

# The association of the blood lymphocytes to neutrophils ratio with overtraining in endurance athletes

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## ABSTRACT

*Overtraining often compromises competitive performance in distance runners. A means for predicting overtraining in time to take corrective action may help improve training effectiveness. Overtraining is characterised by a deregulated autonomic response of the cardiovascular system and therefore assessment of the autonomic nervous system may provide the basis for such a means. The authors examined whether alterations in the ratio of blood lymphocytes to neutrophils (L/N), believed to reflect the activities of sympathetic and parasympathetic sub-systems of the autonomic nervous system, was associated with performance in four female endurance runners over an eight-month period. Blood was sampled and analysed monthly. The results were compared to performances in a series of time trials plus the runners' subjective assessments of their condition. Those who performed well had lower L/N ratios before the time trials while those who did not showed higher ratios. Although the number of subjects in the study was small, the authors conclude that the L/N ratio shows a close relationship with performance and that an increased L/N ratio may be associated with fatigue, exhaustion or stress in endurance runners.*

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## Introduction

**C**ompetitive long-distance runners train with the aim of achieving better performance in races. However, overtraining, which is characterised by a deregulated autonomic response of the cardiovascular system, frequently compromises their competitive results<sup>1,2,3</sup>. A means for predicting overtraining or indicating the condition in time to take corrective action may help these athletes to improve the effectiveness of their training and their performances in competition. Assessment of the autonomic nervous system may provide the basis for such a means.

It is known that the distribution of circulating lymphocytes and neutrophils, white blood cells that play an important part in the human immune system, is under autonomic control<sup>4,5,6</sup>. In other words, the body regulates them through a system below the level of consciousness. Moreover, a number of observations suggest that the levels of lymphocytes and neutrophils in circulation may reflect the activities of the sympathetic and parasympathetic nervous systems, two subsystems of the autonomic nervous system:

- there is an increase in the proportion of circulating lymphocytes after electroacupuncture, which stimulates the parasympathetic nervous system as detected by decreased heart rate<sup>4</sup>;
- platelet activating factor-induced neutrophilia, a high level of neutrophils associated with bacterial infection, inflammation

and other forms of stress, is dependent on the stimulation of beta-adrenoceptors by catecholamines released from the adrenal glands, the "fight or flight" response mechanism<sup>7</sup>;

- blood granulocytes and lymphocyte subsets that increase in the daytime, show a high density of adrenergic receptors while lymphocyte subsets that increase at night show a high proportion of cholinergic receptors<sup>8</sup>.

With these points in mind, we decided to examine whether the proportion of lymphocytes and neutrophils could be associated with the athletic performance of long-distance runners over an eight-month period to see if this parameter could be used as an indicator of overtraining.

## Methods

### Subjects

The subjects, runners A-D, were four female high-school Ekiden (road relay) participants aged 15 to 18 years old. All had more than three years of experience in Ekiden competition ( $3.8 \pm 1.0$  year; mean  $\pm$  SD) and were members of a high-school Ekiden team that competed in the All Japan High School Ekiden Championships in November every year.

As shown in Table 1, in July the subjects' height was  $156.6 \pm 7.1$ cm, body weight was  $44.3 \pm 5.1$ kg, %body fat was  $13.4 \pm 0.5\%$  and maximal oxygen uptake was  $61.2 \pm$

Table 1: Physical characteristic of the study subjects

Runner	A	B	C	D	mean $\pm$ SD
Age (yr)	18	15	15	16	16 $\pm$ 1.4
Height (cm)	151.8	155.0	152.6	167.0	156.6 $\pm$ 7.1
Body weight (kg)	46.9	40.6	39.5	50.3	44.3 $\pm$ 5.1
Body fat (%)	14.1	13.3	13.0	13.3	13.4 $\pm$ 0.5
VO <sub>2</sub> max (ml/kg/min)	62.7	–	65.8	55.0	61.2 $\pm$ 5.5
Training years (yr)	5	3	3	4	3.8 $\pm$ 1.0
Personal best at 3000m	10:16	11:07	11:02	10:26	10:42 7 $\pm$ 25

5.5ml/kg/min. The table also gives their years of training experience and personal best time for 3000m.

### *Performance Assessment*

The results in a series of 3,000m time trials were used as an indicator of athletic performance. The subject's condition was considered "good" when her performance improved on her personal best or the time was within 2% of her personal best. Each runner's condition was also assessed on the basis of her subjective comments after time trials and races and on the basis of the time achieved for her segment of the Ekiden Championship race, taking into consideration the course profile (percentages of uphill, downhill and flat).

### *Blood sampling and analysis*

Blood was sampled in the morning within two hours of breakfast once or twice per month from April to November, and analysed for the numbers of leucocytes, lymphocytes and granulocytes. The performances in the time trials were compared to the lymphocyte to neutrophil (L/N) ratio of the blood samples obtained within the five days before each time trial (Figure 2), and the correlation coefficient was calculated. Statistical significance was determined as  $P < 0.05$ .

## **Results**

### *Case Study - Runner A*

Runner A was the only one of the subjects considered well-conditioned throughout the study. She had a good performance in a race in June, when lymphocyte count was dominant. In the autumn, when the lymphocyte count was still dominant, she achieved a time close to her personal best in the 3000m time trial before the Ekiden Championships and was then ranked 4th in her section in the championship. See Figure 1A.

### *Case Study - Runner B*

Runner B suffered anaemia in May, and did not participate in competitions until the summer. At the end of the summer, she was still anaemic despite iron supplementation. Lym-

phocyte count became dominant one month before the Ekiden Championships but then returned to neutrophil predominance. Her performance in the championships was not satisfactory, which was probably due to her anaemia. See Figure 1B.

### *Case Study - Runner C*

In spring, Runner C's neutrophil count was predominant but her record in an 800m time trial was satisfactory. In the autumn, a predominant lymphocyte count lasted for a considerably long period and she broke her personal record in a time trial 10 days before the Ekiden Championships. However, the blood test just before the championships showed a predominant neutrophil count. Runner C was not informed of this result, but her performance in the championships was unsatisfactory. See Figure 1C.

### *Case Study - Runner D*

The initial blood test for Runner D showed predominance in neutrophil count, but it soon showed lymphocyte predominance lasting nearly three months. Soon after the neutrophils count again became predominant at the end of July, she had an unsatisfactory result in a 3000m competitive time trial. Lymphocyte count became predominant again in October when her record at the pre-championship trial was close to her personal best. Five days before the Ekiden Championships, she not only became neutrophil predominant but also developed a fever. Runner D participated in the championships but her performance was unsatisfactory.

### *Relationship between 3,000m performance and L/N ratio*

Two of the subjects, Runner A and Runner D, showed good condition in the 3000m time trial performances over the eight-month period of the study, while the other two did not. The two who showed improvements had a higher L/N ratio before the time trials and the Ekiden Championships in November while those who failed to improve showed a lower L/N ratio before the time trials and the championships. Figure 2 shows the relationship between per-

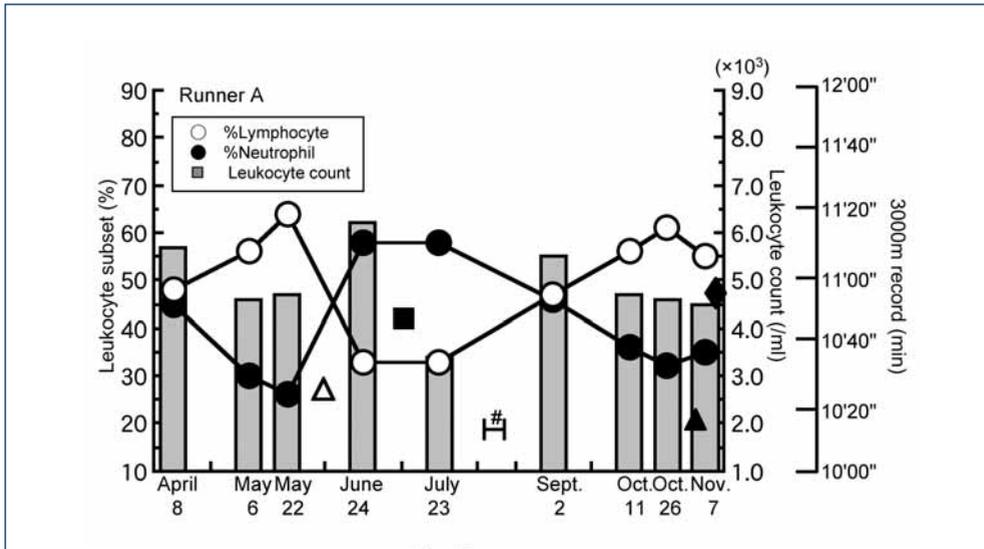


Figure 1A: Changes in leukocyte counts, leukocyte subset and performance of Runner A

- # Participation in an elite-level endurance training camp (14 days)
- △ June 4: competitive time trial: time 10:23; subjective condition: good
- July 2: competitive time trial: time 10:46; subjective condition: leg soreness
- ▲ Nov.3: non-competition time trial: time 10:17; subjective condition: good
- ◆ Nov.1: Ekiden Championships (3km with up and down hills: time 10:51)

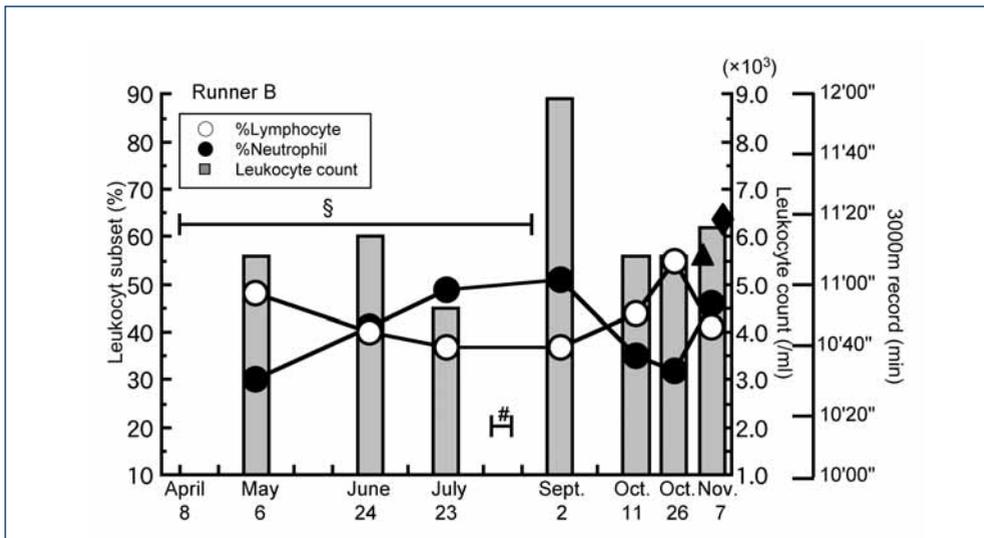


Figure 1B: Changes in leukocyte counts, leukocyte subset and performance of Runner B

- # Participation in an elite-level endurance training camp (14 days)
- § Ferrotherapy for iron deficiency anemia
- ▲ Nov. 3: non-competition time trial: time 11:07; subjective condition: good
- ◆ Nov. 12: Ekiden Championships (3km with up and down hill: 11:19)

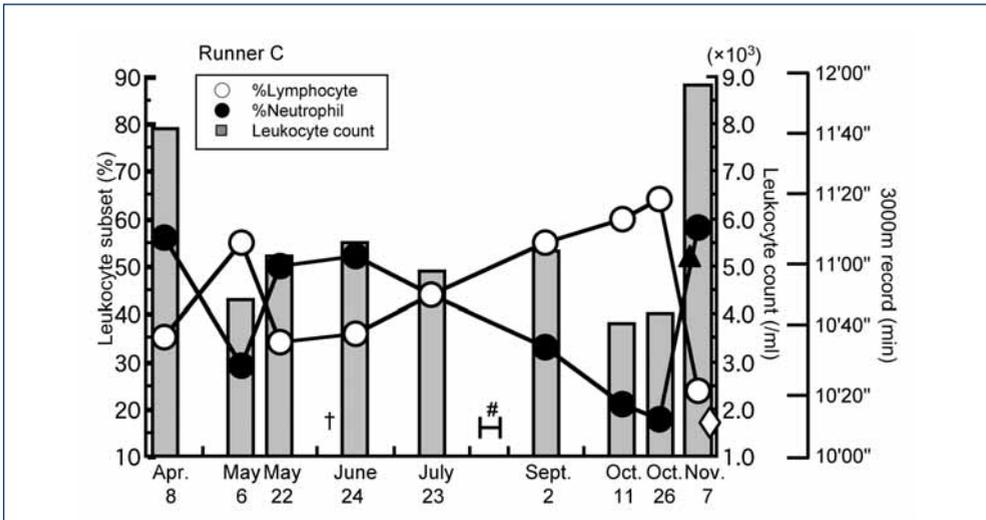


Figure 1C: Changes in leukocyte counts, leukocyte subset and performance of Runner C  
 # Participation in an elite-level endurance training camp (14 days)  
 † June 4: competitive time trial (800m): time 2:34 subjective condition: good  
 ▲ Nov. 3: non-competition time trial: time 11:02; subjective condition: good  
 Nov. 12: Ekiden Championships (4.0975km flat: time 15:27)

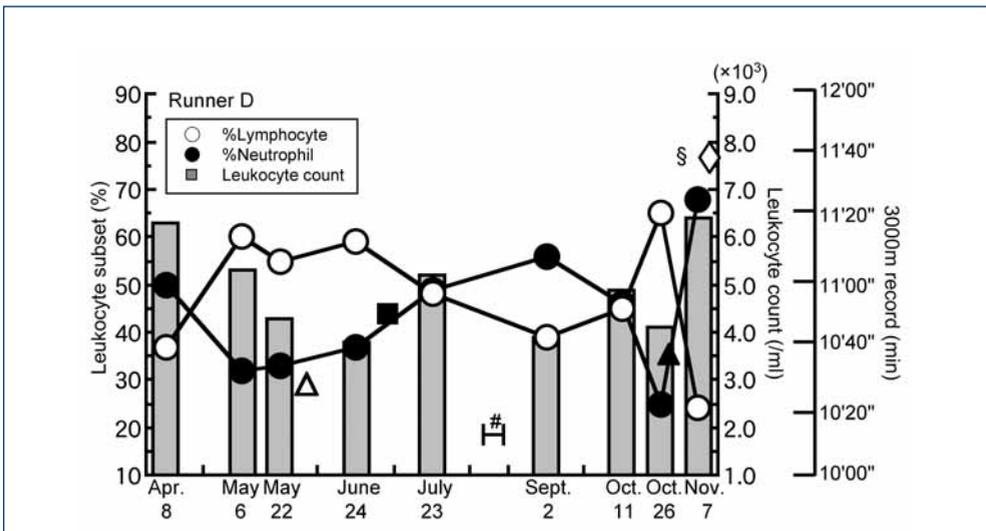


Figure 1D: Changes in leukocyte counts, leukocyte subset and performance of runner D  
 # Participation in an elite-level endurance training camp (14 days)  
 • June 4: competitive time trial: time 10:27; subjective condition: good  
 ■ July 2: competitive time trial: time 10:46; subjective condition: leg soreness  
 ▲ Nov. 3: non-competition time trial: time 10:36; subjective condition: good  
 § Fever from 5 days before Ekiden championship (38?)  
 ◆ Nov. 12: Ekiden Championships (5km with up and down hills: time 19:18)

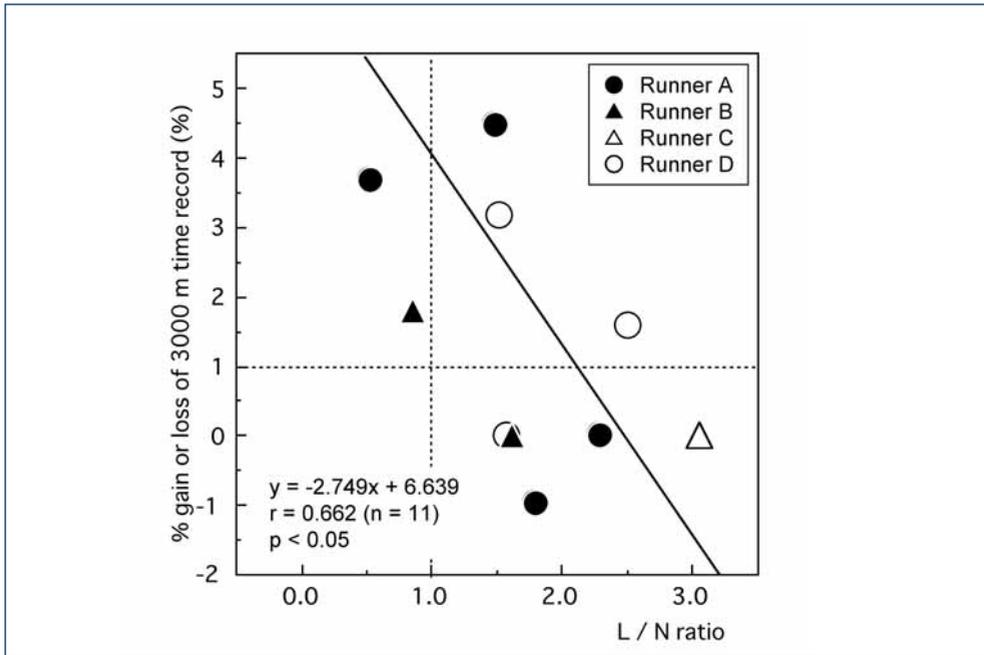


Figure 2: The relationship between 3000m performance (% gain or loss compared to personal record) and blood lymphocytes to neutrophils (L/N) ratio

centage gain or loss in the 3000m time trials and the L/N ratio of 11 data points from the four runners over the eight-month study period. A statistically significant correlation with a coefficient of 0.662 was found ( $P < 0.05$ ).

### Discussion

Performance in the 3000m time trials or the subjective condition of the subjects tended to be better when lymphocyte count was predominant, and poorer when neutrophil count was predominant. Although these are preliminary observations in a small number of runners, we can assume that the L/N ratio is a good reflection of the physical and possibly psychological condition of individual runners. A statistically significant correlation between the 3000m time trial result index and L/N ratio with a correlation coefficient of 0.66 implies not only an intra-individual relation but also a general trend among runners.

It is possible that this correlation may represent a dichotomous distribution of blood lympho-

cytes and neutrophils, as suggested in a previous study that demonstrated a predominance of killer-type lymphocytes and neutrophils during the day and of helper type lymphocytes at night<sup>8</sup>. Further studies are required to confirm this relationship.

The night-time profile of leucocytes, which is thought to be parasympathetic-driven<sup>8</sup>, may be a representation of better recovery from fatigue or exhaustion in the morning. Therefore, the L/N ratio may a useful factor to determine the correct daily training volume (running distance per day) to prevent overtraining syndrome in an endurance runner.

DHABHAR et al. reported a decrease in the number of circulating lymphocytes in rats induced by acute stress, which was reversed by adrenalectomy (surgical removal of the adrenal glands)<sup>5,9</sup>. Administration of a glucocorticoid blocker also inhibits the decrease in circulating lymphocyte number caused by acute stress<sup>9</sup>. We have also suggested that endogenous glucocorticoid (glucocorticoid

naturally produced by the body) may account for lymphopenia (abnormally low level of lymphocytes) through up-regulation of chemokine receptors on T lymphocytes<sup>10</sup>. Taken together, the present observations of the poorly performing runners with decreased proportions of circulating lymphocytes suggests that increased levels of physical or psychological stress induces adrenocortical hormones as well as sympathetic activity<sup>11,12</sup>.

### Conclusions and future perspective

1. Performance in 3000m time trials and the subjective condition of female high-school runners tends to be better when lymphocyte count is predominant, and poorer when blood neutrophil count is predominant.
2. These results suggested that the ratio of peripheral neutrophil to lymphocyte may

reflect the physical and possibly the psychological condition of individual runners. The L/N ratio may serve as one of the considerations for determining daily training volume (running distance per day) to prevent overtraining syndrome in an endurance runner.

3. Although the number of subjects in the study population was small, the L/N ratio showed a close relationship with the competitive performance. Increased L/N ratio may be associated with fatigue, exhaustion or stress in endurance runners.
4. Autonomic and HPA axis (hypothalamo-pituitary-adrenal axis) dependent regulation of L/N balance need to be further investigated.

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