Abstract
The aim of this study was to investigate the effects of vegetable tablets containing Gamma-Aminobutyric Acid (GABA) intake on cardiovascular response and the autonomic nervous system in young adults. In a double-blind, randomized controlled trial, 7 healthy subjects were assigned to take vegetable tablets (10 g/trial) or control tablets (10 g/trial). We measured heart rate (HR), systolic and diastolic blood pressure, stroke volume, cardiac output, total peripheral resistance index, and the low- and high-frequency oscillatory components of heart rate variability (HRV). Two major spectral components were examined at low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.4 Hz) bands to indicate HRV. There were significant interactions in HR \((p < 0.01)\) and in LF/HF of HRV \((p < 0.05)\). HR increased after intake of control tablets, but not after that of vegetable tablets. LF/HF increased rapidly after intake of control tablets and rose slightly after vegetable tablet intake. There was no significant difference between the vegetable and control tablet trials in stroke volume, cardiac output, total peripheral resistance, systolic or diastolic blood pressure, HF, or LF. In conclusion, these results suggest that single administration of vegetable tablets containing GABA suppresses the sympathetic nervous activity leading to an elevation of blood pressure. J Physiol Anthropol 28(3): 101–107, 2009 http://www.jstage.jst.go.jp/browse/jpa2 [DOI: 10.2114/jpa2.28.101]

Keywords: vegetable tablet, heart rate variability, GABA, blood pressure, autonomic nervous system

Introduction
Humans are omnivorous and have particular nutritional requirements (Leonard, 2000) that cannot be obtained from a meat diet alone. Most of the foods consumed to meet those requirements are thought to come from non-meat sources (Baker, 1988), and this reflects adaptations to diets with large amounts of fruit and vegetable material (Leonard, 2000). However, most people living in relatively developed countries are generally able to obtain a sufficient amount and variety of food (Ministry of Agriculture, Forestry and Fisheries, 2007; The State of Food and Agriculture, 2007; World Health Report-Reducing Risks, 2002), which raises the possibility of a rise in the prevalence of chronic diseases related to stress, such as physiological, social, psychological, and technological stresses.

Epidemiological studies have revealed that the intake of vegetables has beneficial effects on age-related diseases, such as cardiovascular disease (Nöthlings et al., 2008). In fact, vegetable consumption can reduce levels of serum triglycerides, total serum cholesterol, and low-density lipoprotein (LDL) cholesterol in hypertensive patients (Adebawo et al., 2006). In interventional studies, a significant inverse association between blood pressure and a vegetarian diet rich in fibers, magnesium, potassium, calcium, and protein has been reported (Appel et al., 1997), and Fan et al. (2000) showed that vegetable juice intake suppresses oxidative stress. It is well known that hypertension, which is affected by stress as well as by changes in dietary pattern, is related to an imbalance in the activity of the cardiac autonomic nervous system (Guzzetti et al., 1988; Takagi et al., 2006).

Gamma-aminobutyric acid (GABA) is a kind of amino acid distributed widely throughout the natural world (Carratù et al.,...
2008). GABA is contained in vegetables and fruits consumed in daily life. It is reported that GABA reduces blood pressure (Inoue et al., 2003) and has anti-stress effects (Abdou et al., 2006). However, most previous studies focused on the effects of long-term intake of GABA, whereas there was little study of short-term effects, especially of the effects of a single intake of GABA.

On the other hand, most people living in modern society suffer from considerable stress. Variable stresses, such as physical or psychological, affect the autonomic nervous system, and heart rate variability (HRV) is used as an index to measure autonomic nerve activity (Maunder et al., 2006; Yoshino and Matsuoka, 2005).

From the result that long-term intake of GABA attributed to reduction of blood pressure, the possibility is raised that a single intake of GABA also affects autonomic nerve activity, which is related to the regulation of blood pressure. However, there are few reports on the acute effect on autonomic nerve activity from a single intake of vegetables containing GABA.

In this study, we assessed the effects of GABA-enriched vegetable tablet intake on cardiac autonomic nervous system activity in healthy people by measuring HRV in a controlled study design. In addition, the purpose of our study was not to investigate an association of a specific stress and GABA, but to study design. In addition, the purpose of our study was not to investigate an association of a specific stress and GABA, but to investigate the acute effect of GABA-enriched vegetable on humans in modern society who suffer from stress. Therefore, measurement was conducted under the condition of no loading of specific stress on the subjects.

Materials and Methods

Subjects

The subjects were seven healthy males, who were not smokers, had no behavioral disturbances, and had no history of drug or alcohol abuse, diabetes, or other pre-existing medical condition. The average age was 22.7 ± 1.0 years (ranging from 21 to 24 years), weight was 58.9 ± 4.4 kg, height was 171.9 ± 4.0 cm, and body mass index (BMI) was 19.9 ± 1.2 kg/m². Informed consent was obtained from all participants. All procedures were approved by the ethics committees of the Graduate School of Science and Technology, Shizuoka University.

Protocol

All subjects participated in two experimental sessions according to a double-blind, randomized crossover design. The washout period between sessions was 1 year in order to avoid both seasonal effects and a carryover effect from the first session. During the first session, the subjects were randomly divided into two trial groups: a vegetable trial (n=3) and a control trial (n=4). For the second session, the trials were reversed (control trial, n=3; vegetable trial, n=4).

All subjects were instructed to refrain from consuming stimulating food and drink, and to lead a regular life from several weeks before any experimentation commenced. On the experiment day, the subjects had the same lunch at 12.00 h, to avoid the influence of lunch on the collected data. The contents were two rice balls and a bowl of miso soup, containing 76.8 g carbohydrate, 9.7 g protein, and 1.5 g fat. The total digestible energy was 369 kcal. After lunch, the subjects took no food or drink, and the experiment began between 13.30 h and 14.00 h in a room where the temperature was 24°C to 26°C and the humidity was about 42%. Immediately after the subjects entered the experimental room, they were prepared with electrocardiogram (ECG) electrodes using a CM5 lead, a pulse wave sensor placed on the left index finger, and a blood-pressure sensor placed on the left upper arm. Then they rested in a reclining chair for 1 hour. Thirty minutes after the resting time was finished, each subject took either 50 vegetable tablets (10 g) or 50 control tablets (10 g) with water (200 ml) within a period of 3 minutes. This time period was based on the time actually spent on the intake of the 50 tablets. Thirty minutes before and 20, 40, and 60 minutes after tablet intake, ECG, pulse wave, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were recorded for a period of 6 minutes.

Vegetable and control tablets

Vegetable and control tablets (Enseki Aojiru Co., Ltd., Toon, Japan). The vegetable tablets were made from organically cultivated kale (Brassicaceae). Each vegetable or control tablet was bright green, circular, 8 mm in diameter, and 3.8 mm thick. The gross weight of either tablet per intake was 10 g (50 tablets). This intake amount was determined by referring to the results of our preliminary study (unpublished), which confirmed that autonomic nerve activity had changed by the intake of at least 30 mg of GABA in an acute study design. Each intake contained 1.93 g carbohydrate, 2.41 g protein, 0.47 g fat, 1.49 mg vitamin E, 0.041 mg vitamin B1, 0.154 mg vitamin B2, 520 mg potassium, 3.06 g dietary fiber, 232 mg calcium, 0.605 mg iron, 50 mg magnesium, 0.296 mg zinc, 1870 IU vitamin A, 3.36 mg beta-carotene, 43.7 mg vitamin C, 31.8 mg GABA, 50 mg chlorophyll, and 3.46 mg lutein. The total energy was 21.6 kcal per intake. The control tablets were made from potato starch, maltodextrin, and gardenia. Each intake contained 9.39 g carbohydrate, less than 0.01 g protein, 0.03 g fat, less than 0.01 mg vitamin E, less than 0.01 mg vitamin B1, less than 0.01 mg vitamin B2, 3.4 mg potassium, 0 g dietary fiber, 1 mg calcium, 0.06 mg iron, 0.6 mg magnesium, 0 mg zinc, 0 IU vitamin A, 0 mg beta-carotene, 0 mg vitamin C, 0 mg GABA, 0 mg chlorophyll, and 0 mg lutein. The total energy was 33.0 kcal per intake. All of the subjects ingested tablets of each type without problems, and none reported any nausea, dislike of the tablets, or other side effects.

Measurement methods

The stroke volume (SV) was obtained by calculating the pulse pressure (beat-to-beat basis) using a sphygmomanometer (GP-303S, Parama-Tech, Fukuoka, Japan). Cardiac output (CO) was calculated as the product of SV and heart rate (HR).
Total peripheral resistance (TPR) was calculated as mean blood pressure (MBP) divided by cardiac output.

To evaluate the autonomic nervous system activity, we used a power spectral density analysis of the temporal interval between heart beats (R-R intervals).

The ECG signals and pulse waves were digitized on-line by a 16-bit analog-to-digital converter (ADM-687zPCI, Micro Science, Tokyo, Japan) at a sampling rate of 1kHz. The digitized ECG signals were discriminated, and the resulting QRS spikes and R-R intervals were stored sequentially on a hard disk. The stored R-R interval data were displayed and interpolated sequentially to obtain equally spaced sample points with an effective sampling frequency of 2Hz (Rompeleman et al., 1977). The interpolation method of R-R interval data used three-dimensional spline interpolation. The power spectral density analysis using the Minimum Entropy Method (MEM) (Marple, 1987) was then performed on successive 360-second time series of the obtained R-R interval data. The MEM was conducted by using the model order determined by the method of AIC (Akaike’s information criteria) (Akaike, 1969).

To evaluate the autonomic nervous system activity, we analyzed the low frequency (LF: 0.04–0.15 Hz) as reflected in both sympathetic and parasympathetic nerve activity (Murray DR, 2003; Srinivasan et al., 2002; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), and the high frequency (HF: 0.15–0.4 Hz) as reflected in parasympathetic nerve activity (Akselrod et al., 1981; Pagani et al., 1986; Pomeranz et al., 1985). We calculated the LF/HF ratio as a measure of cardiovascular sympathovagal balance (Furlan et al., 1990; Pagani et al., 1988).

**Statistical analysis**

Data are expressed as means±S.E.M. To compare data at baseline between the vegetable and control trials, we used Student’s t-test. The effects of time, tablet intake, and time×tablet intake were evaluated by repeated measures analysis of variance (ANOVA) tests. For post-hoc analysis, we used the Bonferroni test to compare the pre-intake data (30 minutes before intake) and post-intake data (20, 40, 60 minutes after intake), and we used paired t-tests with Bonferroni correction to compare the two trials at corresponding time points. Statistical analysis was performed using SPSS® 12.0J for Windows (SPSS Inc., Chicago, IL). Differences with values of $p<0.05$ were considered significant.

**Results**

**Baseline data**

There were no significant differences between the vegetable and control trials in the baseline pre-intake data on cardiovascular variables (Table 1).

In addition, there were no significant differences at baseline between the first and second sessions on all measurement parameters.

**Cardiovascular responses**

Changes in SBP are shown in Fig. 1. ANOVA showed that time had a significant main effect on systolic blood pressure (SBP) ($p<0.05$). There were no significant interactions among SBP. In SBP, $p<0.05$ at 40 min after intake compared with 30 min before and 20 min after intake under the vegetable trial; $p<0.05$ at 60 min after intake compared with 40 min after intake under the vegetable trial.
intake compared with 30 minutes before and 20 minutes after intake. At 60 minutes after intake, HR also fell compared with 20 minutes after intake, but no significant change was shown at 60 minutes compared with 30 minutes before intake. In the vegetable trial, post-hoc analysis found no significant change (Fig. 2A). After a post-hoc test, SV in the vegetable trial significantly fell at 40 minutes after tablet intake compared with 30 minutes before and 20 minutes after intake (Fig. 2B). CO in the control trial significantly fell at 40 minutes after tablet intake compared with 20 minutes after intake (Fig. 2C).

Cardiac interval variability
Changes in LF, HF, and the LF/HF ratio are shown in Figs. 3A, 3B, and 3C, respectively. There was significant interaction in the LF/HF ratio (time×tablet intake, F=3.28, p<0.05), but not in LF or HF. In the post-hoc test, the LF/HF ratio in the control trial significantly rose 20, 40, and 60 minutes after control tablet intake compared with 30 minutes before intake. In the vegetable trial, the LF/HF ratio significantly rose at 40 minutes after tablet intake compared with 30 minutes before intake. In addition, at 20 and 40 minutes after intake, there was a significant difference between the two trials. Further, time showed a significant main effect on LF and on the LF/HF ratio. LF in the control trial significantly rose at 20 minutes, and then fell at 60 minutes after tablet intake compared with 30 minutes before and at 20 minutes after intake. On the other hand, LF in the vegetable trial showed a significant change at 20 minutes after and 60 minutes after intakes compared with 30 minutes before intake (Fig. 3A). HF in both trials tended to rise at 20 minutes, and then fell until 60 minutes after tablet intake, but time did not have a significant main effect.

Discussion
In this study, we assessed the effects of GABA-enriched vegetable tablet intake on the cardiac autonomic nervous system in healthy people. Thus far, there have been reports of the effect of long-term intake of GABA on change of blood pressure (Inoue et al., 2003), but little is known about the effect of short-term or single intake of vegetable on HRV or on blood pressure. To the best of our knowledge, this is the first experimental report on the effect of GABA-enriched vegetable intake in a well-controlled study design. The main finding of our study was that both HR and the LF/HF ratio in the control trial were remarkably higher than in the vegetable trial.

In the present study, the intake of control tablets significantly raised HR and sympathovagal balance, but the intake of vegetable tablets showed only a weak rise in sympathovagal balance. After intake of control or vegetable tablets, the HF component was increased at 20 minutes after intake and then returned to the baseline. Although there has been no report of a relationship between the autonomic nervous system and the acute effect of vegetable intake, Fu et al. (2006) reported that long-term vegetarians show low blood pressure, low lipid concentrations, facilitated vagal regulation of
HRV, and increased baroreflex sensitivity compared with nonvegetarian healthy postmenopausal women, without increasing the sympathetic modulations of the cardiovascular system in either group. This result was not in agreement with ours. This difference may have been caused by the difference in observation period, such as short- vs. long-term, or by differences in study design. On the other hand, in their report on acute effects on the autonomic nervous system, Hayano et al. (1990) reported that HF power decreased and LF power increased after meal intake. Routledge et al. (2002) reported that both HF and LF increased after water ingestion. These results are not necessarily consistent with our results. The reason for this discrepancy is unclear, but it may be due to the difference in nutrient components and quantity among meal, water, and vegetable tablets.

This study revealed sympathetic activation after control tablet intake. Heretofore, sympathetic tone was thought to be activated throughout the entire human body after food intake. Indeed, sympathetic activity has been reported to be activated after food intake in humans and animals, as evidenced by an increased postprandial plasma noradrenalin spillover response of the whole body, forearm, kidney, and skeletal muscle (Vaz et al., 1995). In addition, Schwarz et al. (1988) reported that about 30% of the thermic effect of food was responsible for the activation of sympathetic activity, and Mathias (1991) reported that the thermic effect helps maintain postprandial blood pressure. On the other hand, the total energy contained in a vegetable or control tablet in the present study was 21.6 kcal or 33.0 kcal, respectively, considerably smaller than in the above-mentioned studies. The total energy of the lunch was 369 kcal, but more than 90 minutes passed between lunchtime and the beginning of our experiment. Therefore, in the present study, there seems to be little possibility of thermic effect as a cause of sympathetic activation after tablet intake.

In the present study, contrast in results of sympathovagal balance was shown between the control and vegetable trials. Concretely, HR was reduced and the LF component and LF/HF ratio of HRV were increased very weakly by the vegetable trial, compared with the control trial, although there was no difference in parasympathetic nerve response between the two trials. The underlying mechanism was not made clear in the present study, but there may be several possibilities in the relationship between the nutritional component of vegetables and autonomic nerve activity (Inoue et al., 2003; Manzella et al., 2001; Rylander and Arnaud, 2004). The vegetable tablets used in this study include a large amount of GABA. GABA intake in humans reduces blood pressure, based indirectly on the effect of the autonomic nervous system (Inoue et al., 2003). In addition, the acute effect of orally administered GABA on relaxation has been investigated in humans (Abdou et al., 2006). That acute study reported that in electroencephalography, alpha waves and beta waves were increased and decreased, respectively, 60 minutes after GABA administration. These results in the central nervous system indirectly support the acute effects of GABA on our results.

**Fig. 3** Changes in low frequency (LF) (A) and high frequency (HF) (B) cardiac interval variabilities (power) and the low frequency/high frequency (LF/HF) ratio (C). Repeated measure ANOVA showed a significant main effect of time on LF and the LF/HF ratio \( (p<0.05) \) but not on HF. There was significant interaction in the LF/HF ratio (time\times tablet intake, \( F=3.28, \ p<0.05 \)), but not in LF. After the Bonferroni test, LF, \( p<0.01 \) at 20 min after intake compared with 30 min before intake under the control trial; \( p<0.05 \) at 60 min after intake compared with 30 min before intake under the control trial; \( p<0.05 \) at 20 min after intake compared with 30 min before intake under the vegetable trial (A). LF/HF ratio in the control trial significantly rose at 20, 40, and 60 minutes after tablet intake compared with 30 minutes before tablet intake. In the vegetable trial, the LF/HF ratio significantly rose at 40 minutes after tablet intake compared with 30 minutes before tablet intake. In addition, at 20 and 40 minutes after tablet intake, there was a significant difference between the two trials (C).
in the autonomic nervous system, although the amount of the single GABA that they used was more than twice that contained in the vegetable tablets we used. The detailed mechanism underlying this difference should be studied. Our finding is that HR was reduced, and that the LF component and LF/HF ratio of HRV were increased very weakly, by the vegetable trial compared with the control trial, although there was no difference in parasympathetic nerve response between the two trials. From these reports, GABA, which reduces blood pressure or increases alpha waves, may have some role in the suppression of sympathetic nerve activity by an acute intake of GABA-enriched vegetable tablets, but the physiological mechanism was still unclear.

There were differences in the quantities of vitamins and minerals, such as vitamin E, potassium, magnesium, and calcium, between the vegetable and control tablets. This discrepancy might contribute to the contrasting HRV results between the control and vegetable trials.

In SBP, MBP, DBP, SV, CO, and TPR, there was no significant interaction (time \( \times \) tablet intake), whereas time showed main effects on SBP, SV, and CO. The mechanisms underlying these effects were unclear. Thus, further study is needed to clarify the detailed mechanism underlying these responses.

In the present study, the washout time between the first and second sessions was 1 year in order to avoid seasonal effects and to avoid a carryover effect from the first session. There were no statistical significances at baseline between sessions for any measurement parameter. However, it remains possible that lifestyle changes during the one-year period, especially dietary habits, had some influence on the present results.

**Conclusion**

We examined various autonomic nervous system activities in healthy persons following the ingestion of vegetable tablets. HR showed a rise in the control trial but no change in the vegetable trial. LF/HF showed an increase in the control trial but only a slight rise in the vegetable trial. In LF and HF, significant interaction between time and tablet intake was not found. These results suggest the possibility that single administration of vegetable tablets containing GABA suppresses the sympathetic nervous activity leading to an elevation of blood pressure.

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