pressure of at least 140 mmHg, diastolic blood pressure of at least 90 mmHg, and/or using antihypertensive agents; (ii) dyslipidemia: low-density lipoprotein cholesterol concentration of at least 140 mg/dL, high-density lipoprotein cholesterol concentration of at least 40 mg/dL, triglyceride concentration of at least 150 mg/dL, and/or using antihyperlipidemic agents; (iii) diabetes: fasting blood sugar concentration of at least 126 mg/dL, hemoglobin A1c at least 6.5%, and/or using antidiabetic agents; (iv) hyperuricemia: uric acid concentration of at least 7.0 mg/dL; and (v) chronic kidney disease: estimated glomerular filtration rate below 60 mL/min/1.73 $m^2$.

The study was performed in accordance with the Declaration of Helsinki and was approved by Toranomon Hospital Ethics Review Committee (Approval no. 2294).

### Measurement of IMT

Mean IMT, C-max IMT, and max IMT were measured using an ultrasound system (Aplio 400; Canon Medical Systems, Tochigi, Japan) and a linear electronic scanning probe (PLT-704SBT; Canon Medical Systems, Tochigi, Japan). Mean IMT was defined as the average of 3-points of IMT taken at 10-mm intervals at the far-side against the probe on the common carotid artery proximal to the carotid bifurcation (**Fig. 2A**). C-max IMT was defined as the greatest IMT in the common carotid artery, and max IMT was defined as the greatest IMT in the entire carotid artery, including the internal carotid artery, external carotid artery, bifurcation region, and common carotid artery. In the present study, only the thickness of the carotid artery wall was considered, and no distinction was made between plaque and IMT. IMT data were obtained from the thicker of the left and right carotid arteries.

### Measurement of CACS and definition of CACS for high-risk CAD

CACS was determined by calculating the Agatston score[21] using ZIO station software (Ziosoft Inc., Redwood City, CA, USA) and images were obtained by electrocardiography gated multidetector CT with a slice thickness of 3.0 mm and field of view of 260 mm, with a single breath-hold. From October 2017, CACS was calculated using the software built into the CT system. Images were obtained using a 64-row multidetector CT scanner (Aquilion 64; Toshiba Medical Systems, Tokyo, Japan) until September 2017, and an 80-row multidetector CT scanner (Aquilion Prime SP; Canon Medical

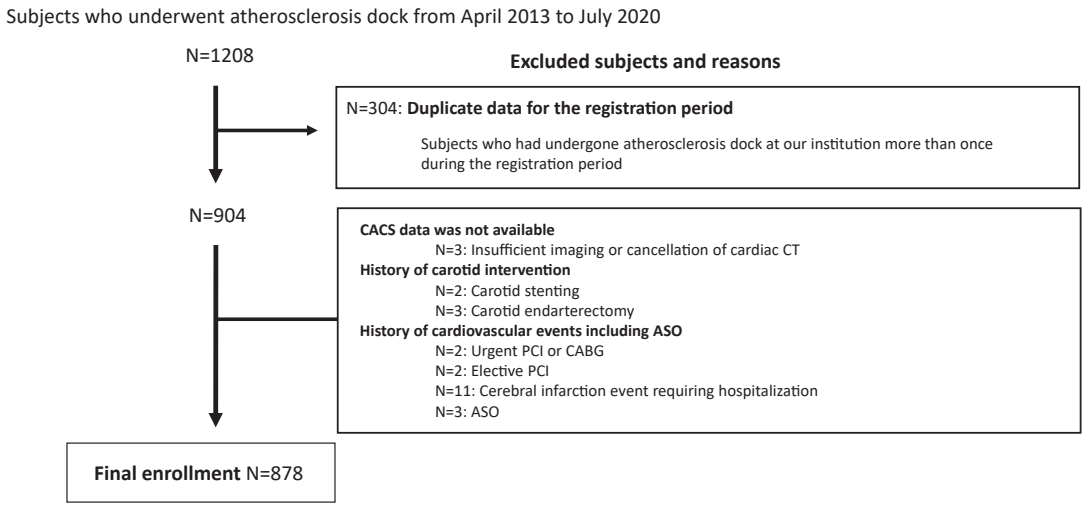



**Fig. 1. Registration of Study Subjects**

Of the 1,208 patients who underwent the atherosclerosis dock, including cardiac CT and carotid US, at our institution from April 2013 to July 2020, 878 subjects who did not meet the exclusion criteria were included.
ASO: arteriosclerosis obliterans, CABG: coronary artery bypass grafting, CACS: coronary artery calcium score, CT: computed tomography, PCI: percutaneous coronary intervention, US: ultrasonography

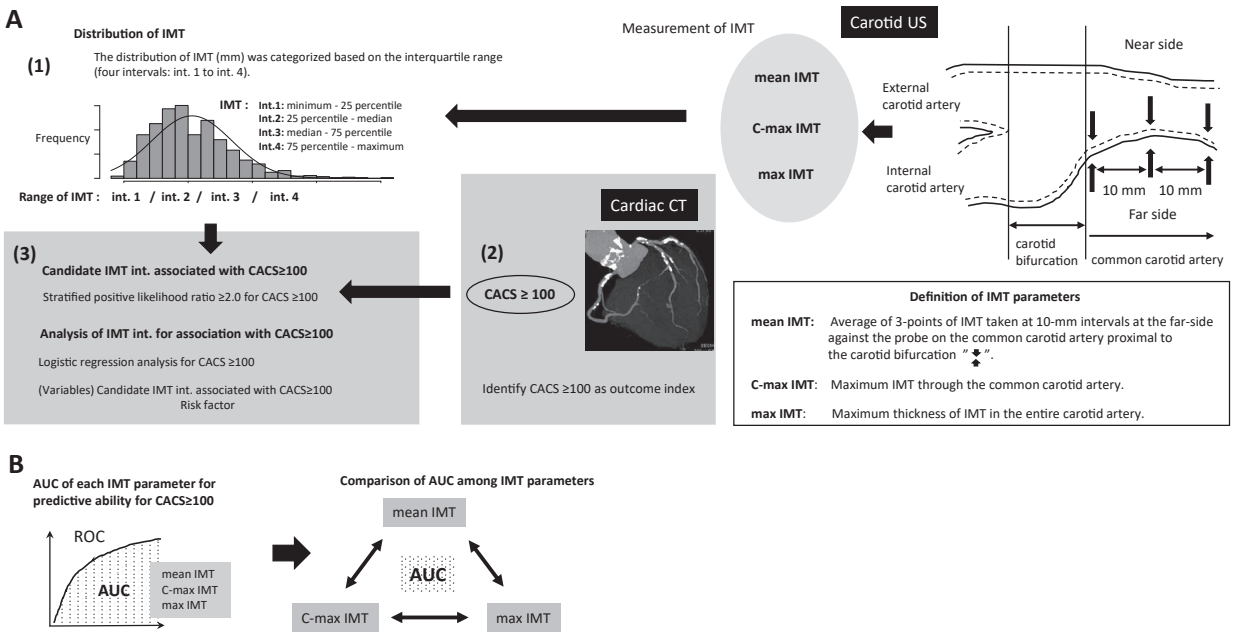



**Fig. 2. Study Protocol**

A: Determination of IMT intervals associated with CACS≥100. (1) Classify the distribution of IMT (mean IMT, C-max IMT, max IMT) obtained using carotid US into four intervals based on the interquartile range (int.1–int.4). (2) Identify subjects with CACS≥100 from CACS obtained from cardiac CT. (3) Identify candidate IMT quartiles associated with CACS≥100 based on a stratified positive likelihood ratio ≥2.0. Test the association between the identified IMT quartile and CACS≥100 for significance in a logistic regression model adjusted for CAD risk factors.
B: Difference in discriminative power between IMT parameters for CACS≥100. AUC calculated from ROC curves is used as an indicator of the discriminative power of mean IMT, C-max IMT, and max IMT for CACS≥100. Further, whether there is a significant difference among the IMT indices in these AUCs is verified.
AUC: area under the curve, CACS: coronary artery calcium score, C-max IMT: maximum thickness of IMT within the common carotid artery, CT: computed tomography, IMT: intima-media thickness, int.: interval in the interquartile range of IMT, max IMT: maximum thickness of IMT in the entire carotid artery, mean IMT: mean IMT at any 3 points in the common carotid artery within 10 mm from the bifurcation, ROC: receiver operating characteristic, US: ultrasonography

Systems, Tochigi, Japan) from October 2017.

We defined CACS≥100 as the threshold for high-risk CAD according to a previous report by Yamamoto *et al.*[20] and a consensus of national and international guidelines on CAD risk[22,23]. Furthermore, as it is well known that age and gender affect CACS, we compared clinical characteristics between a threshold of CACS≥100 and CACS≥400, and found that CACS≥400 was more affected by age (See supplemental data, **Table S1**, **Fig. S1**). We therefore defined CACS≥100 as the threshold for high-risk CAD to eliminate the influence of age.

### Statistical analysis

All data are expressed as mean±standard deviation, median (interquartile range), or number (and percentage). Continuous variables were compared using the Mann–Whitney test, and categorical variables were compared using the $\chi^2$ test. The Cochran–Armitage trend test was used to test for a trend in the IMT interquartile range (int.1 to int.4) with the frequency of CACS≥100. The Bonferroni method was used to test for multiple comparisons. For each quartile of mean IMT, C-max IMT, and max IMT, we examined the relationship with CACS≥100 and calculated the stratified likelihood ratio of predicting CACS≥100. Logistic regression analysis was performed with CACS≥100 as the dependent variable. The independent variables were significant risk factors for CACS≥100 in the study population and parameter quartiles that resulted in an IMT-positive likelihood ratio of at least 2.0 for CACS≥100[24] (**Fig. 2A**(2)(3)).

The discrimination abilities of the three IMT parameters (mean IMT, C-max IMT, and max IMT) in relation to CACS≥100 are presented as areas under the curve (AUC), calculated using receiver operating characteristic (ROC) analysis. AUCs were compared between the following pairs of parameters: mean IMT vs. C-max IMT; mean IMT vs. max IMT; and C-max IMT vs. max IMT (**Fig. 2B**).

A *p*-value below 0.05 was considered significant. All statistical analyses were performed with EZR[25], a modified version of R Commander that offers additional statistical functions frequently used in biostatistics.

## Results

### Clinical characteristics of subjects and prevalence of risk factors

**Table 1** shows the clinical characteristics of the 878 subjects (age, 61.4±11.0 years; 68.8% male) in this study. Median [interquartile range] values were 1 [0 to

**Table 1. Clinical Characteristics of the Study Subjects (*n*=878)**

| | | |
|---|---|---|
| Age, y | 61.4±11.0 | |
| Male, *n* (%) | 604 (68.8) | |
| BMI, kg/m$^2$ | 23.9±3.6 | |
| Total cholesterol, mg/dL | 209.1±34.2 | |
| LDL-C, mg/dL | 120.7±31.5 | |
| HDL-C, mg/dL | 62.0±15.8 | |
| Triglycerides, mg/dL | 117.5±71.0 | |
| Fasting blood sugar, mg/dL | 104.0±18.6 | |
| Hemoglobin A1c, % | 5.8±0.6 | |
| eGFR, mL/min/1.73 m$^2$ | 68.9±13.4 | |
| Uric acid, mg/dL | 5.7±1.3 | |
| BNP, pg/mL | 26.1±28.6 | |
| Hypertension | 376 (42.8) | |
| Diabetes | 94 (10.7) | |
| Dyslipidemia | 385 (43.9) | |
| Medication for hypertension, *n* (%) | 273 (31.1) | |
| Medication for diabetes, *n* (%) | 58 (6.6) | |
| Medication for dyslipidemia, *n* (%) | 212 (24.2) | |
| Smoking habit, packs/y | 8.7±15.0 | |
| Smoking habit, packs/year ≥ 20 | 190 (21.6) | |
| Systolic BP, mmHg | 125±15 | |
| Diastolic BP, mmHg | 77±11 | |
| baPWV, cm/s | 1530±309 | |
| CACS | 118±358, | 1 [0–55] |
| mean IMT, mm | 0.84±0.26, | 0.79 [0.68–0.94] |
| C-max IMT, mm | 1.04±0.45, | 0.90 [0.77–1.17] |
| max IMT, mm | 1.67±0.80, | 1.50 [1.20–2.00] |

Data are expressed as mean ± standard deviation, median [interquartile range] or number of cases (%). Total cholesterol, LDL-C, HDL-C, triglycerides, fasting blood sugar, hemoglobin A1c, eGFR, uric acid and BNP were measured in blood samples taken after a 12-h fast.
baPWV: brachial-ankle pulse wave velocity, BP: blood pressure, BMI: body mass index, BNP: brain natriuretic peptide, CACS: coronary artery calcium score, C-max IMT: maximum thickness of IMT within the common carotid artery, eGFR: estimated glomerular filtration rate, HDL-C: high-density lipoprotein cholesterol, IMT: intima-media thickness, LDL-C: low-density lipoprotein cholesterol, max IMT: maximum thickness of IMT in the entire carotid artery, mean IMT: mean IMT at any 3 points in the common carotid artery within 10 mm from the bifurcation.

55] for CACS, 0.79 [0.68 to 0.94] mm for mean IMT, 0.90 [0.77 to 1.17] mm for C-max IMT, and 1.50 [1.20 to 2.00] mm for max IMT.

**Table 2** shows the prevalence of CAD risk factors in all subjects and in the CACS≥100 and CACS<100 groups, as well as the frequency of high-risk CAD cases (predicted from the Suita score[26] and Framingham risk score[27]; future event rate: at least 14.0%/10 years). Compared with the 705 subjects (80.3%) with a CACS below 100, the 173 subjects (19.7%) with a CACS≥100 were more likely to be at least 60 years old; male; have hypertension, diabetes, chronic kidney disease, a smoking habit of at least 20 packs/year, B-type natriuretic peptide concentration of at least 100 pg/mL, and brachial-ankle pulse wave velocity of at least 1,800 cm/s[28]. The Framingham risk score was higher than the Suita score for the frequency of an estimated risk score of at least 14.0% of CAD events within ten years.

## Association of CACS≥100 with IMT

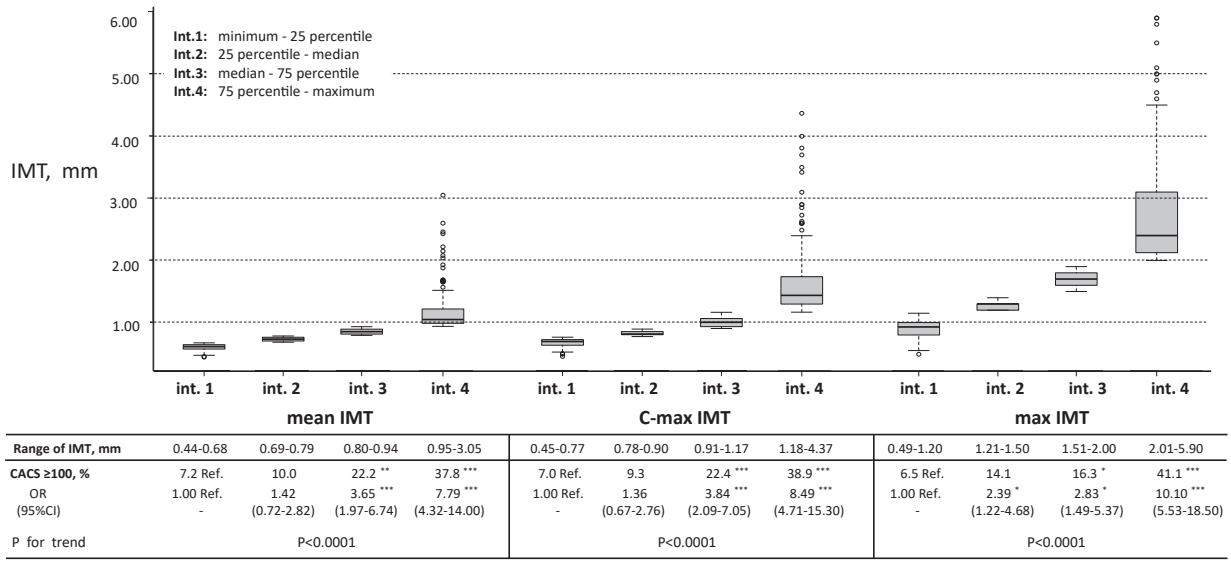
### CACS≥100 and IMT distribution

**Fig. 3** shows the range (mm) of each IMT parameter and the frequency of CACS≥100 for each class width (int.1 to int.4). For each IMT parameter, int.1 was used as the reference. The frequency of CACS≥100 significantly increased in int.3 and higher for all IMT parameters. The odds ratio significantly increased in int.3 and higher for mean IMT and C-max IMT, and in int.2 for max IMT. The rate of CACS≥100 for each IMT parameter showed a trend toward a significant increase with increasing intervals ($p$ for trend: <0.0001). **Fig. 4** shows the stratified likelihood ratio for predicting CACS≥100 in each class. The results showed that intervals with

**Table 2. Prevalence of CAD Risk Factors and High Risk of Adverse Events Related to CAD over 10 Years**

| Risk factor, *n* (%) | Overall *n*=878 | CACS≥100 *n*=173 | CACS<100 *n*=705 |
|---|---|---|---|
| Age ≥60 years | 507 (57.7) | 151 (86.7)*** | 357 (50.6) |
| Male | 604 (68.8) | 138 (79.8)** | 466 (66.1) |
| Hypertension | 376 (42.8) | 114 (65.9)*** | 262 (37.2) |
| Diabetes | 94 (10.7) | 39 (22.5)*** | 55 (7.8) |
| Dyslipidemia | 385 (43.9) | 67 (38.7) | 318 (45.1) |
| CKD: eGFR <60 mL/min/1.73 $m^2$ | 215 (24.5) | 60 (34.7)** | 155 (22.0) |
| Hyperuricemia: uric acid >7.0 mg/dL | 110 (12.5) | 23 (13.3) | 87 (12.3) |
| Smoking habit: packs/year ≥20 | 190 (21.6) | 58 (33.5)*** | 132 (18.7) |
| BMI ≥25.0 kg/$m^2$ | 310 (35.3) | 63 (35.0) | 247 (36.4) |
| Family history of myocardial infarction | 89 (10.1) | 23 (13.3) | 66 (9.4) |
| BNP ≥100 pg/mL | 20 (2.3) | 10 (5.8)* | 10 (1.4) |
| baPWV ≥1800 cm/s | 154 (17.5) | 68 (39.3)*** | 86 (12.2) |
| Development of CAD ≥14.0%/10y estimated Risk-scoring system: | | | |
| Suita score | 52 (5.9) | 28 (16.2)*** | 24 (3.4) |
| Framingham risk score | 197 (22.4) | 73 (42.2)*** | 124 (17.6) |

Data are expressed as the number of subjects in the group (%). The study population (overall) was dichotomized into two groups, CACS≥100 (173 cases, 19.7%) and CACS <100 (705 cases, 80.3%). Risk factors for CAD and the distribution of a high risk of future CAD-related adverse events estimated using published risk scoring systems[26,27] was compared between the groups.
baPWV: brachial-ankle pulse wave velocity, BMI: body mass index, BNP: brain natriuretic peptide, CACS: coronary artery calcium score, CAD: coronary artery disease, CKD: chronic kidney disease, eGFR: estimated glomerular filtration rate




| Range of IMT, mm | 0.44-0.68 | 0.69-0.79 | 0.80-0.94 | 0.95-3.05 | 0.45-0.77 | 0.78-0.90 | 0.91-1.17 | 1.18-4.37 | 0.49-1.20 | 1.21-1.50 | 1.51-2.00 | 2.01-5.90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CACS ≥100, % | 7.2 Ref. | 10.0 | 22.2 ** | 37.8 *** | 7.0 Ref. | 9.3 | 22.4 *** | 38.9 *** | 6.5 Ref. | 14.1 | 16.3 * | 41.1 *** |
| OR (95%CI) | 1.00 Ref. - | 1.42 (0.72-2.82) | 3.65 *** (1.97-6.74) | 7.79 *** (4.32-14.00) | 1.00 Ref. - | 1.36 (0.67-2.76) | 3.84 *** (2.09-7.05) | 8.49 *** (4.71-15.30) | 1.00 Ref. - | 2.39 * (1.22-4.68) | 2.83 * (1.49-5.37) | 10.10 *** (5.53-18.50) |
| P for trend | | P<0.0001 | | | | P<0.0001 | | | | P<0.0001 | | |

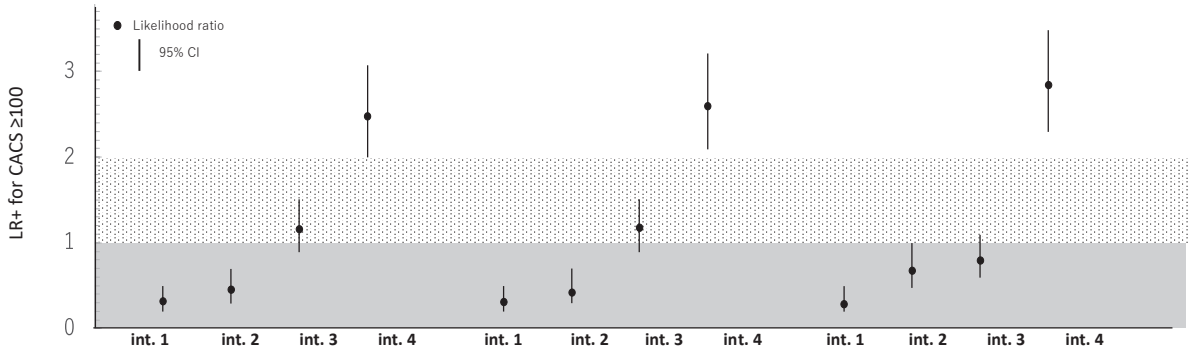
**Fig. 3. Relationship Between the Distribution of IMT and Frequency of CACS≥100**

The distribution of each IMT parameter was classified according to quartiles (int.1: min-25%, int.2: 25%-median, int.3: median-75%, int.4: 75%-maximum). All int. are presented in a boxplot of the distribution. The frequency of CACS≥100 and OR of each int. are also presented. The frequency of CACS≥100 and OR of int.2, int.3, and int.4 were compared with those of int.1.
The Cochran–Armitage trend test was used to analyze the relationship between the frequency of CACS≥100 and each int. of IMT values (int.1 to int.4).
CACS: coronary artery calcium score, C-max IMT: maximum IMT within the common carotid artery, 95%CI: 95% confidence interval, IMT: intima-media thickness, int.: interval in the interquartile range of IMT, max IMT: maximum thickness of IMT in the entire carotid artery, mean IMT: mean IMT at any 3 points in the common carotid artery within 10 mm from the bifurcation, OR: odds ratio

positive likelihood ratios of 2.0 or higher[24], which is clinically meaningful for identifying CACS≥100, were int.4 or higher for each IMT parameter (mean IMT≥0.95 mm; C-max IMT≥1.18 mm; and max IMT≥2.01 mm).

### Association of CACS≥100 with IMT int.4 (**Fig. 5**)

Associations between risk factors and IMT in int.4 or higher and CACS≥100 were evaluated using logistic regression analysis (**Fig. 5**). In logistic regression analysis, the dependent variable was CACS≥100, and independent variables were significant risk factors for CACS≥




| | mean IMT | | | | C-max IMT | | | | max IMT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LR+ (95%CI) | 0.32 (0.19-0.52) | 0.45 (0.30-0.68) | 1.16 (0.89-1.52) | 2.48 (2.01-3.05) | 0.31 (0.19-0.50) | 0.42 (0.27-0.65) | 1.17 (0.91-1.52) | 2.60 (2.10-3.21) | 0.28 (0.17-0.47) | 0.67 (0.47-0.97) | 0.80 (0.59-1.07) | 2.84 (2.31-3.50) |
| Sensitivity (95%CI) | 0.09 (0.05-0.14) | 0.13 (0.08-0.19) | 0.28 (0.22-0.36) | 0.50 (0.43-0.58) | 0.09 (0.05-0.14) | 0.11 (0.07-0.17) | 0.31 (0.24-0.38) | 0.50 (0.42-0.57) | 0.08 (0.05-0.13) | 0.16 (0.11-0.23) | 0.23 (0.17-0.30) | 0.53 (0.46-0.61) |
| Specificity (95%CI) | 0.73 (0.69-0.76) | 0.72 (0.68-0.75) | 0.76 (0.72-0.79) | 0.80 (0.77-0.83) | 0.72 (0.68-0.75) | 0.74 (0.70-0.77) | 0.74 (0.71-0.77) | 0.81 (0.78-0.84) | 0.71 (0.68-0.75) | 0.76 (0.73-0.79) | 0.72 (0.68-0.75) | 0.81 (0.78-0.84) |

**Fig. 4. Stratified Positive Likelihood Ratio for Predicting CACS ≥ 100 According to the Distribution of IMT**

The positive likelihood ratio (+LR), sensitivity, and specificity of the IMT parameters (int.1–int.4) in predicting CACS≥100 are shown. In this study, +LR>2.0 is defined as clinically meaningful[24].
CACS: coronary artery calcium score, 95%CI: 95% confidence interval, C-max IMT: maximum thickness of IMT within the common carotid artery, IMT: intima-media thickness, int.: interval in the interquartile range of IMT, LR+: positive likelihood ratio, max IMT: maximum thickness of IMT in the entire carotid artery, mean IMT: mean IMT at any 3 points in the common carotid artery within 10 mm from the bifurcation.

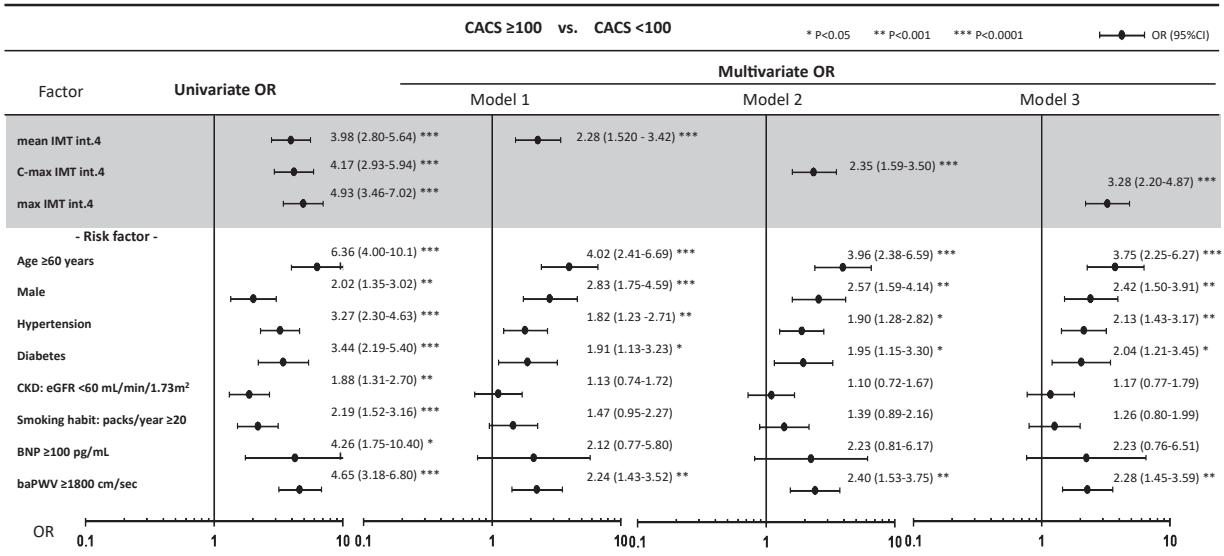



**Fig. 5. Association of CACS ≥ 100 with the Lowest Quartile of IMT Values**

The forest plot shows the univariate and multivariate ORs (95%CI) of each variable at CACS≥100.
Risk factors were defined as items that were significantly associated with CACS≥100 in this study (Table 2). For each IMT parameter, int. 4 was a useful predictor of CACS≥100 (Fig. 4). The association between CACS≥100 and each IMT parameter (int.4) was tested using different logistic regression models (Model 1: mean IMT int.4, Model 2: C-max IMT int.4, Model 3: max IMT int.4) adjusted for risk factors.
baPWV: brachial-ankle pulse wave velocity, BNP: brain natriuretic peptide, CACS: coronary artery calcium score, 95%CI: 95% confidence interval, CKD: chronic kidney disease, C-max IMT: maximum thickness of IMT within the common carotid artery, eGFR: estimated glomerular filtration rate, IMT: intima-media thickness, int.: interval in the interquartile range of IMT, max IMT: maximum thickness of IMT in the entire carotid artery, mean IMT: mean IMT at any 3 points in the common carotid artery within 10 mm from the bifurcation, OR: odds ratio

100 (**Table 2**), mean IMT int.4, C-max IMT int.4, and C-max IMT int.4, which showed IMT likelihood ratios of 2.0 or higher (**Fig. 4**). These independent variables were analyzed according to the following models: Model 1 (risk factors + mean IMT int.4), Model 2 (risk factors + C-max IMT int.4), and Model 3 (risk factors + max IMT int.4). The results showed that mean IMT int.4, C-max IMT int.4, and max IMT int.4 were associated with CACS≥100 independently of the risk factors.

### Discrimination ability of each IMT parameter for CACS≥100

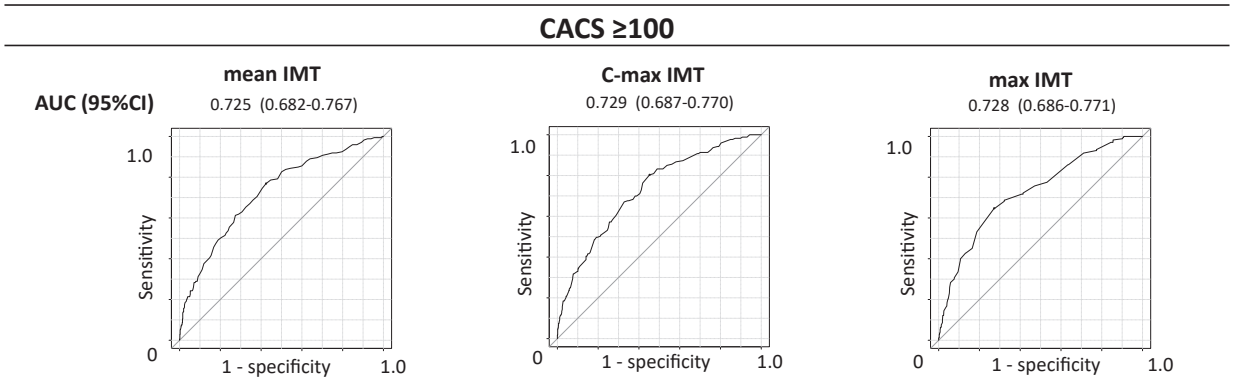
**Fig. 6** shows the ROC curves against CACS≥100 for each IMT parameter. AUCs (95% confidence interval) were 0.725 (0.682 for 0.767) for mean IMT, 0.729 (0.687 to 0.770) for C-max IMT, and 0.728 (0.686 to 0.771) for max IMT. Comparison of the AUCs between IMT parameters showed no significant differences from the reference (mean IMT) for C-max IMT ($p$=0.721) and max IMT ($p$=0.885).

## Discussion

This study examined the relationship between mean IMT and CACS≥100, and compared the predictive ability of mean IMT with that of C-max IMT and max IMT among individuals with moderate-to-severe coronary calcification (CACS≥100), who constitute a population at high risk for CAD. The principal findings were that (i) the 75th percentile values of IMT parameters (mean IMT ≥0.95 mm, C-max IMT ≥1.18 mm, and max IMT ≥2.01 mm) were useful threshold values for detecting CACS≥100 in a population of atherosclerosis dock participants (mean age: 61.4 years) with no history of ischemic heart disease living in urban areas of Japan; and (ii) the discrimination ability of mean IMT for CACS≥100 was not significantly different from that of C-max IMT or max IMT.

While several previous studies have shown that max IMT[1,2] and C-max IMT[3,4] are important risk markers of CAD, mean IMT has been considered to be of low importance with little, if any, clinical significance. However, meta-analyses[7–9] that reported the limited importance of mean IMT for CAD risk contained data from only Europeans and Americans. Caucasian populations generally show high incidences of myocardial infarction. Therefore, it is uncertain whether these meta-analysis findings are applicable to Japanese. The MESA study[11], which included multiple ethnic groups, showed that differences in carotid artery thickness between ethnic groups were actually greater for mean IMT than for max IMT or C-max IMT. We searched for similar studies in Japanese individuals, but only found a small-scale study comparing mean IMT and max IMT in patients with diabetes, and a few reports with large numbers of members of the general population, as in the present study. In a recent Japanese study, Zaid *et al.* examined the relationship between carotid echocardiography and coronary artery calcification in males aged 40 to 79 living in Shiga, Japan[29] and showed that the maximum mean carotid thickness at each locus was related to the degree of coronary artery calcification. However, the participants in that study were older than those in the present study, and were all male. Furthermore, the method used to measure carotid artery thickness differed from conventional methods. In addition, Nagai *et al.* investigated the effectiveness of using mean IMT to predict cardiovascular events in elderly study participants. They divided mean IMT into three classes and found that the third class, with the greatest thickness, was linked to the highest rate of cardiovascular events[13].

Compared to other ethnic groups, the higher carotid bifurcation position in Japanese makes it more difficult




| Factor | AUC | 95%CI | P value ( vs. Ref. ) | |
|---|---|---|---|---|
| mean IMT | 0.725 | 0.682-0.767 | Ref. | - |
| C-max IMT | 0.729 | 0.687-0.770 | 0.721 | Ref. |
| max IMT | 0.728 | 0.686-0.771 | 0.885 | 0.980 |

**Fig. 6. Discrimination Power of IMT Parameters for CACS≥100**

The ROC curve shows the discriminative power of IMT parameters, as determined by the AUC, for CACS≥100. Comparison of AUC among the IMT parameters (mean IMT, C-max IMT, and max IMT) showed no significant differences.
AUC: area under the curve, CACS: coronary artery calcium score, 95%CI: 95% confidence interval, C-max IMT: maximum thickness of IMT within the common carotid artery, IMT: intima-media thickness, max IMT: maximum thickness of IMT in the entire carotid artery, mean IMT: mean IMT at any 3 points in the common carotid artery within 10 mm from the bifurcation, ROC: receiver-operating characteristic

to evaluate the distal position of carotid artery in this population[10]. With this in mind, the absence of a significant difference in the ability of mean IMT to predict cardiovascular events compared to max IMT and C-max IMT in individuals with high calcium scores is a significant finding.

**Can mean IMT be used for risk stratification for CAD?**

This study compared the predictive ability of mean IMT for CAD risk (CACS≥100) with that of max IMT and C-max IMT. We found that mean IMT had comparable predictive ability for CACS≥100 to the other two indices. Given the different vasculature structures of the CCA and carotid bifurcation to the internal carotid artery, different risk factors can affect the thickening of the IMT of these arteries. Polak *et al.*[30] showed that while hypertension and diabetes contribute to CCA thickening, several overlapping risk factors contribute to the thickening of the IMT of each artery, which ultimately lead to CAD events. In the present study, 83.4% of max IMT values were measured at the carotid bifurcation and subjects who had hypertension and diabetes had markedly high CACSs in the CACS>100 population. This evidence may explain the lack of a significant difference in the prediction ability between mean IMT and max IMT for CACS>100.

Automatic tracing can be used to determine the mean IMT[16], and mean IMT is more reproducible than max IMT[15]. Therefore, in the absence of a significant difference between mean and max IMT for predicting CACS≥100, the high precision and shorter examination time required to determine mean IMT may make it a useful screening index for ischemic heart disease in dock participants.

It is well known that IMT and CACS are influenced by age and gender. We showed that fourth quartile values of IMT were significantly associated with CACS≥100 after adjustment for age and gender in multivariate analysis. We also showed that there is a relationship between IMT and CACS.

## Limitations

This study had two main limitations. First, we did not directly evaluate the predictive ability of mean IMT for CAD events. However, cohort studies showing a relationship between CAD onset and CACS≥100 are supported by numerous prospective studies, including cohort studies of patients with asymptomatic CAD[19,20], and studies on the risk of future coronary events in asymptomatic individuals with no history of CAD (1.5% to 2.4% per year)[17,18]. Second, using our study design, we found no significant difference in discrimination ability in relation to CACS≥100 among mean IMT, max IMT, and C-max IMT. However, this study was not designed to prove non-inferiority. Data from prospective cohort studies are needed to address the above limitations.

## Conclusion

We showed that the highest quartile values of mean IMT may be useful for predicting CACS≥100 in human dock participants with no history of cardiovascular disease living in urban Japan. The discrimination ability of mean IMT for CACS≥100 was not significantly different from that of C-max IMT or max IMT.

## Funding

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## Conflicts of Interest

There are no conflicts of interest to declare concerning this study.

## Acknowledgments

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**Table S1. Prevalence of CAD Risk Factors in CACS ≥ 400 and CACS ≥ 100**

| Risk factor, *n* (%) | Overall *n*=878 | CACS ≥ 100 *n*=173 | CACS < 100 *n*=705 | CACS ≥ 400 *n*=70 | CACS < 400 *n*=808 |
|---|---|---|---|---|---|
| Age ≥ 60 years | 507 (57.7) | 151 (86.7)*** | 357 (50.6) | 66 (94.3)††† | 441 (54.6) |
| Male | 604 (68.8) | 138 (79.8)** | 466 (66.1) | 60 (85.7)† | 544 (67.3) |
| Hypertension | 376 (42.8) | 114 (65.9)*** | 262 (37.2) | 49 (70.0)††† | 327 (40.5) |
| Diabetes | 94 (10.7) | 39 (22.5)*** | 55 (7.8) | 17 (24.3)†† | 77 (9.5) |
| Dyslipidemia | 385 (43.9) | 67 (38.7) | 318 (45.1) | 26 (37.1) | 359 (44.4) |
| CKD: eGFR < 60 mL/min/1.73 $m^2$ | 215 (24.5) | 60 (34.7)** | 155 (22.0) | 30 (42.9)†† | 185 (22.9) |
| Hyperuricemia: Uric acid > 7.0 mg/dL | 110 (12.5) | 23 (13.3) | 87 (12.3) | 11 (15.7) | 99 (12.3) |
| Smoking habit: packs/year ≥ 20 | 190 (21.6) | 58 (33.5)*** | 132 (18.7) | 31 (44.3)††† | 159 (19.7) |
| BMI ≥ 25.0 kg/$m^2$ | 310 (35.3) | 63 (35.0) | 247 (36.4) | 28 (40.0) | 282 (34.9) |
| Family history of myocardial infarction | 89 (10.1) | 23 (13.3) | 66 (9.4) | 8 (11.4) | 81 (10.0) |
| BNP ≥ 100 pg/mL | 20 (2.3) | 10 (5.8)* | 10 (1.4) | 6 (8.6)† | 14 (1.7) |
| baPWV ≥ 1800 cm/s | 154 (17.5) | 68 (39.3)*** | 86 (12.2) | 34 (48.6)††† | 120 (14.9) |
| Development of CAD ≥ 14.0%/10y estimated Risk-scoring system : | | | | | |
| Suita score | 52 (5.9) | 28 (16.2)*** | 24 (3.4) | 17 (24.3)††† | 35 (4.3) |
| Framingham risk score | 197 (22.4) | 73 (42.2)*** | 124 (17.6) | 40 (57.1)††† | 157 (19.4) |

Data indicate the number of subjects in the group (%). Risk factors for CAD and the distribution of a high risk of future CAD-related adverse events estimated using published risk scoring systems[26,27] were compared between groups.
* $p<0.05$, ** $p<0.001$, *** $p<0.0001$: CACS ≥ 100 vs CACS < 100
† $p<0.05$, †† $p<0.001$, ††† $p<0.0001$: CACS ≥ 400 vs CACS < 400
baPWV: brachial-ankle pulse wave velocity, BMI: body mass index, BNP: brain natriuretic peptide, CACS: coronary artery calcium score, CAD: coronary artery disease, CKD: chronic kidney disease, eGFR: estimated glomerular filtration rate

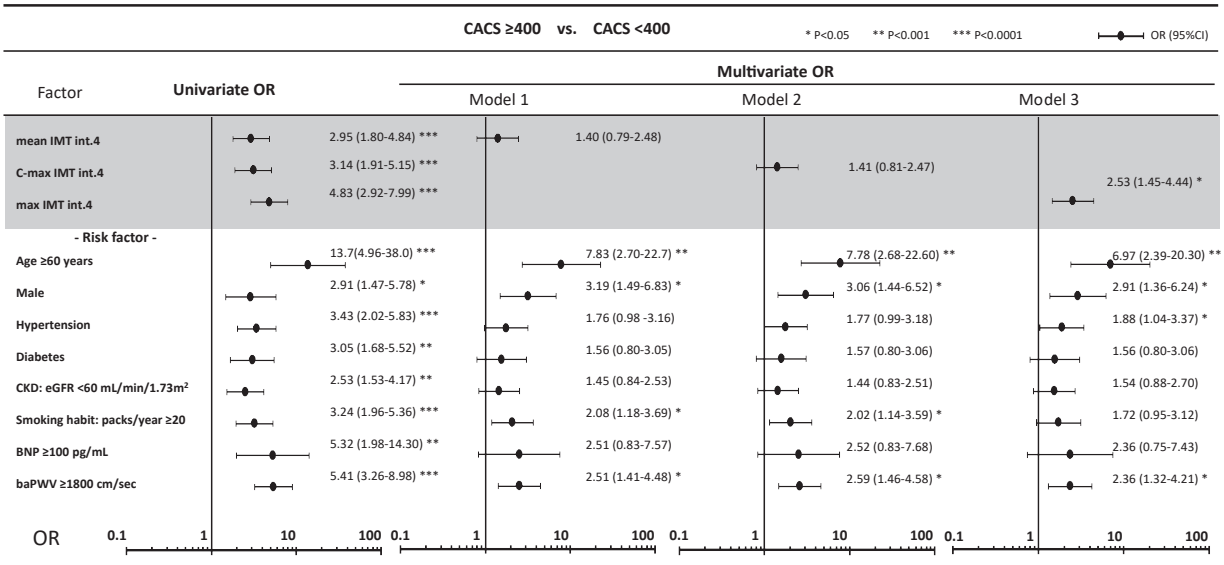


**Fig. S1. Factors Associated with CACS ≥ 400**

The forest plot shows the univariate and multivariate ORs (95%CI) of each variable at CACS ≥ 400. Risk factors were defined as items that were significantly associated with CACS ≥ 400 in this study (Table S1). The association between CACS ≥ 400 and each IMT parameter (int.4) was tested using different logistic regression models (Model 1: mean IMT int.4, Model 2: C-max IMT int.4, Model 3: max IMT int.4) adjusted for risk factors.
baPWV: brachial-ankle pulse wave velocity, BNP: brain natriuretic peptide, CACS: coronary artery calcium score, 95%CI: 95% confidence interval, CKD: chronic kidney disease, C-max IMT: maximum thickness of IMT within the common carotid artery, eGFR: estimated glomerular filtration rate, IMT: intima-media thickness, int.: interval in the interquartile range of IMT, max IMT: maximum thickness of IMT in the entire carotid artery, mean IMT: mean IMT at any 3 points in the common carotid artery within 10 mm from the bifurcation, OR: odds ratio

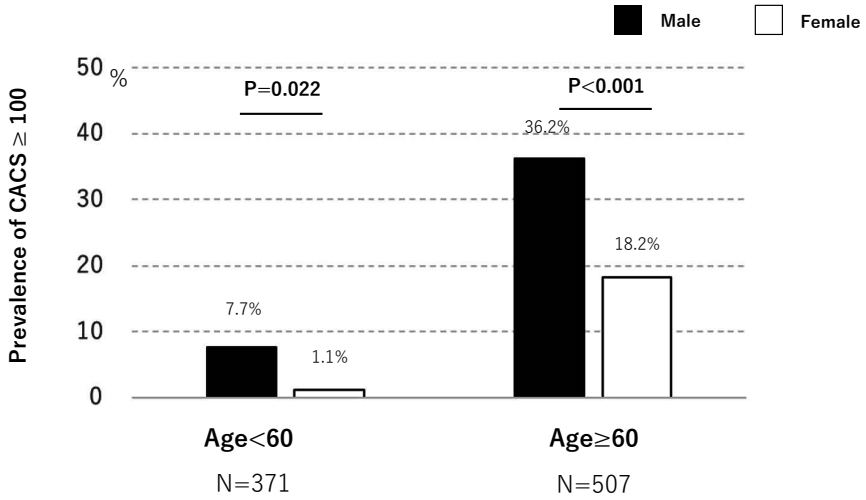


**Fig. S2. The Prevalence of CACS ≥ 100 Was Affected by Age and Sex**

Subjects with CACS ≥ 100 were more likely to be 60 years and older and male regardless of age.
CACS: coronary artery calcium score

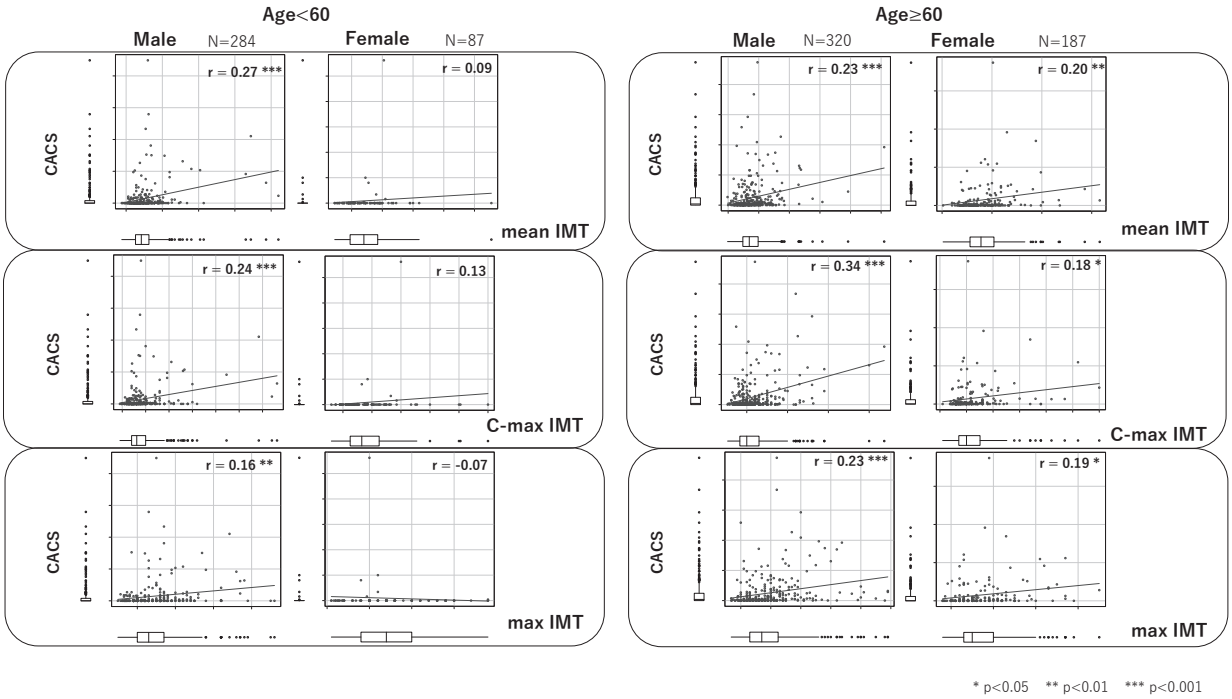


**Fig. S3. Relationship Between CACS and IMT According to Age and Sex**

Scatter plots of CACS and IMT stratified by age (60 years) and sex.

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# Exploring Time Period-, Sex-, and Age-related Changes in the Results of Health Checkups Before and During the COVID-19 Pandemic

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## Abstract

**Objective:** People's daily lives have changed during the coronavirus disease 2019 pandemic. Our study revealed several changes in metrics indicative of general health and well-being during the two years of the pandemic compared to the previous three years, based on annual health examinations. We explored time period-, sex-, and age-related changes in health status under pandemic-related circumstances.

**Methods:** We used a dataset of 56,368 participants aged 30–69 years who underwent health checkups between March 2017 and February 2022. Data were divided into five periods. Body weight, body mass index, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), and uric acid (UA) levels were measured. A three-way ANOVA was conducted to examine the effects of the time period, sex, and age.

**Results:** During the pandemic, SBP and DBP of the two sexes showed opposite trends. Women showed an increase throughout the five periods, whereas men showed a decrease. In all samples, FPG and HbA1c levels increased and then decreased during the pandemic, and UA levels increased before the pandemic but decreased in the first year of the pandemic.

**Conclusions:** SBP and DBP showed opposite trends in men and women, whereas other results showed no significant difference. However, prolongation of the pandemic could further impact societal behavior and lead to changes in future outcomes. We need to consider new methods to improve people's lives through information-sharing and support tailored to the individual.

**Keywords** health checkups, COVID-19, three-way ANOVA, new normal

Due to the global pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) infections, our behavior has been restricted and our lifestyles changed. In Japan, two years have passed since the first COVID-19-positive case was reported in February 2020. Since then, the infection rate has fluctuated seven times, and the Tokyo Olympic Games were delayed by one year. Depending on the number of infected people, severe cases, and hospital bed capacity, the Japanese government or prefectural governments declared a state of emergency or quasi-emergency to prevent the spread of the virus. This involved asking the public to refrain from nonessential travel (self-quarantine), reducing the number of people going to work (work from home and telecommuting), restricting restaurant opening times, including for those serving alcoholic beverages, and restricting access to gyms.

Restrictions are enforced, and citizens are expected to practice a new lifestyle proposed by the Ministry of Health, Labor, and Welfare (MHLW) in a bid to contain the spread of COVID-19. The MHLW also encourages behavioral changes such as washing hands, wearing a mask, and avoidance of the three Cs (close contact settings, closed spaces, and crowded places)[1].

There have been many reports on the health effects of COVID-19. In this general context, the MHLW has proposed six recommendations for a "new healthy lifestyle". One of these is the "Recommendations for Health Checkups[2]." The role of Ningen Dock (Comprehensive Health Checkup System) has become even more significant.

Lifestyle changes differ among individuals. For example, the long-term health effects of a new lifestyle are likely to vary depending on whether one works remotely, whether one uses the gym, how often one eats out,

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how much time one spends doing housework, the composition of and relations within the family, and various other personal circumstances.

This study analyzed changes in health checkup outcomes of men and women in four age groups (30s, 40s, 50s, and 60s) over five time periods (the three years before the COVID-19 pandemic and the two years during the pandemic).

## Methods

### Subjects

Of the 60,133 people who underwent annual health checkups at our institution between March 2017 and February 2022, we used a dataset of 56,368 of those between 30 and 69 years of age. Our facility is in a suburb of Tokyo.

The study was approved by the ethics committees of our institution.

### Data categorization

Because the first COVID-19 patient in our city was reported on March 18, 2020, we defined the pandemic periods as period D from March 2020 to February 2021 and period E from March 2021 to February 2022. The three years prior to these periods were defined as follows: period A, from March 2017 to February 2018; period B, from March 2018 to February 2019; and period C, from March 2019 to February 2020. Subjects in the five periods A–E were divided into men and women in their 30s, 40s, 50s, and 60s, respectively.

### Laboratory measurements

The test items were as follows: BW (kg), BMI (kg/m$^2$), WC (cm), SBP (mmHg), DBP (mmHg), TG (mg/dL), HDL-C (mg/dL), LDL-C (mg/dL), FPG (mg/dL), HbA1c (%) and UA (mg/dL). The test results are expressed as means±standard deviations.

### Statistical analysis

Three-way analysis of variance (ANOVA) was performed in which time periods, age, and sex were not matched. Cases in which each variable factor was significant were subjected to further analysis including simple main effect tests, and those with significant outcomes were subjected to multiple comparisons using Tukey's HSD.

Statistical analyses were performed using SPSS ver. 23.0 for Windows (IBM Japan, Tokyo, Japan). Significance was set at $p<0.05$.

## Results

The definitions of the time periods and baseline characteristics of the 56,368 participants are shown in **Table 1**. For periods A, B, C, D, and E, there were 11,380, 11,469, 11,463, 10,768, and 11,288 participants, respectively. Mean ages were 50.9±9.1, 50.9±9.1, 51.3±9.1, 51.2±9.0, and 51.5±8.9 years, and the proportion of men was 60%, 59%, 58%, 59%, and 59%, respectively.

Subsequently, for each time period participants were divided into mens and womens and categorized into the following age groups: 30–39, 40–49, 50–59, and 60–69 years old. Graphs of the mean test values are shown in **Fig. 1**, and the data are presented in **Table 2**. We performed an ANOVA including the three factors time period (A, B, C, D, E), age group (30s, 40s, 50s, 60s), and sex (men, women), and then analyzed the

**Table 1. Definition of the Time Periods and the Baseline Characteristics of the Subjects**

| Before the COVID-19 pandemic | | *n* | | Age (years) | Age groups (year) | Men (*n*) | Women (*n*) |
|---|---|---|---|---|---|---|---|
| Period A | March 2017–February 2018 | Overall | 11380 | 50.9±9.1 | 30–39 | 736 | 485 |
| | | | | | 40–49 | 2374 | 1659 |
| | | Men | 6792 | | 50–59 | 2301 | 1525 |
| | | Women | 4588 | | 60–69 | 1381 | 919 |
| Period B | March 2018–February 2019 | Overall | 11469 | 50.9±9.1 | 30–39 | 742 | 501 |
| | | | | | 40–49 | 2362 | 1654 |
| | | Men | 6795 | | 50–59 | 2325 | 1529 |
| | | Women | 4674 | | 60–69 | 1366 | 990 |
| Period C | March 2019–February 2020 | Overall | 11463 | 51.3±9.1 | 30–39 | 661 | 479 |
| | | | | | 40–49 | 2277 | 1693 |
| | | Men | 6672 | | 50–59 | 2275 | 1595 |
| | | Women | 4791 | | 60–69 | 1459 | 1024 |
| During the COVID-19 pandemic | | | | | | | |
| Period D | March 2020–February 2021 | Overall | 10768 | 51.2±9.0 | 30–39 | 621 | 444 |
| | | | | | 40–49 | 2215 | 1547 |
| | | Men | 6371 | | 50–59 | 2198 | 1527 |
| | | Women | 4397 | | 60–69 | 1337 | 879 |
| Period E | March 2021–February 2022 | Overall | 11288 | 51.5±8.9 | 30–39 | 605 | 442 |
| | | | | | 40–49 | 2206 | 1606 |
| | | Men | 6628 | | 50–59 | 2382 | 1638 |
| | | Women | 4660 | | 60–69 | 1435 | 974 |

Variables are given as means ± SD. COVID-19: Coronavirus disease 2019.

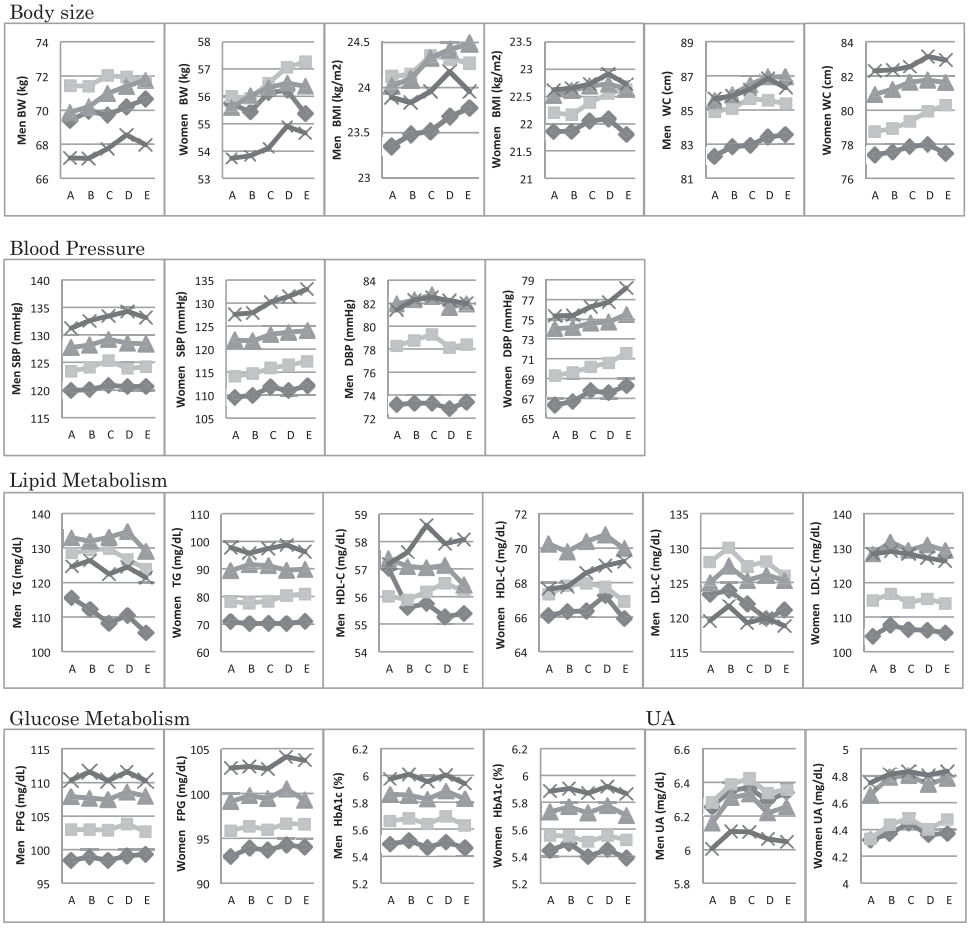


**Fig. 1. Average of Test Results**

Horizontal Axis: Periods (A, B, C, D, E). Lines: Age groups (◆: 30s, ■: 40s, ▲: 50s, ×: 60s).

changes across the time periods.

Significant main effects of time period were found for the variables BW, BMI, WC, SBP, DBP, LDL-C, FPG, HbA1c, and UA. For all data, multiple comparisons were performed to look for significant differences between the five pairs of consecutive periods (A–B, B–C, etc.). Simple interaction effects that included time period were found for SBP (period × age and period × sex) and DBP (period × sex). Simple main-effect comparisons were performed to interpret the interaction. As there were significant differences in each case, multiple comparisons were performed. Conversely, none of the items showed a significant two-way interaction. The effect size $\eta^2$ was calculated, and for each result it was less than 0.01. The results of the three-way ANOVA are presented in a **Supplementary Table**.

The main effect of time period on BW was highly significant ($F(4, 56{,}328)=14.183$, $p<0.001$). Multiple comparisons revealed significant differences ($p=0.001$ and $p=0.036$, respectively).

The main effect of time period on BMI was highly significant ($F(4, 56{,}328)=9.801$, $p<0.001$). Multiple comparisons revealed a significant difference between periods B and C ($p=0.013$).

The main effect of time period on WC was highly significant ($F(4, 56{,}263)=15.999$, $p<0.001$). Multiple comparisons revealed significant differences between periods B–C and C–D (both $p=0.019$).

The main effect of time period on SBP was highly significant ($F(4, 56{,}325)=33.428$, $p<0.001$). Simple interaction effects including time period, were also significant: period × age ($F(12, 56{,}325)=2.523$, $p<0.01$), and period × sex ($F(4, 56{,}325)=9.737$, $p<0.001$). To interpret the interactions, we then conducted simple main effect tests for each period and age and each period and sex. Because all the results were significant ($p<0.001$), multiple comparisons were performed for age and sex in each period, A through E. Significant differences were found between periods A and B, B and C, C and D, C and E, and D and E. Concerning period × age, there was a significant increase in the 60s group from periods A to B ($p=0.018$) and a significant increase in the 30s, 40s, 50s, and 60s groups from periods B to C ($p=0.005$, 0.002, 0.001, and <0.001, respectively). There were no significant differences between periods C and D, C and E, and D and E. Regard-

**Table 2. Characteristics of Study Subjects in Each Periods**

| | Period A | | | Period B | | | Period C | | | Period D | | | Period E | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Men | Women | Overall | Men | Women | Overall | Men | Women | Overall | Men | Women | Overall | Men | Women | Overall |
| BW (kg) | 69.8±10.7 | 55.4±9.7 | 64.0±12.5 | 70.0±11.0 | 55.5±9.7 | 64.1±12.7 | 70.5±11.1 | 55.9±10.0 | 64.4±12.9 | 70.9±11.3 | 56.3±10.2 | 64.9±13.0 | 70.8±11.4 | 56.2±10.3 | 64.8±13.1 |
| BMI (kg/m$^2$) | 24.0±3.3 | 22.4±3.7 | 23.3±3.6 | 24.0±3.4 | 22.4±3.7 | 23.3±3.6 | 24.2±3.5 | 22.5±3.9 | 23.5±3.7 | 24.3±3.5 | 22.6±3.9 | 23.6±3.8 | 24.2±3.5 | 22.6±3.9 | 23.6±3.8 |
| WC (cm) | 85.0±8.8 | 80.0±9.7 | 83.0±9.5 | 85.3±9.1 | 80.3±9.7 | 83.2±9.7 | 85.8±9.2 | 80.6±9.9 | 83.6±9.8 | 86.1±9.4 | 81.0±10.1 | 84.0±10.0 | 86.0±9.4 | 81.0±10.0 | 83.9±10.0 |
| SBP (mmHg) | 126.1±15.0 | 118.9±16.5 | 123.2±16.0 | 126.8±15.2 | 119.3±16.8 | 123.7±16.3 | 128.0±15.1 | 120.9±16.9 | 125.0±16.3 | 127.4±15.5 | 121.4±17.1 | 124.9±16.4 | 127.3±15.5 | 122.4±17.6 | 125.3±16.6 |
| DBP (mmHg) | 79.6±11.9 | 71.8±11.8 | 76.4±12.5 | 80.1±11.9 | 72.0±12.1 | 76.8±12.6 | 80.6±11.7 | 72.7±12.0 | 77.3±12.4 | 79.7±11.8 | 72.9±12.1 | 76.9±12.3 | 80.0±11.8 | 74.0±12.0 | 77.5±12.2 |
| TG (mg/dL) | 127.8±98.7 | 85.0±49.0 | 110.5±85.0 | 127.8±99.6 | 85.2±48.4 | 110.4±85.2 | 127.2±95.5 | 85.9±56.6 | 109.9±84.0 | 127.4±96.0 | 86.1±52.3 | 110.6±83.5 | 123.5±87.7 | 86.3±51.0 | 108.2±77.0 |
| HDL-C (mg/dL) | 56.8±13.7 | 68.2±14.8 | 61.4±15.3 | 56.6±13.5 | 68.3±15.3 | 61.4±15.4 | 56.9±13.8 | 68.7±15.4 | 61.9±15.6 | 56.9±13.7 | 69.0±15.2 | 61.8±15.5 | 56.6±13.7 | 68.4±15.4 | 61.5±15.5 |
| LDL-C (mg/dL) | 124.8±30.2 | 121.0±29.5 | 123.2±29.9 | 126.8±30.4 | 123.3±30.8 | 125.3±30.6 | 124.3±30.1 | 121.5±30.0 | 123.2±30.1 | 124.9±29.7 | 122.2±31.1 | 123.8±30.3 | 123.7±29.9 | 121.2±29.7 | 122.7±29.9 |
| FPG (mg/dL) | 105.7±19.0 | 98.0±13.7 | 102.6±17.5 | 105.9±17.9 | 98.6±13.2 | 102.9±16.6 | 105.6±18.8 | 98.3±13.3 | 102.6±17.1 | 106.6±20.0 | 99.3±13.5 | 103.6±18.0 | 105.9±18.4 | 98.8±12.8 | 103.0±16.7 |
| HbA1c (%) | 5.8±0.7 | 5.7±0.5 | 5.7±0.6 | 5.8±0.7 | 5.7±0.5 | 5.7±0.6 | 5.8±0.7 | 5.6±0.5 | 5.7±0.6 | 5.8±0.7 | 5.7±0.5 | 5.8±0.6 | 5.8±0.7 | 5.6±0.5 | 5.7±0.6 |
| UA (mg/dL) | 6.2±1.2 | 4.5±1.0 | 5.5±1.4 | 6.3±1.2 | 4.6±1.0 | 5.6±1.4 | 6.3±1.2 | 4.7±1.0 | 5.6±1.4 | 6.2±1.2 | 4.6±1.0 | 5.6±1.4 | 6.3±1.2 | 4.6±1.0 | 5.6±1.4 |

Variables are given as mean ± SD.
BW: body weight, BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, UA: uric acid.

ing time period × sex, there was a significant increase in men from period A to B ($p$=0.001), significant increases in both mens and womens between periods B and C ($p$<0.001), significant decreases in men from periods C to D and C to E ($p$=0.007 and 0.01, respectively), significant increases in women from periods C to D and C to E ($p$=0.013 and <0.001, respectively), and a significant increase in women from period D to E ($p$=0.013).

The main effect of time period on DBP was highly significant ($F$(4, 56,325)=13.216, $p$<0.001). The simple interaction between period and sex was also significant ($F$(4, 56,325)=12.721, $p$<0.001). To interpret this interaction, simple main effect tests for period and sex were performed. Because all results were significant ($p$<0.001), multiple comparisons of age and sex were conducted for periods A through E. We found significant differences between periods A and B, B and C, C and D, C and E, and D and E, respectively. There was a significant increase in men from period A to B ($p$=0.013), a significant increase in men and women between periods B and C ($p$=0.033, $p$=0.012), and a significant decrease in men between periods C and D, and C and E ($p$<0.001, $p$=0.002), in contrast to a significant increase in women ($p$=0.013, $p$<0.001) and a significant increase in both men and women from periods D to E ($p$=0.045, $p$=0.001).

There were no significant differences involving time period concerning TG or HDL-C levels.

The main effect of time period on LDL-C level was highly significant ($F$(4, 56,320)=10.403, $p$<0.001). Multiple comparisons showed significant differences between periods A and B, B and C, and D and E ($p$<0.001, $p$<0.001, $p$=0.041).

The main effect of time period on FPG was highly significant ($F$(4, 56,211)=5.199, $p$<0.001). Multiple comparisons revealed significant differences between periods C and D, and D and E ($p$<0.001 and $p$=0.029, respectively).

The main effect of time period on HbA1c was highly significant ($F$(4, 56,321)=16.455, $p$<0.001). Multiple comparisons showed significant differences between periods B and C, C and D, and D and E (all $p$<0.001). The main effect of period on UA was also highly significant ($F$(4, 56,322)=16.611, $p$<0.001), with multiple comparisons revealing significant differences between periods A and B, and C and D ($p$<0.001, $p$=0.001).

## Discussion

### Societal changes and health checkup scores

In this study, we compared health checkup scores across two years of the COVID-19 pandemic and three years before the pandemic by performing a three-way analysis of variance including the factors time period, age, and sex. Differences in SBP and DBP between men

and women were observed during the pandemic, as were changes in glucose metabolism and UA levels. No significant differences were observed between the age groups at this time point. However, some age- and sex-related changes showed different trends, and we predict further significant differences if the COVID-19 pandemic persists.

Our study did not allow us to take account of specific changes in the lifestyle of the health checkup participants. However, we assumed that, during the two years of the pandemic, "new normal lifestyle," emergency situation, quasi-emergency, or other COVID-19-related requests from the government issued to everyone. In the area where our facility is located, a state of emergency, quasi-emergency, or other requests from the government were enacted during periods D and E. During these time periods, the government asked all Japanese citizens to stay at home and work from home (telecommuting), bars and restaurants to comply with the ban on the sale of alcoholic beverages, and other businesses (e.g., fitness clubs and gyms) to shorten their hours. These conditions applied to 56 days in period D and 108 days in period E. We examined the relationship between these social effects and the outcomes of health checkup.

**Specific laboratory measurements**

Body size (BW, BMI, and WC) increased before the pandemic but did not significantly increase during the pandemic. Looking at participant groups, the trend for these three measures was downward for 40-year-old men in period D, 60-year-old men, and 30- and 60-year-old women in period E. Although these changes were not significant, some age groups showed unique trends. Although the result is not evident, it can be estimated that the degree of adjustment to the new normal varies from group to group. Previous studies showed that in Niigata Prefecture, WC and BMI increased from 2017 to 2020[3]; in Slovenia, BW and BMI increased after lockdown compared to February 2020[4]; and in Turkey, BW and BMI increased, but WC did not change in October 2020 compared to before lockdown[5]. We suggest that in this study the three body size measures increased in the first year of the pandemic not due to COVID-19-related changes, but rather as an extension of the pre-existing trend toward weight gain.

The results for SBP and DBP showed a simple interaction effect, characterized as follows: SBP showed significant interactions between period and age, and between period and sex. Further analysis showed that the age difference concerned only periods A and B; that is, there were no differences during the pandemic. By contrast multiple comparisons involving period and sex showed remarkable results. Before the pandemic, hypertension increased in both sexes. While the increase persisted in women during the pandemic, it decreased in men during the same period. It declined in men in their 40s and 50s. Similarly, DBP increased in both men and women before the pandemic (periods A–C), continued increasing during the pandemic in women, but decreasing significantly in men in the first year before increasing again in the second year. A significant decrease was observed in men in their 40s and 50s.

This study revealed that there was a sex difference in blood pressure changes during the pandemic. These effects were particularly strong in middle-aged men. As the pandemic continues, we expect to see further improvements in blood pressure in men and the persistence of increased blood pressure in women. In a report from Niigata, blood pressure increased in both sexes in 2020[3]. In contrast, reports from Italy and France showed that the blood pressure of patients with uncontrolled hypertension decreased during lockdown[6,7]. In this study, blood pressure decreased mainly in men, which is different from previous studies and may be due to regional differences. Furthermore, it may be useful to compare the results for each health checkup classification. It is well known that blood pressure increases with stress, examples being high blood pressure in the presence of white coat professionals and increased blood pressure immediately after the Great East Japan Earthquake[8]. By contrast, in the present context, it could be suggested that decreases in frequency of restaurant visits and in stress at work contributed in part. Comparing these results with stress monitoring conducted in the workplace might provide useful further information.

TG, HDL-C, and LDL-C levels showed no significant trends. Some sex and age groups showed opposite changes, as in previous reports[3–5].

FPG and HbA1c levels showed similar movements in all groups. There are reports from Italy that the lockdown did not affect FPG levels in type 1 or type 2 DM[9,10]. In our study, although significant differences were noted for each year, the increases and decreases alternated year by year, making it challenging to determine whether and how the pandemic affected either parameter.

UA levels showed tended to increase from periods A to C, but decreased significantly from periods C to D. In particular, the decline, that is, the improvement in men in their 50s, was greater than that in other groups. However, this improvement could be transient, because there was an increase between periods D and E, albeit a non-significant one. In our study, only UA levels improved in period D, whereas BW, BMI, WC, and other blood test results did not. Although there are reports that BMI and TG decrease in parallel with UA decreases[11] or UA level after weight loss[12], our study showed an

improvement in UA value without weight loss, which is unique in this pandemic situation.

### Societal changes, blood pressure and a uric acid value

The pandemic has significantly affected the food industry. Sales decreased by 15%[13], and food industry spending decreased by 28%[14] compared with FY2019. In particular, the decline in sales in "pub restaurants and taverns" in 2021 was significant. According to the "National Health and Nutrition Survey" in 2019, more men aged 30 to 60 years than women answered "frequently eat out" to the question "What disturbs healthy eating habits?" and the percentage of "frequently eating out" was high among younger men[15]. Not eating out reportedly greatly impacts these groups[16]. Reports show that the more frequently you eat out, the fewer vegetables you eat[17], and the more frequently you eat out, the more energy you consume daily[18]. According to a report on the lifestyle of male industrial workers, frequent eating out could be a risk factor for hyperuricemia[19].

The fitness industry experienced a 34% decrease in sales and a 25% decrease in users in 2021 compared to 2019[20]. Our lifestyles have also changed because of the pandemic. For example, the proportion of remote work increased to 32.2%, time spent on housework and childcare increased by 38% for men and 43.9% for women[21], and time spent on physical activity decreased[22,23]. In the report that examined the "Specific Health Examination Questionnaire that tested health performance and lifestyle" during the pandemic, the number of people "with adequate sleep time" increased, and that of "people having dinner within two hours before going to bed" decreased in men, while "drinking habits," "drinking amount," and "snack amount" showed no significant changes during the pandemic[3]. Based on these societal changes and the results of this study, we hypothesized that the reduced opportunities to eat out due to shortened restaurant hours and not eating out affected men's health. As a result, blood pressure and UA levels decreased.

### Gender gap

Although the situation has improved in recent years, there is still a gender gap in the Japanese workplace. According to the results of the Ministry of Internal Affairs and Communications' Survey on Time Use and Leisure Activities (2016), the daily time spent at home is 44 minutes for men, compared to 3 hours and 28 minutes for women[24]. In terms of exercise and sports activities, it was reported that women are more likely to be non-exercisers than men. In addition, women living with children are both positively and negatively affected by the pandemic[25]. Therefore, there is a concern that women will spend more time on housework if their families stay home longer because of remote work and the closure of nursery schools, kindergartens, and schools.

### Prospects for this study

The effect sizes were calculated; however, they were very small. There are two possible reasons for this. First, this study was not an active intervention study, and second, the overall health effects of COVID-19 are not currently large enough to show up in the statistics. Although there are currently no significant differences, some age and sex groups show different trends. These groups could see significant changes in the future if the pandemic continues for a long time.

During this pandemic, people have not been going out, and they have been limiting their contact with others, which has resulted in more "personal time". The question is how that time is used. People are also asked to self-manage their health even more actively. To adapt to such a new environment with a view to maintaining health, healthcare providers need to know the trends in test results and advise individuals on how best to maintain their health.

### Limitations

This study had the following limitations: 1) It was a retrospective observational study, and no causal relationship between social changes and test results has been shown. 2) The participants' social background and actual life changes were unknown, and the analysis did not account for confounding factors. 3) The results were obtained at a single institution, and there was a regional selection bias. 4) Age-related changes were not considered. More large-scale, multi-facility prospective studies are needed. According to the abstract collection of the 62nd Annual Scientific Meeting of the Japan Society of Ningen Dock, there were 14 reports of general presentations on "WITH CORONA" and health checkup results[26]. An analysis that incorporates these reports is also required.

## Conclusions

With the onset of a new lifestyle during the pandemic, blood pressure, which had been rising over the previous three years, continued to rise in women whereas it fell in men. UA levels, which previously showed an increasing trend, showed a slightly decreasing trend in both sexes. Glucose metabolism, which had previously increased or decreased every two years, worsened in the first year of the pandemic and improved in the second year. Weight gain occurred before the pandemic. The changes in laboratory results before and during the pandemic were overall unremarkable. Our findings indicate that the impact of the COVID-19 pandemic on health outcomes is not statistically significant at this time point.

## Conflict of interest

The authors have no conflict of interest to declare.

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**Supplementary Table. Results of Three-way Analysis of Variable**

| Variable: BW | | | | | | |
|---|---|---|---|---|---|---|
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 6352.744 | 4 | 1588.186 | 14.183 | <0.001*** | <0.01 |
| Age Group | 73957.188 | 3 | 24652.396 | 220.159 | <0.001*** | <0.01 |
| Sex | 2198949.129 | 1 | 2198949.129 | 19637.818 | <0.001*** | <0.01 |
| Period/Age Group | 900.498 | 12 | 75.041 | 0.670 | 0.782 | <0.01 |
| Period/Sex | 172.913 | 4 | 43.228 | 0.386 | 0.819 | <0.01 |
| Age Group/Sex | 5187.400 | 3 | 1729.133 | 15.442 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 2348.426 | 12 | 195.702 | 1.748 | 0.050811 | <0.01 |
| error | 6307340.452 | 56328 | 111.975 | | | |
| Overall | 243306678.610 | 56368 | | | | |
| Variable: WC | | | | | | |
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 5640.045 | 4 | 1410.011 | 15.999 | <0.001*** | <0.01 |
| Age Group | 83278.545 | 3 | 27759.515 | 314.980 | <0.001*** | <0.01 |
| Sex | 255970.616 | 1 | 255970.616 | 2904.433 | <0.001*** | <0.01 |
| Period/Age Group | 393.905 | 12 | 32.825 | 0.372 | 0.973 | <0.01 |
| Period/Sex | 69.931 | 4 | 17.483 | 0.198 | 0.939 | <0.01 |
| Age Group/Sex | 10142.242 | 3 | 3380.747 | 38.360 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 1558.471 | 12 | 129.873 | 1.474 | 0.126 | <0.01 |
| error | 4958514.431 | 56263 | 88.131 | | | |
| Overall | 398554451.080 | 56303 | | | | |
| Variable: DBP | | | | | | |
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 7029.141 | 4 | 1757.285 | 13.216 | <0.001*** | <0.01 |
| Age Group | 443846.341 | 3 | 147948.780 | 1112.648 | <0.001*** | <0.01 |
| Sex | 500330.787 | 1 | 500330.787 | 3762.734 | <0.001*** | <0.01 |
| Period/Age Group | 1116.969 | 12 | 93.081 | 0.700 | 0.753 | <0.01 |
| Period/Sex | 6766.023 | 4 | 1691.506 | 12.721 | <0.001*** | <0.01 |
| Age Group/Sex | 15050.775 | 3 | 5016.925 | 37.730 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 931.806 | 12 | 77.650 | 0.584 | 0.857 | <0.01 |
| error | 7489535.916 | 56325 | 132.970 | | | |
| Overall | 342900481.000 | 56365 | | | | |
| Variable: HDL-C | | | | | | |
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 1413.823 | 4 | 353.456 | 1.730 | 0.140 | <0.01 |
| Age Group | 45604.433 | 3 | 15201.478 | 74.385 | <0.001*** | <0.01 |
| Sex | 1391777.216 | 1 | 1391777.216 | 6810.382 | <0.001*** | <0.01 |
| Period/Age Group | 3548.131 | 12 | 295.678 | 1.447 | 0.137 | <0.01 |
| Period/Sex | 1168.972 | 4 | 292.243 | 1.430 | 0.221 | <0.01 |
| Age Group/Sex | 16432.274 | 3 | 5477.425 | 26.803 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 2680.167 | 12 | 223.347 | 1.093 | 0.361 | <0.01 |
| error | 11509618.391 | 56320 | 204.361 | | | |
| Overall | 227244674.000 | 56360 | | | | |

| Variable: BMI | | | | | | |
|---|---|---|---|---|---|---|
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 507.410 | 4 | 126.853 | 9.801 | <0.001*** | <0.01 |
| Age Group | 2155.048 | 3 | 718.349 | 55.499 | <0.001*** | <0.01 |
| Sex | 26750.159 | | 26750.159 | 2066.710 | <0.001*** | <0.01 |
| Period/Age Group | 72.896 | 12 | 6.075 | 0.469 | 0.933 | <0.01 |
| Period/Sex | 18.868 | 4 | 4.717 | 0.364 | 0.834 | <0.01 |
| Age Group/Sex | 698.617 | 3 | 232.872 | 17.992 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 197.628 | 12 | 16.469 | 1.272 | 0.227 | <0.01 |
| error | 729073.159 | 56328 | 12.943 | | | |
| Overall | 31781492.770 | 56368 | | | | |
| Variable: SBP | | | | | | |
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 30941.658 | 4 | 7735.414 | 33.428 | <0.001*** | <0.01 |
| Age Group | 1378346.895 | 3 | 459448.965 | 1985.469 | <0.001*** | <0.01 |
| Sex | 471180.243 | 1 | 471180.243 | 2036.165 | <0.001*** | <0.01 |
| Period/Age Group | 7004.873 | 12 | 583.739 | 2.523 | <0.01** | <0.01 |
| Period/Sex | 9013.227 | 4 | 2253.307 | 9.737 | <0.001*** | <0.01 |
| Age Group/Sex | 74000.009 | 3 | 24666.670 | 106.595 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 2709.421 | 12 | 225.785 | 0.976 | 0.469 | <0.01 |
| error | 13033928.868 | 56325 | 231.406 | | | |
| Overall | 887647036.000 | 56365 | | | | |
| Variable: TG | | | | | | |
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 31568.942 | 4 | 7892.236 | 1.227 | 0.297 | <0.01 |
| Age Group | 2221762.131 | 3 | 740587.377 | 115.134 | <0.001*** | <0.01 |
| Sex | 16403601.855 | 1 | 16403601.855 | 2550.145 | <0.001*** | 0.02 |
| Period/Age Group | 19216.308 | 12 | 1601.359 | 0.249 | 0.996 | <0.01 |
| Period/Sex | 48748.523 | 4 | 12187.131 | 1.895 | 0.108 | <0.01 |
| Age Group/Sex | 858162.596 | 3 | 286054.199 | 44.471 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 51966.677 | 12 | 4330.556 | 0.673 | 0.779 | <0.01 |
| error | 361559816.586 | 56209 | 6432.419 | | | |
| Overall | 1067249298.000 | 56249 | | | | |
| Variable: LDL-C | | | | | | |
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 36301.454 | 4 | 9075.363 | 10.403 | <0.001*** | <0.01 |
| Age Group | 929820.497 | 3 | 309940.166 | 355.267 | <0.001*** | <0.01 |
| Sex | 183005.990 | 1 | 183005.990 | 209.770 | <0.001*** | <0.01 |
| Period/Age Group | 9285.016 | 12 | 773.751 | 0.887 | 0.560 | <0.01 |
| Period/Sex | 2424.696 | 4 | 606.174 | 0.695 | 0.595 | <0.01 |
| Age Group/Sex | 1269805.282 | 3 | 423268.427 | 485.169 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 6555.002 | 12 | 546.250 | 0.626 | 0.822 | <0.01 |
| error | 49134379.321 | 56320 | 872.414 | | | |
| Overall | 912844477.000 | 56360 | | | | |

| Variable: FPG | | | | | | |
|---|---|---|---|---|---|---|
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 5623.062 | 4 | 1405.765 | 5.199 | <0.001*** | <0.01 |
| Age Group | 623687.938 | 3 | 207895.979 | 768.839 | <0.001*** | <0.01 |
| Sex | 506043.735 | 1 | 506043.735 | 1871.446 | <0.001*** | <0.01 |
| Period/Age Group | 859.919 | 12 | 71.660 | 0.265 | 0.994 | <0.01 |
| Period/Sex | 398.675 | 4 | 99.669 | 0.369 | 0.831 | <0.01 |
| Age Group/Sex | 12956.500 | 3 | 4318.833 | 15.972 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 2030.173 | 12 | 169.181 | 0.626 | 0.822 | <0.01 |
| error | 15199600.606 | 56211 | 270.403 | | | |
| Overall | 612565266.000 | 56251 | | | | |

| Variable: UA | | | | | | |
|---|---|---|---|---|---|---|
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 84.096 | 4 | 21.024 | 16.611 | <0.001*** | <0.01 |
| Age Group | 166.401 | 3 | 55.467 | 43.824 | <0.001*** | <0.01 |
| Sex | 29325.085 | 1 | 29325.085 | 23169.520 | <0.001*** | 0.02 |
| Period/Age Group | 6.814 | 12 | 0.568 | 0.449 | 0.944 | <0.01 |
| Period/Sex | 3.325 | 4 | 0.831 | 0.657 | 0.622 | <0.01 |
| Age Group/Sex | 1034.406 | 3 | 344.802 | 272.425 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 2.620 | 12 | 0.218 | 0.173 | 0.999 | <0.01 |
| error | 71285.354 | 56322 | 1.266 | | | |
| Overall | 1865704.880 | 56362 | | | | |

| Variable: HbA 1c | | | | | | |
|---|---|---|---|---|---|---|
| Source | SS | df | MS | *F* | *p* | $\eta^2$ |
| Period | 23.000 | 4 | 5.750 | 16.455 | <0.001*** | <0.01 |
| Age Group | 1253.292 | 3 | 417.764 | 1195.550 | <0.001*** | <0.01 |
| Sex | 94.022 | 1 | 94.022 | 269.070 | <0.001*** | <0.01 |
| Period/Age Group | 1.606 | 12 | 0.134 | 0.383 | 0.970 | <0.01 |
| Period/Sex | .266 | 4 | 0.067 | 0.190 | 0.944 | <0.01 |
| Age Group/Sex | 6.278 | 3 | 2.093 | 5.989 | <0.001*** | <0.01 |
| Period/Age Group/Sex | 2.823 | 12 | 0.235 | 0.673 | 0.779 | <0.01 |
| error | 19680.388 | 56321 | 0.349 | | | |
| Overall | 1871878.660 | 56361 | | | | |

SS: sum of square, df: degree of freedom, MS: mean square. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. $\eta^2$: effect size (0.01: small, 0.06: medium, 0.14: large).

Original Article

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# Quality Assessment of a Review by the Quality Evaluation for Ningen Dock and Health Screening Institute for Diagnosing Auscultation in Patients with Atrial Fibrillation

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## Abstract

**Objective:** To evaluate the quality of a review by the Quality Evaluation for Ningen Dock and Health Screening Institute (QENDI), approved by the Japan Society of Ningen Dock, using examination findings.

**Methods:** We investigated the difference between QENDI and non-QENDI-reviewed hospitals affiliated with the same corporation using the rate at which an irregular heartbeat was indicated on auscultation as an index in patients with atrial fibrillation, a condition that is characterized by an irregular heartbeat.

**Results:** The indication rate in the QENDI-reviewed hospital was 88.0%, which was significantly higher than that in the non-QENDI-reviewed hospital (45.5%) ($p<0.05$). The rate at which an irregular heartbeat was indicated by non-Health Evaluation and Promotion Specialists (non-HEPSs) in the QENDI-reviewed hospital was 87.2%. This rate did not differ from that indicated by HEPSs (88.6%).

**Conclusion:** Diagnostic accuracy may depend more on certification by QENDI than examination by a HEPS. Thus, a review by QENDI may improve the standard of Ningen Dock institutes and the quality of clinical practice through continuing education.

**Keywords** atrial fibrillation, Quality Evaluation for Ningen Dock and Health Screening Institute, Ningen Dock, Health Evaluation and Promotion Specialist

Atrial fibrillation, also known as absolute arrhythmia, is characterized by an irregular heartbeat on auscultation. Atrial fibrillation which leads to cerebral thrombosis is one of the most important types of arrhythmia in clinical settings. We previously examined the auscultation findings of 25 physicians in 514 patients diagnosed with atrial fibrillation based on electrocardiography during Ningen Dock conducted over a period of 9 years[1,2] and found that auscultation showed irregular heartbeat in 400 patients (77.8%). The proportion of patients diagnosed with irregular heartbeat by eight Ningen Dock specialists was 89.5%, which was significantly higher than that by non-specialists (67.9%, $p<0.001$).

The "Quality Evaluation for Ningen Dock and Health Screening Institute (QENDI)" system was approved by the Japan Society of Ningen Dock on September 1, 1994[2] to enhance the calibre of comprehensive health checkup (Ningen Dock) institutes. While various standards exist for Ningen Dock institutes, review by the QENDI system is expected to help improve the performance of these facilities. The review is performed by a third party from the perspective of the overall operation and management of the organization as well as the Ningen Dock services provided. The institutes reviewed must perform more than 500 examinations per year on examinees undergoing the basic physical examination items recommended by the Japan Society of Ningen Dock, have a full-time doctor, and fully understanding the purpose of the guidelines and philosophy of the QENDI system.

Here, we investigated the rate at which an irregular heartbeat was indicated with respect to the presence or absence of a QENDI review. No study has examined whether a QENDI review guarantees quality at a Ningen Dock institute.

## Methods

A total of 25 physicians were responsible for conducting Ningen Dock between 2010 and 2018. The experience and specialty of these physicians are summarized in **Table 1**. To avoid identification of the physicians, the

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**Table 1. Specialty and Years of Experience of the 25 Doctors in the Present Study**

| Doctor | Specialty | Years of experience | Full-time or part-time |
|---|---|---|---|
| A | gastroenterologist | 15–19 | full-time |
| B | cardiologist | 25–29 | part-time |
| C | cardiologist | 25–29 | full-time |
| D | internist | 15–19 | full-time |
| E | internist | 20–24 | part-time |
| F | gastroenterologist | 20–24 | part-time |
| G | gastroenterologist | 5–9 | full-time |
| H | gastroenterologist | 10–14 | full-time |
| I | surgeon | 10–14 | full-time |
| J | cardiologist | 25–29 | full-time |
| K | internist | 35–39 | full-time/part-time |
| L | gastroenterologist | 20–24 | full-time |
| M | gastroenterologist | 1–4 | part-time |
| N | gastroenterologist | 20–24 | full-time |
| O | gastroenterologist | 25–29 | full-time |
| P | gastroenterologist | 15–19 | full-time |
| Q | internist | 10–14 | full-time |
| R | gastroenterologist | 20–24 | full-time |
| S | internist | 10–14 | full-time |
| T | cardiologist | 20–24 | full-time |
| U | internist | 30–34 | full-time |
| V | internist | 20–24 | full-time |
| W | gastroenterologist | 25–29 | full-time |
| X | gastroenterologist | 25–29 | part-time |
| Y | gastroenterologist | 5–9 | part-time |

25 doctors are labeled from A to Y.

We extracted patients with irregular heartbeats detected on auscultation by the physicians. The background and profiles of these patients were described in a previous study[3]. Of 86,030 patients (57,936 male, 28,094 female) aged ≥30 years who underwent Ningen Dock at a QENDI and a non-QENDI-reviewed hospital, both of which are affiliated with Hospital A, over nine years (from 2010 to 2018), 514 were diagnosed with atrial fibrillation based on electrocardiography findings. Atrial fibrillation was diagnosed by an automatic diagnostic function built into the electrocardiograph, and confirmed by a clinician. Medical examinations, including auscultation, were conducted within one hour after electrocardiography in the morning. Details are described elsewhere[1].

We investigated the rate at which an irregular heartbeat was indicated on auscultation in patients with atrial fibrillation in the QENDI and non-QENDI-reviewed hospital. In addition, we examined the rate at which an irregular heartbeat was indicated by a non-Health Evaluation and Promotion Specialist (HEPS)[4] in the QENDI and non-QENDI-reviewed hospitals. The assignment of physicians to the QENDI and non-QENDI-reviewed hospitals was determined by Hospital A.

Differences were analyzed using a proportion-based significance test. Statistical significance was indicated by $p<0.05$.

This study was conducted in compliance with the Declaration of Helsinki and was approved by the institutional board of Jikei University School of Medicine (approval no. 17-015). The results of this study have not been reported previously.

## Results

The 25 physicians evaluated the heartbeats of 400 (77.8%) of the 514 patients with atrial fibrillation as irregular on auscultation. Of the 391 patients with atrial fibrillation in the QENDI-reviewed hospital, 344 (88.0%) had an irregular heartbeat on auscultation. Of the 123 patients with atrial fibrillation in the non-QENDI-reviewed hospital, 56 (45.5%) had an irregular heartbeat on auscultation. The percentage of patients with irregular heartbeat on auscultation was significantly lower in the non-QENDI-reviewed hospital than in the QENDI-reviewed hospital ($p<0.05$) (**Table 2**).

During the survey period, 20 physicians belonged to the QENDI-reviewed hospital. Of these, 8 were HEPSs and 12 were non-HEPSs. In the non-QENDI-reviewed hospital, all 5 physicians were non-HEPSs.

Among the non-HEPSs, 12 physicians working for the QENDI-reviewed hospital diagnosed an irregular heartbeat on auscultation in 150 (87.2%) of 172 patients. This percentage was significantly higher than by the non-HEPSs working for the non-QENDI-reviewed hospital (45.5%) ($p<0.05$). There was no statistically significant difference between the rate of diagnosis of irregular heartbeat on auscultation between HEPSs and

**Table 2. Diagnosis of Irregular Heartbeat on Auscultation in a QENDI and Non-QENDI-reviewed Hospital by HEPSs and Non-HEPSs**

| | QENDI-reviewed hospital | | | | Non-QENDI-reviewed hospital | | | |
|---|---|---|---|---|---|---|---|---|
| | Doctor | AF cases | Irregular HB by auscultation | Rate of diagnosis (%) | Doctor | AF cases | Irregular HB by auscultation | Rate of diagnosis (%) |
| HEPS | 1 | 25 | 25 | 100.0 | | | | |
| | 2 | 7 | 7 | 100.0 | | | | |
| | 3 | 52 | 50 | 96.2 | | | | |
| | 4 | 11 | 10 | 90.9 | | | | |
| | 5 | 46 | 39 | 84.8 | | | | |
| | 6 | 13 | 11 | 84.6 | | | | |
| | 7 | 41 | 33 | 80.5 | | | | |
| | 8 | 24 | 19 | 79.2 | | | | |
| non-HEPS | 1 | 49 | 49 | 100.0 | 1 | 37 | 26 | 70.3 |
| | 2 | 7 | 7 | 100.0 | 2 | 30 | 20 | 66.7 |
| | 3 | 4 | 4 | 100.0 | 3 | 17 | 8 | 47.1 |
| | 4 | 1 | 1 | 100.0 | 4 | 37 | 2 | 5.4 |
| | 5 | 28 | 27 | 96.4 | 5 | 2 | 0 | 0.0 |
| | 6 | 12 | 11 | 91.7 | | | | |
| | 7 | 6 | 5 | 83.3 | | | | |
| | 8 | 9 | 7 | 77.8 | | | | |
| | 9 | 20 | 15 | 75.0 | | | | |
| | 10 | 24 | 17 | 70.8 | | | | |
| | 11 | 10 | 7 | 70.0 | | | | |
| | 12 | 2 | 0 | 0.0 | | | | |
| Total | | 391 | 344 | 88.0 | | 123 | 56 | 45.5 |

QENDI: Quality Evaluation for Ningen Dock and Health Screening Institute, HEPS: Health Evaluation and Promotion Specialist, AF: atrial fibrillation, HB: heart beat

non-HEPSs in the QENDI-reviewed hospital. Given that there were no HEPSs in the non-QENDI-reviewed hospital, we were unable to investigate potential differences in the indication rate by HEPSs between the QENDI and non-QENDI-reviewed hospital.

## Discussion

The purpose of this study was to evaluate the quality of the QENDI system, which is approved by the Japan Society of Ningen Dock, using examination findings. In patients with atrial fibrillation, which is characterized by an irregular heartbeat, we investigated differences in the rate at which an irregular heartbeat was indicated on auscultation between a QENDI and non-QENDI-reviewed hospital, both of which were affiliated with the same corporation. We found that the indication rate in the QENDI-reviewed hospital (88.0%) was significantly higher than that in the non-QENDI-reviewed hospital (45.5%).

The QENDI system aims "to improve the quality of Ningen Dock facilities and promote improvement activities, allowing examinees to have a medical check-up without anxiety", and includes a continuing education system. The evaluation is performed in two steps of writing and visiting, before an evaluation group makes a decision based on the established evaluation standard. The Japan Society of Ningen Dock and Japan Hospital Association subsequently offers the certification. Certified facilities are additionally appointed facilities for training HEPSs. Since 2009, the Japan Society of Ningen Dock has trained Board Certified physicians to become specialists, thus sustainably ensuring the quality of the service provided by HEPSs and their trust by society through lifelong education.

We compared the rate at which an irregular heartbeat was indicated by non-HEPSs and HEPs within and between the QENDI and non-QENDI-reviewed hospitals. In the QENDI-reviewed hospital, 12 non-HEPSs diagnosed an irregular heartbeat in 87.2% of patients. This indication rate was not significantly different from that by HEPSs at the same institute (88.6%). In contrast, in the non-QENDI-reviewed hospital, where all 5 physicians were non-HEPSs, the indication rate was 45.5%, which was significantly lower than that by non-HEPs in the QENDI-reviewed hospital ($p<0.05$). Because QENDI-reviewed hospitals are required to have a system to explain the results of a physician's examination on the day of the checkup, the physician may be more likely to notice a relationship between the electrocardiogram and auscultatory findings.

Reapplication for a review by QENDI is required every 5 years. To obtain a HEPS certification, a physician must pass only one exam that tests their knowledge of preventive medical health. In the present study, we noted the following differences between the QENDI and non-QENDI-reviewed hospitals: compared to the non-QENDI-reviewed facility, the QENDI-reviewed hospital had a system to explain the results of the examina-

tion by the doctor on the day of the checkup, held daily conferences in the facility, and had a director who was a HEPS. The system may thus improve the quality of the hospital, including physicians' auscultation skills.

Atrial fibrillation is also known as absolute arrhythmia, and is characterized by an irregular heartbeat. However, in some patients, the heart rate interval can be almost regular and the heart rate is faster than that in others. As a consequence, irregular heartbeat can be difficult to diagnose in many patients. Thus, the detection rate of irregular heartbeat is rarely 100%. The highest detection rate obtained in this study was 88.0%, which may be an upper limit.

A limitation of this study is that the evaluation was conducted in a single QENDI-reviewed hospital and single non-QENDI-reviewed hospital. However, that the two institutes were managed by the same hospital is significant. Additionally, we only used the rate at which an irregular heartbeat was indicated as an index for assessing the quality of Ningen Dock at the two hospitals. It is possible that even in the event that no irregular heartbeat is detected on auscultation, Ningen Dock may still be advantageous because electrocardiography is performed. In the future, more indices should be used to evaluate the quality of an institute.

## Conclusions

We investigated the quality of the QENDI system, which is approved by the Japan Society of Ningen Dock, based on auscultation findings in patients with atrial fibrillation. The detection rate of irregular heartbeat on cardiac auscultation in the QENDI-reviewed hospital was significantly higher than that in the non-QENDI-reviewed hospital. There was no statistically significant difference in the rate of diagnosis between HEPSs and non-HEPSs by auscultation in the QENDI-reviewed hospital. Among non-HEPSs, the rate of diagnosis was higher in the QENDI-reviewed hospital than in the non-QENDI-reviewed hospital. Thus, a review by QENDI may improve the standard of Ningen Dock institutes and the quality of clinical practice through continuing education.

## Acknowledgments

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## Conflict of Interest

The authors declare no conflicts of interest.

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# Association Between Colorectal Adenoma and Lifestyle-related Diseases

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## Abstract

**Objective:** To elucidate the relationship between colorectal adenoma and lifestyle-related diseases.
**Methods:** We enrolled 227 individuals who underwent a complete medical checkup at Tokyo Women's Medical University between June 2016 and December 2017. Risk factors related to the development of colorectal adenoma were analyzed by contingency tables using multivariate logistic regression. Statistical significance was set at $p<0.05$.
**Results:** Colorectal adenoma was significantly related to metabolic syndrome that comprises three components, namely central obesity with hypertension, dyslipidemia, and impaired fasting glucose.
**Conclusions:** The presence of the three components of metabolic syndrome, hypertension, dyslipidemia, and impaired fasting glucose, significantly increases the risk of colorectal adenoma. Therefore, in addition to medication and lifestyle improvements (e.g., diet, exercise therapy), participants with metabolic syndrome with these three components should undergo screening for colorectal adenoma.

**Keywords** medical checkup, colorectal adenoma, metabolic syndrome

The incidence and mortality rate of colorectal cancer (CRC) has increased in Japan in recent years. In fact, in 2021, CRC was the second leading cause of mortality among malignant neoplasms in Japan. Moreover, in 2019, the colon and rectum were the most common sites of malignant neoplasm among Japanese patients[1].

To decrease the mortality rate of CRC, early detection and treatment are needed. Lifestyle habits and metabolic factors are reported risk factors of CRC[2–5]. The risk of developing CRC increases in patients aged ≥40 years. Further, older age is associated with a higher risk of developing CRC[6].

Given that colorectal adenomatous polyps (adenomas) are precursor lesions for the vast majority of colorectal cancers[7,8], the present study aimed to analyze whether the development of colorectal adenoma (CRA) is related to obesity, lifestyle-related diseases and habits. We hope our findings will improve the ability to identify which patients should be recommended to undergo colonoscopy for early diagnosis of CRA.

## Methods

### Study design

This retrospective cohort study was performed in accordance with the principles stipulated in the Declaration of Helsinki and approved by the Ethics Committee of Tokyo Women's Medical University (approval date: March 16, 2022; approval number: 2021-0209). The study cohort comprised individuals who underwent a complete medical checkup at Tokyo Women's Medical University (Japan) between June 2016 and December 2017. Specifically, we included those who underwent a periodic checkup as part of the "Ningen Dock," a Japanese health checkup system. In this study, we included participants who had previously undergone colonoscopy or polypectomy. We excluded those with inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis) and either hepatitis C or hepatitis B infection.

### Services delivered as part of the Ningen Dock

The periodic Ningen Dock health checkup program is comprehensive. It includes the following assessments: physical characteristics (height, body weight, and waist circumference), complete blood count, blood biochem-

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istry, urinalysis, electrocardiography, abdominal ultrasonography, upper gastrointestinal tract barium meal or endoscopic examination, colonoscopy, visual acuity testing, tonometry, fundic examination (retinal photography), and hearing assessment. Details of participants' medical history and alcohol and tobacco consumption were obtained by interview with a doctor.

### Insulin resistance

Insulin resistance was determined using the homeostasis model assessment-insulin resistance (HOMA-IR) score, which was calculated as follows: [fasting glucose (mg/dL) × fasting insulin (μU/mL)]/405. A score ≥2.5 was used to indicate insulin resistance.

### Risk factors

We included the following potential risk factors in the analysis: alcohol abuse, smoking, metabolic syndrome (MetS), hyperuricemia, impaired renal function and nonalcoholic fatty liver disease (NAFLD). These factors were defined as follows: alcohol abuse (>20 g ethanol/day), smoking (Brinkman Index ≥400) and MetS (diagnosed using the 2005 guidelines of the Evaluation Committee on Diagnostic Criteria for Metabolic Syndrome of Japan)[9]. MetS diagnosis requires the presence of central obesity and at least two of the following three medical conditions: hypertension (including prior treatment for hypertension), dyslipidemia (including prior treatment for dyslipidemia) and impaired fasting glucose (IFG) (including prior treatment for diabetes mellitus). Central obesity was defined as a waist circumference ≥85 cm for men and ≥90 cm for women. Hypertension was defined as a systolic blood pressure ≥130 mmHg, and/or diastolic blood pressure ≥85 mmHg. Dyslipidemia was defined as a serum triglyceride level ≥150 mg/dL and/or high-density lipoprotein cholesterol (HDL-C) level <40 mg/dL. IFG was defined as a glucose level ≥110 mg/dL. Hyperuricemia was defined as a uric acid level >7 mg/dL and/or current drug treatment for hyperuricemia. Impaired renal function was defined as an estimated glomerular filtration rate <60 mL/min/1.73 $m^2$. NAFLD was defined as the presence of a fatty liver (FL) in the absence of either hepatitis C or hepatitis B infection, and without alcohol consumption of >20 g ethanol/day. FL was diagnosed using abdominal ultrasonography if any of high hepatorenal echo contrast, liver brightness, or deep attenuation was present.

### Statistical analysis

Statistical analysis was performed using IBM SPSS version 28.0 (IBM Corp., Armonk, NY). Continuous variables are expressed as mean (standard deviation) per group. Statistical differences were determined using two-sided Student's *t*-test (for equal variance) or Welch's *t*-test (for unequal variance). Non-normally distributed variables were compared using the Mann–Whitney *U* test. Variables reported as proportions were compared using the chi-squared test. The relationships between risk factors and CRA were examined using multivariate logistic regression analysis and reported as odds ratios (ORs). A $p$ value <0.05 was considered statistically significant. Inclusion of variables in the models was based on existing knowledge of risk factors for CRA and hyperuricemia, impaired renal function, and NAFLD. The variables considered in the models were age; sex; MetS comprising the three components hypertension (including prior treatment for hypertension), dyslipidemia (including prior treatment for dyslipidemia), and impaired fasting glucose (IFG) (including prior treatment for diabetes mellitus); HOMA-IR; hyperuricemia; impaired renal function; NAFLD; alcohol abuse; and smoking.

## Results

### Study population

We included 227 participants. Of these, 167 were men aged 62.5 (standard deviation 11.2) years and 60 were women aged 62.2 (standard deviation 10.1) years. CRA

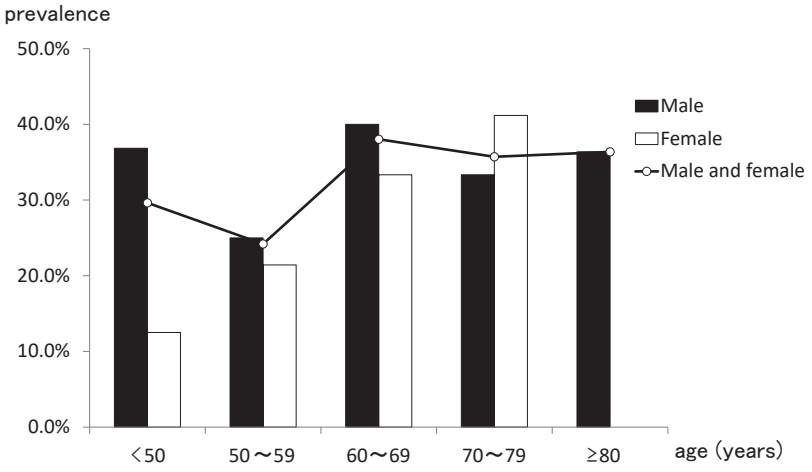


**Fig. 1. Age- and Sex-specific Proportion of Individuals with Colorectal Adenoma**

was detected in 74 (32.6%) participants. Overall, 33.5% of men and 30.0% of women participants had CRA.

**Fig. 1** shows the age- and sex-specific rates of CRA. The prevalence of CRA in women tended to increase gradually with age.

**Table 1. Clinical Data of Participants With or Without Colorectal Adenoma**

| Characteristic | Subjects with CRA | Subjects without CRA | *p* value |
|---|---|---|---|
| | Mean (Standard deviation) [Number] | | |
| Age | 63.6 (11.0) [74] | 61.8 (10.9) [153] | 0.251 |
| Sex, male/female | [56/18] | [111/42] | 0.618 |
| Systolic blood pressure (mmHg) | 126.2 (17.3) [74] | 125.0 (16.9) [153] | 0.615 |
| Diastolic blood pressure (mmHg) | 77.9 (10.5) [74] | 76.9 (12.3) [153] | 0.548 |
| Total cholesterol (mg/dL) | 207.0 (39.4) [74] | 207.1 (35.5) [153] | 0.989 |
| LDL cholesterol (mg/dL) | 119.8 (31.3) [74] | 121.4 (30.5) [153] | 0.722 |
| HDL cholesterol (mg/dL) | 64.2 (20.7) [74] | 63.2 (18.2) [153] | 0.718 |
| Triglycerides (mg/dL) | 125.3 (89.5) [74] | 121.8 (72.8) [153] | 0.755 |
| Uric acid (mg/dL) | 5.8 (1.3) [74] | 5.8 (1.3) [153] | 0.654 |
| FPG (mg/dL) | 107.7 (17.0) [74] | 103.9 (13.2) [153] | 0.093 |
| HbA1c (%) | 6.0 (0.5) [74] | 6.0 (0.6) [153] | 0.852 |
| Fasting insulin (μIU/mL) | 5.5 (3.1) [74] | 5.8 (4.2) [153] | 0.533 |
| HOMA-IR | 1.5 (1.0) [74] | 1.5 (1.2) [153] | 0.857 |
| eGFR | 69.6 (14.7) [74] | 68.5 (13.9) [153] | 0.577 |
| Waist circumference (cm) | 87.8 (8.6) [74] | 87.3 (10.4) [153] | 0.715 |
| MetS with 3 components (%) | 21.6 [16/74] | 9.2 [14/153] | 0.022 |
| NAFLD (%) | 24.3 [18/74] | 26.1 [40/153] | 0.770 |
| Alcohol overuse (%) | 43.2 [32/74] | 36.6 [56/153] | 0.338 |
| Smoking (BI ≥400) (%) | 16.2 [12/74] | 11.8 [18/153] | 0.355 |

CRA: colorectal adenoma; LDL: low-density lipoprotein; HDL: high-density lipoprotein; FPG: fasting plasma glucose; HbA1c: hemoglobin A1c; HOMA-IR: homeostasis model assessment-insulin resistance; eGFR: estimated glomerular filtration rate; MetS with three components: metabolic syndrome with hypertension, dyslipidemia and impaired fasting glucose; NAFLD: nonalcoholic fatty liver disease; BI: Brinkman index.

**Table 2. Multivariate Logistic Analysis of Risk Factors for the Development of Colorectal Adenoma**

| Characteristic | Subjects with CRA (*n*=74) | Subjects without CRA (*n*=153) | Adjusted OR | 95%CI | *p* value |
|---|---|---|---|---|---|
| | Number (%) | | | | |
| Age (over 60 years old) | 47 (63.5) | 86 (56.2) | 1.323 | 0.719–2.437 | 0.368 |
| Male | 56 (75.7) | 111 (72.5) | 1.239 | 0.591–2.599 | 0.571 |
| Female | 18 (24.3) | 42 (27.5) | | | |
| MetS with 3 components (%) | 16 (21.6) | 14 (9.2) | 2.951 | 1.278–6.817 | 0.011 |
| HOMA-IR | 12 (16.2) | 21 (13.7) | 0.974 | 0.412–2.303 | 0.953 |
| HU | 35 (47.3) | 80 (52.3) | 0.633 | 0.322–1.244 | 0.185 |
| eGFR<60 | 16 (21.6) | 35 (23.0) | 0.914 | 0.438–1.904 | 0.809 |
| NAFLD | 18 (24.3) | 40 (26.1) | 1.055 | 0.460–2.421 | 0.900 |
| Alcohol overuse | 32 (43.2) | 56 (36.6) | 1.350 | 0.659–2.767 | 0.412 |
| Smoking (BI ≥400) | 12 (16.2) | 18 (11.8) | 1.607 | 0.704–3.669 | 0.260 |

CRA: colorectal adenoma; OR: odds ratio; CI: confidence interval, MetS with 3 components: metabolic syndrome with hypertension, dyslipidemia and impaired fasting glucose; HOMA-IR: homeostasis model assessment-insulin resistance; HU: hyperuricemia; eGFR: estimated glomerular filtration rate; NAFLD: nonalcoholic fatty liver disease; BI: Brinkman index.

**Table 3. Relationship Between the Number of MetS Components and Colorectal Adenoma**

| Characteristic | Subjects with CRA (*n*=74) | Subjects without CRA (*n*=153) | Adjusted OR | 95%CI | *p* value |
|---|---|---|---|---|---|
| | Number (%) | | | | |
| Age (over 60 years old) | 47 (63.5) | 86 (56.2) | 1.254 | 0.696–2.258 | 0.451 |
| Sex, male/female | 56 (75.7) | 111 (72.5) | 1.101 | 0.553–2.192 | 0.784 |
| MetS with 3 components | 16 (21.6) | 14 (9.2) | 2.631 | 1.128–6.135 | 0.025 |
| MetS with 2 components | 16 (21.6) | 37 (24.2) | 1.009 | 0.477–2.133 | 0.981 |
| One component of MetS | 8 (10.8) | 18 (11.8) | 1.025 | 0.396–2.656 | 0.959 |

CRA: colorectal adenoma; OR: odds ratio; CI: confidence interval; MetS with 3 components: metabolic syndrome with hypertension, dyslipidemia and impaired fasting glucose; MetS with two components: metabolic syndrome with hypertension and dyslipidemia, metabolic syndrome with hypertension and impaired fasting glucose, or metabolic syndrome with dyslipidemia and impaired fasting glucose; one component of MetS: central obesity with hypertension, central obesity with dyslipidemia, or central obesity with impaired fasting glucose.

### Clinical characteristics of the study participants and risk factors for colorectal adenoma

**Table 1** shows the clinical characteristics of the 74 and 153 participants with and without CRA, respectively. Notably, the proportion of patients with MetS that comprised the three components of interest (hypertension, dyslipidemia, and IFG) was significantly higher in those with CRA. To investigate which risk factors were significantly associated with CRA, we performed a contingency table analysis to compare participants with and without CRA via a multivariate logistic regression analysis. The analysis showed that MetS that comprises the three components (OR=2.951, $p$=0.011) was a statistically significant risk factor for CRA (**Table 2**).

We also compared the relationship between CRA and MetS according to the number of MetS components participants were diagnosed with. While MetS that comprises all three components significantly increased CRA risk (OR=2.631, $p$=0.025), MetS that comprises two components (hypertension and dyslipidemia, hypertension and IFG, dyslipidemia and IFG) or one component (hypertension or dyslipidemia or IFG) was not correlated with CRA (**Table 3**).

## Discussion

This study investigated the relationship between CRA and MetS. Obesity and visceral adiposity, which are pathological conditions of MetS, induce insulin resistance and cause abnormal secretion of adipocytokines, which induce insulin resistance. Insulin resistance increases the secretion of insulin from pancreatic β-cells. It also increases the secretion of insulin-like growth factor, inducing cell proliferation and angiogenesis, which is thought to trigger oncogenesis[10]. Abnormal secretion of adipocytokines itself also reportedly contributes to oncogenesis[11].

Insulin resistance is a pathogenic mechanism of MetS and a common condition used to identify the risk of diabetes and MetS[12]. According to Matthews *et al.*, the HOMA-IR method is based on fasting glucose and

**Table 4. Relationship Between the Number of MetS Components and HOMA-IR**

1)

| Characteristic | Subjects with MetS (*n*=75) | Subjects without MetS (*n*=152) | Adjusted OR | 95%CI | *p* value |
|---|---|---|---|---|---|
| | Number (%) | | | | |
| Age (over 60 years old) | 53 (70.7) | 80 (52.6) | 2.283 | 1.231–4.232 | 0.009 |
| Male | 66 (88.0) | 101 (64.4) | 3.457 | 1.562–7.653 | 0.002 |
| Female | 9 (12.0) | 51 (33.6) | | | |
| HOMA-IR | 19 (25.3) | 14 (9.2) | 2.766 | 1.259–6.078 | 0.011 |

2)

| Characteristic | Subjects with MetS with 3 components (*n*=30) | Subjects without MetS with 3 components (*n*=197) | Adjusted OR | 95%CI | *p* value |
|---|---|---|---|---|---|
| | Number (%) | | | | |
| Age (over 60 years old) | 22 (73.3) | 111 (56.3) | 2.105 | 0.873–5.077 | 0.097 |
| Male | 25 (83.3) | 142 (72.1) | 1.543 | 0.542–4.395 | 0.417 |
| Female | 5 (16.7) | 55 (27.9) | | | |
| HOMA-IR | 11 (36.7) | 22 (11.2) | 4.205 | 1.728–10.233 | 0.002 |

3)

| Characteristic | Subjects with MetS with 2 components (*n*=53) | Subjects without MetS with 2 components (*n*=174) | Adjusted OR | 95%CI | *p* value |
|---|---|---|---|---|---|
| | Number (%) | | | | |
| Age (over 60 years old) | 33 (62.3) | 100 (57.5) | 1.245 | 0.649–2.388 | 0.509 |
| Male | 49 (92.5) | 118 (67.8) | 5.589 | 1.908–16.371 | 0.002 |
| Female | 4 (7.5) | 56 (32.2) | | | |
| HOMA-IR | 11 (20.8) | 22 (12.6) | 1.417 | 0.624–3.215 | 0.405 |

4)

| Characteristic | Subjects with one component of MetS (*n*=26) | Subjects without one component of MetS (*n*=201) | Adjusted OR | 95%CI | *p* value |
|---|---|---|---|---|---|
| | Number (%) | | | | |
| Age (over 60 years old) | 18 (69.2) | 115 (57.2) | 1.726 | 0.712–4.185 | 0.227 |
| Male | 23 (88.5) | 144 (71.6) | 3.133 | 0.895–10.965 | 0.074 |
| Female | 3 (11.5) | 57 (28.4) | | | |
| HOMA-IR | 4 (15.4) | 29 (14.4) | 0.880 | 0.278–2.784 | 0.827 |

OR: odds ratio; CI: confidence interval; HOMA-IR: homeostasis model assessment-insulin resistance; MetS with 3 components: metabolic syndrome with hypertension, dyslipidemia and impaired fasting glucose; MetS with 2 components: metabolic syndrome with hypertension and dyslipidemia, metabolic syndrome with hypertension and impaired fasting glucose, or metabolic syndrome with dyslipidemia and impaired fasting glucose; one component of MetS: central obesity with hypertension, central obesity with dyslipidemia, or central obesity with impaired fasting glucose.

insulin plasmatic levels[13] and has been used to define insulin resistance for clinical and research purposes in several populations[14]. Thus, we analyzed the relationship between CRA and HOMA-IR. While we initially found no significant association between CRA and HOMA-IR (**Table 2**), a more detailed investigation into the mechanisms of the relationship showed a significant link between HOMA-IR and MetS. MetS that comprises the three components hypertension, dyslipidemia and IFG was significantly related to HOMA-IR, indicating risk of insulin resistance (OR=4.205, $p$=0.002), whereas MetS that comprises two or one component was not correlated with HOMA-IR (**Table 4**). Thus, the current study suggested that insulin resistance is one of the factors indicating the relationship between CRA and MetS.

MetS is indicated in individuals with the coexistence of two or more risk factors that could precipitate ischemic cardiac disease, although each risk factor alone may not be serious or life-threatening[15]. Further, Inoue *et al.*[16] and Hu *et al.*[17] showed that MetS was associated with an increased risk of CRA, and that this risk increased with the number of metabolic components. These reports support our study finding that MetS that comprises three components was a statistically significant risk factor for the development of CRA, while MetS that comprises two or one component of MetS was not correlated with CRA (**Table 2** and **3**).

In addition to insulin resistance, peroxisome proliferator-activated receptor gamma (PPARγ) is also thought to be linked to oncogenesis. PPARγ is expressed mainly in fat cells and controls lipid and glucose metabolism. Normally, a large amount of PPARγ is expressed in the intestinal tract, thereby reducing the development of carcinogenesis in the stomach and colon[18]. However, PPARγ has been suggested to reduce the suppression of carcinogenesis in patients with MetS[19]. Furthermore, oxidative stress leads to insulin resistance, elevated blood pressure, and impaired plasma glucose[20]. Thus, oxidative stress is a probable mechanism governing the relationship between components of MetS and the development of colorectal tumor[11,16]. Future studies should investigate the link between PPARγ and oxidative stress.

MetS is accompanied by several concomitant factors, such as obesity, hypernutrition, and insufficient exercise[15]. Thus, it is important that individuals with MetS receive medication and undergo lifestyle improvement (e.g., diet, exercise therapy). In addition, our findings suggest that individuals with MetS that comprises the three components hypertension, dyslipidemia and IFG should undergo screening for CRA.

## Conclusions

MetS that comprises the three components hypertension, dyslipidemia and IFG significantly increased the risk of CRA. Therefore, in addition to medication and lifestyle improvements (e.g., diet, exercise therapy), individuals with MetS with the three components should undergo screening for CRA. Our data are expected to contribute greatly to the early detection and treatment of not only CRA but also CRC.

## Conflict of Interest

The authors have no conflict of interest to declare.

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Case Report

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# Incorrect Placement of the Ground Electrode in the 12-lead Electrocardiogram: Its Clinical Implications and a Simple Identification Strategy in Healthcare Fields

Osamu Saitoh[1], Kyogo Fuse[1], Takumi Kasai[1], Minoru Tagawa[2], Masaomi Chinushi[1]

## Abstract

The twelve-lead electrocardiographic (ECG) examination is used in various healthcare fields, and its automatic diagnosis functions play an important role in enabling the handling of a large number of ECGs in a short period of time. Incorrect placement of the ground electrode, while rare, can happen due to human error, and can impede the automatic diagnosis functions from identifying erroneous ECG recordings. This study assessed the ECG characteristics of incorrect ground electrode placement and its clinical implications in two patients. The results showed that (1) an isoelectrical line in either lead II or III is a simple way to identify incorrect electrode placement, and that two pairs of identical limb ECGs, or one pair of identical and one pair of polarity reversed symmetric limb ECGs additionally aid in identifying such a mistake; and (2) incorrect ground electrode attachment can result in the loss of electrocardiographic information corresponding to several frontal axis directions. All healthcare staff in charge of ECG examinations should to be aware of the unique characteristics of incorrect ground electrode placement to ensure proper recording of ECGs and improve the accuracy of ECG examinations.

**Keywords** electrode misplacements, automatic diagnosis, isoelectric lead, electrocardiogram

The twelve-lead electrocardiographic (ECG) examination is widely used not only in clinical treatment fields but also in health checks for the general population, and the automatic diagnosis functions of ECG recorders play an important role in enabling the handling of a large number of ECGs in a short period of time[1,2]. While recent advancements in medical technology have enhanced the abilities of the automatic diagnosis functions, they remain unable to correctly diagnose several findings[3–5]. All healthcare professionals involved in ECG examinations should be aware of the characteristics of these difficult-to-diagnose findings to ensure they obtain results that reflect the visual diagnosis made by physicians.

Although placing the recording electrodes in the correct position is a fundamental technique, incorrect electrode placement occasionally happens due to human error and can lead to misdiagnosis by ECG[6]. Since the automatic diagnosis functions cannot discriminate between incorrect ECG recordings and actual pathological findings, it is important for healthcare staff in charge of ECG examination to instantly recognize incorrect electrode placement at the time of recording. Unlike incorrect placement of the three active limb electrodes (e.g., reversed placement of right- and left-hand electrodes), the ECG characteristics and clinical implications of incorrect ground electrode (right foot electrode) placement have not been well studied.

To minimize misdiagnoses due to incorrect electrode placement, we compared ECGs recorded with the ground electrode placed in either the correct position or incorrect positions in two patients. The two patients provided signed consent for the anonymous publication of their medical cases.

## Case Report

ECGs with electrodes correctly attached (ECG-1) were recorded from two patients (Cases A and B) (**Figs. 1**, **2**). Case A was a 51-year-old man admitted to hospital for the treatment of coronary artery disease,

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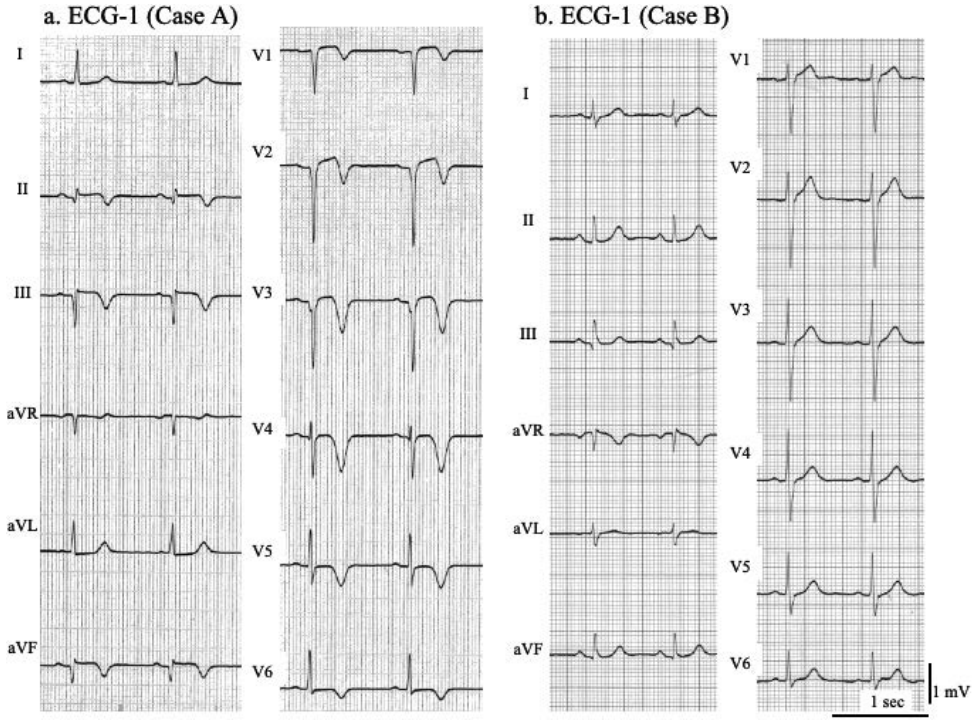


**Fig. 1. 12-lead Electrocardiograms (ECG)**

Panels (a) and (b) were obtained from Case A, who had myocardial infarction, and Case B, who did not have any structural heart diseases, respectively. The two ECGs were recorded with correct attachment of all electrodes (ECG-1). See details in the text.

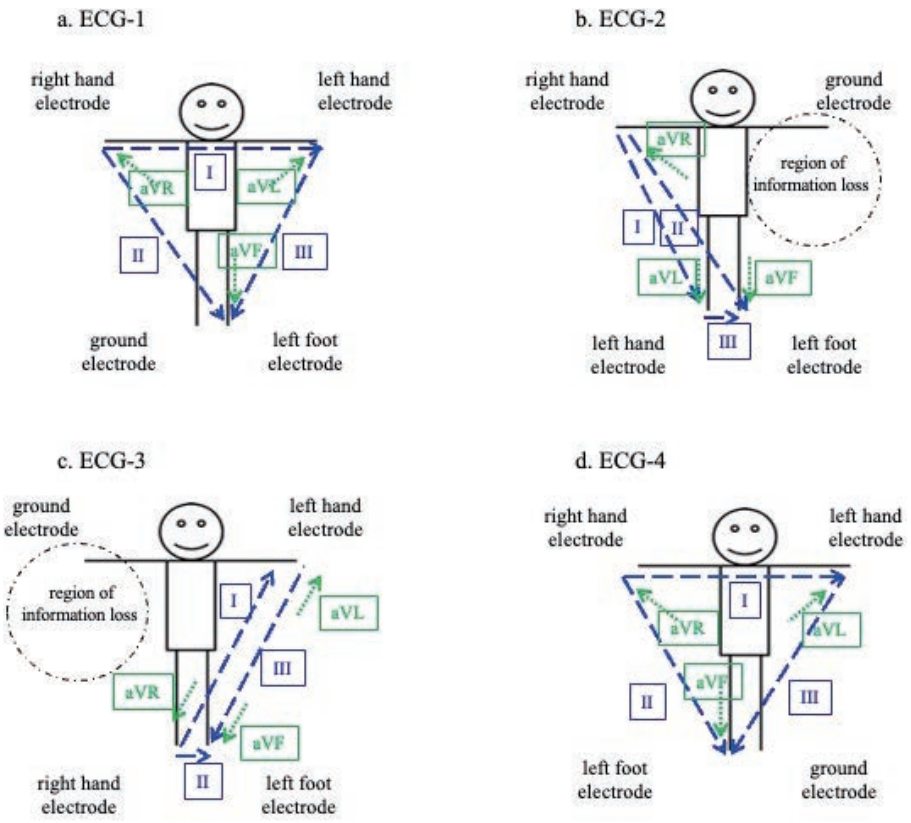


**Fig. 2. Illustrations of Correct and Incorrect Ground Electrode Attachment**

Thick blue dotted lines indicate the recording directions of bipolar limb leads (I, II and III). Thin green dotted lines are the recording directions of unipolar limb leads (aVR, aVL and aVF). The region enclosed by the black dotted line shows loss of ECG information. See details in the text.

and Case B was a 60-year-old man treated for dyslipidemia without structural heart diseases. ECG-1 in Case A showed sinus rhythm and abnormal Q wave, ST elevation, and inverted T wave in leads II, III, aVF, and V1–V3. Inverted T wave was also observed in the remaining precordial leads V4–V6. These findings suggested inferior and anterior wall myocardial infarction. Indeed, the coronary angiogram of Case A showed 99% stenosis in the right coronary artery and chronic total occlusion in the left anterior descending coronary artery. ECG-1 in Case B showed normal sinus rhythm, and neither QRS complex nor ST-T abnormalities was recorded.

ECGs were also recorded with electrodes attached incorrectly as follows: the right foot electrode was replaced with the left hand electrode (ECG-2), the right foot electrode was replaced with the right hand electrode (ECG-3), and the right foot electrode was replaced with the left foot electrode (ECG-4) (**Fig. 2**). ECG-2 in Case A showed mild ST elevation accompanied by T wave inversion in several leads (I, II, aVL, aVF, V1–V3) (**Fig. 3**), and T wave inversion was also recorded in the remaining leads (V4–V6). Although these findings suggested broad myocardial ischemia including in the inferior, anterior and lateral walls, lateral myocardial ischemia was not demonstrated by other cardiac examinations (echocardiogram and coronary angiogram) in this patient. In addition, several unique characteristics were noted on this ECG-2, namely the presence of (1) an isoelectric line in lead III and (2) almost identical electrocardiograms in two pairs of ECGs (I and II, aVL and aVF). The same two unique characteristics were observed on ECG-2 in Case B (**Fig. 3**). Compared to ECG-1, electrocardiographic information toward the left-hand direction (I and aVL) was lost in ECG-2 in both patients (**Figs. 1–3**). ECG-3 showed similar unique characteristics to those observed in ECG-2 in both patients: the presence of (1) an isoelectric line in lead II and (2) almost identical electrocardiograms in leads aVR and aVF, and polarity reversed symmetric electrocardiograms in leads I and III (**Fig. 4**). Compared to ECG-1, electrocardiographic information toward the right-hand direction (II and aVR) was lost in ECG-3 in both patients (**Figs. 1**, **2**, **4**). ECG-4 was almost identical to ECG-1 in both patients, so much so that ECG-1 and ECG-4 could not be distinguished from each other (**Figs. 1**, **5**). ECG-4 included almost all directional information observed in ECG-1 (**Fig. 2**). There were no specific differences in the precordial leads among the four ECGs (from ECG-1 to ECG-4) in either patient.

## Discussion

This study suggests that (1) an isoelectrical line in either lead II or III is a simple way to identify incorrect placement of the ground electrode, and two pairs of

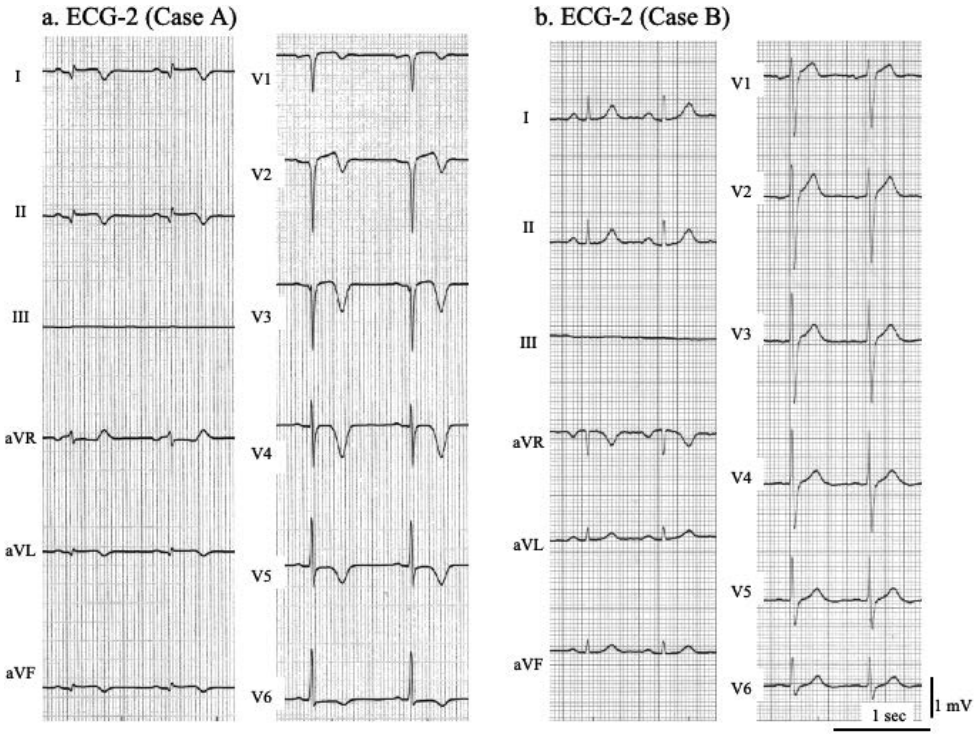


**Fig. 3. 12-lead ECG with an Incorrect Ground Electrode Position**

ECG was recorded with the right foot electrode replaced with the left hand electrode (ECG-2) in Case A and Case B. A unique isoelectric line was observed in lead III. See details in the text.

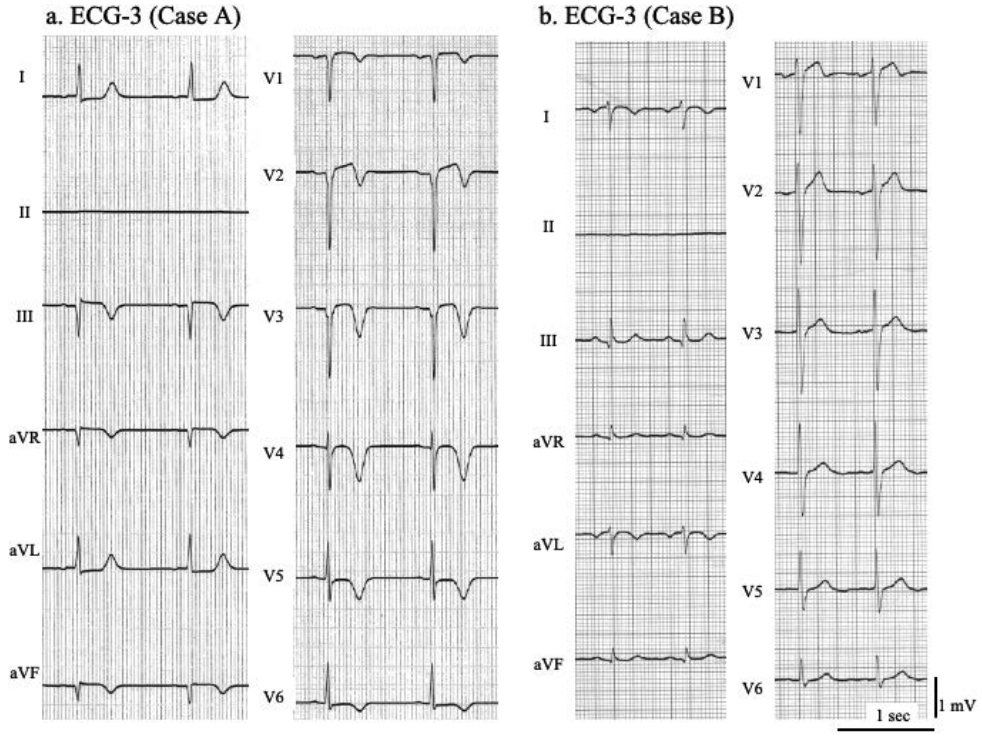


**Fig. 4. 12-lead ECG with Incorrect Ground Electrode Position**

ECG was recorded with the right foot electrode replaced with the right hand electrode (ECG-3) in Case A and Case B. A unique isoelectric line was observed in lead II. See details in the text.

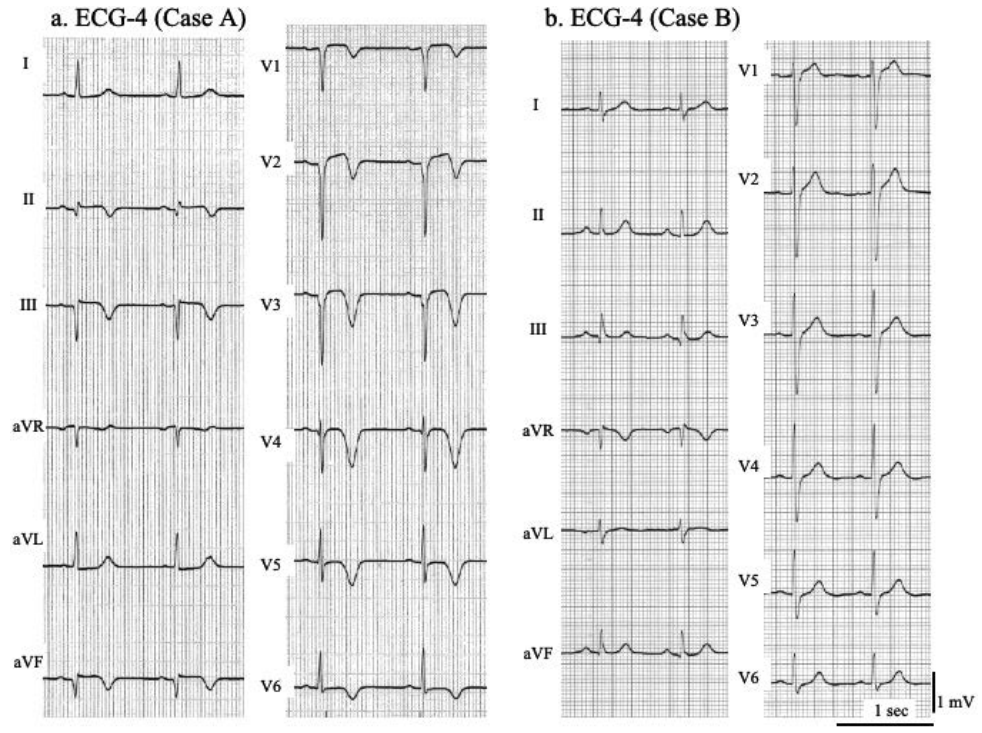


**Fig. 5. 12-lead ECG with Incorrect Ground Electrode Position**

ECG was recorded with the right foot electrode replaced with the left foot electrode (ECG-4) in Case A and Case B. See details in the text.

identical limb ECGs, or one pair of identical and one pair of polarity reversed symmetric limb ECGs additionally aid in identifying incorrect attachment at either the left or right hand, and (2) incorrect ground electrode attachment to the left or right hand can result in the loss of electrocardiographic information represent-

ing several frontal axis directions (**Fig. 2**).

The ground electrode of an ECG is responsible for reducing either noise or static electricity and determining the reference voltage. Incorrect attachment of the ground electrode to either the left or right hand leads to placement of two of the three active limb electrodes at either the right or left foot (e.g., left hand and left foot electrodes in ECG 2) (**Fig. 2**). Since the voltage gradient between the right and left foot should be minimal, an isoelectrical line was recorded on the ECG lead (II or III), where two corresponding electrodes were placed at the right and left foot[7]. Disconnection of one of the active limb electrodes was not the cause of the isoelectric line, because an electrical signal was recorded on all three unipolar limb leads (aVR, aVL and aVF). Instead, we predict that the occurrence of a similar voltage gradient between two leads of the electrocardiogram was why two pairs of electrocardiograms showed almost identical morphologies in ECG-2 (leads I and II: representing the voltage gradient between either foot and the right hand; leads aVL and aVF: representing the voltage gradient between either foot and Goldberger's terminal) and one pair showed identical and the other showed polarity reversed symmetric morphology in ECG-3 (leads I and III: representing the voltage gradient between either foot and the left hand; leads aVR and aVF: representing voltage the gradient between either foot and Goldberger's terminal) (**Fig. 2**). In ECG-3, a reversed voltage gradient between leads I and III resulted in polarity reversed symmetric morphologies in the two leads. Accordingly, a negative *P* wave appeared in lead I in both cases. Voltage gradients created in ECG-1 and ECG-4 closely resembled each other, resulting in almost identical electrocardiograms in ECG-1 and ECG-4 (**Figs. 1**, **2**, **5**).

The ECG characteristics of incorrect ground electrode placement were reported in several articles in the 1980s and 2000s[7–9]. Therefore, this study does not present new ECG findings related to incorrect ground electrode placement. However, these previous reports did not emphasize the clinical implications or identify a simple way to identify this problem, probably because ECG examinations based on automatic diagnosis have only recently become more common in various healthcare fields, including health checks for the general population. Remembering the ECG features of incorrect ground electrode placement will help to remind medical staff that they should first reconfirm and then promptly reposition the electrodes in the correct place when they notice the unique features at ECG recording. Although incorrect ground electrode placement at the left foot (ECG-4) may not have any clinical implications in adults, placing the electrodes in the correct position is a fundamental issue in ECG examinations.

In the case of incorrect placement of active limb electrodes (e.g., reversed attachment of right- and left-hand electrodes), the correct ECG pattern can be subsequently reconstructed based on the incorrect ECG recording if the healthcare staff who conducted the ECG examination are aware of their mistake. In contrast, ECG recorded with incorrect ground electrode placement leads to loss of information in several directions (left-hand direction in ECG-2 and right-hand direction in ECG-3) at the time of the ECG recording (**Fig. 2**), preventing reconstruction of the correct ECG pattern after the examination. Therefore, careful attention is essential to avoid this type of error. This case report reveals the unique ECG characteristics of incorrect ground electrode placement. Given the simplicity of these characteristics, it may be possible to incorporate this algorithm into the automatic diagnostic functions of ECG recorders to further improve the accuracy of ECG examinations. As this case report focused only on incorrect placement of the ground electrode, further studies are needed to identify the simple ECG characteristics of other types of incorrect electrode placement.

## Conflict of Interest

No financial support was received for this study from any specific company.

## Disclosure

The authors have no potential conflict of interest to disclose.

## Ethical Consensus and Patients Consent Statement

The two patients provided signed consent for the anonymous publication of their medical cases.

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# The Regulations of the International Society of Ningen Dock

**Article 1**
Name
The name of the association shall be the International Society of Ningen Dock.

**Article 2**
Office
The Society has its principal office in Japan Society of Ningen Dock.

**Article 3**
Aims
The Society, an organization of Japan Society of Ningen Dock for international operations, aims to contribute to global health promotion by enhancing the development of ningen dock as a medical check-up system.

**Article 4**
Tasks
The Society conducts the following tasks to achieve the aims described in the preceding section.
1. Holds congress (World Congress on Ningen Dock), board meetings, lectures, and committee meetings
2. Publishes journals and news magazines
3. Communicates and cooperates with related academic societies both in Japan and overseas
4. Promotes research activities in ningen dock and related fields
5. Does whatever is necessary to achieve the aims of the Society

**Article 5**
Membership
1. The Society consists of the following members
   1) Regular member
      A regular member shall be a member of the International Society of Ningen Dock who agrees to the aims of the Society, and has expert knowledge, techniques, or experience in the areas associated with the Society.
   2) Supporting member
      A supporting member shall be a person, a corporation, or a group that agrees to the aims of the Society, and supports its programs.
   3) Honorary member
      An honorary member shall be recommended, from those who have significantly contributed to the areas associated with the Society, by the executive board.
2. Those who want to apply for regular or supporting membership of the Society shall submit the prescribed application form with the membership fee.
3. The board meeting will process applications mentioned in the preceding section, and promptly notify the applicants of its decision.

**Article 6**
Officials
1. The Society shall appoint the following honorary advisors and officials.

Honorary advisor: Number not decided
Congress president: 1
President: 1
Vice president: 3 (from Japan : 2, overseas: 1)
Board members: up to 25 (from Japan : 15 or less, overseas : 10 or less)
Auditor: 2

**Article 7**
Honorary advisor
1. An honorary advisor shall be appointed by the president from those who have contributed to the development of the Society for a long period, and approved by the executive board.
2. Honorary advisors shall be eligible to attend the board meeting, and to express opinions; honorary advisors will not have voting rights.

**Article 8**
Congress president
1. The congress president shall be recommended by the executive board and appointed by the president.
2. The congress president shall represent the Society and host the World Congress on Ningen Dock as a scientific meeting.

**Article 9**
President
1. The president shall be selected by and from among board members and delegated by the president of Japan Society of Ningen Dock.
2. The president shall preside the Society.

**Article 10**
Vice president
1. The vice president shall be appointed, from among board members, by the president.
2. The vice president shall assist the president. In the case of accident, one of the vice presidents will be appointed by the president and will temporarily take over the duties.

**Article 11**
Board members
1. Board members from Japan shall be selected among candidates from regular members at Japan Society of Ningen Dock.
2. Overseas board members shall be selected at the recommendation of the executive board.
3. Board members execute duties for the Society under the orders from the president.
4. Board members, together with the president and the vice president, comprise the executive board.

**Article 12**
Board meeting
1. The president will call a board meeting on an as-needed basis, and serves as the chairman of the meeting.
2. The board meeting will pass resolutions on important matters of the Society.
3. The board meeting shall have the right to start proceedings if the majority of all the board members (including a letter of proxy) attend the meeting.
4. The board meeting shall pass resolutions with the majority votes of attendances.

**Article 13**
Auditor
Auditors shall audit accounts of the Society, and report to the board meeting.

**Article 14**
Commissioner
For the aims of successful programs of the Society, the president will set up committees and divisions through the resolutions of the executive board, and delegate the commissioners to regular members or other members of the Society.

**Article 15**
Accounting
1. The fiscal year for the Society starts on April 1 every year and ends on March 31 the following year.
2. Expenses required for the Society shall be covered by the following revenues.
   1) Membership fees
   2) Grants
   3) Donations
   4) Others

**Article 16**
Modification of rules
The rules of the Society can be amended by the resolution of the executive board.

**Article 17**
Miscellaneous provisions
Detailed regulations necessary for the enforcement of the rules of the Society are defined elsewhere by the president with the approval of the executive board.

**Article 18**
Additional clause
The Regulations of the International Society of Ningen Dock will come into effect on September 15, 2006.

# Detailed Regulations of the International Society of Ningen Dock

Detailed regulations of the International Society of Ningen Dock are defined as follows:

**(Detailed regulations on members)**

**Article 1**

1. Members shall pay the following annual membership fee; honorary members will be exempt from membership fee.
   1) Regular member : 2,000 yen
   2) Supporting member : from one unit (unit: 20,000 yen)
2. Annual membership fee paid shall not be refunded for any reason.
3. Members with foreign citizenship shall pay a 3-year membership fee of 50 dollars.

**Article 2**

Members will be given priority in the following events :

1) Participation in scientific meetings hosted by the Society;
2) Contributions of articles to and receipt of the journal of the Society.

**Article 3**

Members shall lose their memberships in the event of the following:

1) Withdrawal from membership;
2) Adjudication of incompetence or quasi-incompetence;
3) Death or adjudication of disappearance, or dissolution of the group in the case of a member of a supporting group;
4) Delinquency in payment of membership fee for over three year.

**Article 4**

Those intending to withdraw from the Society must submit the notice of withdrawal in the prescribed form to be approved by the executive board.

**Article 5**

The Society can expel a member to whom either of the following would apply, with a resolution of the executive board:

1) Those who violate their duty as members of the Society;
2) Those who damage the honor of members of the Society or act against the aims of the Society.

**Article 6**

Those who satisfy Sections 1 and 2 of Article 5 of the Regulations of the International Society of Ningen Dock will be accepted as members of the Society.

**(Detailed regulations on officials)**

**Article 7**

1. The president will be selected from the board members of Japan Society of Ningen Dock.
2. In principle, the majority of board members from Japan will be selected from among the board members of Japan Society of Ningen Dock.

3. Overseas board members will essentially be selected from Asia, Pacific Rim, North America, or Europe.

**Article 8**

1. The term of the congress president will be from the end of the congress of which he/she is in charge to the next congress.
2. The term of board members will be six years (two terms of three years).

**(Detailed regulations on congress and board meeting)**

**Article 9**

Congress and board meeting will be held as follows :

1) The title of the congress will be World Congress on Ningen Dock.
2) In principle, the congress and the board meeting will be held once every three years; with the resolution of the executive board, however, the congress and the board meeting will be held as needed.
3) The congress and the board meeting will be held at the same time.
4) The name of the congress president and the location of the next congress will be announced.

**Article 10**

1. Those who want to take part in the congress shall pay the participation fee, which is defined separately.
2. Participation fee for the congress will be defined accordingly by the congress president.
3. Only regular members shall be allowed to present the results of their studies, except those who have been approved by the congress president.

**(Enforcement of the detailed regulations)**

**Article 11**

1. The detailed regulations will come into effect on September 15, 2006.

# INSTRUCTIONS TO AUTHORS

## Ningen Dock International
## Official Journal of Japan Society of Ningen Dock

**Ningen Dock International** is the official journal of Japan Society of Ningen Dock, in which original articles, case reports, short reports, review articles, and clinical experience or practice report in English are published. Ningen Dock accepts only manuscripts that are original work in the field of ningen dock and related areas not previously published or being considered for publication elsewhere, except as abstracts. The manuscripts published in Ningen Dock will appear on the website of our society.

If the manuscript concerns a clinical study, it must be in accordance with the Declaration of Helsinki of 1964 (subsequent revisions included). Therefore, for a manuscript whose content is epidemiological or clinical research, the approval of the facility's Institutional Review Board (IRB) or the Ethics Committee of Japanese Society of Ningen Dock must have been obtained for the study described. Also, in the text, it should be indicated that informed consent has been obtained from subjects. Additionally, for case reports, it should be stated that adequate care has been taken to ensure the privacy of the subject concerned.

### Online submission system

Ningen Dock International uses an online submission system called ScholoarOne Manuscripts.
Please access https://mc.manuscriptcentral.com/ndi

### Preparation of manuscript

All manuscripts must be written in English with MS-Word, Excel, PowerPoint and/or a common graphic format. Authors who are not fluent in English must seek the assistance of a colleague who is a native English speaker and is familiar with the field of the manuscript.

The title, abstract, text, acknowledgments, references, tables, and figure legends should begin on separate sheets, with pages numbered, and be typed double-spaced using the 12-point font size in MS-Word.

Files for submission should be prepared in English in a Microsoft Word or other file format that may be uploaded to the online system.

Available formats for files to be uploaded: doc (docx), xls (xlsx), ppt (pptx), jpg, tiff, gif, ai, eps, psd File names must consist of alphanumeric characters and an extension.

Example file names: Manuscript.doc, Fig 1 .jpg, Table 1 .xls, etc.

Please indicate the version of Microsoft Office used in a cover letter accompanying the uploaded files.

All measurements should be expressed in SI units. Less common abbreviations should be spelled out at first usage and the abbreviated form used thereafter.

### Title page

Titles should be concise and informative. Include the full names of authors, names and addresses of affiliations, and name and address of a corresponding author to whom proofs are to be sent, including a fax number, telephone number and e-mail address. Running title should not be more than 50 characters.

## Abstract

The abstract should not exceed 250 words, and should be arranged under the following subheadings: Objective, Methods, Results, Conclusions, and have up to 4 keywords.

## Types of articles

**Original articles**: An original article should not exceed 4,000 words, and should be arranged as follows: Abstract, Objective, Methods, Results, Discussion, (Limitations), (Conclusions), (Acknowledgments), and References.

**Case reports:** A case report should not exceed 3,000 words, and be arranged as follows: Abstract (which should be a brief summary of the content without headings), Introduction, Case report, Discussion, and References.

**Short reports:** A short report should not exceed 3,000 words.

**Review articles:** Review articles should not exceed 5,000 words. Review articles are usually by invitation. However, articles submitted without an invitation may also be considered by the Editorial Board.

**Clinical experience or Practice report:** Clinical experience or Practice report should not exceed 4,000 words.

## References

References should be numbered consecutively in order of appearance in the text and cited in the text using superscript numbers. For example, according to the study by Sasamori[1]. For journals, the names and initials of the first three authors, followed by "*et al.*" if there are other coauthors, the complete title, abbreviated journal name according to Index Medicus, volume, beginning and end pages, and year should be included. For books, the names and initials of the first three authors, followed by "*et al.*" if there are other coauthors, the complete title, book name, edition number, beginning and end pages, name and city of publisher, and year should be included. For websites, the names and initials of the first three authors, followed by "*et al.*" if there are other coauthors, title of cited page/the document, year of posting, URL, and accessed date in parentheses should be included. Examples of references are given below.

**Journal:** Frías JP, Davies MJ, Rosenstock J, *et al.*: Tirzepatide versus semaglutide once weekly in patients with type 2 diabetes. N Engl J Med 2021; 385: 503–515.

**Book:** Kaplan NM: Measurement of blood pressure. In: Kaplan NM(ed), Kaplan's Clinical Hypertension. 7th ed., Lippincott William & Wilkins, Philadelphia, 2002, 25–55.

**Websites:** Ministry of Health, Labour and Welfare: The National Health and Nutrition Survey in Japan. 2013, http://www.mhlw.go.jp/bunya/kenkou/eiyou/dl/h25-houkoku.pdf (in Japanese) (accessed March 1, 2022)

## Tables

Tables should be cited in the text, and numbered sequentially with Arabic numerals. Each table should be given a number and a brief informative title, and should appear on a separate page. Explain in footnotes all abbreviations used.

## Figures

Figures should be cited in the text, and numbered sequentially with Arabic numerals. A brief descriptive legend should be provided for each figure. Legends are part of the text, and should be appended to it on a separate page. Color figures can be reproduces if necessary, but the authors will be expected to contribute towards the cost of publication.

**Conflict of Interest (COI)**

All authors are required to disclose any conflict of interest (COI) on the form designated by the Japan Society of Ningen Dock.

If no author has any COI, this should be indicated in the manuscript.

**Page proofs**

The corresponding author will receive PDF proofs, the author should correct only typesetting errors. After correcting, page proofs must be returned promptly.

**Reprints**

Thirty reprints of each paper are free, and additional reprints are available at charge in lots of 10, but for a minimum order of 50. Reprints should be ordered on submission of the manuscript as follows: For example, "I order 100 reprints: 30 (free) + 70."

**The Editorial Board** considers only manuscripts prepared according to the Instructions to Authors, and makes decisions regarding the acceptance of manuscripts as well as the order of printing them. All published manuscripts become the permanent property of Japan Society of Ningen Dock, and may not be published elsewhere without written permission from the Society.

**Appendix 1:** Use of figures, tables, images, etc. from other sources

Please exercise caution in the use or quotation of figures, tables, images, etc. from other sources when submitting to "Ningen Dock International".

- When using figures, tables, images, etc., by either direct quotation or modification, it is the author's responsibility to obtain permission from any copyright holders, such as the original author, publisher, and academic society, before submission. As part of this process, authors may be required to pay copyright royalties.
- The number of figures, tables, images, etc. that are used from other sources should be within an objectively valid range (as determined by the ethical consideration of the author).
- The reputation of the original author should not be disparaged or prejudiced, and the material should not be used in a manner contrary to the intention of the original author.
- Specify that the use is a quotation or modification, and document the source.

Updated: March 3, 2023

# Check list for submission of papers to Ningen Dock International
# Official Journal of Japan Society of Ningen Dock

Categories of manuscript:

- ☐ Original article (not more than 4,000 words)
- ☐ Case report (not more than 3,000 words)
- ☐ Short report (not more than 3,000 words)
- ☐ Review article (not more than 5,000 words)
- ☐ Clinical experience or Practice report (not more than 4,000 words)

Typing:

- ☐ Manuscript on A4 paper with wide margins
- ☐ Type double space using 12-point

Title page:

- ☐ Title of paper
- ☐ Full names of authors and affiliations without title of MD, PhD, etc
- ☐ Full name and address of a corresponding author including fax number, telephone number and e-mail address.
- ☐ Running title not more than 50 characters.

Abstract:

- ☐ Not more than 250 words.
- ☐ Arranged in the order of Objective, Methods, Results, and Conclusions.
- ☐ Up to 4 key words.

Text of paper:

- ☐ Manuscript is arranged in the order of Objective, Methods, Results, Discussion, (Limitations), (Conclusions), (Acknowledgments), and References.
- ☐ Papers involving ethical considerations, particularly with regard to the methods, have described these considerations in the Methods section.
- ☐ Measurements are expressed in SI units.
- ☐ Abbreviations are spelled out at first usage.

References:

- ☐ References are numbered consecutively in order of appearance in the text and cited in the text using superscript numbers.
- ☐ Format is consistent with examples in Instructions for Authors.

Tables, figures, images:

☐ Each table is given a number and a brief informative title, and appears on separate page.

☐ All abbreviations used are explained in footnotes.

☐ Figure legends are appended to the text on a separate page.

☐ Permission to quote or modify figures, tables, images, etc., from the copyright holder (original author, publisher, academic society, etc.) has been obtained before submission.

Submission:

☐ Agreement, cover letter, manuscript (title page, abstract, text, acknowledgments, and references), figure legends, tables, figures and/or photos prepared in due form.

☐ This paper has been submitted to this journal only, and has not been submitted to or published in other journal.

☐ All pages are numbered.

Date: _____

Name (print) _____ Signature _____

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If the author is a member of Japan Society of Ningen Dock, please write the membership number on the right of the signature line.

# Abbreviations

| | | | | | |
|---|---|---|---|---|---|
| 1 | **1,5-AG** | 1,5-anhydroglucitol | 61 | **hCG** | human chorionic gonadotropin |
| 2 | **17-OHCS** | 17α-hydroxycorticosteroid | 62 | **HCV** | hepatitis C virus |
| 3 | **95% CI** | 95% confidence interval | 63 | **HDL-C** | high-density lipoprotein cholesterol |
| 4 | **α-GI** | α-glucosidase inhibitor | 64 | **HLA** | histocompatibility [leucocyte] antigen |
| 5 | **$\beta_2$-MG** | $\beta_2$-microglobulin | 65 | **HPLC** | high-performance liquid chromatography |
| 6 | **γ-GTP** | γ-glutamyl transpeptidase | 66 | **Ht** | hematocrit |
| 7 | **A/G ratio** | albumin-globulin ratio | 67 | **ICD** | International Classification of Disease |
| 8 | **ABI** | ankle-brachial index | 68 | **ICU** | intensive care unit |
| 9 | **ACTH** | adrenocorticotropic hormone | 69 | **IFG** | impaired fasting glucose |
| 10 | **ADL** | activities of daily living | 70 | **IGT** | impaired glucose tolerance |
| 11 | **AFP** | α-fetoprotein | 71 | **IMT** | intima-media thickness |
| 12 | **ALP** | alkaline phosphatase | 72 | **LAP** | leucine aminopeptidase |
| 13 | **ALT** | alanine aminotransferase | 73 | **LDH** | lactate dehydrogenase |
| 14 | **Apo (a)** | apolipoprotein (a) | 74 | **LDL-C** | low-density lipoprotein cholesterol |
| 15 | **APTT** | activated partial thromboplastin time | 75 | **Lp(a)** | lipoprotein (a) |
| 16 | **AST** | aspartate aminotransferase | 76 | **LPL** | lipoprotein lipase |
| 17 | **BMI** | body-mass index | 77 | **MCH** | mean corpuscular hemoglobin |
| 18 | **CA125** | carbohydrate antigen 125 | 78 | **MCHC** | mean corpuscular hemoglobin concentration |
| 19 | **CA19-9** | carbohydrate antigen 19-9 | 79 | **MCV** | mean corpuscular volume |
| 20 | **cAMP** | cyclic adenosine 3', 5'-monophosphate | 80 | **METs** | meatbolic equivalent |
| 21 | **CAPD** | continuous ambulatory peritoneal dialysis | 81 | **MetS** | metabolic syndrome |
| 22 | **CBC** | complete blood cell count | 82 | **MMG** | mammography |
| 23 | **Ccr** | creatinine clearance | 83 | **MRA** | magnetic resonance angiography |
| 24 | **cDNA** | complementary deoxyribonucleic acid | 84 | **MRI** | magnetic resonance imaging |
| 25 | **CEA** | carcinoembryonic antigen | 85 | **mRNA** | messenger RNA |
| 26 | **cGMP** | cyclic guanosine 3', 5'-monophosphate | 86 | **MRSA** | methicillin-resistant *Staphylococcus aureus* |
| 27 | **ChE** | cholinesterase | 87 | **MSW** | medical social worker |
| 28 | **CKD** | chronic kidney disease | 88 | **NMR** | nuclear magnetic resonance |
| 29 | **COI** | conflict of interest | 89 | **PET** | positron emission tomography |
| 30 | **COPD** | chronic obstructive pulmonary disease | 90 | **PSA** | prostate-specific antigen |
| 31 | **CK** | creatinine kinase | 91 | **PTH** | parathyroid hormone |
| 32 | **CRP** | c-reactive protein | 92 | **PWV** | pulse wave velocity |
| 33 | **CT** | computed tomography | 93 | **QOL** | quality of life |
| 34 | **CVA** | cerebrovascular accident | 94 | **RBC** | red blood cell |
| 35 | **D-Bil** | direct bilirubin | 95 | **RF** | rheumatoid factor |
| 36 | **DBP** | diastolic blood pressure | 96 | **RI** | radioactive isotope |
| 37 | **DNA** | deoxyribonucleic acid | 97 | **RIA** | radioimmunoassay |
| 38 | **DRG** | diagnosis-related group | 98 | **RNA** | ribonucleic acid |
| 39 | **dsDNA** | double stranded deoxyribonucleic acid | 99 | **SBP** | systolic blood pressure |
| 40 | **EBM** | evidence-based medicine | 100 | **SD** | standard deviation |
| 41 | **ECG** | electrocardiogram | 101 | **SEM** | standard error of the mean |
| 42 | **eGFR** | estimated glomerular filtration rate | 102 | **STD** | sexually transmitted disease |
| 43 | **EIA** | enzyme immunoassay | 103 | **T-Bil** | total bilirubin |
| 44 | **ELISA** | enzyme-linked immunosorbent assay | 104 | **$T_3$** | triiodothyronine |
| 45 | **EPO** | erythropoietin | 105 | **$T_4$** | thyroxine |
| 46 | **ESR** | erythrocyte sedimentation rate | 106 | **TC** | total cholesterol |
| 47 | **FBG** | fasting blood glucose | 107 | **TG** | triglyceride |
| 48 | **FDA** | Food and Drug Administration | 108 | **TIA** | transient (cerebral) ischemic attack |
| 49 | **FEV** | forced expiratory volume | 109 | **TIBC** | total iron binding capacity |
| 50 | **$FEV_1$** | forced expiratory volume in one second | 110 | **tPA** | tissue plasminogen activator |
| 51 | **$FEV_1$%** | forced expiratory volume % in one second | 111 | **TPHA** | *Treponema pallidum* hemagglutination assay |
| 52 | **FPG** | fasting plasma glucose | 112 | **TSH** | thyroid stimulating hormone |
| 53 | **FSH** | follicle stimulating hormone | 113 | **TTT** | thymol turbidity test |
| 54 | **FT3** | free triiodothyronine | 114 | **UCG** | ultrasonic echocardiography |
| 55 | **FT4** | free thyroxine | 115 | **UIBC** | unsaturated iron binding capacity |
| 56 | **FVC** | forced vital capacity | 116 | **UN** | urea nitrogen |
| 57 | **GFR** | glomerular filtration rate | 117 | **VLDL** | very-low-density lipoprotein |
| 58 | **GH** | growth hormone | 118 | **WBC** | white blood cell |
| 59 | **Hb** | hemoglobin | 119 | **WHO** | World Health Organization |
| 60 | **HbA1c** | hemoglobin A1c | 120 | **ZTT** | zinc sulfate (turbidity) test |

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Japan Society of Ningen Dock

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## The International Society of Ningen Dock (ISND)
## ISND Membership Application Form

Please type or print legibly and complete all information requested and FAX to the International Society of Ningen Dock (FAX: +81-3-3265-0083)

1. Name and principal professional mailing address

Last (Family) Name　First Name　Middle Initial　Degree

Affiliation

Address
Street　City　State　Country　Postal Code

Telephone Number　Facsimile

E-mail Address

2. Specialty (Circle one)

Doctors (internal medicine, primary care, surgery, gynecology, ophthalmology, pediatrics, radiology, orthopedics, pharmacology, epidemiology, other:　)

Nurse, Public Health Nurse, Dietician, Clinical Technologist,

Clinical Radiological Technologist, Pharmacist, Other:

3. Annual Dues

☐Regular Member

Annual dues in Japanese yen ……2,000

☐Supporting Member

Annual dues in Japanese yen …… 20,000

☐Regular Member -International

3-year dues in US$ ……50.00

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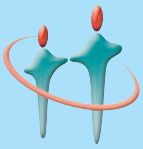
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