Cardiovascular Autonomic Function in Normotensive Men with High Salt and Low Salt Intakes

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ABSTRACT
Background and Aim: The autonomic nervous system may play an important role in salt-induced increases in blood pressure (BP). The aim of this study is to assess and compare the heart rate (HR) responses to Valsalva maneuver, deep breathing and standing and BP responses to standing and sustained handgrip between normotensive men with habitual high salt intake (HSI) and low salt intake (LSI). Methods: Twenty-fourty years old normotensive men (n=34; n=17 for HSI and n=17 for LSI) were recruited by collecting 4 early morning spot urine samples. Average estimated 24-hr sodium (Na⁺) excretion of 4 urine samples of ≥166 mmol/day was regarded as HSI and <115 mmol/day as LSI. Heart rate responses to Valsalva maneuver, deep breathing and standing (parasympathetic function tests) and BP responses to standing and sustained handgrip (sympathetic function tests) were investigated in both groups. Results: Valsalva ratio (VR) [1.64±0.36 (HSI) vs 1.78±0.49 (LSI)], deep breathing difference (DBD) [24.51±9.56 beats/min (HSI) vs 22.93±6.37 beats/min (LSI)] and 30:15 ratio [1.34 (1.12–1.46) (HSI) vs 1.19 (1.13–1.52) (LSI)] were not significantly different in both groups. Systolic blood pressure (SBP) change from lying to standing [-0.67 (-4.00–9.00) mmHg (HSI) vs 0.00 (-3.34–2.34) mmHg (LSI)] was not significantly different between the two groups whereas diastolic blood pressure (DBP) rise in sustained handgrip [18.20±3.49 mmHg (HSI) vs 14.77±4.71 mmHg (LSI)] was significantly higher in the HSI (p=0.022). Conclusion: Cardiovascular sympathetic activity appears to increase in normotensive men with habitual high salt intake. Key words: Cardiovascular autonomic function, Normotensive, Salt intake, Sympathetic activity, Parasympathetic activity

INTRODUCTION
Salt is a ubiquitous constituent of diets.¹ Preference for salt increases the quantity of salt consumed and the incidence of hypertension is more in salt-prefering individuals than in no salt-prefering persons.² Consumption of high dietary Na⁺ may increase BP.³ Hypertension is associated with increased sympathetic outflow.⁴ The major determinant of BP variability is sympathovagal balance of cardiovascular regulation.⁵ High dietary Na⁺ can adversely affect the heart and areas of the brain that control autonomic outflow.⁶ The autonomic nervous system may play an important role in Na⁺-induced increases in BP.⁷ Pal and colleagues reported that salt predilection is associated with sympathovagal imbalance in the form of sympathetic overactivity and vagal withdrawal in young Indian normotensives.⁸ However, Luft and associates discovered that sympathetic nervous system activity appears to decrease with salt loading in normal subjects.⁹ McNeely and his co-workers found the increased vagal tone in normotensive persons on high salt intake.¹⁰ A meta-analysis of randomized controlled trials demonstrated that there was no change in sympathetic tone with long-term moderate Na⁺ restriction in normotensives.¹¹ The mechanisms priming Na⁺-induced rise in BP are not completely understood. Previous studies stated that salt intake may be associated with changes in the cardiovascular autonomic nervous system, but the results are diverse, especially in normotensives. Many studies were undertaken by giving high and low sodium diet interventions.¹²⁻¹³ Studies with habitual sodium intake on heart rate and blood pressure responses to various stressors meant to elicit autonomic responses in normotensives are limited. We assumed that study utilizing habitual sodium intake may reflect the lifelong cardiovascular autonomic function. Therefore, we aimed to investigate the cardiovascular autonomic function in normotensive men with habitual high and low salt intakes.

MATERIALS AND METHODS
Participants
The study was approved by the Protocol Board of the University of Medicine 2, Yangon and carried out in July 2016 to February 2017 with written informed consent. Twenty to fourty years aged 106 participants with 18.5-25 kg/m² of body mass index (BMI), resting HR between 50 and 100 beats/min, SBP <120 mmHg.

DBP < 80 mmHg, serum creatinine < 1.5 mg/dl and fasting blood sugar (FBS) < 110 mg/dl were recruited from the office staff of the University of Medicine 2, Yangon and from surrounding areas. Exclusion criteria included: diabetes mellitus, cardiovascular diseases, neurological disorders, syncpe, chronic alcoholics and smokers.

Experimental Protocol

Dietary sodium can be accessed by 24-hr urine collection or a spontaneously voided morning urine sample.[12,13] The spot urine sample reflects the 24-hr urinary Na+ excretion.[14-17] In Myanmar, ICNND (Interdepartmental Committee on Nutrition for National Defense) reported that average daily individual salt consumption is 11.8 g in Myanmar military in 1963.[18] Aye-Aye-Myint also found that mean daily salt intake was 11.08±0.62 g of sodium chloride in 1992.[19] The estimation of salt consumption by Tanaka's formula was adequate when the consumption was between 9-12 g/day (Mill et al. 2015).[20] Therefore, in our study, 24 hr urinary Na+ excretion was calculated from the spot urine sample by using the Tanaka's formula.[21]

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[XNa] = \frac{Na^+ \text{ concentration in the spot urine (mg/L)}}{creatinine concentration in the spot urine sample (mg/L)} \times \text{predicted value of 24 hr urinary Cr excretion (PrU}_C^{24})
\]

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\text{PrU}_C^{24} = 2.04 \times \text{Age (years)} + 14.89 \times \text{Body weight (kg)} + 16.14 \times \text{Height (cm)} - 2244.45
\]

A meta-analysis based on 23 cohort studies and 2 randomized controlled trials investigated the incidence of cardiovascular disease events in populations exposed to dietary intakes of low sodium (< 115 mmol/day), usual sodium (low usual sodium: 115-165 mmol/day; high usual sodium: 166 – 215 mmol/day) and high sodium (> 215 mmol/day). Both low and high sodium intakes are associated with increased incidence of cardiovascular disease compared with usual sodium intakes.[22] Therefore, we defined the average 24 hr urinary Na+ excretion 166 mmol/day or more as HSI and < 115 mmol/ day as LSI.

There is tremendous day-to-day variability in urinary sodium excretion and therefore to get the habitual dietary sodium, 4 early morning spot urine samples were collected. First and 2nd samples were collected on Monday and Tuesday and two weeks later, 3rd and 4th samples were collected on Monday and Tuesday again. The average 24 hr urinary Na+ excretion calculated from the 4 spot urine samples was used to identify the salt intake of the subject.

Among the recruited 106 men, 15 withdrew voluntarily from the study before completion of the collection of 4 urine samples according to their personal matters. Ninety one subjects completed the collection of 4 urine samples. Among the 91 subjects, 22 men for HSI and 19 men for LSI were obtained. The remaining participants were excluded from the study. To reduce the confounding effect of age and BMI on cardiovascular autonomic function, age and BMI-matched 17 HSI and 17 LSI subjects were selected to assess the cardiovascular autonomic function. The interval between the day of taking the last urine sample and the day of cardiovascular autonomic function assessment was in the range of 1 to 71 days.

On the day of cardiovascular autonomic function assessment, the subject was requested not to take drugs and caffeinated beverages and to refrain from heavy exercise. The tests were performed at least 2 hr after last meal. The examination room was kept quiet with temperature 22-25°C. After instrumentation, the subject was resting on a chair for 15 min. Baseline HR was calculated from the average of 10 consecutive RR intervals of ECG. BP was measured 3 times at one-minute interval and the average was taken as the baseline. Valsalva maneuver, deep breathing, orthostasis and sustained handgrip (SHG) were then carried out in series resting 10 to 15 min between each test until the HR returned to the baseline.[23]

Measurements

BMI was measured with a bathroom scale and a flexible inch tape. FBS was measured by Glucometer (Prodigy Preferred Blood Glucose Meter, Prodigy Diabetes Care, Taiwan). Serum and urine creatinine were determined by Humalyzer Primus (Human, Germany) and auto creatinine liquidolor (Human, Germany). Na+ concentration of urine was measured by the use of graphite furnace atomic absorption spectrometer (GBC 932plus, Serial number A4982, GBC Scientific Equipment Pty. Ltd., Australia). HR was recorded in lead II ECG and analysis was done by computerized ECG system (containing PowerLab 8/35 – 8 Channels (PL3508) data acquisition system with FEI132 BioAmp and LabChart software, AD Instruments, Australia). BP was measured with mercury sphygmomanometer (Kenxin, Hong Kong) and stethoscope (3M Littmann, classic II S.E, USA). Handgrip dynamometer (CH. Stoelting, USA) was used for the SHG.

HR Response and Valsalva Maneuver

The subject blew into a disposable mouthpiece (a 5cc disposable syringe) connected to a modified sphygmomanometer holding at a pressure of 40 mmHg for 15 sec while ECG was recording continuously. The maneuver was performed 3 times with one-minute interval in between. The ratio of the longest RR interval after the maneuver (reflecting overshoot bradycardia following release) to the shortest RR interval during the maneuver (reflecting the tachycardia during strain) was taken as VR.[23] The mean of 3 VRs was taken as the final value.

HR Variation and Deep Breathing

The subject was sitting quietly and breathing deeply at six breaths per minute. The minimum and maximum HR during the breathing cycle was measured. The result (DBD) was expressed as the mean of the differences between maximum and minimum HR for 6 breathing cycles.

HR Response and Standing

After lying quietly on a couch for 15 min, the subject stood up unaided within 5 sec and remained motionless for two minutes. To get the shortest RR interval around 15th beat and the longest RR interval around 30th beat after standing up, the RR interval of 5 heart beats before and after the 15th beat (i.e. from 10th to 20th beats) and the 30th beat (i.e. from 25th to 35th heart beats) were measured. The HR response, 30:15 ratio, was expressed by the ratio of the longest RR interval around 30th beat to the shortest RR interval around 15th beat.

SBP Response and Standing

After lying quietly on a couch for 15 min, BP was measured 3 times with one-minute interval and the average was taken as lying BP. Then the subject stood up unaided (within 5 sec) and remained motionless for 2 min. BP was measured at one minute after standing up. The difference in SBP one minute after standing up and that in lying position was taken as postural change in BP. The Consensus Committee of the American Autonomic Society and the American Academy of Neurology defines that orthostatic hypotension is reduction of SBP of at least 20 mmHg or DBP of at least 10 mmHg, within 1-3 min.[24] Therefore, BP was assessed at one minute after standing.

DBP Response and SHG

The participants were sitting down during the handgrip trial. In handgrip dynamometer, one dial hand “freezes” at the maximum grip achieved, while the other follows the grip up or down to show the current squeeze
strength. Handgrip was maintained at the workload of 30 percent of maximum voluntary contraction for 5 min by using handgrip dynamometer with dominant hand. To verify the workload throughout 5 min, the dial hand that freezes at the maximum grip achieved was set at 30% of maximal voluntary contraction while the target workload was monitored by the other dial hand. BP was measured 3 times just before and at one-minute interval during handgrip. The difference between the highest DBP during handgrip and the mean of the 3 DBP readings just before handgrip was taken as the response.

### Statistical Analysis of Data

Statistical analysis was done with SPSS software, version 22 (IBM Corporation, New York, United States). Mean ± SD was used to present normally distributed data and comparison was done by independent t test. Skewed data were expressed as median and interquartile range and compared by Mann-Whitney U test. Statistical significance was set as p values of less than 0.05.

### RESULTS

#### General Characteristics

BMI, serum creatinine, FBS, resting HR, resting SBP, DBP and MAP were not significantly different. Average 24-hr urinary Na+ excretion was significantly different (p<0.001) (Table 1).

#### HR Response and Valsalva Maneuver

In HSI group, the mean longest RR interval was 1.02 ± 0.20 sec and mean shortest RR interval was 0.63 ± 0.11 sec. In LSI group, the mean longest RR interval was 0.99 ± 0.16 sec and the mean shortest RR interval was 0.58 ± 0.11 sec. Mean VRs were 1.64 ± 0.36 (HSI) and 1.78 ± 0.49 (LSI) and were not significantly different (p = 0.355, independent t test).

#### HR Variation and Deep Breathing

In the HSI group, maximum HR was 89.57±12.87 beats/min and minimum HR was 65.05±12.32 beats/min. In the LSI group, maximum HR was 93.38±6.70 beats/min and minimum HR was 70.46±8.79 beats/min. Deep breathing differences were 24.51±9.56 beats/min (HSI) and 22.93±6.37 beats/min (LSI) and not statistically significant (p = 0.574, independent t test).

#### HR Response and Standing

Mean HRs during lying in subjects were 66.23 ± 11.97 beats/min (HSI) and 68.82 ± 9.47 beats/min (LSI). In the HSI group, mean HRs were 101.63 ± 12.01 beats/min around 15th beat and 79.02 ± 15.97 beats/min around 30th beat on standing.

In the LSI group, the mean HR was 103.45 ± 9.75 beats/min around 15th beat and 80.80 ± 14.61 beats/min around 30th beat. In HSI group, the median (IQR) of the longest RR interval around 30th beat during standing was 0.82 (0.69-0.91) sec and that of the shortest RR interval around 15th beat during standing was 0.60 (0.53-0.65) sec. In LSI group, the longest RR interval around 30th beat was 0.73 (0.63-0.87) sec and the shortest RR interval around 15th beat was 0.60 (0.53-0.62) sec. The 30:15 ratios [median (IQR)] were 1.34 (1.12-1.46) (HSI) and 1.19 (1.13-1.52) (LSI) and not significantly different (p = 0.756, Mann-Whitney U test).

#### SBP Response and Standing

In HSI group, SBP during lying was 109.84 ± 11.02 mmHg and mean SBP at one minute after standing was 111.88 ± 13.86 mmHg. In the LSI group, mean SBP during lying was 109.02 ± 7.58 mmHg and mean SBP at one minute after standing was 108.94 ± 8.84 mmHg. SBP changes in response to standing were less than 10 mmHg. The changes in SBP at one minute after standing [median (IQR)] were -0.67 (-4.00 – 9.00) mmHg (HSI) and 0.00 (-3.34 – 2.34) mmHg (LSI) and not significantly different (p = 0.593, Mann-Whitney U test).

### BP Response to Sustained Handgrip

In HSI group, the mean SBP before SHG was 103.61 ± 10.80 mmHg and the mean highest SBP during SHG was 118.59 ± 14.21 mmHg. In LSI group, the mean SBP before SHG was 104.00 ± 8.80 mmHg and the mean highest SBP during SHG was 116.94 ± 9.06 mmHg. The mean changes in SBP were 14.98 ± 5.55 mmHg (HSI) and 12.94 ± 6.53 mmHg (LSI). Increase in SBP was higher in HSI group than in LSI group but not statistically significant (p = 0.334, independent t test).

All 34 participants completed the 5 min of handgrip exercise. In HSI group, the mean DBP before SHG was 68.75 ± 8.29 mmHg and the mean highest DBP during SHG was 86.94 ± 10.05 mmHg. In LSI group, the mean DBP before SHG was 72.06 ± 7.44 mmHg and the mean highest DBP during SHG was 86.82 ± 9.06 mmHg. The mean changes in DBP were 18.20 ± 3.49 mmHg (HSI) and 14.77 ± 4.71 mmHg (LSI). Increase in DBP was significantly higher (p = 0.022) in HSI group (Table 2).

### DISCUSSION

In 1982, a battery of autonomic function tests, heart rate responses to Valsalva manoeuvre, deep breathing and standing (tests of parasympathetic function) and BP responses to standing and sustained handgrip (tests of sympathetic function), are devised by Ewing and Clarke.[23] The American Academy of Neurology has recommended this battery of tests as the standard tests for assessment of autonomic dysfunction.[24] In the INTERSALT study in the late 1980’s, Na+ intake higher by 100 mmol/day
increased SBP by only ~3 to 6 mmHg and DBP by only 0 to 3 mmHg.\cite{25}

In our study, the mean Na+ intake of the HSI group, (174.53 ± 8.71 mmol/day) and that of the low salt intake group (108.83 ± 7.89 mmol/day) differ only ~65.7 mmol/day. The results of 3 parameters (Valsalva ratio, DBD and 30:15 ratio) which reflects the cardiac parasympathetic function were not significantly different between the HSI group and the LSI group in the present study. HR variation during deep breathing reflected cardiac parasympathetic activity.\cite{22} The 30:15 ratio, commonly used to check the parasympathetic function, had been recommended as an index of cardiovagal function.\cite{26} The approximation of salt intake of the subjects in the present study was 108.83 – 174.53 mmol/day of sodium with the usual range of human dietary Na+ intake (114 and 210 mmol/day)\cite{21} and it might be the basis for no different findings of cardiac parasympathetic function between the two groups.

Cardiac parasympathetic activity was decreased in hypertensive patients when compared with normotensive subjects.\cite{27} Baroreceptor reflex may involve in the regulation of the HR response to Valsalva maneuver\cite{20} and standing.\cite{29} Baroreflex sensitivity has a negative correlation with age.\cite{28,30}

In this regard, the age-related decrease in baroreflex sensitivity may take part some role in decreased cardiac parasympathetic function with aging. Rossi and colleagues found that parasympathetic function tests decreased significantly in obese subjects than in the non-obese subjects.\cite{31} The participants in our study were 20 to 40 years aged normotensives and with normal BMI.

In normotensives, to maintain BP, the heart and peripheral vessels seem to accommodate to the changes in plasma volume that follow changes in Na+ consumption.\cite{8} When Na+ intake is high, there will be increase in extracellular fluid volume, increase in blood volume, increase in wall stretch in the heart and blood vessels, increase in the discharge rate of baroreceptors and the resultant decrease in cardiac activity and vascular tone. There will be the reverse mechanism when the Na+ intake is low.\cite{22,23}

The cardiac parasympathetic function of the HSI group was not different from that of the LSI group in the present study. It might be due to the fact that the participants in both groups of the present study were healthy normotensive men and hence their baroreflex activity might be adequate to accommodate the effect of Na+ consumption on plasma volume.

Postural hypotension (fall in SBP more than 20 mmHg between lying and one minute of standing) was found in none of the subjects in our study and therefore the subjects might be free from sympathetic insufficiency. The SBP changes between lying and standing in the HSI group was not significantly different from that of the LSI group. Therefore, the effect of SI in the range of 108.83 – 174.53 mmol/day of Na+ might not be related to SBP changes from lying to standing in the present study.

In this study, the mean SBP and DBP rose in both groups during sustained handgrip. Sustained muscle contraction caused a rise in SBP and DBP and HR. The stimulus derived from exercising muscle and central command. During a voluntary muscle contraction, a release from vagal tone initiates an increase in HR and the rise in arterial pressure could be ascribed both to HR acceleration and to an effect on systemic vascular resistance mediated by the sympathetic nervous system.\cite{24} Pal and colleagues concluded that an increase in DBP during sustained handgrip in the salt-prefering group indicated increased sympathetic reactivity in salt-prefering subjects, as the rise in DBP during sustained handgrip reflected the state of sympathetic reactivity.\cite{33} Previous discoveries pointed out the effect of HSI on sympathetic activity.\cite{7,26} In healthy individuals, urinary excretion is the primary mechanism for maintaining Na+ balance.\cite{27} Normally about, 99% of the filtered Na+ was reabsorbed by the kidney.\cite{30} High salt diet might increase plasma and cerebrospinal fluid Na+ concentration.

Elevated plasma or cerebrospinal fluid Na+ concentration contributes to elevated sympathetic nerve activity and arterial BP in animals and humans.\cite{19-22} Both in vivo and in vitro electrophysiological studies have reported that the organum vasculosum of the lamina terminalis and the subfornical organ contained neurons that were intrinsically osmosensitive.\cite{23,24}

Hypothalamic paraventricular neurons influenced sympathetic nerve activity and arterial BP through mono- and polysynaptic pathways to several sympathetic brain regions such as the rostral ventrolateral medulla (RVLM) and spinal intermedulillary column cord.\cite{42,43}

Elevated dietary Na+ may sensitize sympathetic neurons in the RVLM,\cite{44,45} causing a greater sympathetic response to a variety of stimuli,\cite{46} including skeletal muscle contraction.\cite{31} This increased responsiveness might be associated with a higher DBP response to sustained handgrip in normotensive men with HSI in the present study.

Campese and associates evaluated and reported that plasma norepinephrine level was significantly higher in the hypertensives than normotensives during high Na+ intake. They suggested that there was hyperactivity of the sympathetic nervous system in these patients.\cite{32} Pal and coworkers assessed the link of salt preference to sympathovagal imbalance measured by HR variability analysis in healthy prehypertensive subjects and they found that salt preference is associated with sympathovagal imbalance caused by sympathetic overactivity and vagal withdrawal.\cite{38} These studies indicated that HSI may cause sympathovagal imbalance and sympathetic hyperactivity which would lead to prehypertension and hypertension. According to our study, even in normotensive men, high salt intake may lead to greater cardiovascular sympathetic reactivity than low salt intake.

Limitations of the Study
Concerning limitations of present study, the cross-sectional design obstructs the establishment of causality between SI and cardiovascular autonomic function. Salt sensitivity and resistance of the participants was not assessed. Serum Na+ level of the participants were not determined. There are limitations to using a spot urine sample to assess 24-hr urinary sodium excretion but this weakness is partially offset by performing a spot urine collection on 4 separate days. It has been well known that there is increase in salt sensitivity with aging. Our subjects were young in both groups and it is possible that the redundant mechanisms that control the cardiovascular system are able to tolerate wider ranges of sodium intake in young adults than older adults. This may be the reasons that the VR, DBD, 30:15 ratio and SBP change from lying to standing were not significantly different between the two groups. The blood pressure response to handgrip exercise is an indirect index of sympathetic responses.

CONCLUSION
Regarding two tests of sympathetic function, although the SBP response to standing was not significantly different, the DBP response to sustained handgrip was significantly higher in the high salt intake group. High salt intake might increase cardiovascular sympathetic reactivity even in normotensive men. Future studies need to confirm an association between the degree of salt consumption and cardiovascular autonomic function and underlying mechanisms.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.
ABBREVIATIONS

HR: Heart rate; BP: Blood pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; FBS: Fasting blood sugar; ECG: Electrocardiogram; Na+: Sodium; HSI: High salt intake; LSI: Low salt intake; VR: Valsalva ratio; DBD: Deep breathing difference; SHG: Sustained handgrip.

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