Reactive hyperemia index can screen endothelial dysfunction in obese subjects with non-alcoholic fatty liver disease

Shozo Yano\(^1\), Hiroshi Tobita\(^2\), Chikako Watano\(^3\), Seiji Mishima\(^3\), Yutaka Ishibashi\(^4\), Abdullah M. Sheikh\(^1\) and Atsushi Nagai\(^1\)\(^3\)

Abstract:
Background and Aims: Reactive hyperemia-peripheral arterial tonometry (RH-PAT) has been developed to detect early stage vascular dysfunction. Recent studies have suggested that Reactive hyperemia index (RHI) assessed using RH-PAT could be used to predict the mortality of subjects with high risks for cardiovascular events. On the other hand, carotid intima-media thickness (IMT), pulse wave velocity (PWV), and/or cardio-ankle vascular index (CAVI) have been used as screening tools for vascular disorders. Since obesity and metabolic syndrome are the risk factors for cardiovascular events, we aimed to examine the association of RHI, CAVI, and carotid artery plaque scores with the body mass index (BMI).

Methods: The participants of this study were 39 Japanese subjects who were admitted at the Department of Liver Disease of Shimane University Hospital between August and December 2016 (mean age, 62 years). RH-PAT findings, CAVI, and carotid IMT were evaluated, and their associations with BMI were analyzed. Carotid IMT and plaque score (PS) were evaluated by ultrasonography following the standard protocol. A score of less than 1.67 was defined as a low RHI. Results: The PS was associated with age (r=0.511, p<0.0001) but not with BMI. Interestingly, CAVI had a positive association with age (r=0.706, p<0.0001) and an inverse association with BMI (r=-0.511, p<0.0001). In contrast, RHI had no significant association with age and BMI. Among 14 patients with non-alcoholic steatohepatitis (NASH) or non-alcoholic fatty liver disease (NAFLD) with BMI >25, only one patient had high CAVI (>9.0) whereas 6 patients had low RHI. Conclusions: NASH/NAFLD subjects, especially those with obesity, are good candidates for RH-PAT examination to detect early stage of vascular failure.

Key words: Reactive hyperemia index (RHI), Non-alcoholic fatty liver disease (NAFLD), Obesity, Cardio-ankle vascular index (CAVI), Intima-media thickness (IMT)

1. Introduction
Non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD) are highly associated with obesity and metabolic syndrome. Although obesity and metabolic syndrome are the risk factors of cardiovascular events, they are often associated with NASH/NAFLD\(^1\)\(^2\). In addition, patients with NASH/NAFLD have been reported to have cardiovascular risks including hypertension, dyslipidemia, and diabetes, which may lead to increased mortality\(^3\)\(^4\). Significant decrease in brachial artery flow mediated dilatation (FMD) and increase in carotid artery intima-media thickness (IMT) were observed in these patients\(^5\)\(^6\). These findings indicate that NASH/NAFLD patients are good candidates for screening examinations of vascular function.

Reactive hyperemia index (RHI) that is assessed by reactive hyperemia-peripheral arterial tonometry (RH-PAT) may be a sensitive marker for vascular endothelial function\(^7\). RH-
PAT is non-invasive and requires no special techniques. The results are consistent and objective. Furthermore, it takes less than 30 minutes including the resting time. Nowadays, RH-PAT is commercially available and recommended as a screening tool for vascular failure\(^\text{10}\). Recent study demonstrated that RHI can be a good predictor to detect the early stage of vascular dysfunction\(^\text{11}\). RH-PAT may contribute to identification of individuals with high risk for cardiovascular (CV) events\(^\text{12}\).

On the other hand, pulse wave velocity (PWV) and cardio-ankle vascular index (CA VI), which represent vascular function, the measurement is affected by blood pressure. The CA VI, which is highly reproducible and easy to measure\(^\text{15,16}\), is independent of the blood pressure at the time of the measurement\(^\text{17}\).

In this study, we therefore examined vascular function using RH-PAT to compare conventional examinations in patients with NASH/NAFLD.

## 2. Methods

### 2.1. Subjects

The participants of the present study included all individuals who were admitted in the Department of Liver Diseases of Shimane University Hospital and diagnosed with NASH or NAFLD between August and December 2016. Exclusion criteria were patients with severe disorders such as advanced cancer, liver cirrhosis, heart failure, and renal failure with serum creatinine $>2$ mg/dl. Based on these criteria, a total of 39 subjects (20 males and 19 females) were consecutively assigned to this study. This study is part of the cohort study conducted by the Department of Liver Diseases of Shimane University Hospital.

### 2.2. Ethics

Written informed consent was obtained from each participant. The study protocol was approved by the ethics committee of Shimane University.

### 2.3. Data collection

Patients’ information such as anthropometric and laboratory data were obtained from medical reports. Smoking status was classified into 2 groups ($0$=never smoke, $1$=past or present smoke). Measurements of RHI, CA VI, and IMT were performed at the same day, early in the morning after overnight fasting.

IMT in the carotid artery was measured with a high-resolution real-time ultrasonography with a 7.5-MHz transducer (Vivid I or LOGIQ e, GE, Tokyo, Japan). The measurement of the arterial wall thickness was performed at four segments in the bilateral carotid arteries: at 1.5 cm distal to the bifurcation in the internal carotid artery (S1), at the bifurcation (S2), and at 0-1.5 cm (S3) and 1.5-3.0 cm (S4) proximal to the bifurcation in the common carotid artery. Eight measurements at S1-S4 of the bilateral carotid arteries were summed up as plaque score (PS) if they were 1.1 cm or more.

CAVI was measured using VaSera VS-1500 (Fukuda Den-shi Co. Ltd., Tokyo, Japan) that is basically the same as the method of measuring PWV. In short, electrocardiogram and monitoring of heart sounds were performed with the subjects in a supine position. PWV from the heart to the ankle was obtained by measuring the length from the origin of the aorta to the ankle and by calculating time taken for the pulse wave to propagate from the aortic valve to the ankle. Blood pressure was measured in the brachial artery. CAVI was automatically calculated using the equation published previously\(^\text{15,16}\).

Reactive hyperemia was evaluated using the endothelial peripheral arterial tone (EndoPAT) 2000 (Itamar Medical, Israel). RH-PAT measures the pulsatile volume changes to a reactive hyperemia challenge at the fingertip using proprietary non-invasive finger PAT probes. The reactive hyperemia procedure consisted of a 10-min baseline recording with the participant in a relaxed seated position with both arms resting on grooved arm rests. A blood pressure cuff was used to occlude blood flow to the left arm for 5 minutes. Resting systolic blood pressure was used to determine the appropriate level of cuff inflation. Finally, post-occlusion pulsatile volume changes were recorded for 5 minutes. The ratio between the post- to pre-occlusion average signal size was calculated to determine each participant’s RHI.

### 2.4. Statistics

Data were expressed as mean±S.D. The Student’s $t$-test or the Chi-square test was employed in comparison of values or ratios between patients with NASH and the others. Then, the simple linear regression analyses were performed to examine the association of age or BMI with the values of PS, CAVI, and RHI. Gender and age-referenced CAVI value (CAVI $>9.0$ in most cases) were automatically categorized as “high CAVI” while RHI=1.67 or more was defined as “low RHI”\(^\text{17}\). The Chi-square test was performed to examine the normal distribution of “high CAVI” and “low RHI” among NASH patients. All statistical analyses were performed using the IBM SPSS Statistics software (SPSS Statistics 21). Statistical significance was defined as $p<0.05$.

## 3. Results

### 3.1. Demographic data of the studied population

The baseline characteristics of the 39 participants (20 males and 19 females) were shown in Table 1. Patients in the NASH/NAFLD (-) group suffered from hepatitis due to virus, alcohol, drugs and undetermined reasons. Patients with NASH/NAFLD tend to be younger than the others. In addition, the BMI was higher and the CAVI value was significantly lower in NASH/NAFLD patients than the others.
No difference was observed in gender, smoking history, systolic and diastolic blood pressure, medication for hypertension, dyslipidemia and diabetes mellitus, and history of vascular diseases between the two groups. Vascular diseases include cerebral infarction, subarachnoidal hemorrhage, aneurysm in the cerebral artery, and angina.

### 3.2. Relationship between age, BMI, and vascular function

A simple regression analysis showed that PS was significantly associated with age ($r=0.511$, $p<0.001$) but not with BMI (Figure 1A). On the other hand, CA VI had a positive association with age ($r=0.706$, $p<0.001$) and an inverse association with BMI ($r=-0.511$, $p<0.001$). However, no association was found between RHI and age or between RHI and BMI (Figure 1B).

### 3.3. Impact of BMI on PS and RHI

We compared CAVI and RHI values in NASH/NAFLD patients with BMI≥25 or BMI<25. CAVI value in patients with BMI≥25 (N=14) was significantly lower than that in patients with BMI<25 (N=12) (7.3±1.6 vs 8.5±1.1, $p<0.05$); however, no difference was found in the RHI (1.93±0.59 vs 1.85±0.45, $p=0.70$) and PS values (2.3±2.1 vs 3.8±4.0, $p=0.22$) and in age (56.7±14.0 vs 63.3±12.3, $p=0.22$). Furthermore, we checked the distributions of CAVI and RHI scores in NASH/NAFLD patients with BMI≥25 and BMI<25. Among 26 NASH/NAFLD patients, 14 (53.8%) individuals showed either high CAVI or low RHI (Table 2), suggesting that these individuals are highly susceptible to vascular dysfunction. In 12 patients with BMI<25, four had high CAVI values and four had low RHI values. However, in 14 patients with BMI≥25, only one had high CAVI value whereas six had low RHI values. Although the Chi-square test did not show statistical significance ($\chi^2 = 0.14$, $p=0.70$), patients with low RHI were detected at higher rates (42.9%), compared to high CAVI patients (7.1%). Interestingly, there was only one patient duplicated with high CAVI and low RHI. This suggests that, especially in obese subjects, early stage of vascular disease can be effectively detected using the RHI in addition to CAVI.

### 4. Discussion

We found that CAVI, but not RHI or PS, was inversely associated with BMI in all subjects examined, suggesting that the measurement of CAVI alone might overestimate or underestimate vascular function in obese or lean subjects, respectively. In addition, NASH/NAFLD patients, especially those with obesity, are good candidates for RH-PAT examination to detect early stage of vascular failure.

NASH/NAFLD is associated with increased risk of death and ischemic stroke and heart diseases. A recent meta-analysis demonstrated that NAFLD patients have a significantly higher risk for clinical CV events with a relative risk of 1.77 (95% CI: 1.26-2.48, $p<0.001$). NASH/NAFLD patients are associated with high risk of insulin resistance and obesity. In addition to dyslipidemia and insulin resistance, endothelial dysfunction and inflammation are thought to be related to the pathophysiology of atherosclerosis and CV event in NASH/NAFLD patients. Shiotani et al. performed brachial-ankle PWV (baPWV) measurements in young students to show that baPWV in male students with NASH was significantly higher than those without NASH. Colak et al. reported that NASH/NAFLD patients showed increased IMT and decreased FMD compared to the control healthy subjects. A more recent study demonstrated that 61 male patients with NASH showed decreased FMD in addition to increased IMT and carotid-femoral PWV (cPWV) compared to 41 healthy male volunteers. Furthermore, in a total of 2,954 subjects, arterial stiffness was increased by the presence and severity of NAFLD when...
evaluated by CAVI, and the difference was significant even after adjustment for age, sex, BMI and other CV risks\(^{13}\). These findings support the notion that NASH/NAFLD patients are at high risk of CV disease and should be screened by vascular function examinations.

Here, we first report comparative findings of RH-PAT, CAVI, and PS in a small retrospective study and found that there was a marked discrepancy between RH-PAT and CAVI results in NASH/NAFLD patients. To date, growing evidences support the significance of RHI to determine subjects with high risk of CV events. Hirata et al. demonstrated that chronic kidney disease patients with RHI<1.69 had higher risk of CV events than those with RHI≥1.69\(^{32}\). Matsuzawa et al. reported in their prospective study that among high risk patients, those with RHI<1.70 had higher risk of CV events, compared to those with RHI≥1.70\(^{11}\). Akiyama et al. conducted an observational cohort study in heart failure patients with normal left ventricular ejection fraction and showed that those with RHI<1.64 had higher risk of future CV events\(^{33}\). Since RHI depends on endothelial function, low
RHI may be observed in patients with interstitial pneumonia or pulmonary fibrosis\(^1\).

Since PWV is known to be associated with blood pressure, CAVI has been developed to measure arterial stiffness, and the measurement is basically independent of blood pressure. In a recent study, however, CAVI is inversely associated with the BMI of both male and female\(^2\). This association is consistent with our findings, although the reason is still uncertain. Since PWV is inversely associated with BMI in children and adolescents, arterial diameter and stiffness can be affected by the fat mass or may be physiologically adapted to the hyperemic state of adiposity\(^3\,4\). In obese subjects, CAVI=7.8 has been reported as a cutoff value of CVD events\(^5\). This may be resulted from the characteristics of the participants, where they are much younger and more obese compared to the other studies’ population. On the other hand, successful body weight reduction in obese subjects ameliorates vascular function, where the CAVI value is decreased\(^6\,7\). Thus, CAVI can be comparable in each person at different time points, although it is not suitable for comparison between persons with different BMI. In contrast, RHI is not affected by BMI. Therefore, early stage of vascular endothelial dysfunction can be detected by RH-PAT in NAFLD patients with obesity, in addition to other conventional examinations.

Limitations of this study include small number of participants, mixture of various background of the control group, and thus lack of statistical analysis using multiple logistic regression analysis. Since the present pilot study has too small sample size and was conducted among patients with liver diseases, future studies with large scale and with healthy control of similar age are absolutely needed to confirm our findings. In addition, Lack of adjustment with confounding factors is also serious limitation in the present study. Recent studies demonstrated that alcohol consumption and anti-hypertensive drugs affect endothelial function, suggesting that these factors should be addressed\(^8\,9\).

In conclusion, RH-PAT could be a suitable tool to detect early stage of vascular failure especially in obese subjects. However, further study on a larger population is necessary to validate the effectiveness of RH-PAT as a diagnostic tool for vascular dysfunction.

**References**

17. Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, Kuvit JT, Lerman A. Noninvasive identification of patients with early coro-