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Original article

Noninvasive measurements to evaluate the effects of military training on the human autonomic nervous system

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Background: Chinese university freshmen receive 4-weeks military training that involved moderate to intense physical exercise. Studies have demonstrated heterogeneous effects of exercise on the autonomic nervous system.

Objective: To evaluate the effects of training on the autonomic nervous system noninvasively using electrogastrograms, heart rate variability (HRV), pulse rate, and the sympathetic skin response (SSR).

Methods: Twenty freshmen received all assessments in the fasting state and after a standard meal: (1) one week before the training, (2) at the end of the second week of the training, and (3) one week after the training.

Results: (1) The training had a significant effect on meal-induced gastric pacemaker activity. Before the training, a standard meal did not increase the dominant frequency of gastric slow waves, but the frequency increased during and after the training; (2) The preprandial high frequency (HF), low frequency (LF), and very low frequency (VLF) components of heart rate variability decreased significantly after the training. The ratio of the LF and HF (LF/HF) of the heart rate variability (HRV) did not significantly change after a meal or training condition. The basal pulse rate did not change. The latencies of the sympathetic skin response (SSR), as measured in the arm muscle, increased in response to the training.

Conclusion: Military training affects meal-induced changes in gastric pacemaker activity, causes a marked reduction of the vagal tone to the heart with maintenance of the vagal-sympathetic balance, and its effects on SSR may reflect a reduction in sympathetic tone.

Keywords: Autonomic nervous system, electrogastrogram, heart rate variability, sympathetic skin response, sympathovagal balance

Military training is routine in China for first-year students at universities before their regular courses start. Each year, millions of freshmen participate in this 4-week compulsory program that involves moderate to intense physical exercises including standing at ease, standing to attention, striding, marching, running, and military boxing. The vast number and the special identity (university students) of the participants in this nation-wide event have aroused interest in its influence on the young students from both psychological and physiological perspectives. This study focuses on the functioning of the autonomic nervous system (ANS), using three independent methods.

A number of investigators have studied the effects of exercise on ANS investigating the electrogastrogram (EGG) and/or heart rate variability (HRV) [1-4] with widely variable results. The EGG records the myoelectrical activity of the stomach musculature [5], which is equivalent to the gastric slow wave or gastric pacemaker activity, can be influenced by the ANS. HRV, defined as the variations in the heart rate and RR intervals, the interval from the peak of one QRS complex to the peak of the next as shown on an electrocardiogram [6], are broadly-available and

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low-cost measures of cardiac function associated with the sympathovagal balance [7]. Because of different methodologies used, and differences in the test meals, exercise intensity, and ethnic backgrounds of the subjects, these studies using EGG or HRV, or both, have reported controversial results so that no consensus on the changes in sympathetic and parasympathetic nervous activity after exercises have been reached thus far.

The sympathetic skin response (SSR) is a noninvasive method that assesses sympathetic nervous activity. It measures the skin potential changes generated by sweat glands as the activated effectors of the reflex arch consisting of postganglionic sympathetic fibers [8].

To our knowledge, the effects of military training on the ANS have not yet been explored nor has there been a study combining EGG, HRV, and SSR. The aim of the present study was to investigate the influence of the routine one-month military training on the function of the ANS using these independent assessments.

Materials and methods

Subjects

Twenty healthy male college freshmen of Wuhan University (aged from 17 to 22 years, mean age 18.6 years), who had no history of gastrointestinal surgery nor any chronic disease requiring regular medical care were randomly recruited into this study. Participants were excluded for a history of any chronic or recurrent abdominal pain or discomfort, nausea, vomiting, diarrhea, constipation, or bloating. All students participated in the one-month military training program before start of regular classes. Training took place from 8 AM to 5 PM every day with a 2 h break at noon, and included: standing at ease, standing at attention, striding, drill, running, marching, and military boxing, which was equivalent moderate exercise. Measurement were conducted: (1) the week before the training, (2) the end of the second week of the training, and (3) the week after the training was completed. Approval for this study was obtained from the Ethics Committee of Renmin Hospital of Wuhan University. The written informed consent was obtained from each participant before the study.

Experimental protocol

Meal-related electrogastrogram (EGG), heart rate variability (HRV), pulse rate, and SSR were assessed in each subject in the three periods. After at least 4 hours' fasting, each subject received 30 min or longer EGG and HRV recordings simultaneously. After a test meal that was composed of 450 kcal (20% protein, 15% fat, and 65% carbohydrate, without water) was given and finished in 15 min, EGG and HRV were resumed for another 30 min or longer with the same electrodes. During the entire recording period, the subject was in a supine position in a quiet room, and remained as still as possible [9]. Meal-related SSR of each subject was tested before the preprandial recording and after the postprandial recording of EGG and HRV. The pulse rate was taken during the test periods manually.

EGG recording and analysis

Surface electrogastrography (Medtronic-Synectics, Shoreview, Minnesota, USA) was used to measure the gastric myoelectrical activity of the subjects. Before the recording, the abdominal surface was shaved, cleaned with a sandy skin paste to reduce electrical impedance before electrode attachment. The electrogastrography used six electrodes. Four active surface electrodes (channels 1 to 4) were positioned over the stomach. Electrode 3 was placed first, which was 2 cm above the middle point between the xiphoid process and the umbilicus, therefore positioned approximately at the distal antrum. Electrode 4 was placed 4 cm horizontally to the right of electrode 3, and therefore positioned approximately at the pylorus region. Electrode 2 and 1 were placed 45° superior and to the left of electrode 3, respectively, with a 4- to 6-cm space in between, depending on subject size, positioned approximately at the proximal antrum and the corpus. The common reference electrode was placed at the cross point of two lines, one horizontal, connecting electrode 1, and another vertical, connecting electrode 3. The ground electrode was placed on the left costal margin horizontal to electrode 3 [10].

After EGG recording, segments of the recording with motion artifacts were deleted. The following parameters from electrode 3, which was placed at the approximate site of the distal antrum, were computed using spectral analysis methods [11]: (1) EGG dominant frequency, was defined as the frequency with the highest power in the EGG power spectrum in the range of 0.5"9.0 cycles per minute (cpm). (2) EGG dominant power was defined as the power of the dominant frequency in the power

spectrum. (3) Percentage of normal gastric slow wave, defined as the percentage of time during which regular 2–4 cpm slow waves were present over the entire analyzed period.

HRV recording and analysis

Following a 15-min rest period, a 30 min electrocardiogram (ECG) was obtained before and after the meal, while the subjects were in the supine position. The ECG data were obtained by a Holter monitoring at 250 kHz and analyzed using a Marquette Laser SXP Holter analysis system (Milwaukee, WI, USA) [12]. The HRV analysis methods included time domain analysis and frequency domain analysis, both reflecting the parasympathetic and sympathetic activity [13]. The following indices of the frequency domain were measured: (a) a high frequency component (HF), ranging from 0.15 to 0.4 Hz, which corresponds to the respiratory modulation and is an indicator of the performance of the vagus nerve on the heart; (b) a low frequency component (LF), ranging between 0.04 and 0.15 Hz, which reflects activity of the vagal and sympathetic components with a predominance of the sympathetic activity; (c) a very low frequency component (VLF), ranging from 0.01 to 0.04 Hz, which can reflect the activity of the sympathetic components. (d) The LF/HF ratio, reflecting the relative changes between the sympathetic and parasympathetic components of the ANS characterizes the balance of the sympathetic-vagal activity on the heart [12].

Recording of Sympathetic Skin Responses (SSR) and analysis

Sympathetic skin responses were studied using a standard method. The skin temperature was maintained at 33°C (room temperature >20°C). The electromyography was recorded and analyzed using a 2-channel electromyogram evoked-potential system (Medelec Synergy model M153635; Viasys HealthCare, Old Woking, Surrey, UK) with 2 pairs of recording electrodes that were placed on the palms of the hands and on the soles of the feet, while reference electrodes were placed on the dorsal side of the hand or foot, and a ground electrode that was placed on an arm or leg. A single stimulation (larger or equal to 70 mA, pulse width: 0.2 ms, frequency: 3 Hz, analyzed time: 10 s, sensitivity: 2 mV) was given unwittingly after the baseline became stable while the subjects were relaxed. The evoked potential latency was measured from the onset of the stimulus artifact to the onset of the first negative deflection and expressed in seconds. The amplitude was measured from the baseline to the negative peak and expressed in microvolts.

Statistical analysis

Each index of EGG, HRV, and SSR was compared between the preprandial period and postprandial periods of the same training period. Each preprandial index and postprandial index was analyzed in all training periods. Student's *t* test was used to make inferences about potential differences regarding EGG characteristics (dominant power, dominant frequency, and the percentage of normal slow waves). Comparisons of HRV parameters (very low frequency, low frequency, high frequency, and the LF/HF ratio) and SSR parameters (amplitudes and latencies) during training periods were performed using non-parametric tests. All data are presented as mean values ± SD unless otherwise stated. A probability value of $P < 0.05$ was considered to indicate statistically signi cant difference between means.

Results

Effects of military training on gastric electrical activity and relationship with a meal

As shown in **Table 1** and **Figure 1**, a standard meal increased the dominant frequency of the slow wave during the training (3.07 ± 0.20 vs. 2.87 ± 0.16, $P < 0.001$) and after the training (3.08 ± 0.20 vs. 2.87 ± 0.26, $P < 0.001$), but not before training. No significant differences of preprandial gastric electrical activity were found between the three periods ($P > 0.05$)

Effects of military training on HRV frequency domain indices and relationship with a meal

As shown in **Figure 2**, preprandial VLF, LF, and HF decreased significantly after the 4-weeks military training. Before the training, a meal caused a significant decrease in HRV frequency domain indices. However, after training, with all components being much lower; the meal did not decrease the HRV frequencies any further. The LF/HF ratio did not significantly change by the meal or by the training.

Table 1. Effects of military training on gastric electrical activity

	Before training	During training	After training
Preprandial			
Dominant power (dB)	47.27 ± 5.03	48.59 ± 7.54	47.42 ± 6.34
Dominant frequency (cpm)	2.90 ± 0.19	2.87 ± 0.16	2.87 ± 0.26
% Normal slow wave	84.65 ± 16.14	87.17 ± 14.99	87.81 ± 15.53
Postprandial			
Dominant power (dB)	50.98 ± 6.04	49.87 ± 5.56	50.69 ± 5.91
Dominant frequency (cpm)	2.94 ± 0.21	3.07 ± 0.20*	3.08 ± 0.20
% Normal slow wave	85.83 ± 14.05	83.91 ± 14.01	84.62 ± 17.86

*P <0.001 vs. preprandial period, P <0.001 vs. preprandial period, P <0.05 vs. before training

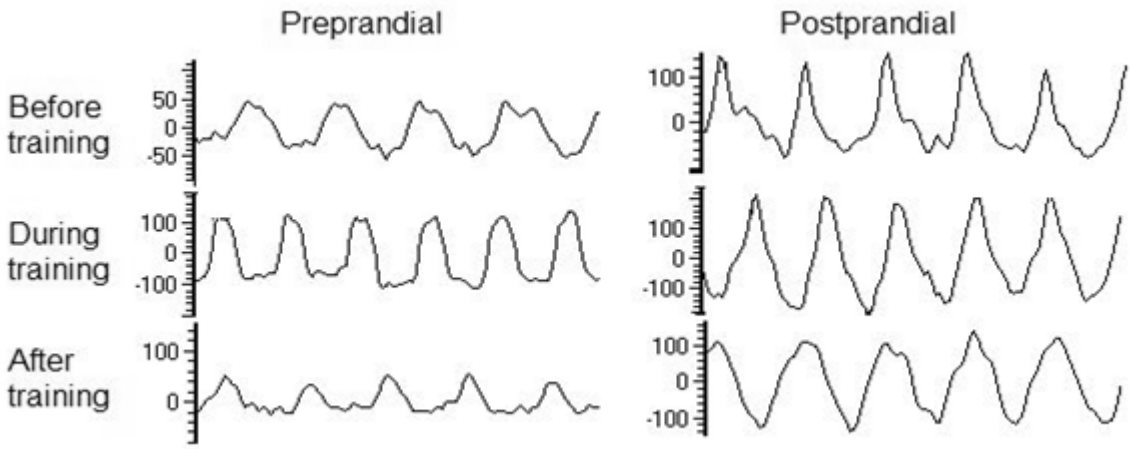

Figure 1. Meal-related typical tracings of gastric electrical activities before, during, and after the military training

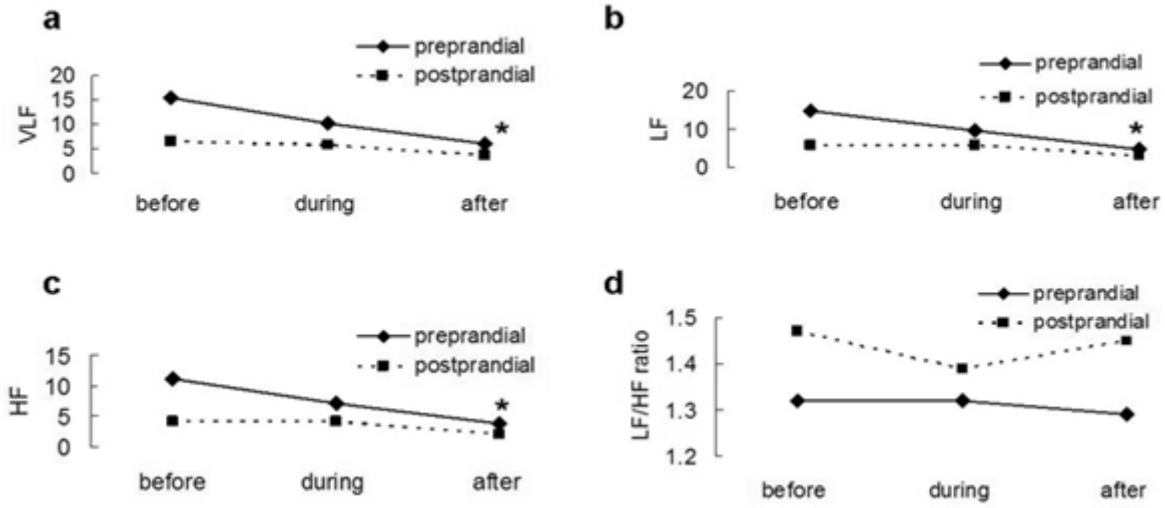

Figure 2. Effects of a standard meal and military training on HRV frequency domain indices: very low frequency, VLF (**A**); low frequency, LF (**B**); high frequency, HF (**C**); the ratio of low frequency to high frequency, LF/HF ratio (**D**). Preprandial VLF, LF, and HF (mean ± SEM) decreased significantly after 4-weeks military training (VLF: 15.54 ± 3.81 vs. 5.98 ± 2.21 ms^2, *P = 0.005; LF: 14.77 ± 3.92 vs. 4.77 ± 2.17 ms^2, *P = 0.012; HF: 11.19 ± 3.10 vs. 3.83 ± 1.57 ms^2, *P = 0.029).

Effects of military training on pulse rate and relationship with a standard meal

Before training the pulse rate was 64.9 ± 2.1 ppm before a standard meal and 69.1 ± 2.1 ppm ($P = 0.018$) after the meal. During training the pulse rate was 60.1 ± 1.3 ppm before a standard meal and 65.9 ± 1.7 ppm ($P = 0.006$) after the meal. After the training, the pulse rate was 60.3 ± 2.0 ppm before a standard meal and 67.7 ± 2.2 ppm ($P = 0.002$) after the meal. The training did not affect basal preprandial heart rates. The standard meal increased the heart rate, but the training did not change this.

Effects of military training on SSR latencies and amplitudes and relationship with a standard meal

As shown in **Tables 2 and 3**, all standard meal- and training-related SSR latency values were within normal ranges, although the training prolonged preprandial latencies of SSR as measured in the arm; and the standard meal decreased the latencies as measured in the legs, but not in the arm. The preprandial amplitudes were not markedly affected by training although a standard meal reduced the amplitudes during and after training, but not before training. Typical tracings of SSR triphase and diphase waves are shown in **Figure 3**.

Table 2. Effects of a standard meal and military training on sympathetic skin response latencies(s)

	Before training	During training	After training
Preprandial			
Left arm	1.2 ± 0.3	1.4 ± 0.7	1.5 ± 0.5*
Right arm	1.2 ± 0.3	1.4 ± 0.9	1.5 ± 0.5*
Left leg	1.6 ± 0.5	2.0 ± 0.6	1.8 ± 0.6
Right leg	1.6 ± 0.5	2.1 ± 0.6	1.8 ± 0.6
Postprandial			
Left arm	1.3 ± 0.4	1.3 ± 0.3	1.4 ± 0.4
Right arm	1.3 ± 0.5	1.4 ± 0.7	1.4 ± 0.4
Left leg	2.0 ± 1.7	1.8 ± 0.4	2.5 ± 0.5
Right leg	1.9 ± 0.9	1.8 ± 0.4	2.4 ± 0.8#

*$P < 0.05$ vs. before training; $P < 0.001$ vs. preprandial period; $P < 0.05$ vs. before training; #$P < 0.05$ vs. preprandial period

Table 3. Effects of meal and military training on sympathetic skin responses amplitudes (μV)

	Before training	During training	After training
Preprandial			
Left arm	4.9 ± 2.6	4.5 ± 1.9	4.4 ± 3.3
Right arm	4.9 ± 2.7	4.5 ± 1.9	3.9 ± 2.7
Left leg	2.3 ± 1.6	2.2 ± 1.2	1.7 ± 1.2
Right leg	2.6 ± 1.8	2.2 ± 1.3	2.0 ± 0.9
Postprandial			
Left arm	4.1 ± 2.8	4.2 ± 2.9	3.0 ± 1.7
Right arm	3.7 ± 2.8*	4.3 ± 2.6	3.1 ± 1.9
Left leg	2.4 ± 2.0	1.6 ± 0.8*	1.2 ± 0.8*
Right leg	2.2 ± 1.9	1.5 ± 0.8*	1.4 ± 0.9*

*$P < 0.05$ vs. preprandial period

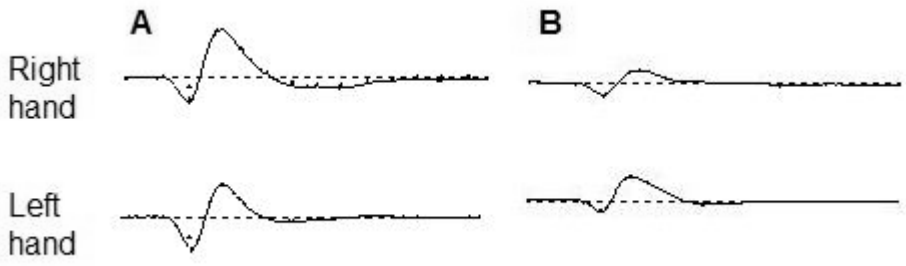

Figure 3. Typical tracings of sympathetic skin response (SSR) triphase waves (**A**) and diphase waves (**B**). Following a negative deflection, a positive deflection with high amplitude appears, and another negative deflection shows at the end, which is the characteristic shape of the triphase wave (A). The diphase wave is a negative deflection followed by a positive deflection (B).

Discussion

Military training has been a mandatory activity for university freshmen in China for more than 30 years. Its positive effects on both physical and psychological conditions have been generally accepted, although not many studies have provided data. Our interest was to study the effects on the autonomic nervous system and we employed three independent techniques to investigate this, EGG, HRV, and SSR.

The military training was shown to have a highly significant effect on the EGG response to a meal. During and after training, but not before training, a meal caused a significant increase in EGG frequency. The EGG reflects the activity of the gastric pacemaker cells, which are the interstitial cells of Cajal (ICC) [14-16]. An increase in pacemaker frequency will lead to an increase in the frequency of peristaltic contractions when the stomach is maximally activated. The human stomach pacemaker activity is myogenic in origin [17], but can be influenced by the parasympathetic and sympathetic nervous system to increase or decrease its duration and frequency. The present study does not allow us to make conclusions about effect of the training on autonomic innervation of the gastric pacemaker cells. However, because noradrenaline causes a marked decrease in duration and increase in frequency of the human gastric pacemaker [18], the increase in EGG frequency is consistent with a meal-induced increase in sympathetic activity. In the guinea pig, stimulation of excitatory vagal fibres causes an increase in slow-wave frequency [19]. Therefore, the meal induced an increase in frequency could be the result of increased vagal cholinergic activity. However, inhibitory vagal fibers also innervate the stomach such that a decrease in vagal inhibitory activity could be responsible as well. Interestingly, the communication between the vagus and ICC is bidirectional in that the vagal afferent fibers have synapse-like contact with ICC [20, 21]. Our observation that the meal induced an increase in heart rate, consistent with findings in another recent study [22], could also the result of a meal-induced increase in sympathetic activity.

During and after exercise, the average values of the dominant power (DP) of the dominant frequency (DF) tended to increase, but the difference was not significant. Although the DP increased significantly before training, because the values before and after training were similar, the conclusion that exercise did not markedly affect dominant power characteristics appears warranted. Different exercise regimes result in different outcomes. Lu et al. reported that postprandial DP and DF were larger than preprandial DP and DF with and without a 30 min cycling exercise [2]. They also found that the percentage of normal gastric slow waves increased after exercise. Kato et al. observed that exercise alone led to a greater increment in DP than a meal alone and that DF decreased under fasting conditions after exercise [23]. These discrepancies may be the result of different protocols used in the three studies.

The military training has a marked effect on the HRV. The HF component was significantly reduced, which indicates a marked reduction in vagal tone. The HF/LF ratio did not change, which is commonly interpreted as an indication of a maintained balance between the vagal and sympathetic activity. The decrease in LF likely reflects a decrease in both vagal and sympathetic tone. The general reduction in vagal tone may be responsible for the abolishment of the effect of a meal on HRV by the training that was found in the present study. A reduction in vagal tone in healthy volunteers after a meal was noted by Chang et al. [24].

Although HRV is an established method to assess autonomic innervation of the heart, the effects of exercise on HRV vary widely. Moderate exercise was reported to cause a reduction in the high-frequency component (HF) [23], in both the HF and low-frequency component (LF) [25], in HRV as a whole [26], or no significant change in HF [2]. Whereas exercise training lasting for weeks or months was shown to have no impact on HRV [27], cause an increase in HRV [26, 28], or lead to decreased LF and increased HF [29]. Moreover, the change in LF/HF ratio after exercise also varied: some found that it shifted to a sympathetic dominance [25], some reported a parasympathetic activation [30], or that there was no significant change [28]. Population-based studies have shown that leisure time physical activity of greater intensity is associated with higher levels of LF and HF [31]. The duration and intensity of the exercise therefore seem to have an obvious influence on HRV, in addition to variables like respiration, body posture, age, and sex [32].

The third technique, SSR, was employed as a noninvasive method of observing the neural afferent activity of the sympathetic nervous system. Although training did not affect the basal SSR amplitudes, a significant increase in latency was observed when measuring the responses using the arm at the end of the training. This effect was not seen in the legs, but the arms are usually found to give more reliable data [8]. This suggests a decreased sympathetic activity, although clearly the values remained fully within the normal range. A meal reduced the latencies as measured in the legs and also the amplitudes. This was not observed when measured in the arms. In a study of subjects with Huntington disease, palm latency values increased from 1.4 s to 1.8 s which was interpreted as abnormal activity. Our study indicates a significant change from 1.2 s to 1.5 s, which is within normal range [8]. Although some investigators suggest only placing a value on an absence of SSR values, Kucera et al. [8] argue for the value of quantitative evaluation. Nevertheless, the physiological significance of rather small changes needs further study. Further, the physiological significance of the response of SSR parameters to a meal needs further study.

Conclusion

In summary, the military training of university freshman in China has significant effects on their autonomic nervous system. The data can best be interpreted as a reduction in vagal and sympathetic activity with maintenance of the sympathovagal balance. Because the literature on the effect of training on HRV shows marked variability, interpretation of the data are best restricted to the specific conditions of each study. It is unlikely that the vagal tone to the heart has a simple relationship with the vagal tone to the stomach, therefore no direct conclusions can be made related to a possible relationship between autonomic activity to the heart and the increased EGG frequency in response to a meal. The significant increase in stomach pacemaker frequency seen in response to a meal indicates that training improves the peristaltic efficiency.

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