Title

Oxidative stress and heart rate variability in patients with vertigo

Short title

OS and HRV in patients with vertigo

Author

Keiichiro Ohara, M.D., Yoshiaki Inoue, M.D., Yuka Sumi, M.D., Miki Morikawa, M.D., Shigeru Matsuda, M.D., Ken Okamoto, M.D., Hiroshi Tanaka, M.D.

Affiliation

Department of Emergency and Critical Care Medicine, Juntendo University Urayasu Hospital
2-1-1, Tomioka, Urayasu City, Chiba, 279-0021, Japan

Corresponding author

Keiichiro Ohara, M.D.
Department of Emergency and Critical Care Medicine, Juntendo University Urayasu Hospital
2-1-1, Tomioka, Urayasu City, Chiba-ken 279-0021, Japan
TEL: +81-47-353-3111, FAX: +81-47-353-3138,
E-mail: keioha@juntendo-urayasu.jp

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Abstract

【Aims】Peripheral vertigo has been reported to result from oxidative stress (OS) or autonomic nervous dysfunction. Recently, heart rate variability (HRV) has been used to evaluate autonomic nervous activity (ANA). Parasympathetic nervous dysfunction is associated with peripheral vertigo; however, the relationships among vertigo, OS, and ANA have not been investigated. The aim of this study was to elucidate the changes in OS and ANA in vertigo patients compared with healthy volunteers (HVs). 【Methods】 OS was assessed by evaluating biological antioxidant potential (BAP) and reactive oxygen metabolites (dROM), and HRV was measured to evaluate ANA. Thirty-four patients who complained of peripheral vertigo and were treated in our emergency department between January and August 2011 were enrolled in study 1. OS and HRV were measured and compared with those of HVs (N = 23). In study 2, OS in 18 vertigo patients and HRV in 41 patients were measured between January and August 2012 before and after the conventional treatment of vertigo to evaluate the effect of the treatment on OS and ANA.【Results】dROM were higher in vertigo patients than in HVs. On the other hand, parasympathetic nervous activity was lower and the sympathetic/parasympathetic nervous activity ratio (ANA ratio) was higher in vertigo patients than in HVs. After the treatment of vertigo, dROM decreased significantly and the ANA ratio became much similar to that observed in HVs.【Conclusions】Bedside monitoring of OS and HRV may be useful for the diagnosis of vertigo and evaluation of the effect of treatment.

Background

Patients who complain of vertigo are often transported to an emergency department (ED). Multiple factors induce vertigo, because the sense of balance requires proper functioning of multiple body parts including the inner ears, eyes, muscles, skeleton, and nervous system. In the United States, the common causes of vertigo have been reported as aural (32.9%), cardiological (21.1%), neurological (11.2%), cerebrovascular (4%), and others (34.8%) including injury, psychiatric disease, or infectious disease[1].

The pathophysiological mechanism of vertigo has been studied and physiological stress appears to play an important role. House et al. reported that Meniere's disease was not caused by psychological disorders but by biological stress; patients with Meniere's disease are in stressful situations[2]. Most of the previous studies evaluating physiological stress used patient interviews because physiological stress was difficult to measure quantitatively. Recently, methods for quantitative evaluation of physiological stress have become available in the clinical setting. In particular, oxidative stress (OS) and autonomic nervous activity (ANA) are major targets for clinical evaluations of physiological stress. For example, protein carbonyl (PC) levels were previously used as an indicator of protein oxidation. It was revealed that PC was higher in patients with Meniere's disease than in controls[3]. These findings suggested that antioxidant therapy may be useful for patients with Meniere's disease[4,5]. It has been reported that ANA could be evaluated by assessing heart rate variability (HRV)[6]. HRV can reflect the dynamic interplay between ongoing perturbations in circulatory function and the compensatory responses of short-term cardiovascular control systems. Analysis of HRV[7-9] includes low-frequency (LF) fluctuations, which reflect both parasympathetic

and sympathetic activity, and high-frequency (HF) fluctuations, which reflect parasympathetic activity. These findings have been used for the evaluation of ANA in vertigo patients. Although some reports have described the relationship between vertigo and physiological stress[2-4], to the best of our knowledge, no study has examined OS and ANA in vertigo patients. In particular, changes in physiological stress have not been compared before and after the treatment of vertigo. In this study, OS and HRV were evaluated in vertigo patients compared with healthy volunteers (HVs) and the effect of treatment was evaluated.

Patients and Methods

Overall protocol

This study was approved by the Institutional Review Board of Juntendo University (approval number 23-32) and informed consent was obtained from each patient or a close relative. Subjects were recruited from patients transferred by ambulance to the ED of Juntendo University Urayasu Hospital. The exclusion criteria included age of <15 years, vertigo due to central nervous system disease, or other injuries.

Measurements

For each patient, whole blood samples (10 mL) were collected from a peripheral vein into heparin-coated tubes within 30 min of arrival to the ED to assess OS by measuring biological antioxidant potential (BAP) and reactive oxygen metabolites (dROM). Blood samples were centrifuged for 5 min at 12000 rpm, and the

collected serum samples were divided into to 2 tubes of at least 2 mL each; these samples were rapidly frozen at −80°C. These samples were defrosted within 72 h and BAP and dROM were measured using Free Radical Analytical System 4 (FRAS4^{TM}, Health & Diagnostics Limited Co., Parma, Italy). BAP reflected the blood level of antioxidant substances. The BAP test uses a colored solution containing ferric (Fe^{3+}) ions bound to a special chromogenic substrate that changes color when the Fe^{3+} ions are reduced to ferrous ions (Fe^{2+}). Then, 10 μL of the serum sample was added to the cuvette. After incubating for 5 min at 37°C, absorbance at 505 nm was recorded. The dROM test reflected the blood level of reactive oxygen metabolites, particularly that of hydroperoxides, which are markers and amplifiers of free radical-induced oxidative damage. In this test, the ROM level is proportional to the intensity of red coloration. In brief, 20 μL of blood and 1 mL of buffered solution were mixed in a cuvette, and 10 μL of the chromogenic substrate was added to the cuvette. After mixing and centrifugation for 60 s, the cuvette was incubated in a thermostatic block for 5 min at 37°C. Thereafter, absorbance at 505 nm was recorded. The results were expressed as U.CARR.

HRV was assessed using a sphygmograph (TAS9^{TM} Pulse Analyzer Plus, YKC Corporation, Tokyo, Japan) attached to the left forefinger while the patients lay silently on a bed in the supine position with their eyes closed. It was recorded for 2.5 min and the frequency domain information was analyzed automatically with a fast Fourier Transformation. The technical details of HRV analysis have been presented in detail previously[8-10]. In brief, the power spectral components of the R–R interval between 0.04–0.15 Hz were considered LF components and those between 0.15–0.40 Hz were considered HF components. The heart rate data was sampled immediately after each heart beat and was transferred to a personal computer and analyzed with supplied

software. The heart rate values were averaged, and the LF and HF power values were calculated by integrating each frequency band every 2.5 min; these measurements were then subjected to further analysis[10]. Patients with arrhythmias were excluded from HRV analysis, because HRV could not be measured correctly with an irregular heart rhythm.

Study protocol and evaluation

Using these OS (BAP, dROM) and HRV measurements, we established 2 study protocols. In the first study (study 1), OS and HRV were compared between vertigo patients treated at our ED between January to August 2011 and HVs (N = 23). In the second study (study 2), OS and HRV in vertigo patients were compared before and after the conventional treatment of vertigo. This treatment included a 2-h infusion of Sordem 3A^{TM} (200 mL, Ohtsuka, Tokyo, Japan) mixed with adenosine triphosphate (ATP) disodium hydrate (40 mg) and 8.4% sodium bicarbonate (20 mL). If the patient complained of nausea, 10 mg of metoclopramide was injected through the intravenous line. Whole blood samples (10 mL) were collected immediately after visiting the ED for the "before treatment" data and then collected immediately after the 2-h infusion for the "after treatment" data. Patients included in the second study were treated in our ED between January and August 2012.

Statistics

Data are expressed as mean ± standard deviation (SD). Welch's t-test was used for comparisons of groups in study 1, and the paired t-test was used in study 2. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). *P* values of <0.05 were considered statistically significant.

Results

Patient background

The patients selected for studies 1 and 2 are shown in Figure 1. In study 1, BAP and dROM were measured in 34 patients (age: 64 ± 15 years, 12 males and 22 females), and HRV in 24 patients (age: 56 ± 17 years, 9 males and 15 females). Twenty-three HVs (age: 36 ± 11 years, 15 males and 8 females) were included in this study as a control group. Ten patients were excluded because of arrhythmia. In study 2, HRV was measured in 41 patients (age: 59 ± 14 years, 15 males and 26 females) before and after the treatment of vertigo, whereas BAP and dROM were evaluated in 18 patients (age: 65 ± 15 years, 6 males and 12 females). Twenty-three patients were excluded because they did not give consent (Fig. 1).

Oxidative stress in vertigo patients

We measured BAP, dROM, and the BAP/dROM ratio in vertigo patients compared with HVs (Fig. 2). The BAP/dROM ratio was evaluated to investigate the balance of OS. dROM were significantly higher in vertigo patients than in HVs (HVs: 295 ± 51 U.CARR, Pt: 337 ± 60 U.CARR, Fig. 2B, $p < 0.01$). There was no significant difference in BAP (HVs: 2183 ± 207 μM, Pt: 2207 ± 429 μM, Fig. 2A) or the BAP/dROM ratio (HVs: 7.74 ± 2.15, Pt: 6.79 ± 1.91, Fig. 2C). These results indicate that superoxide and oxygen metabolites were higher in vertigo patients than in HVs.

Autonomic nervous activity in vertigo patients

Sympathetic nervous activity (LF/HF), parasympathetic nervous activity (HF), and the sympathetic/parasympathetic nervous activity ratio, which is reflective of ANA balance (LF/HF^2, defined as the ANA ratio), were assessed (Fig. 3). All data were logarithm transformed and compared with the values of HVs. There was no significant difference in sympathetic nervous activity between HVs and vertigo patients (HVs: 1.02 ± 0.23, Pt: 1.14 ± 0.30, Fig. 3A). However, parasympathetic nervous activity in vertigo patients was significantly suppressed (HVs: 5.27 ± 1.00, Pt: 4.13 ± 2.34, Fig. 3B, $p < 0.05$) and the ANA ratio was significantly elevated compared with HVs (HVs: 0.20 ± 0.08, Pt: 0.47 ± 0.51, Fig. 3C, $p < 0.05$). These results suggest that ANA balance was disturbed in vertigo patients.

Effect of treatment on oxidative stress in vertigo patients

The changes in BAP, dROM, and the BAP/dROM ratio before and after the 2-h treatment of vertigo are shown in Figure 4. dROM were significantly reduced after treatment (before: 349 ± 60 U.CARR, after: 331 ± 60 U.CARR, Fig. 4B, $p < 0.01$). However, no significant difference was observed in BAP (before: 1985 ± 325 μM, after: 1941 ± 278 μM, Fig. 4A) or the BAP/dROM ratio (before: 5.88 ± 1.47, after: 6.07 ± 1.44, Fig. 4C). The symptoms improved in 16 patients after treatment, and 2 patients were admitted to our hospital for observation.

Effect of treatment on autonomic nervous activity in vertigo patients

Figure 5 shows ANA, expressed as HRV, in 41 vertigo patients before and after treatment. There was no change in sympathetic nervous activity (before: 1.21 ± 0.42, after: 1.14 ± 0.32, Fig. 5A) or parasympathetic nervous activity (before, 4.08 ±

1.96, after: 4.07 ± 1.11, Fig. 5B) after treatment. The ANA ratio after treatment had a tendency to be similar to that in HVs (before, 0.51 ± 0.66, after: 0.32 ± 0.20, HVs: 0.20 ± 0.08, Fig. 5C, $p = 0.06$). Although there was no statistical difference in the ANA ratio before and after treatment, ANA imbalance may be attenuated by the treatment.

Discussion

In our study, we quantitatively evaluated physiological stress in vertigo patients by measuring OS and ANA. As an OS biomarker, dROM were significantly higher in vertigo patients than in HVs (Fig. 2B). Parasympathetic nervous activity, as quantified by HF of HRV, was significantly suppressed in vertigo patients compared with HVs (Fig. 3B).

Similar to our findings, some studies have reported an elevation of OS in vertigo patients[3,4,11]. The production of dROM results from several mechanisms, including oxidative phosphorylation in the mitochondria as a product of normal cellular aerobic metabolism[12,13]. Thus, dROM can be produced by the major process from which the body derives energy[13]. The balance between dROM production and activation of the antioxidant defense system is crucial in human physiology and the control of cellular homeostasis[14]. While dROM play an important role in signaling processes, their overproduction generates OS. dROM can regulate cellular functions during immune and inflammatory processes[15], which cause the overproduction of OS. Therefore, it is difficult to determine the source of production of dROM. It is possible that OS promotes vasculitis of the vertebrae and endolymphatic hydrops in vertigo patients[3,4]. Measurement of OS could evaluate not only the severity of vertigo but also the cause of

vertigo.

Previous studies[7,8,11,16] have reported significant parasympathetic nervous hypofunction in vertigo patients, which is similar to the findings of our study (Fig. 3B). It was considered that the suppression of parasympathetic activity and the relative hyperfunction of sympathetic activity in vertigo patients influenced the vertebrobasilar arterial system. These pathophysiological mechanisms may produce laterality of peripheral vestibular function, thus resulting in vertigo[9]. Therefore, one possible mechanism of vertigo is change in blood flow and pressure in the vertebrobasilar artery and cochleovestibular organs.

Our research also evaluated the effect of the treatment of vertigo on biological stress. Conventionally, 8.4% sodium bicarbonate and ATP disodium hydrate have been used for the treatment of vertigo. It is believed that sodium bicarbonate improves vertigo by acting on the central and peripheral vestibular system and correcting acidosis[17], while ATP disodium hydrate improves vertigo by increasing cerebral blood flow and cerebrovascular extension[18]. After treatment, dROM decreased significantly (Fig. 4B) and ANA balance was also attenuated (Fig. 5C). Possibly, adding antioxidants to our medication protocol would enhance the effect of the conventional treatment. Our management of these patients now includes bedside monitoring of HRV and measurement of oxidative activity, which is very useful and can be measured repeatedly.

Our study has some limitations. First, patients with arrhythmia were excluded because accurate HRV analysis could not be performed in these patients. However, some patients complaining of vertigo have synchronizing paroxysmal arrhythmia. Second, the age and sex of HVs and vertigo patients were different. Vertigo patients were older and included a higher number of females. This background difference could

have induced a bias. Further studies are necessary using age- and sex-matched HVs.

Conclusion

We quantitatively evaluated physiological stress in vertigo patients using OS and HRV. We found that OS was significantly higher and parasympathetic activity was significantly suppressed in vertigo patients. After the conventional treatment of vertigo, dROM was reduced and ANA balance was improved. Bedside monitoring of OS and HRV may be useful for the diagnosis of vertigo and evaluation of the effect of treatment.

Conflict of Interest

There is no conflict of interest that should be disclosed.

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Figure Legends

Figure 1. Patient selection in study 1 and study 2.

A: In study 1, BAP, dROM (N = 34), and HRV (N = 24) were compared between vertigo patients and HVs (N = 23).

B: In study 2, BAP, dROM (N = 18), and HRV (N = 41) were measured in vertigo patients before and after treatment.

Figure 2. Comparison of oxidative stress in vertigo patients and healthy volunteers.

A: BAP, B: dROM, and C: BAP/dROM ratio. Open circles show HVs (N = 23), and closed circles show vertigo patients (N = 34). $^{**}p < 0.01$, Welch's t-test.

Figure 3. Comparison of autonomic nervous activity in vertigo patients and healthy volunteers.

A: Sympathetic nervous activity, B: Parasympathetic nervous activity, and C: ANA ratio expressed as the sympathetic/parasympathetic nervous activity ratio. Open circles show HVs (N = 23), closed circles show vertigo patients (N = 24). $^{*}p < 0.05$, Welch's t-test.

Figure 4. Effect of the treatment of vertigo on oxidative stress.

A: BAP, B: dROM, and C: BAP/dROM ratio before (left) and after (right) treatment in vertigo patients (N = 18). Gray area indicates mean ± SD of HVs (N = 23). $^{**}p < 0.01$, paired t-test.

Figure 5. Effect of the treatment of vertigo on autonomic nervous activity.

A: Sympathetic nervous activity, B: parasympathetic nervous activity, and C: ANA ratio

expressed as the sympathetic/parasympathetic nervous activity ratio in vertigo patients (N = 41) before (left) and after (right) treatment. Gray zone indicates the mean ± SD of HVs (N = 23).

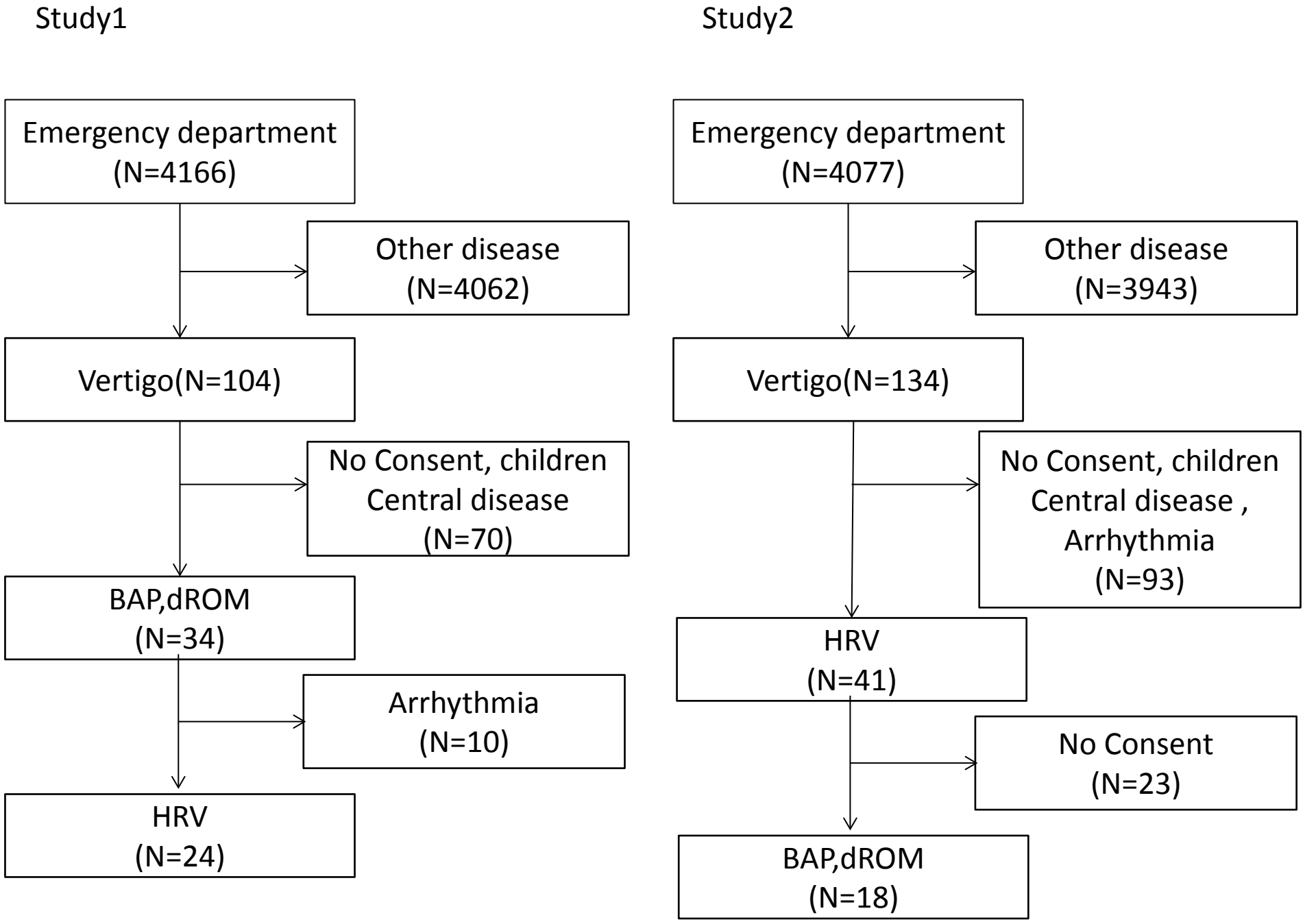
Study1
Emergency department
(N=4166)
Other disease
(N=4062)
Vertigo(N=104)
No Consent, children
Central disease
(N=70)
BAP,dROM
(N=34)
Arrhythmia
(N=10)
HRV
(N=24)
Study2
Emergency department
(N=4077)
Other disease
(N=3943)
Vertigo(N=134)
No Consent, children
Central disease ,
Arrhythmia
(N=93)
HRV
(N=41)
No Consent
(N=23)
BAP,dROM
(N=18)

Figure. 1

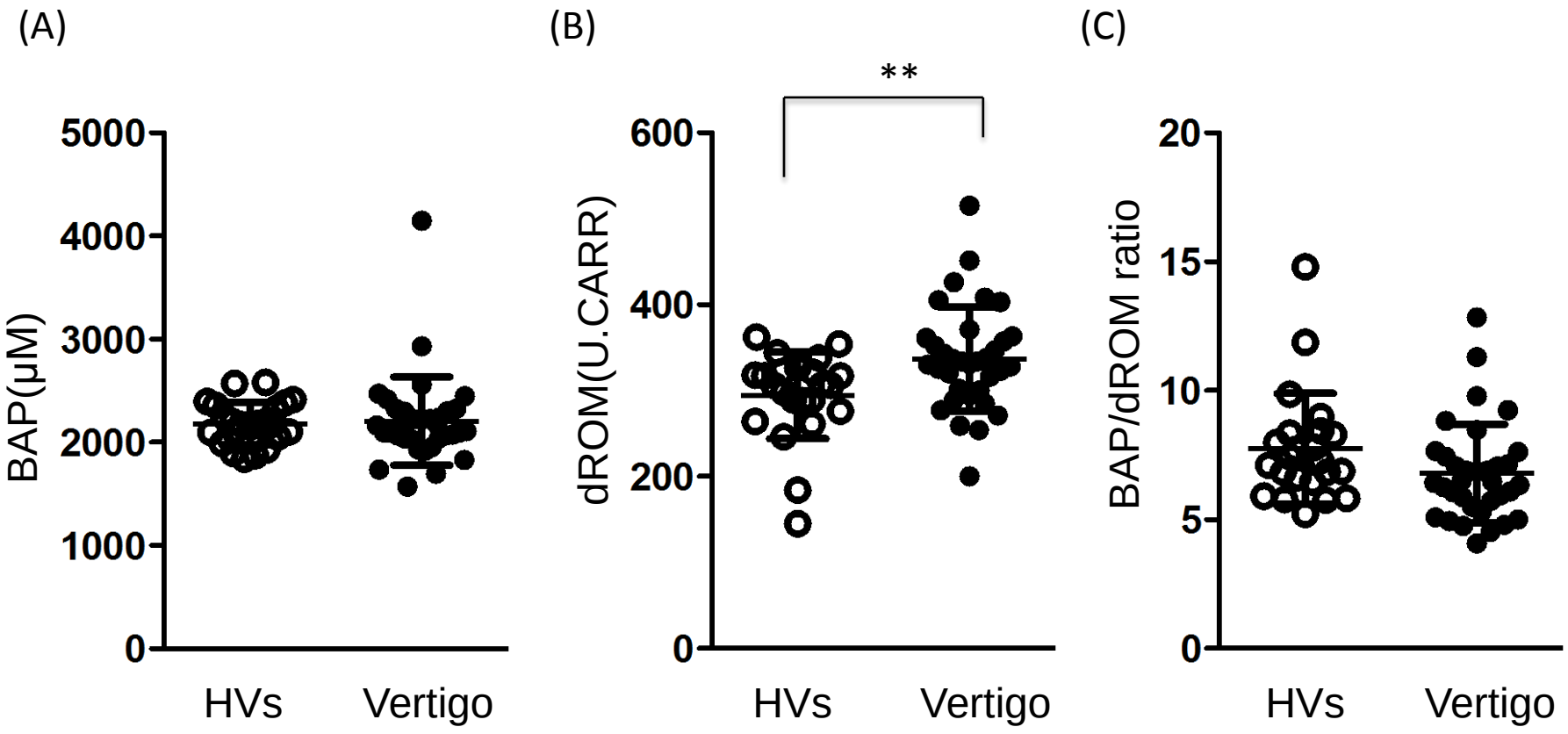
(A)
BAP(μM)
5000
4000
3000
2000
1000
0
HVs
Vertigo
(B)
**
dROM(U.CARR)
600
400
200
0
HVs
Vertigo
(C)
BAP/dROM ratio
20
15
10
5
0
HVs
Vertigo

Figure. 2

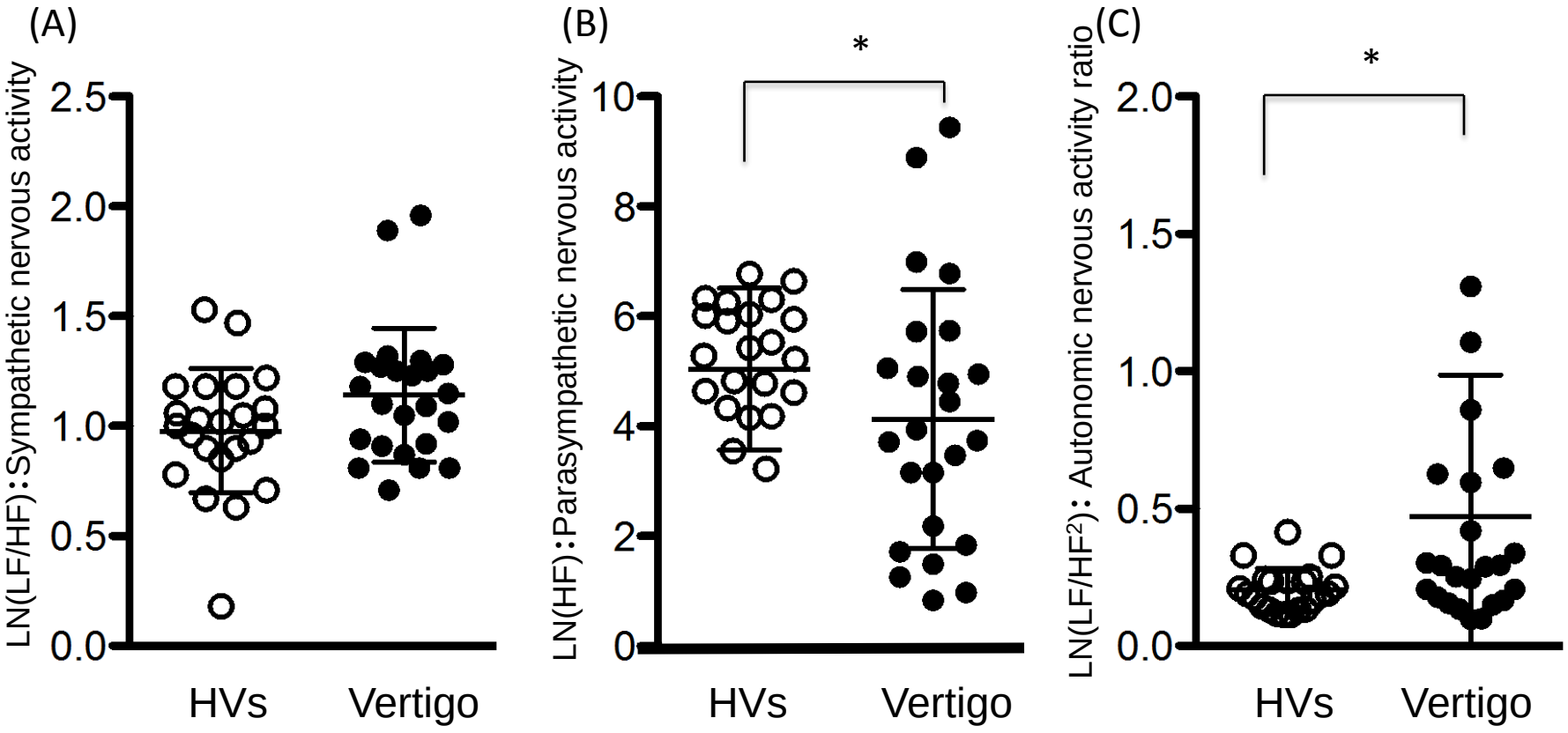
(A)
LN(LF/HF):Sympathetic nervous activity
2.5
2.0
1.5
1.0
0.5
0.0
HVs
Vertigo
(B)
LN(HF):Parasympathetic nervous activity
*
10
8
6
4
2
0
HVs
Vertigo
(C)
LN(LF/HF²): Autonomic nervous activity ratio
*
2.0
1.5
1.0
0.5
0.0
HVs
Vertigo

Figure. 3

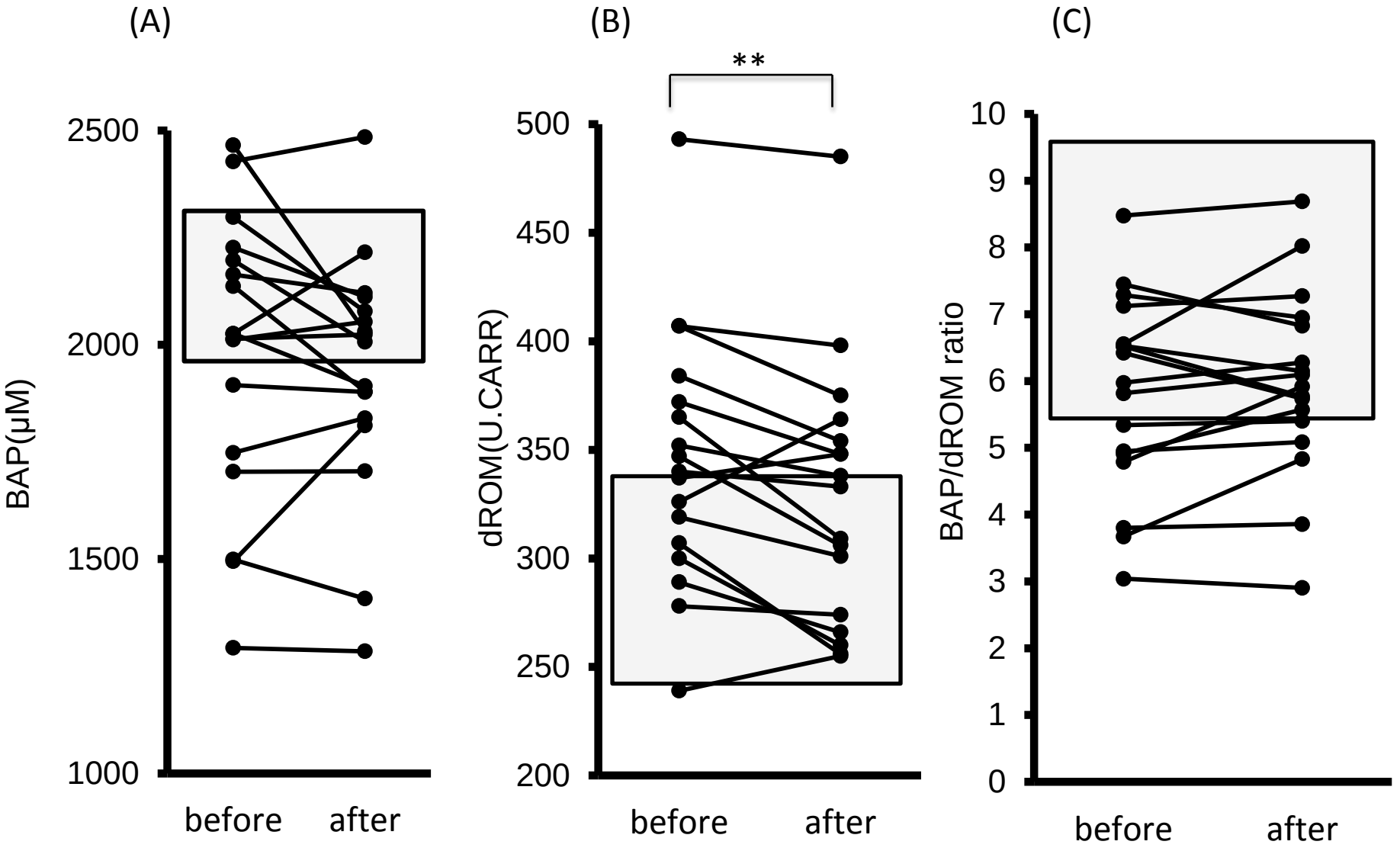
(A)
(B)
(C)
**
BAP(μM)
2500
2000
1500
1000
before
after
dROM(U.CARR)
500
450
400
350
300
250
200
before
after
BAP/dROM ratio
10
9
8
7
6
5
4
3
2
1
0
before
after

Figure. 4

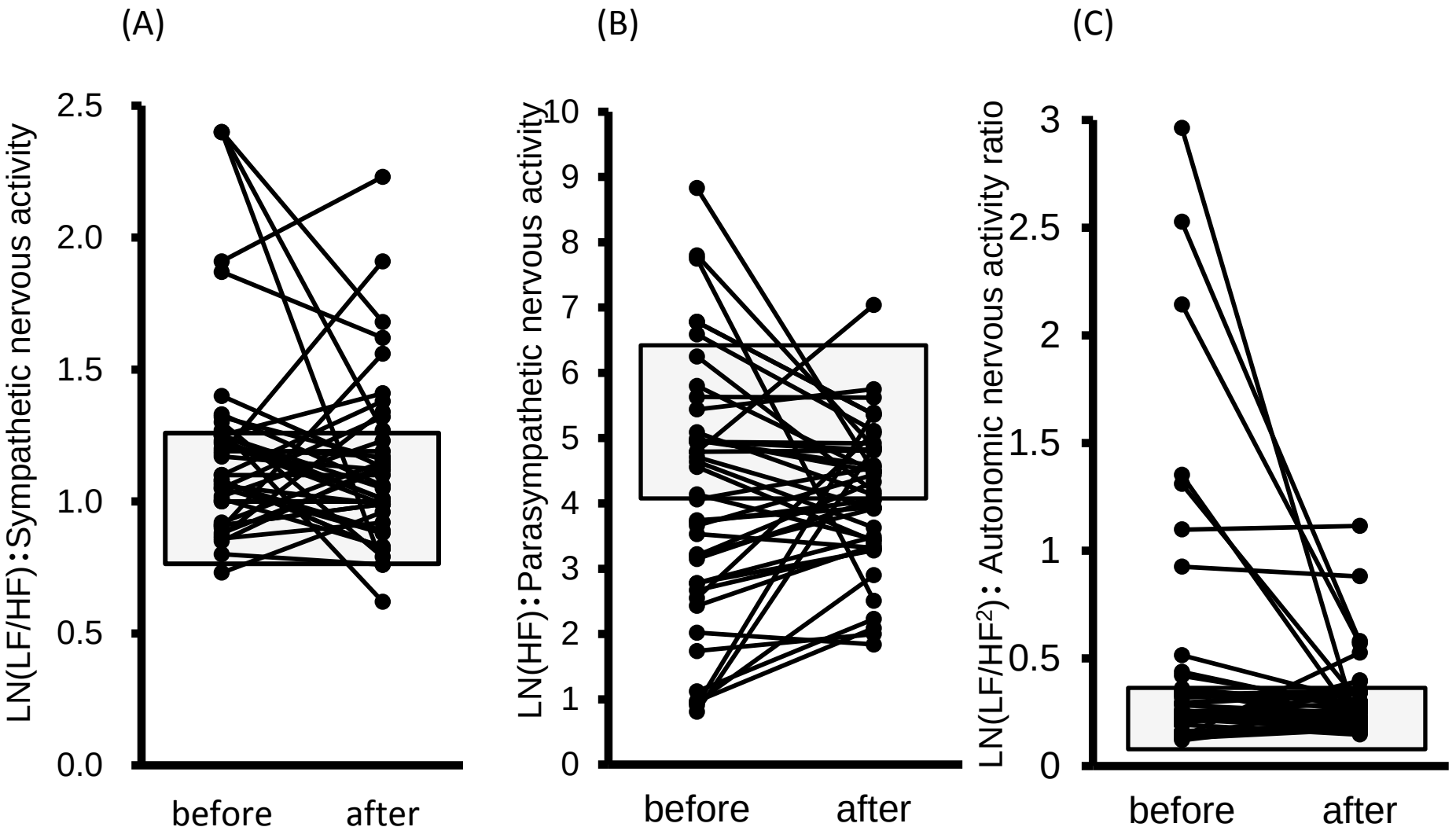
(A)
(B)
(C)
LN(LF/HF)：Sympathetic nervous activity
LN(HF)：Parasympathetic nervous activity
LN(LF/HF²)：Autonomic nervous activity ratio
2.5
2.0
1.5
1.0
0.5
0.0
10
9
8
7
6
5
4
3
2
1
0
3
2.5
2
1.5
1
0.5
0
before
after
before
after
before
after

Figure. 5