



## SUBJECTS AND METHODS

### Subjects

Ten healthy subjects (5 men, age 21 to 28; 5 women, age 19 to 27), all non-athletes, provided written informed consent to participate in the endurance exercise training.

### Training sessions

All subjects were instructed to exercise two days per week for 10 weeks according to pre-programmed schedule. A training session was held each day. Training consisted of 20 sessions. Each exercise session consisted of a warm-up of three to five min, continuous running for 25 to 40 min, and a cooling-down period of three to five min<sup>10</sup>. The training was conducted at an outdoor track in good weather and at an indoor sports facility on inclement days.

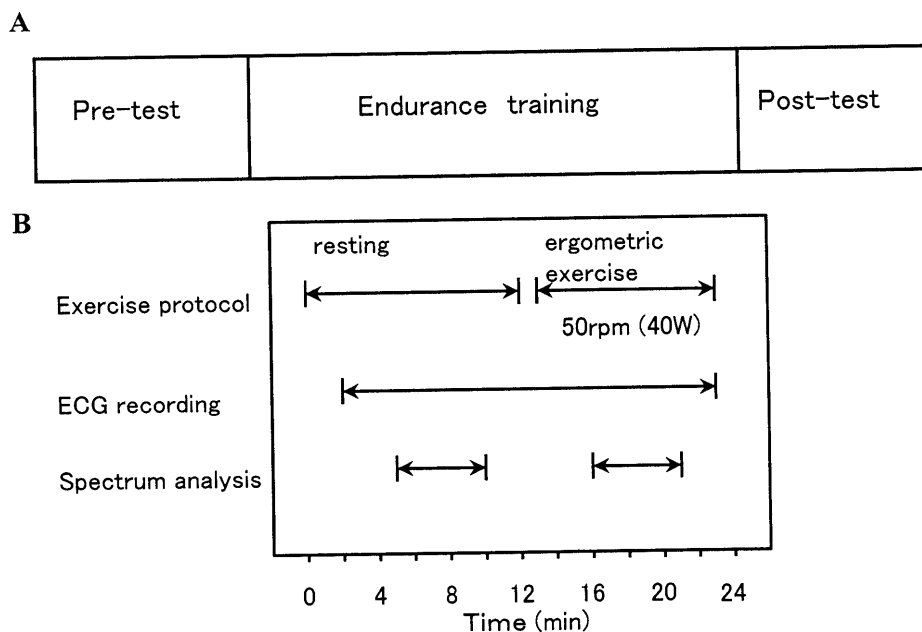
We instructed subjects to run for 35 min, the running time being controlled by one of the authors on the basis of their exhaustion. Maximum heart rate was estimated by the formula,  $220 - \text{age}^{11}$ . The first day of training served as an acclimatization day when the subjects became accustomed to the initial stress of running and recognized his/her maximum

heart rate and running speed at 80% of his/her maximum heart rate. We instructed each subject to adjust his/her running speed to 80% of his/her maximum heart rate. This running speed was controlled by the subjects. We instructed them to record their heart rate immediately after each running session by touching his/her radial artery him/herself. No other parameter was recorded during running sessions. There was no difference in conditions for running between males and females. The heart rate and running time for each subject were converted into calories (Kcal) of energy expenditure (EE) per session<sup>9,11</sup>.

### Test sessions

Before and after the training sessions, test sessions were performed (Fig. 1). In the test sessions, parameters of the autonomic nervous function were measured, and blood samples were collected under the same conditions and at the same time for each subject. Room temperature was kept constant during the test sessions by an air conditioner ( $24.3 \pm 1.8^\circ\text{C}$  and  $24.9 \pm 1.6^\circ\text{C}$ ) before and after exercise training, respectively. Room temperature was measured from 10 a.m. through 8 p.m.

We used a mechanically-braked cycle ergometer



**Fig. 1.** Experimental protocol. Upper figure (A) indicates the protocol which contains a pre-test, endurance training, and post-test. The pre and post-test consist of the autonomic nervous test and blood analyses. Lower figure (B) indicates the autonomic nervous test.

(TUNTURI W1, Canon, Tokyo, Japan) for exercise loading. The heart rate for each subject was recorded using a heart-rate monitoring system (VANTAGE XL, Canon) before and after the training sessions, respectively.

Fig. 1 shows the protocol for the test sessions for exercise and electrocardiography (ECG)<sup>7)</sup>. The total time for executing the test protocol was 23 min. After 12 min of supine rest, all subjects were instructed to exercise on a bicycle for 10 min. Steady state exercise was maintained at a 50 rpm (40W) load. The heart rate was stable in every subject during the 10-min test sessions before and after training.

### Data analysis

We defined the low frequency (LF) component as 0.06 to 0.13 Hz, which contains both sympathetic and parasympathetic nervous activities. The high frequency (HF) component was 0.16 to 0.50 Hz, reflecting primarily the parasympathetic nervous activity<sup>10,12-15)</sup>. We also calculated the LF/HF ratio, which represents an index in the predominance of sympathetic nervous activity.

Blood samples were obtained from the subjects before breakfast after an overnight fast as was necessary for hematological and serum analyses.

The data were analyzed using a paired Student's *t*-test. Statistical significance was established at the  $p < 0.05$  level.

## RESULTS

### Subjects

Data on subjects during the training sessions are shown in Table 1. Significant differences between males and females were observed in height and weight ( $p < 0.05$ ). There was no significant difference between males and females in any other parameters such as running time or energy expenditure during the total sessions.

### Training sessions

In training sessions, subjects performed the running task according to the pre-programmed task. Subjects were instructed to adjust their speed of running as to maintain their heart rate at 80% maximum, determined by the duration of the running based on the degree of their exhaustion. The average running time is listed in Table 1.

### Autonomic nervous activities in test sessions

Typical trendgrams of heart rates during the steady state exercise are shown in Fig. 2. For measurements during the ergometric exercise, the LF component and LF/HF decreased significantly ( $p < 0.05$ ), while the HF component increased significantly, after their training sessions ( $p < 0.05$ ) (Table 2). By these measurements, however, no significant change was observed in the female subjects. Hematological analysis revealed a significant decrease in RBC ( $p < 0.01$ ),

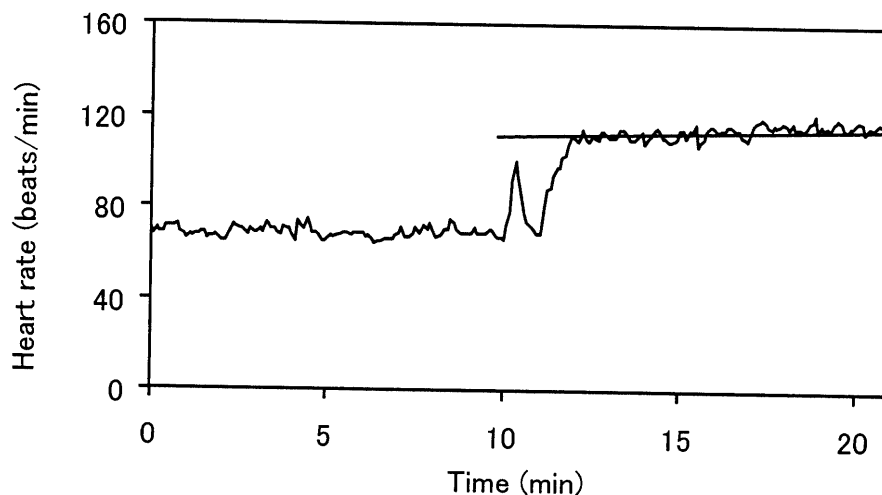


Fig. 2. Heart rate of a 19-year-old woman before training. The short horizontal line is a regression line during exercise ( $y = 0.1x + 110.6$ ).

**Table 1.** Data from subjects during the training sessions

	Subjects					Total <sup>†2</sup>
	1	2	3	4	5	
Age (years)						
Males	21	24	27	28	31	26.2±3.8
Females	19	20	21	23	27	22.0±3.2
Height (cm)						
Males	176	173	170	166	166	170.2±4.4*
Females	152	164	158	167	158	159.8±5.8
Weight (kg)						
Males	52	64	55	55	70	59.2±7.5*
Females	46	55	45	54	44	48.8±5.3
BMI (kg/m <sup>2</sup> )						
Males	16.8	21.4	19.0	20.0	25.4	20.5±3.2
Females	19.9	20.4	18.0	19.3	17.6	19.1±1.2
Per session						
Maximum heart rate (beats/min)						
Males	199	196	193	192	189	193.8±3.8
Females	201	200	199	197	193	198.0±3.2
Mean heart rate (beats/min) <sup>†1</sup>						
Males	162.9	158.4	157.4	151.4	150.2	156.1±5.2
Females	177.6	167.7	159.6	166.2	155.5	165.3±8.5
Percent of maximum heart rate (%) <sup>†1</sup>						
Males	81.9	80.8	81.6	78.9	79.5	80.5±1.3
Females	88.4	83.6	80.2	84.4	80.6	83.4±3.3
Running time (min) <sup>†1</sup>						
Males	34.5	34.5	34.5	37.3	31.2	34.5±2.2
Females	34.5	34.4	34.5	34.5	34.5	34.5±0.04
Energy expenditure (Kcal) <sup>†1</sup>						
Males	270.1	319.5	273.7	279.3	296.6	287.8±20.4
Females	272.8	298.7	227.0	290.8	215.4	260.9±37.7
Total session						
Running time (min)						
Males	690	690	690	745	635	690.0±38.9
Females	690	687	690	690	690	689.4±1.3
Energy expenditure (Kcal)						
Males	5401.1	6389.8	5474.1	5585.5	5931.2	5756.4±408.3
Females	5455.1	5973.5	4540.9	5816.2	4308.4	5218.8±753.4

<sup>†1</sup>Means of total sessions. <sup>†2</sup>Data on all subjects are expressed as mean±SD. SD is standard deviation. \*Statistically significant difference between male and female ( $p < 0.05$ ).

**Table 2.** Data in the test sessions performed before and after training

Parameter	Before training <sup>†3</sup>	After training <sup>†4</sup>
Heart rate (beats/min)		
During rest	63.5±9.2	61.3±6.3
During exercise	107.4±10.2	101.8±9.6
SD <sup>†1</sup>		
During rest	2.2±0.7	2.0±1.1
During exercise	2.6±0.6	2.4±0.6
CV <sup>†2</sup>		
During rest	3.5±0.9	3.4±2.4
During exercise	2.4±0.5	2.3±0.6
LF component		
During rest	0.6±0.2	0.5±0.2
During exercise	0.6±0.1	0.4±0.2*
HF component		
During rest	0.3±0.2	0.4±0.1
During exercise	0.3±0.1	0.4±0.1*
LF/HF		
During rest	2.2±1.4	1.3±0.7
During exercise	1.9±0.9	1.1±0.9*
WBC ( $\times 10^2/\mu\text{L}$ )	60.8±30.3	56.1±16.7
RBC ( $\times 10^4/\mu\text{L}$ )	478.2±52.1	451.8±55.4**
HGB (g/dL)	14.5±1.9	13.8±2.1*
HCT (%)	43.1±4.6	40.6±5.3**
MCV (fL)	90.2±2.9	89.8±3.7
MCH (pg)	30.3±1.4	30.6±1.8
MCHC (g/dL)	33.6±1.1	34.0±0.8
PLT ( $\times 10^4/\mu\text{L}$ )	21.1±4.4	22.2±4.9
LYP ( $\times 10^2/\mu\text{L}$ )	20.5±6.1	20.2±6.7
NU ( $\times 10^2/\mu\text{L}$ )	35.4±25.0	31.3±10.6
OGR ( $\times 10^2/\mu\text{L}$ )	4.9±2.5	4.7±2.1
TP (g/dL)	7.2±0.3	6.9±0.8
ALB (g/dL)	4.7±0.2	4.7±0.2
A/G	2.0±0.3	2.0±0.3
UN (mg/dL)	12.6±3.3	14.5±3.7
CRN (mg/dL)	0.7±0.1	0.7±0.1
UA (mg/dL)	5.0±1.5	5.0±1.1
CRT (mg/dL)	0.4±0.2	0.4±0.1
Na (mEq/L)	139.8±1.0	139.3±0.9
K (mEq/L)	4.2±0.3	4.4±0.4
Cl (mEq/L)	101.6±1.4	102.1±1.0
Ca (mg/dL)	9.3±0.4	9.2±0.3
Mg (mEq/L)	2.1±0.1	2.0±0.1
IP (mg/dL)	3.7±0.6	3.9±0.8
Fe ( $\mu\text{g}/\text{dL}$ )	96.8±38.7	91.4±34.2
GOT (IU/L)	20.0±6.7	21.5±5.7
GPT (IU/L)	22.9±16.6	21.3±13.5
LDH (IU/L)	347.7±88.7	361.9±52.6

ALP (IU/L)	133.9±28.7	140.4±29.0
$\gamma$ -GTP (IU/L)	25.5±19.0	22.6±13.0
LAP (IU/L)	52.8±5.7	52.2±5.9
ChE (IU/L)	294.9±57.9	287.6±51.2
AMY (IU/L)	133.9±86.5	119.9±59.2
CK (IU/L)	97.9±55.2	138.1±73.1
TBIL (mg/dL)	0.7±0.3	0.8±0.2
TTT (Mc. U)	1.3±0.6	1.3±0.6
ZTT (K. U)	6.0±2.4	6.1±2.2
TCHOL (mg/dL)	202.9±35.4	194.8±20.2
PL (mg/dL)	227.9±35.5	227.0±22.0
TG (mg/dL)	93.9±37.9	73.6±38.9
HDLC (mg/dL)	66.1±18.6	70.3±18.6
LDLC (mg/dL)	118.3±23.8	110.3±17.0

WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin concentration; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet count; LYP, lymphocyte; NU, neutrophile leukocyte; OGR, and the other granulocytes: TP, serum composition was analyzed for total protein; ALB, albumin; A/G, albumin/globulin; UN, urea nitrogen; CRN, creatinine; UA, uric acid; CRT, creatine; Na, sodium; K, potassium; Cl, chloride; Ca, calcium; Mg, magnesium; IP, inorganic phosphorus; Fe, iron; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LDH, lactic dehydrogenase; ALP, alkaline phosphatase;  $\gamma$ -GTP,  $\gamma$ -glutamyltranspeptidase; LAP, leucine aminopeptidase; ChE, cholinesterase; AMY, amylase; CK, creatine kinase; TBIL, total bilirubin; TTT, thymol turbidity test; ZTT, zinc sulfate turbidity test; TCHOL, total cholesterol; PL, phospholipids; TG, triglyceride; HDLC, high density lipoprotein cholesterol; and LDLC, low density lipoprotein cholesterol.

<sup>†1</sup>SD and <sup>†2</sup>CV is standard deviation and coefficient of variation, respectively. Data are expressed as mean±SD. N=10. <sup>†3</sup>Before 10-week endurance training; <sup>†4</sup>after 10-week endurance training; \*statistically significant ( $p<0.05$ ); \*\*statistically significant ( $p<0.01$ ).

HGB ( $p<0.05$ ), and HCT ( $p<0.01$ ), after their training sessions. By these hematological measurements, no significant change was observed in the male subjects. Blood chemistry revealed no significant changes after the training sessions.

## DISCUSSION

New findings in this study showed changes in autonomic nervous activities from 10 days of physical training. The results were obtained by comparison of the parameters in non-athletes before and after physical training, which was repeated for 20 days over 10 weeks with sufficient intensity. In our previous study, we found no difference in autonomic nervous activ-

ities and hematological values before and after physical training. While height, weight, and BMI of the non-athletes subjects did not significantly differ in the previous and present studies, a higher intensity of exercise was found in the protocol of the present study, judging from the higher heart rate, longer running time, and greater energy expenditure. This exercise intensity seems a fairly moderate one compared with those of other reported protocols<sup>16,17</sup>. The following four factors may influence the effect of physical training: athletic experience, intensity of training, frequency of training, and duration of training<sup>11</sup>.

In the present study, the spectral analysis of heart rate variability revealed a decrease in the LF component, with a concomitant increase in the HF compo-

nent, both of which lead to a decrease in LF/HF during exercise as a result of training. These changes were accompanied by decreased RBC, HGB, and HCT.

An important prerequisite for obtaining a power spectrum is a stable heart rate during recording. In the present study, the regression coefficients of heart rate during exercise were almost zero for all subjects both before and after the training (Fig. 2); thus, the experimental protocol in the present study is applicable to FFT. In addition, although we observed no significant change in spectral patterns during rest, there were significant changes in the LF power, HF components, as well as LF/HF during exercise. These findings show that this exercise loading is useful for detecting the influence of endurance training on autonomic nervous activity.

The administration of propranolol, a well known  $\beta$ -sympathetic blocker, significantly increases R-R interval variability and also influences its spectral components such that the LF component and LF/HF decrease<sup>15</sup>). Similarly, the administration of atropine, a parasympathetic blocker, decreases the LF component<sup>18</sup>). These experimental results show that the LF component reflects mixture of sympathetic and parasympathetic nervous activity. In contrast, atropine administration diminishes the HF component, whereas propranolol administration has no effect, indicating that the HF component reflects parasympathetic nervous activity alone. Since the LF component reflects a mixture of sympathetic and parasympathetic nervous activity, LF/HF must also be an index of sympathetic nervous activity. Many researchers have suggested that the power spectrum of heart rate variability provides a simple noninvasive method to assess cardiac neuroregulatory response and disorders.<sup>10,12-15,18</sup>

In the present study, ten-week endurance training decreased RBC, HGB, and HCT. Under hypoxic conditions, HGB synthesis is enhanced, and the production and release of RBC from the bone marrow (erythropoiesis) are increased<sup>9</sup>). EPO secretion is facilitated by catecholamines via  $\beta$ -adrenergic mechanism. Therefore, the observed decreases in RBC, HGB, and HCT may be related to changes in autonomic nervous activity as a result of the endurance training.

This study did not analyze catecholamines, since it is well known that the secretion of catecholamines is related to the intensity of training<sup>19,20</sup>). Although blood flow to the liver and kidney, and also thrombopoietin excretion from the liver during exercise are important, it does not seem to be any previous report on these issues, which are far beyond the scope of the

present study.

The limitations of this study includes such factors as the lack of any data on the correlation between changes in heart rate and blood pressure during the training sessions. This should be clarified in the future. Also, we have to confirm through long-term studies whether or not the present results contribute to the prevention of future diseases.

## CONCLUSION

Physical training apparently decreased sympathetic nervous function and increased parasympathetic nervous function in non-athletes. This was accompanied by decreases in RBC, HGB, and HCT after training. A moderate training regimen would seem to lead to an improved autonomic nervous balance in non-athletes.

**Acknowledgments.** This study was supported by a Grant-in-Aid for Science Research from the Ministry of Education, Science and Culture of Japan (No. 09922045; 1997).

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