Influence of Reapportionment of Daily Salt Intake on Circadian Blood Pressure Pattern in Normotensive Subjects

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Summary The aim of this study was to investigate whether there is variation in blood pressure when we reapportion the percentage of total daily salt intake consumed at each of three regular meals. The study was conducted on seven clinically healthy normotensive female subjects who, in Stages LH and DH, consumed two-thirds of the normal daily salt intake (12 g/day) at lunchtime or at dinnertime, respectively. The total daily amounts of nutrients and dietary salt were similar in Stage-R (regulated salt intake), Stage-LH (a high-salt intake at lunchtime), and Stage-DH (a high-salt intake at dinnertime). The blood pressure response to the variation of sodium content in the meals was examined by means of noninvasive automated blood pressure monitoring (ABPM-630; readings every 15 or 30 min over 48 h) and chronobiologic analysis. A significant shift of blood pressure circadian rhythm was observed in Stage-LH. Additionally, the 24-h mean level of blood pressure significantly increased when the prevailing salt intake was at lunch, and significantly decreased when the prevailing intake was at dinner. These opposite responses corroborate the view that the blood pressure susceptibility of human beings to salt intake varies during the day, showing its maximal expression at midday. Such a time-dependent sensitivity may be exploited for better nutritional prevention and treatment of arterial hypertension by reapportioning the salt intake so that two-thirds is consumed at dinner.

Key Words acrophase, ambulatory blood pressure monitoring, amplitude, blood pressure, circadian rhythm, cosinor method, mesor, normotensive subjects, timing of salt intake, sodium

Many fundamental studies provide evidence that salt intake influences blood pressure (1–14). Most of these studies have been performed by means of the "casual" blood pressure (BP) measurements. Recently, the relationship between salt intake and BP has been investigated by means of automated non-invasive
monitoring (15). In these studies, however, salt intake was computed as the total consumption of dietary sodium over 24 h. As far as our knowledge is concerned, no human study has investigated whether varying the proportion of salt taken at lunch or at dinner will have an observable influence on the 24-h BP pattern.

We hypothesize that dietary salt might induce tensiogenic effects depending on the meal in which the larger proportion of salt is ingested. Such a postulated time-dependent reaction seems plausible when we consider that BP shows a circadian variability (16–18) which depends on a biological rhythm (19–22). Dietary salt could, thus, act as a synchronizer of the BP circadian rhythm.

To explore such a hypothesis, we performed a study with the aim of better exploring the relationships between salt intake and BP for the nutritional prevention and non-pharmacological treatment of arterial hypertension.

SUBJECTS AND METHODS

1. Subjects and protocol This study was conducted on 7 clinically healthy normotensive female subjects, ranging in age from 21 to 22 years, who gave their informed consent to participate. The clinical health status of the subjects investigated was assessed by means of a medical check-up including a physical examination and extensive laboratory data. The profile of the investigated subjects is briefly shown in Table 1. Their BP was proven to be normal by means of “casual” sphygmomanometric measurements, and subsequently confirmed by 24-h BP monitoring. A randomized crossover study was performed according to a protocol based on the three different stages, and each stage was separated by an interval of 9 days. During each interval, the subjects consumed an essentially unrestricted diet which we assume to contain approximately 12 g of salt per day. They were requested to avoid consuming any items “out of the ordinary” during this interval.

1) Stage-R: Regulated meal-time salt intake: During this stage, the investigated subjects ingested daily amounts of dietary salt (12 g/day) divided among three regulated meals (breakfast at 08:00–08:20; lunch at 12:00–12:30; dinner at 18:00–18:30) for 8 days. Customarily, dietary salt intake was divided into the following proportions: 2 g at breakfast and 5 g at both lunch and dinner. The

| Table 1. Subjects’ profile. |
|-----------------------------|
| No. of subjects             | 7 women                      |
| Age (years)                 | 21.1 ± 0.4                   |
| Height (cm)                 | 158 ± 2.8                    |
| Weight (kg)                 | 53 ± 5.2                     |
| Body mass index (kg/m²)     | 21.4 ± 2.2                   |
| Systolic BP (mmHg)          | 108 ± 7.6                    |
| Diastolic BP (mmHg)         | 63 ± 5.9                     |
| Heart rate (beats/min)      | 69 ± 6.5                     |

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Table 2. Nutrient intakes and nutritional ratios.

|                          | Total intake | Breakfast | High-salt | Low-salt |
|--------------------------|--------------|-----------|-----------|----------|
| Energy (kcal)            | 1,810±47     | 496±18    | 679±49    | 626±44   |
| Protein (g)              | 78.3±3.8     | 22.7±1.9  | 23.5±9.6  | 23.8±1.6 |
| Fat (g)                  | 56.6±8.0     | 17.9±1.6  | 17.1±4.2  | 18.3±5.3 |
| Crude fiber (g)          | 4.0±0.4      | 1.0±0.2   | 1.3±0.1   | 1.7±0.3  |
| Sodium (mg)†             | 4,742±308    | 861±290   | 3,102±397 | 779±280  |
| Potassium (mg)†          | 2,345±788    | 777±375   | 958±354   | 589±259  |
| Calcium (mg)†            | 658±110      | 272±16    | 240±148   | 146±34   |
| Magnesium (mg)†          | 275±34       | 69±35     | 108±18    | 98±25    |
| A/F ratio (%)            | 46.9±2.4     | 52.1±17.2 | 39.4±7.8  | 44.2±22.0|
| P/S ratio                | 0.92±0.17    | 0.60±0.14 | 1.44±0.53 | 1.45±0.62|

† Analyzed value. M±SD. A/F ratio: the ratio of animal fat to fat intake. P/S ratio: the ratio of polyunsaturated to saturated fatty acid. Stage-LH: breakfast, high-salt at lunch and low-salt at dinner. Stage-DH: breakfast, low-salt at lunch and high-salt at dinner. Volunteers took “total intake” on Stage-R.

composition of the diet was normal in calories, 34 kcal/kg BW being contributed by 4.65 g/kg BW of carbohydrates, 1.06 g/kg BW of lipids, and 1.47 g/kg BW of proteins, as shown in Table 2.

2) Stage-LH: Highest salt intake at lunchtime: This stage refers to the increase of salt intake at lunchtime. In this stage, two-thirds of the dietary salt intake (8 g) were consumed at lunchtime, and 2 g at both breakfast and dinner. Food composition, including total daily salt intake, and meal timing were left unchanged, as shown in Table 2. This stage lasted for 8 days.

3) Stage-DH: Highest salt intake at dinnertime: In this stage, the dietary salt intake was shifted so that two-thirds (8 g) of the total daily intake occurred at dinnertime, and 2 g were consumed at both breakfast and lunch. This stage lasted for 8 days, during which time no change was made in food composition (Table 2) and meal timing.

2. Methods. The study was carried out in March and April, months in which the interval of daytime and nighttime is almost equal. In each stage, BP monitoring was performed by an automated, non-invasive device (ABPM-630, Nippon-Colin Co., Ltd., Komaki, Japan) programmed to take sphygmomanometric measurements (oscillometric technique) (23, 24) at 15-min intervals during the daytime span (06:00–23:00) and at 30-min interval during the nighttime span (23:00–06:00) from a cuff attached to the left upper arm. During BP recording, all subjects stayed 2 nights on each occasion at the Institute of Health Science, Kyushu University, so that they could follow similar patterns of activity during each stage. The timing of meals was precise and constant. Room temperature (20–24°C) and the light-dark cycle (light on at 06:00; light off at 23:00) were also controlled. Smoking, coffee, caffeinated beverages, mineral water, alcohol, and food intake other than the 3 planned meals were not allowed during the study span.
The volume of each 24-h urine specimen was measured and urinary Na⁺ and K⁺ concentrations were determined by flame photometry (Hitachi 775: Hitachi Co., Ltd., Hitachi, Japan) and urinary creatinine (Cr) concentration was measured by the picric acid method using an autoanalyzer (Olympus CRE-1: Olympus Optical Co., Ltd., Tokyo, Japan).

3. Data series analysis. Systolic (S) and diastolic (D) BP time series were analyzed according to the following steps. First, individual SBP and DBP were grouped according to each span of daytime and nighttime, and the averages of SBP and DBP for the 48-h, daytime and nighttime span were calculated in each stage to obtain stage-specific mean values. In a second order of inferential statistics, individual time series of SBP and DBP were processed by means of single cosinor analysis (25) to estimate the properties of BP circadian rhythm. Individual rhythmometric estimates were summarized by population mean cosinor for stage-related characteristics of BP circadian rhythm (26).

We estimated the effects of salt intake shift on the circadian rhythm's influence on BP by Bingham's test (27), which is particularly effective for detecting differences in rhythm parameters: mesor (rhythm-adjusted mean), amplitude (extent of variability from mesor), and acrophase (timing of oscillatory crest related to local midnight).

RESULTS

The average values and the rhythm characteristics (mesor, amplitude, and acrophase) of SBP and DBP were not statistically significantly different between the 1st and 2nd days in each stage.

As shown in Table 3, the average values of SBP for both 48-h and daytime span did not significantly vary during the three stages of the investigation. During the nighttime, however, the SBP during Stage-DH was significantly lower than during Stage-R and Stage-LH. On the other hand, the average values of DBP for both the 48-h and daytime spans were significantly higher during Stage-LH than during

|                | SBP (mmHg) | DBP (mmHg) |
|----------------|------------|------------|
|                | 48-h       | Daytime    | Nighttime |
| Stage-R        | 108±0.8    | 110±1.0    | 102±1.9   |
| Stage-LH       | 110±0.9    | 112±0.9    | 101±1.9***|
| Stage-DH       | 104±0.7    | 107±0.8    | 97±1.5***|
|                | 48-h       | Daytime    | Nighttime |
|                | 64±0.5     | 65±0.7     | 60±1.3    |
|                | 65±0.7***  | 67±0.7***  | 58±1.5    |
|                | 61±0.6***  | 63±0.5***  | 56±1.3    |

**p<0.01, ***p<0.001 (by paired test). M±SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure. Daytime: 07:00–23:00; nighttime: 23:00–07:00. Stage-R: regulated mealtime salt intake. Stage-LH: highest salt intake at lunchtime. Stage-DH: highest salt intake at dinnertime.

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### Table 4. Summary of properties of systolic and diastolic blood pressure circadian rhythm in clinically healthy subjects monitored on three occasions.

| Occasion | Variable | \( p^1 \) | Mesor ±SE (mmHg) | Amplitude (95% CL) (mmHg) | Acrophase (95% CL) (degrees) (hour:minute) |
|----------|----------|-----------|-----------------|--------------------------|-----------------------------------------------|
| Stage-R  | SBP      | 0.038     | 105             | 4 (-256)                 | 17:04 (12:12; 21:28)                          |
|          | DBP      | 0.019     | 61              | 3 (-248)                 | 16:32 (12:04; 19:32)                          |
| Stage-LH | SBP      | 0.034     | 107             | 4 (-235)                 | 15:40 (13:00; 22:00)                          |
|          | DBP      | 0.034     | 63              | 5 (-231)                 | 15:24 (11:16; 19:30)                          |
| Stage-DH | SBP      | 0.001     | 103             | 6 (-249)                 | 16:36 (14:40; 18:56)                          |
|          | DBP      | 0.001     | 60              | 6 (-247)                 | 16:28                                         |

\( ^1 \)Statistical significance for validation of circadian rhythm. Stage-R: regulated mealtime salt intake; Stage-LH: highest salt intake at lunchtime; Stage-DH: highest salt intake at dinnertime. SE, standard error; 95% CL, 95% confidence limits; SBP, systolic blood pressure; DBP, diastolic blood pressure. *\( p < 0.001 \), **\( p < 0.001 \).

### Stage-DH.

A summary of rhythmometric estimates is given in Table 4. The cosinor-derived parameters document that the temporal variability of SBP and DBP can be contained within a sinusoidal oscillation at a \( p \) level of significance which is statistically acceptable in each stage. The SBP and DBP circadian rhythms show stage-related characteristics. Two findings are prominent: The mean level of 24-h BP (mesor) is increased during Stage-LH (from 105 to 107 mmHg for SBP; from 61 to 63 mmHg for DBP), and lowered during Stage-DH (107 to 103 mmHg for SBP; from 63 to 60 mmHg for DBP). The acrophase timing as well shows a stage-dependence, being clearly anticipated in Stage-LH (from 17:04 to 15:40 for SBP; from 16:32 to 15:24 for DBP), and resets toward the ordinary time in Stage-DH (from 15:40 to 16:36 for SBP; from 15:24 to 16:28 for DBP). Bingham’s test found the differences between mesor and acrophase in Stage-LH and Stage-DH to be statistically significant (\( p < 0.01 \) and \( p < 0.001 \) for SBP; \( p < 0.001 \) and \( p < 0.01 \) for DBP, respectively).

There were no significant differences in 24-h urinary Na\(^+\), K\(^+\), and Cr excretion in the three different stages.
DISCUSSION

We investigated the hypothesis that consuming two-thirds of the total daily dietary sodium at either lunch or dinner might induce different effects on daily BP. We hypothesized that a phase entrainment of BP circadian rhythm might occur which might be analogous to changes occurring in some biorhythmic variables related to digestion and metabolism as a result of shifting caloric intake (28, 29). Our study showed that the 24-h BP mean level increased or decreased when two-thirds of daily dietary salt was ingested at lunchtime or dinnertime, respectively. Entrainment of BP circadian rhythm seems to be a likely cause because BP acrophase varied not because of a change in total intake but because of the time at which the largest percentage of salt was consumed.

Many previous studies (10–12, 14, 30) show that susceptibility to a hypertensive reaction to salt consumption is a genetically inherited trait. This is also true for hypotensive conditions (31). However, our preliminary results are based on a study of normotensive young women. Obviously, our hope is that hypertensive subjects will show improvement on a diet in which the total daily consumption of salt is limited, and about two-thirds of that total is consumed during dinner. Subsequent studies may help to determine the most beneficial division of salt intake per meal; for now, the two-thirds figure provides a benchmark for other researchers. However, whatever the results, further studies on such hypertensive subjects could raise a variety of interesting questions. A genetically inherited susceptibility to salt-induced hypertension apparently has some relation to circadian rhythms (15). Suppose the stage-DH consistently lowers BP in normotensive subjects but not in hypertensive subjects. This might suggest the existence of altered circadian rhythms in these hypertensive subjects, and point out the need for further research on the genetic basis of the circadian BP rhythm.

Assuming that BP susceptibility to salt critically changes in a circadian manner, one can postulate that by adjusting the amounts of dietary sodium which are ingested at lunch and at dinner, it is possible not only to encourage better nutritional prevention of high BP, but also to offer a novel means of nutritional control of hypertension. Both goals would be promoted by suggesting that persons regulate their daily salt intake so that two-thirds will be consumed at dinnertime. We intend to follow up with another study using one or two control groups in order to eliminate other possible causes of the results discussed here. However, we wish to make our preliminary results available to other researchers immediately, in hopes that additional studies will help to clarify in statistical terms the benefits of taking two-thirds of the daily dietary salt during the evening meal.

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