1 Analysis of cardiorespiratory phase coupling and cardiovascular autonomic responses
2 during food ingestion
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12 Cardiorespiratory phase coupling during food ingestion
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Abstract

The present study analyzed whether the phase coherency (λ) of respiratory sinus arrhythmia (RSA) is altered by food ingestion in healthy young subjects. After 5 min of resting control, 13 healthy volunteers were asked to eat a solid meal with access to water at their own pace, followed by 5 min of the postprandial state. The R-R interval (RRI), beat-to-beat blood pressure (BP), and respiratory activity were recorded using electrocardiography, a Finapres device, and inductance plethysmography, respectively. The stroke volume was calculated by the pulse-contour method from continuous BP measurement, and the cardiac output (CO) was obtained by multiplying the stroke volume by the heart rate. From the oscillatory signals of RSA and respiration, λ was computed; additionally, frequency domain indexes of the heart rate variability (HRV) were calculated using a short-time Fourier transform. A steady-state 3-min resting period (R), food ingestion period (FOOD), and the first 2-min and the last 3-min of the post prandial period were analyzed separately. We also compared the responses to gum chewing (GUM) and water intake (WATER) using the same protocol on separate days. A shortening of RRI and increases in BP and CO were observed in FOOD compared to R, suggesting a shift of sympathovagal balance toward sympathetic activation. Similar responses but smaller magnitudes were observed in the GUM condition, whereas only transient shortening of RRI was observed in the WATER condition. The HRV indexes did not show any significant changes in response to GUM and WATER but sympathovagal balance was shifted in favor of sympathetic dominance in FOOD. λ decreased during all of the conditions. There was a significant negative correlation between λ and the indirect measure of sympathovagal balance. These results suggest that

1 ingestion of food induces enhanced cardiac sympathetic activity and that a phase coherence of RSA
2 could provide a sensitive measure for evaluating the cardiac autonomic profile.

3 **Keywords:** mastication, swallowing, respiratory sinus arrhythmia, sympathovagal balance

4 5 **1. Introduction**

6 Changes in heart rate variability (HRV) induced by respiration are known as respiratory sinus
7 arrhythmia (RSA), described as an acceleration and deceleration of the heart rate corresponding to the
8 phases of the respiratory cycle. In our previous study, we found that mental stress in humans exerts
9 an influence on the oscillations of RSA, inducing an incoherent phase lag with respect to breathing
10 [i.e., disruption of the phase coherence (λ) between RSA and respiration] in addition to causing a
11 decrease in the amplitude of RSA [21]. The results of the study suggested that the enhanced
12 sympathetic nerve activity during mental stress may modulate the transduction property of the cardiac
13 vagal efferent nerve. Interestingly, λ was not susceptible to changes in the respiratory frequency;
14 however, such an influence is critical for evaluating the tonicity of the autonomic nervous system
15 (ANS) from the heart rate variability (HRV) in humans [3], [4], [9]. Therefore, it was expected that λ
16 could act as an alternative index that allows for standardization when measuring ANS activity.
17 However, it remains unclear how changes in λ may relate to ANS activity under situations other than
18 mental stress. It would also be of interest to examine whether λ is altered by natural interventions
19 during activities of daily life, depending on changes in ANS tonicity.

20 One such primary activity of humans that affects hemodynamic changes and ANS activity is eating.

1 Eating behavior has been documented to increase the heart rate (HR), cardiac output (CO), and
2 systolic blood pressure (SBP) [8] [12] [16], but at the same time feeding activity is known to induce
3 vasodilation in the visceral arteries by increasing gastrointestinal parasympathetic activity [7] [24].
4 These cardiovascular responses are mediated through the ANS to meet the requirement of greater
5 energy utilization associated with the digestion, absorption, and storage of food in gastrointestinal
6 tracts. Studies conducted using the spectral analysis of HRV to evaluate cardiac autonomic reactions
7 to meal ingestion in the case of young adults showed that high-frequency (HF) power decreased [29]
8 and low-frequency (LF) power increased [28] after a meal, suggesting a suppression of cardiac
9 parasympathetic tone and sympathetic excitation. During mental stress conditions (mental arithmetic
10 task), the decrease in λ has been found to be accompanied by enhanced sympathetic activity and/or
11 decreased parasympathetic activity [21]. However, it is not clear whether the correlations between λ
12 and HRV indexes found in the mental stress condition may also be generalized to the activity of
13 feeding. In this study we examined how the coherent oscillation of RSA is altered by ingestion of
14 food in conjunction with the autonomic indexes of HRV and cardiovascular reactions in a laboratory
15 setting. We hypothesized that similar to the mental stress condition, food ingestion should induce
16 weak cardiorespiratory phase synchronization as reflecting cardiac sympathetic activation and
17 parasympathetic inhibition during food ingestion, if this the case, we further assess whether and to
18 what extent mastication (gum chewing) and water intake affect cardiorespiratory phase
19 synchronization, depending on the degree of cardiovascular and autonomic reactions.

2. Methods

2.1. Subjects

Thirteen young subjects, nine males and four females [mean age: 23 ± 0.2 years, mean height: 170 ± 1.4 cm, mean weight: 65.0 ± 4.6 kg, BMI: 22.0 ± 1.5 (SE) kg/m^2] were recruited for voluntary participation in the study. All subjects were normotensive nonsmokers and were not taking any medication. The experimental protocol was approved by the Yamagata University Institutional Ethics Committee and the study conformed to the Declaration of Helsinki. The subjects gave written informed consent to participate in the study after being provided a verbal explanation of how to noninvasively record the cardiovascular variables, types of foods eaten and gum chewed, and the experimental procedures.

2.2. Experimental procedures

The subjects arrived at the laboratory after overnight fasting, having abstained from caffeine and intensive exercise. The study started at around 10:00 AM in a quiet laboratory room ($23 - 24$ °C). The subjects were comfortably seated in upright position. After a 5-min rest period, the subjects were asked to eat two pieces of solid food, with each piece weighing 20 g (maple-flavored Calorie Mate; total caloric content of 200 kcal, with 20.1 g of carbohydrates, 11.3 g of fat, and 4 g of protein; Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) as the FOOD condition, along with 100 ml of water at their own pace. This was followed by 5 min of postprandial recording. This food has a crunchy texture and the shear force required for breaking was 3.83 ± 0.12 kg (SE, $n = 6$). The subjects were allowed to drink water ad libitum while eating. The time taken for food intake was recorded as the

1 duration from the beginning of the first bite to swallowing after the last bite. The final swallowing
2 was indicated by a finger movement of the subjects. The separate effects of mastication (gum
3 chewing, abbreviated as GUM) and water drinking (abbreviated as WATER) were also examined. In
4 the GUM condition, the subjects were instructed to chew a piece of low-adhesive tasteless gum base
5 weighing 1.0 g (GC Co. Ltd., Tokyo, Japan) as they liked during a period identical to that of the
6 FOOD condition. In the WATER condition, the experimental procedure was similar to that outlined
7 for the FOOD condition with the exception of providing solid meal. The subjects drank a total of
8 100 ml of pure water three separate times at one-third, two-thirds, and three-thirds of the meal duration
9 for each subject after a 5-min resting control period, followed by a 5-min recovery. These
10 experiments were performed on separate days and the durations of the respective conditions were
11 same for each subject. The order of the conditions was fixed as FOOD, GUM, and WATER because
12 we intended to compare the transient changes in the cardiovascular and autonomic responses with an
13 identical time scale between FOOD, GUM, and WATER for each subject where the time for food
14 consumption by each subject is required to be known in advance, and to avoid unnecessary stress to
15 the subjects by setting a limit on eating time. Time interval between FOOD and GUM testing
16 sessions was 7-10 days for each subject. On a separate occasion (~2 months later) subjects returned
17 to the laboratory and underwent a test for WATER condition. Efforts were made to ensure that all
18 subjects were examined at the same time of day for all conditions.

19 **2.2. Instrumentation**

The subjects wore thoracic and abdominal belts for performing respiratory inductance plethysmography (zRIP; Pro-Tech, Mukilteo, WA) in order to measure breathing movements. Disposable electrodes were attached to the subject's chest for obtaining bipolar electrocardiogram (ECG) leads using a wireless ECG system (ZM-940P; Nihon Kohden, Japan). A Finapres finger sensor (model-2300; Ohmeda, Englewood, CO) was placed around the index or middle finger of the subject's non-dominant hand. During the data collection period, the arm of the subject was supported at the heart level. After a stable BP waveform was achieved, the servo-reset mechanism of the Finapres device was turned off, allowing uninterrupted data collection.

2.4. Signal acquisition and data analyses

During the experiments, the electrocardiograph, beat-by-beat BP, and breathing activity were continuously recorded by means of a wireless ECG system, the Finapres device, and an uncalibrated respiratory inductance plethysmograph, respectively. All signals were digitized with a sampling frequency of 1 kHz with a PowerLab data acquisition system (model-8/SP; ADInstruments, NSW, Australia). The beat-to-beat R-R intervals (RRIs), systolic blood pressure (SBP) values, and diastolic blood pressure (DBP) values were derived as the duration between successive R peaks, the maximum BP, and the minimum BP, respectively, in a cardiac cycle. The beat-to-beat stroke volume (SV) was determined by the pulse-contour method outlined by Wesseling et al. [37]. In this method, the SV was defined by the integration of the pressure wave over the ejection phase divided by the effective characteristic impedance of the aorta. The details for the calculation of the SV have been described

elsewhere [33]. The cardiac output (CO) was calculated as the SV multiplied by the HR, and the total peripheral resistance (TPR) was calculated as the mean BP divided by the CO.

To obtain the RRI with equidistant time steps, the beat-to-beat RRI was resampled at 10 Hz by the spline interpolation method. Before resampling, the calculated RRIs were inspected visually, and outliers were deleted if present. The respiratory movement signals were sampled at a frequency of 10 Hz. These bivariate time series of the RRI and respiration were further band pass filtered with a frequency range of 0.10 - 0.4 Hz, as these frequency bands cover the respiratory frequency range observed in the present study.

From the oscillatory signals of the RSA and respiration, the analytic signal can be constructed from a real signal and its Hilbert transform. The instantaneous phases of the RSA $[\phi_{\text{RSA}}(t_k)]$ and breathing $[\phi_{\text{RESP}}(t_k)]$ at discrete time t_k were calculated from each complex analytic signal as the arctangent of the angle of the vector with respect to the real axis. Then the time-dependent phase coherence (λ) between the RSA and respiration was obtained using respective instantaneous phases as

$$\lambda(t_k) = \left| \frac{1}{N} \sum_{j=k-N/2}^{k+N/2} e^{i\psi(t_j)} \right|^2$$

where $\psi(t_k) = [\phi_{\text{RESP}}(t_k) - \phi_{\text{RSA}}(t_k)] \bmod 2\pi$, and N denotes the number of consecutive data samples to be considered in the computation. This index has been widely used to assess the strength of phase synchronization in the human cardiorespiratory system [15][27]. The λ value was calculated from 100-point windows (10 -s) with a 50-point (5 -s) sliding window. During eating or water drinking, swallowing activity briefly interrupts respiration and therefore influences the RSA oscillation. This

1 may affect the value of λ . An example of this situation is illustrated in Fig. 1, where parts of the RSA
 2 and the simultaneous breathing trace at around the beginning of the food intake are shown (Fig. 1A).
 3 The Hilbert transform of a these traces gives the instantaneous phases of RSA and breathing (Fig. 1B).
 4 When breathing is interrupted by swallowing, RSA terminates the oscillation. This causes phase-lag
 5 distortions of RSA with respect to breathing and thereby decreases λ . Although swallowing has been
 6 suggested to elicit transient inhibition of cardiac vagal activity [31], it is uncertain whether the
 7 decrease in λ calculated during swallowing is caused by the changes in autonomic nervous activity or
 8 is merely a result of misestimation of the instantaneous phases, because it is possible to define the
 9 Hilbert transform meaningfully for the oscillatory signals. To avoid such uncertainty in the
 10 interpretation of phase-lag variations by swallowing activity, λ values derived from the data window
 11 contain swallowing activity were excluded from the analysis. The time of occurrence of swallowing
 12 activity was visually inspected by the trace of breathing activity as a non-oscillatory part.

13 For determining frequency-domain HRV indexes, a short-time Fourier transform was performed
 14 [22]. This method has been used to estimate the time course of transient changes in HRV and was
 15 demonstrated to be effective in quantifying the dynamic pattern of the HRV during nonstationary
 16 conditions [6] [20]. A section of 1024 samples was multiplied by the Hanning window function, and
 17 the fast Fourier transform of their product was taken. The window was then shifted 10 samples
 18 ahead, and same calculations were performed again. This process was repeated until the entire RRI
 19 time series was covered. Then the LF (0.04 - 0.15 Hz) and HF (0.15 - 0.40 Hz) components of HRV

1 were computed by the integration of each spectral component [19] in order to estimate the level of
2 sympathetic and vagal branches of the autonomic nervous system to the heart. The frequency
3 resolution is inversely proportional to the window length, and changes in time resolution compromise
4 the frequency resolution in the short-time Fourier transform. In accord with the Task Force guideline,
5 recording periods of 1-min are recommended for HF variability and 2-min for LF variability [34].
6 Since the present interest was to make an assessment of the time-dependent changes ANS activity
7 during the phase of feeding behavior, we chose 1024 window length (102.4-s time window) to obtain
8 high temporal resolution. This resulted in a frequency resolution of 0.01 Hz, which covers 4 cycles
9 of the lowest frequency of HRV analysis. To evaluate the HF power, we checked whether most of
10 the respiratory power was within the HF region. If the peak frequency of the auto-power spectral
11 power of respiration was outside the HF region (below 0.15 Hz), we modified the lower boundary
12 frequencies of the HF band, where the auto-spectral power of respiration was reduced to 20 % of its
13 maximum power (this modification was applied to one male subject for all conditions because his
14 spontaneous breathing frequency was less than 0.15 Hz). The total spectral power for the LF and HF
15 bands was calculated as a function of time by the integration of each spectral component. The power
16 spectrum for each frequency component was expressed in normalized units $[100 \times (\text{absolute power})^{1/2}$
17 $/ (\text{mean RRI}), \%$]. This normalization has been found to eliminate the influence of the basal level of
18 the cardiac sympathetic tone on HF power [10] [11]. The normalized LF-to-HF ratio (nLF/nHF), an
19 estimate of sympathovagal balance, was also calculated. Data were analyzed using custom-written

1 MATLAB programs (MathWorks)

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5 Figure 1. A: Data segment showing effect of swallowing on changes in RSA (i.e., bandpass filtered
 6 RRI, solid trace) and breathing trace (dotted trace) for 1-min portion around beginning of food intake.
 7 Breathing trace was plotted in arbitrary units. Upward deflections denote expiration. Vertical line
 8 indicates the beginning of food intake. B: Instantaneous phases for RSA (solid trace) and breathing
 9 (dotted trace). C: phase synchronization index λ calculated from 10-s windows with a sliding
 10 window of 5-s (dotted curve). Closed circles shown are λ values calculated from data segment
 11 containing swallowing activity; these were removed from the analysis. Solid curve indicates
 12 interpolated λ values after removing λ values indicated by closed circles. Note that instantaneous
 13 phase for breathing precedes that for RSA, and that during swallowing, both breathing and RSA
 14 oscillations are briefly interrupted. Thereby the phase-lag between RSA and breathing is influenced.

1 **2.4. Statistics.** Since eating pace (i.e., meal duration) was different among the subjects, the time scale
2 during the FOOD, GUM, and WATER portion was normalized to the mean duration of eating by
3 interpolating individual meal durations. Then the cardiovascular variables and HRV indexes as well
4 as λ were interpolated with a cubic spline function and resampled at 0.5 Hz (cardiovascular variables)
5 or 0.2 Hz (HRV and λ indexes) to obtain a time series of equidistantly spaced data points. Next,
6 group-mean responses were derived. To quantify the variability of the parameters, the mean and the
7 SE over the 3-min resting period (abbreviated as R, 1.5 - 4.5 min from the beginning of recording), the
8 solid meal ingestion (FOOD), gum chewing (GUM), or water drinking (WATER) periods, and first
9 2-min (P1) and last 3-min (P2) of the post-conditions were separately calculated for each subject and
10 then averaged to obtain the group mean. Repeated-measures two-way ANOVAs were applied for
11 each variable to test for two main effects: conditions and the stage of the respective time period.
12 When a significant F value was detected, this was further examined using a Dunnett's post hoc test to
13 compare variables to resting baseline in the respective conditions, and using a Bonferroni post hoc test
14 to assess the simple effects at the respective time period between conditions. Linear fits were
15 calculated between λ and the autonomic indexes of HRV according to the Deming regression, which
16 takes into account errors in both coordinates. Statistical analyses were performed using JSTAT
17 (version 13.0) with significance accepted at P less than 0.05.

18 **3. Results**

19 The meal duration and numbers of breaths and swallowing during the respective conditions are

1 summarized in Table 1. The number of breaths did not differ significantly among FOOD, GUM, and
 2 WATER conditions. The number of swallowings in the FOOD condition was greater than that in the
 3 GUM condition ($P < 0.01$).

4 *Cardiovascular responses.* In the FOOD condition, from rest to eating, a hemodynamic transient was
 5 observed with a shortening of RRI, gradual increases in SBP and DBP, and an increase in CO (Fig.
 6 2A). At the termination of eating, these variables tended to return to the level corresponding to the R
 7 condition; however, SBP, DBP, and SV remained elevated. TPR remained relatively constant
 8 throughout the experiment. The trends of the cardiovascular reactions to GUM were the same as
 9 those to FOOD, but the time course of the shortening of RRI was slow and the magnitude of the
 10 changes in variables was smaller than those observed in FOOD (Fig. 2B). In the WATER condition,
 11 a single water intake induced a sharp shortening of RRI with a fast recovery to the baseline level (Fig
 12 2C). This reaction was accompanied by a brief increase in CO and a decrease in TPR.

13 The effects of FOOD, GUM, and WATER on the cardiovascular variables are summarized in Fig. 3.
 14 Significant interactions of condition and time were found for the variables except for the SV and TPR.
 15 All of the cardiovascular variables during the resting baseline (R) did not differ significantly among
 16 the conditions. Compared to the resting baseline, a significant decrease in RRI ($P < 0.01$) was
 17 observed during eating (from 842 ± 27 to 736 ± 18 ms), chewing (from 830 ± 37 to 790 ± 33 ms), and
 18 water intake (from 874 ± 23 to 839 ± 23 ms). SBP, DBP, and CO significantly increased during
 19 eating and chewing compared with the resting baseline (SBP: from 112 ± 5 to 133 ± 3 mmHg, $P <$

0.01; DBP: from 61 ± 4 to 73 ± 4 mmHg, $P < 0.05$; CO: from 5.02 ± 0.19 to 6.19 ± 0.20 L/min, $P < 0.01$ in FOOD and SBP: from 115 ± 3 to 123 ± 3 mmHg, $P < 0.05$; DBP: from 59 ± 2 to 63 ± 2 mmHg, $P < 0.05$; CO: from 5.09 ± 0.18 to 5.45 ± 0.23 L/min, $P < 0.05$ in the GUM conditions). The WATER condition did not produce any significant changes in the variables except for the RRI. The increases in SBP and DBP were significantly larger in FOOD compared with GUM and WATER ($P < 0.05$ vs. GUM; $P < 0.01$ vs. WATER). In addition, the increase in CO was significantly larger in FOOD compared with GUM and WATER ($P < 0.05$ vs. GUM; $P < 0.01$ vs. WATER). The SV and TPR did not change significantly among the conditions, and did not vary in these conditions from the resting baseline.

HRV indexes and λ nHF remained relatively constant and nLF/nHF tended to increase during food ingestion and gum chewing, while λ decreased in both conditions (Figs. 4A and 4B). In the FOOD condition, nHF tended to overshoot after eating. λ undershot at the onset and at the termination of gum chewing. In the WATER condition, λ decreased rapidly after the onset of water intake and returned to its resting control level (Fig. 4C), which was reflected by a sharp shortening of RRI at the onset of water intake. The change in the pattern of nLF/nHF during water intake was a mirror image of the pattern of change in λ .

The effects of the FOOD, GUM, and WATER conditions on the HRV indexes and λ are summarized in Fig. 5. Significant interactions between conditions were not found, but there was a significant main effect of time on all indexes. The nHF index tended to decrease during eating but statistical

1 significance was not reached; rather, nHF significantly increased after eating compared with the
 2 resting baseline ($P < 0.05$). The nLF/nHF index increased from the resting baseline during the eating
 3 period (from 0.75 ± 0.04 to 0.85 ± 0.04 , $P < 0.01$). There was no statistical significance for the HRV
 4 indexes in the GUM and WATER conditions. On the other hand, λ decreased significantly during
 5 eating (from 0.81 ± 0.03 to 0.57 ± 0.05), chewing (from 0.81 ± 0.05 to 0.60 ± 0.06), and water intake
 6 (from 0.84 ± 0.03 to 0.71 ± 0.04) when compared with the resting baseline. λ remained low over the
 7 first 2-min of the post-condition (P1) in the FOOD ($P < 0.05$) and WATER conditions ($P < 0.01$).
 8 Bivariate correlations among λ , nHF, and nLF/nHF are shown in Fig. 6. λ showed a modest positive
 9 correlation with the nHF in the FOOD ($r = 0.312$; $P = 0.024$) but not for the GUM and WATER
 10 conditions. There was a significant negative correlation between λ and nLF/nHF in all conditions (r
 11 $= -0.536$, $P < 0.0001$ in FOOD; $r = -0.655$, $P < 0.0001$ in GUM; and $r = -0.494$, $P = 0.0002$ in
 12 WATER).

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Table 1. Meal duration, number of breaths, and number of swallowings during each condition.

Subj.	meal duration (s)	number of breaths during food ingestion	number of breaths during gum chewing	number of breaths during water intake	number of swallowings during food ingestion	number of swallowings during gum chewing	number of swallowings during water intake
1	274	68 (0.25)	54 (0.20)	58 (0.21)	8	3	3
2	174	55 (0.32)	53 (0.30)	47 (0.27)	9	3	3
3	172	34 (0.20)	44 (0.26)	48 (0.28)	7	2	3
4	145	30 (0.21)	26 (0.18)	33 (0.23)	7	2	3
5	133	25 (0.19)	47 (0.35)	47 (0.35)	6	4	3
6	152	48 (0.32)	48 (0.32)	49 (0.32)	8	1	3
7	156	44 (0.28)	40(0.26)	43 (0.28)	7	3	3
8	187	66 (0.35)	44 (0.24)	54 (0.29)	7	2	3
9	149	32 (0.21)	33 (0.22)	30 (0.20)	6	2	3
10	177	42 (0.24)	51 (0.29)	64 (0.36)	9	3	3
11	264	80 (0.30)	94 (0.36)	67 (0.25)	8	3	3
12	182	50 (0.27)	49 (0.27)	52 (0.29)	7	3	3
13	405	39 (0.10)	34 (0.08)	41 (0.10)	6	5	3
mean	198 ± 22	47.2 ± 4.7	47.5 ± 4.7	48.7 ± 3.1	7.3 ± 0.3** ^{ss}	2.7 ± 0.3	3.0 ± 0

Values are mean ± SE

Values in parentheses are respiratory frequency in Hz.

**P < 0.01 food ingestion vs. gum chewing, ^{ss}P < 0.01 food ingestion vs. water intake

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Figure 2. Group-mean responses of cardiovascular variables in FOOD (A), GUM (B), and WATER (C) conditions. Vertical dashed lines indicate start and termination of food intake, gum chewing, or water drinking, respectively. Time scale during FOOD, GUM, and WATER portion was normalized to the mean duration of meal by interpolating individual meal duration over 100 points. Data were analyzed at the 3-min resting period (R), during FOOD, GUM, or WATER, and in first 2-min (P1) and last 3-min (P2) of post-condition. Values are mean $\pm$ SE.

RRI, R-R interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance.

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Figure 3. Effects of food ingestion, gum chewing, and water intake on cardiovascular variables.

R: resting period, P1: first 2-min of post-condition, P2: last 3-min of post-condition.

Values are mean $\pm$ SE. * $P < 0.05$ and ** $P < 0.01$ vs. resting baseline (R) for each condition, $^{\$}P < 0.05$ and $^{SS}P < 0.01$ FOOD vs. GUM condition, $^{\#}P < 0.05$ and $^{##}P < 0.01$ FOOD vs. WATER condition.

RRI, R-R interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance.

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Figure 4. Group-mean cardiac autonomic indexes (nHF and nLF/nHF) and phase coherence (λ) in FOOD (A), GUM (B), and WATER (C) conditions. Vertical dashed lines indicate start and termination of food intake, gum chewing, or water drinking, respectively. Time scale during FOOD, GUM, and WATER portion was normalized to mean duration of meal by interpolating individual meal duration over 40 points. Data were analyzed at the 3-min resting period (R), during FOOD, GUM, and WATER, and in first 2-min (P1) and last 3-min (P2) of post-condition.

Values are mean $\pm$ SE.

Figure 5. Effects of food ingestion, gum chewing, and water intake on normalized HF component of HRV (nHF, A), normalized LF/HF (nLF/nHF, B), and phase coherence (λ , C). Values are mean $\pm$ SE. R:resting period, P1: first 2-min of post-condition, P2:last 3-min of post-condition.

** P < 0.01 and * P < 0.05 vs. resting baseline (R) for each condition.

Figure 6. Scatter plots of relations between λ and normalized high-frequency power (nHF) and between λ and normalized LF-to-HF ratio in FOOD (A, D), GUM (B, E), and WATER conditions (C, F). Regression line (dotted line) and correlation coefficient for each relation are indicated.

4. Discussion

The present study demonstrated that food ingestion resulted in a significant decrease in λ compared with the resting baseline. The decrease in λ was accompanied by a shortening of RRI and increases in SBP, DBP and CO without any compensatory reduction in TPR and increase in nLF/nHF as an index of sympathovagal balance. These cardiovascular responses, together with an indirect measure of autonomic response, are likely to reflect a combination of cardiac autonomic profile of a sympathetic activation and a parasympathetic withdrawal. Since there was a significant negative correlation between λ and nLF/nHF, a potential link between cardiac autonomic nervous activity and λ could be inferred.

The changes in the direction of cardiovascular variables in response to food ingestion and gum chewing were similar to the increases in BP and CO and the shortening of RRI, although the magnitude of those changes were relatively small in GUM (Fig. 3). This observation suggests that other than mastication involved hemodynamic changes during food ingestion. In the WATER condition, where the same amount of water was drunk as in the FOOD condition, rapid increases in BP and CO were observed immediately after drinking water (Fig. 2C), but they did not reach statistical significance when responses were averaged over the entire period of water intake. Thus water drinking appears not to be a primary cause for the sustained enhancement of cardiovascular reactivity observed in FOOD. Rather, orosensory stimulation, swallowing activity, arrival of food in the stomach, or digestion of food could play a role in increasing BP and CO. Because the number of

1 swallowing in FOOD was greater than that observed in GUM, swallowing might have contributed to
2 the increased cardiovascular reactions, although a direct effect of swallowing has not been examined
3 in the present study. Actually, it was demonstrated that swallowing activity induced tachycardia [31].
4 However, there remains doubt as to whether several instances of swallowing induce a sustained
5 elevation of CO.

6 In addition to its role in swallowing activity, gastric motility (which is regulated in part by the
7 ANS) and insulin that are released during the digestive process [14] might be involved in the
8 enhancement of cardiovascular reaction. Several studies demonstrated the effects of gastric
9 distension on cardiovascular reactivity in healthy humans, including increases in HR and cardiac index
10 [5] and increases in BP and muscle sympathetic activity [23]. As to the effects of insulin, there is
11 evidence that acute, short-term insulin infusion in healthy men under euglycemic clamp conditions
12 stimulate sympathetic nerve activity as determined by measurements of plasma norepinephrine
13 spillover that could elevate arterial pressure [26]. This suggests that insulin itself could induce
14 sympathetic activation. In addition, the elevation of plasma insulin was found to provoke a rise in
15 CO [1], and reflex increases in myocardial contractility are sustained during digestion in humans [13].
16 Increases in plasma insulin during the cephalic phase have been also reported [35], suggesting an
17 effect of orosensory stimulation. An immediate increase in CO (owing to a shortening of RRI),
18 which was not observed in GUM condition, may be induced by orosensory stimulation, whereas the
19 postprandial elevation of CO and BP may cohere to the gastric phase. It is therefore speculated that

1 the increased cardiovascular responses during food ingestion compared with gum chewing could be at
2 least explained by food-induced gastric distension and/or cardiovascular and autonomic actions of
3 insulin.

4 Studies on the effects of solid meal ingestion on autonomic activity using HRV spectral analysis
5 demonstrated increased sympathovagal balance (LF/HF index) following a solid meal in healthy
6 children [8] and in healthy adults [17], which is consistent with the results of the present study.
7 However, we did not observe significant changes in HRV indexes during gum chewing and water
8 intake, although λ decreased in both conditions. In a previous study, the sympathetic nervous activity
9 estimated by a spectral analysis of HRV was found to be increased by the mastication of chewing gum
10 base [32]. We cannot explain this discrepancy, but methodological approaches may contribute to the
11 different results between HRV indexes and λ . The short-time Fourier transform analysis to determine
12 the spectral power of HRV employed in the present study requires successive segmental data and for
13 the processed signal to be stationary in the analyzed temporal window. As we used a successive
14 1024 points of data (102.4 s) for the calculation, HRV indexes are considered to be smoothed or
15 averaged over this period, while λ was calculated with a window of 10 s. This could partly explain
16 why HRV indexes were not changed in the GUM and WATER conditions despite the decrease in
17 λ that was observed.

18 In the FOOD and WATER conditions, λ remained low over the first 2-min of post-condition (P1),
19 but such a reaction was not observed in GUM. This could be related to the gastric inflow of the food

1 and water as mentioned above. It could be inferred that sympathetic activation may not persist in the
2 GUM condition owing to absence of gastric inflow. However, if this is the case, a question may arise
3 as to why nLF/nHF does not change during water intake. Studies investigating the effects of water
4 intake on cardiac autonomic responses have shown different results. In a previous study, water
5 ingestion caused bradycardia and an increase in cardiac vagal activity [25], while in a study using
6 direct measurement of muscle-sympathetic nerve activity, drinking water induced a rise in
7 muscle-sympathetic nerve activity in healthy young subjects [30]. As shown in Fig. 3C, a change in
8 the nLF/nHF index during water intake showed a mirror image of the λ ; high immediately after water
9 intake and quickly returning to the resting baseline. This observation appeared to support the view
10 that water intake may produce an increase in sympathetic activity. Because the response was
11 transient, possibly owing to the small amount of water taken, statistical significance was not achieved
12 when averaging over the entire period of water intake.

13 We observed significant increase in nHF index at postprandial period immediately after eating
14 compared with the resting baseline in FOOD (Fig. 5). We cannot explain this observation
15 physiologically but it may be related to the termination of mastication, because this tendency was also
16 seen in GUM (Fig. 4B) but not in WATER. It is known that change in respiratory frequency
17 indirectly affects spectral parameters of HRV. Because we did not observe significant change in the
18 number of breaths during food ingestion, gum chewing, and water intake (Table 1), the difference, if
19 any, in the autonomic indexes among the conditions was not due to the respiratory influence.

1 As shown in Fig. 6, λ showed a negative correlation with nLF/nHF in all conditions and positive
2 correlation with nHF in FOOD, indicating that λ comprises information on cardiac autonomic activity,
3 preferentially reflecting a sympathovagal balance, in other words, λ is close to unity when the
4 sympathovagal balance shifts toward a parasympathetic activation, but it is close to zero when the
5 sympathovagal balance shifts toward a sympathetic activation. In this respect, λ could be a
6 noninvasive index for evaluating cardiac ANS activity.

7 There are several potential limitations in this study. **First**, because we did not measure any
8 neurohumoral factors, direct evidence for cardiac sympathoexcitation during food ingestion has not
9 been obtained. While it has been well documented that sympathetic tone is activated after food
10 ingestion as evidenced by increased postprandial plasma norepinephrine in the entire body, cardiac
11 plasma norepinephrine spillover has been reported to be unaltered [36]. However, significant
12 elevated BP in response to food ingestion and gum chewing suggests that these behaviors elicit
13 sympathetic activation. **Second**, since we did not control the number of chews between the FOOD
14 and GUM conditions, the separate effects of mastication on the cardiovascular and autonomic
15 responses may be different between the FOOD and GUM conditions. **Third**, the test meal in the
16 present study contained relatively small amounts of energy (200 kcal). It was suggested that the
17 hemodynamic responses were related to the total caloric content and the size of the meal [2]. The
18 changes in λ in response to subjects eating a different size of meal and number of calories warrant
19 further investigation. **Fourth**, as mentioned previously, all subjects were submitted to the same

sequence of conditions and counterbalancing was not realized. For this reason, the order effects cannot be ruled out completely when comparing the results from three conditions. Finally, the study was compromised by the small sample size and narrow age range of the subjects. Therefore, the obtained results cannot be generalized to individuals beyond the study participants. In particular, age may be an important consideration because underlying hemodynamic reactions may differ from those observed in young subjects. For example, postprandial hypotension is known to be more prevalent in healthy elderly people [18].

In summary, we examined the effects of solid-meal eating, as well as gum chewing and water intake, on cardiovascular autonomic responses and the degree of cardiorespiratory phase synchronization (λ). Food ingestion, gum chewing, and water intake caused a significant decrease in λ , but HRV indexes were not changed except for the food ingestion. λ showed an inverse correlation with the index of sympathovagal balance, suggesting that the cardiac sympathoexcitation and/or vagal inhibition can be detected by an incoherent phase lag of RSA. We suggest that the phase coherence of RSA could act as a sensitive measure for evaluating the cardiac autonomic profile. In spite of this potential utility of λ for assessing cardiac autonomic activity, future studies are required in order to elucidate the reliability and validity of the relationship between actual cardiac sympathetic/vagal activity and λ , e.g., by the pharmacological blockade of the ANS.

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References

- [1] Baron AD, Brechtel G. Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am J Physiol endocrinol Metab* 265(1993), pp. E61-E67
- [2] Bagatell CJ, Heymsfield SB. Effect of meal size on myocardial oxygen requirements: implications on postprandial infarction diet. *Am J Clin Nutr* 39(1984), pp. 421-426
- [3] Berntson GG, Cacioppo JT, Quigley KS. Respiratory sinus arrhythmia: Autonomic origins, physiological mechanisms, and psychophysiological implications. *Psychophysiology* 30(1993), pp. 183-196
- [4] Brown TE, Beightol LA, Koh J, Eckberg DL. Important influence of respiration on human R-R interval power spectra is largely ignored. *J Appl Physiol* 75(1993), pp. 2310-2317
- [5] Cantú P, Savojardo D, Barelli MV, Buonamici V, Bertinieri G, Penagini R. Cardiovascular effects of gastric intubation and distension in healthy humans. *Neurogastroenterol Motil* 20(2008), pp. 304-310
- [6] Elsenbruch S, Wang Z, Orr WC, Chen DZ. Time-frequency analysis of heart rate variability using short-time Fourier transform. *Physiol Meas* 21(2000), pp. 229-240
- [7] Fagan TC, Sawyer PR, Gourley IA, Lee JT, Gaffey TE. Postprandial alterations in hemodynamics and blood pressure in normal subjects. *Am J Cardiol* 58(1986), pp. 636-641
- [8] Friesen CA, Lin Z, Schurman JV, Andre L, McCallum RW. Autonomic nervous system response to a solid meal and water loading in healthy children: its relation to gastric myoelectrical activity. *Neurogastroenterol Motil*, 19 (2007), pp. 376-382
- [9] Grossman P, Wilhelm FH, Spoerle M. Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. *Am J Physiol Heart Circ Physiol* 287(2004), pp. H728-H734
- [10] Hayano J, Sakakibara Y, Yamada M, Kamiya T, Fujinami T, Yokoyama K, Watanabe Y, Takata K. Diurnal variations in vagal and sympathetic cardiac control. *Am J Physiol Heart Circ Physiol*. 27(1990), pp. H642-H646
- [11] Hayano J, Sakakibara Y, Yamada M, Ohte N, Fujinami T, Yokoyama K, Watanabe Y, Tanaka K. Decreased magnitude of heart rate spectral components in coronary artery disease. *Circulation* 81(1990), pp. 1217-1224

- 1
- 2 [12] Kearney MT, Cowley AJ, Macdonald IA. The cardiovascular responses to feeding in man. *Exp*
- 3 *Physiol* 80(1995), pp. 683-700
- 4
- 5 [13] Kelbaek H, Munck O, Christensen NJ, Godtfredsen J. Autonomic nervous control of postprandial
- 6 hemodynamic changes at rest and upright exercise. *J Appl Physiol.* 63(1987), pp. 1862-1865
- 7
- 8 [14] Kingston WJ, Livingston LN, Moxley RT. Enhancement of insulin action after oral glucose
- 9 ingestion. *J Clin Invest* 77(1986), pp. 1153-1162
- 10
- 11 [15] Lackner HK, Papousek I, Batzel JJ, Roessler A, Scharfetter H, Hinghofer-Szalkay H. Phase
- 12 synchronization of hemodynamic variables and respiration during mental challenge. *Int J*
- 13 *Psychophysiol* 79(2011), pp. 401-409
- 14
- 15 [16] Lipsitz LA, Ryan SM, Parker JA, Freeman R, Wei JY, Goldberger AL. Hemodynamic and
- 16 autonomic nervous system responses to mixed meal ingestion in healthy young and old subjects
- 17 and dysautonomic patients with postprandial hypotension. *Circulation* 87(1993), pp. 391-400
- 18
- 19 [17] Lu C-L, Zou X, Orr WC, Chen JDZ. Postprandial changes of sympathovagal balance measured
- 20 by heart rate variability. *Digestive Disease Sci.* 44(1999), pp. 857-861
- 21
- 22 [18] Luciano GL, Brennan MJ, Rothberg MB. Postprandial hypotension. *Am J Med*, 123(2010), pp.
- 23 e281-286
- 24
- 25 [19] Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the
- 26 frequency domain. *Circulation* 84(1991), pp. 482-492
- 27
- 28 [20] Martinmäki K, Rusko H, Saalasti Sami, and Kettunen J. Ability of short-time Fourier transform
- 29 method to detect transient changes in vagal effects on hearts: a pharmacological blocking study.
- 30 *Am J Physiol- Heart Circ Physiol.* 290(2006), pp. H2582-H2589
- 31
- 32 [21] Niizeki K, Saitoh T. Incoherent oscillations of respiratory sinus arrhythmia during acute mental
- 33 stress in humans. *Am J Physiol Heart Circ Physiol.* 302(2012), pp. H359-H367
- 34
- 35 [22] Oppenheim AV and Schafer RW. *Discrete-Time Signal Processing.* Engelwood Cliffs, New
- 36 Jersey: Prentice-Hall, (1989).
- 37
- 38 [23] Rossi P, Andriese GI, Oey PL, Wieneke GH, Roelofs JMM, Akkermans LMA. Stomach
- 39 distension increases efferent muscle sympathetic nerve activity and blood pressure in healthy

- 1 humans. *J Neuro Sci* 161(1998), pp 148-155
- 2
- 3 [24] Rokowsk RJ, Spodick DH. Prandial effects on cardiac function and responses. *Am Heart J.*
- 4 118(1989), pp. 1078-1082
- 5
- 6 [25] Routledge HC, Chowdhary S, Coote JH, Townend JN. Cardiac vagal response to water ingestion
- 7 in normal human subjects. *Clin Sci* 103(2002), pp. 157-162
- 8
- 9 [26] Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta JA, Landsberg L. Effect of insulin and
- 10 glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* 30(1981), pp.
- 11 219–225
- 12
- 13 [27] Rosenblum M, Pikovsky A, Kurths J, Schäfer C, Tass PA. Phase synchronization: from theory
- 14 to data analysis. In: *Handbook of Biological Physics*, edited by Moss F and Gielen S. Elsevier
- 15 Science, Amsterdam, 4(2001), pp. 279-321
- 16
- 17 [28] Ryan SM, Goldberger AL, Ruthazer R, Mietus J, Lipsitz LA. Spectral analysis of heart rate
- 18 dynamics in elderly persons with postprandial hypotension. *Am J Cardiol* 69(1992), pp. 201-205
- 19
- 20 [29] Sauder KA, Johnston ER, Skulas-Ray AC, Campbell TS, West SG. Effect of meal content on
- 21 heart rate variability and cardiovascular reactivity to mental stress. *Psychophysiology* 49(2012),
- 22 pp. 470-477
- 23
- 24 [30] Scott EM, Greenwood JP, Gilbey SG, Stoker JB, Mary DA. Water ingestion increases
- 25 sympathetic vasoconstrictor discharge in normal human subjects. *Clin Sci* 100(2001), pp. 335-342
- 26
- 27 [31] Sherozia OP, Ermishkin VV, Lukoshkova EV. Dynamics of swallowing-induced cardiac
- 28 chronotropic responses in healthy subjects. *Bull Exp Biol Med.* 135(2003), pp.322-326
- 29
- 30 [32] Shiba Y, Nitta E, Hirono C, Sugita M, Iwasa Y. Evaluation of mastication-induced change in
- 31 sympatho-vagal balance through spectral analysis of heart rate variability. *K Oral Rehabil*
- 32 29(2002), pp. 856-960
- 33
- 34 [33] Stok WJ, Stringer RC, Karemaker JM. Noninvasive cardiac output measurement in orthostasis:
- 35 pulse contour analysis compared with acetylene rebreathing. *J Appl Physiol* 87(1999), pp.
- 36 2266–2273
- 37
- 38 [34] Task Force of the European Society of Cardiology the North American Society of Pacing
- 39 Electrophysiology, Heart rate variability: Standards of measurement, physiological interpretation,

- 1 and clinical use. *Circulation* 93(1996), pp. 1043-1065
- 2
- 3 [35] Teff KL, Mattes RD, Engelman K. Cephalic phase insulin release in normal weight males:
- 4 verification and reliability. *Am J Physiol* 261(1991), E430-436
- 5
- 6 [36] Vaz M, Turner A, Kingwell B, Chin J, Koff E, Cox H, Jennings G, Esler M. Postprandial
- 7 sympatho-adrenal activity: Its relation to metabolic and cardiovascular events and to changes in
- 8 meal frequency. *Clin Sci* 89(1995), pp. 349-357
- 9
- 10 [37] Wesseling KH, Jansen JR, Settles JJ, Schreuder JJ. Computation of aortic flow from pressure in
- 11 humans using a nonlinear, three-compartment model. *J Appl Physiol* 74(1993), pp. 2566-2573
- 12
- 13