Dietary Thiamin and Riboflavin Intake and Blood Thiamin and Riboflavin Concentrations in College Swimmers Undergoing Intensive Training

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The purpose of this study was to examine the effect of high-intensity physical activity during training on the biochemical status of thiamin and riboflavin in athletes. Thiamin and riboflavin concentrations in whole blood of a group of 19 athletes (6 men and 13 women) were measured during a low-intensity preparatory period and compared with measurements taken during a high-intensity training period. Additional variables measured included anthropometric characteristics, estimated energy expenditure during swim training, distance covered, resting energy expenditure obtained by indirect calorimetry, estimated energy requirement per day, and dietary intake of energy, thiamin, and riboflavin estimated from 3-day food records. For both male and female subjects, no major changes were observed in anthropometric characteristics or dietary intake, but energy expenditure during swim training per day significantly increased in the intensive-training period (496 ± 96 kcal in the preparation period compared with 195 ± 96 kcal in the intensive-training period for male subjects \[ p < .001 \] and 361 ± 27 kcal vs. 819 ± 48 kcal, respectively, for female subjects \[ p < .001 \]). Blood thiamin concentration decreased significantly during the intensive-training period compared with the preparation period (41 ± 6 ng/ml decreased to 36 ± 3 ng/ml for male subjects \[ p = .048 \], and 38 ± 10 ng/ml decreased to 31 ± 5 ng/ml for female subjects \[ p = .004 \]); however, the concentration of riboflavin was unchanged. These results suggest that intense training affects thiamin concentration, but not riboflavin concentration, in the whole blood of college swimmers.

Keywords: energy expenditure, swim training, intense training, preparation period

The relationship between physical activity and vitamins has been of great interest to athletes and other active individuals because of the essential functions of vitamins in metabolic pathways. Thus, many studies have been conducted on the subject (Manore, 2000; van der Beek, 1985; Woolf & Manore, 2006). Depletion studies have indicated that marginal or subclinical deficiencies of some vitamins might cause a decline in athletic performance (van der Beek et al., 1988; van der Beek et al., 1984; van der Beek, van Dokkum, Wedel, Schrijver, & van den Berg, 1994). These studies reported that an 8-week restriction of a single vitamin or a combination of vitamins (thiamin, riboflavin, vitamin B₆, and vitamin C) resulted in deterioration in the biochemical status of these vitamins and was accompanied by a decrease in maximal oxygen uptake (VO₂max) and onset of blood lactate accumulation; however, they did not examine the level of thiamin and riboflavin status that initiated the deterioration of these indicators of athletic performance. These results suggest the importance of maintaining the biochemical vitamin status within a certain range. Nevertheless, several other studies have shown the existence of a marginal biochemical vitamin deficiency in physically active people. Fogelholm, Ruokonen, Laakso, Vuorimaa, and Himberg (1993) revealed that a poor biochemical status of thiamin, riboflavin, and vitamin B₆ is highly prevalent among physically active college students. Biochemical deficiencies in thiamin, riboflavin, vitamin B₆, and vitamin E were found more frequently in athletes than in sedentary people, even though athletes consumed more of these vitamins (Guilland et al., 1989). In a study by Guilland et al. a 30-day supplementation of multivitamins (containing thiamin, riboflavin, niacin, and vitamins B₆, C, and E) restored the biochemical status of all these deficient vitamins in sedentary people to a normal range, but this supplementation was not entirely effective in athletes. A biochemical deficiency of thiamin was also found in female college tennis players but not in control subjects (Sekine, Takahashi, Inoue, & Higuchi, 2001). These studies suggest that intense physical activity affects biochemical vitamin status. To confirm this, it is necessary to investigate a cohort of subjects exposed in succession to different levels of physical activity to eliminate the influence of individual differences. However,
results of studies of this kind have been equivocal. The mean biochemical indices of riboflavin in female university students decreased during a 6-week period of jogging exercise (Belko et al., 1983). On the other hand, a 24-week fitness-type exercise regimen significantly reduced the biochemical index of vitamin B6 in female university students but not the biochemical indices of thiamin or riboflavin (Fogelholm, 1992). In those two studies, the subjects were not athletes, and their physical activities were moderate. Two other studies that examined the biochemical vitamin status in athletes during different training periods reported that the status of biochemical thiamin remained unchanged even though dietary thiamin intake was increased during intensive-training periods (Fogelholm et al., 1992; Hasegawa, Inoue, Ishii, & Higuchi, 2000). However, one of the studies did not precisely describe the changes in variables according to the training period, and the other did not quantify the physical activity. Thus, further study is required to confirm the effect of intense physical activity on the biochemical vitamin status in athletes.

In the current study, we focused on thiamin and riboflavin because they are closely linked with energy metabolism. Hence, we examined the effect of intense physical activity on the biochemical status of these vitamins, including changes in blood thiamin and riboflavin concentration, dietary intake, and energy expenditure during training, in a cohort of athletes during different training periods. The purpose of this study was to provide quantified data on these variables to establish a clear relationship between intense physical activity and biochemical thiamin and riboflavin status.

Method

Subjects

All 20 members of a university swim team participated in this study. All were competitive swimmers who competed at intercollegiate swimming championships, and 1 had participated in the FINA Short Course World Swimming Championships. They underwent swim training for 16 hr/week and dry-land training (running, strength training, and stretching) for 10 hr/week throughout the year, except for a 1-month off-season in September. The subjects received oral and written explanations of the purpose, content, advantages, and disadvantages of the study, including providing blood samples. They were also informed that they could refuse or cease to participate at any time. All the participants signed written consent forms. The results of 19 subjects (6 men and 13 women age 19.5 ± 1.0 and 19.4 ± 1.0, respectively) who successfully completed all the measurements were analyzed in this study. The ethics committee of the Niigata University of Health and Welfare approved the study design.

General Design

The study consisted of two observational periods: a 9-day preparation period in October and a 7-day period during the November–February intensive-training phase (Figure 1). The preparation period directly followed the athletes’ 1-month off-season and was considered a lead-in for the intensive-training phase that started in November.

Figure 1 — General design of the study.
All measurements and blood samples were obtained during each of the two observational periods, except for the height, weight, and percent body-fat measurements, which were obtained during the preparation period, and resting energy expenditure (REE), which was obtained during the preparation and intensive-training periods. These values were sometimes measured at times besides those during the observational periods because of subjects’ conflicting schedules.

**Anthropometric Determination**

Height, weight, and percent body fat of each participant were measured. Height was determined with a fully automatic height scale (AD-6228P, A&D Company, Ltd., Tokyo, Japan). Body weight and percent body fat were simultaneously measured with a body-composition scale (BC-118E, TANITA Corp., Tokyo, Japan) that estimates body fat by bioelectrical impedance. The body-composition scale had eight electrodes, which lessened the influence of circadian variation. To minimize the influence of hydration, body-fat measurements for all subjects were obtained between 2 and 3 p.m. before training when they were not perspiring. Lean body mass was calculated from weight and percent body fat.

**Energy Expenditure**

For each subject, swimming distance and energy expenditure were estimated from the training program during the 9- or 7-day observational periods (preparation or intensive training), which included 7 or 5 training days. The training program, which was designed and individualized by a coach, consisted of components based on swimming style, including arm stroke, leg kick, or whole-body swimming. The program specified the number of sets, repetitions, distance, interval time, and intensity. Energy expenditure for each training item was obtained with the following formula, which is based on the general assumption that 5 kcal of energy production requires 1 L of oxygen (Yoshitake, 2006):

\[
\text{Energy expenditure (kcal)} = \frac{\text{VO}_{2\text{max}} \times \text{intensity} \times \text{duration (min)}}{5 \text{ (kcal/L) }}
\]

\(\text{VO}_{2\text{max}}\) for swimmers is generally expressed by the absolute value of oxygen uptake per minute rather than the relative value per kilogram of body weight because swimmers do not directly carry their body weight as a result of buoyancy (Yamaji, 2001). Moreover, \(\text{VO}_{2\text{max}}\) during swimming is 5–15% lower than \(\text{VO}_{2\text{max}}\) measured on land because of limitations in breathing and a decrease in pulmonary ventilation caused by water pressure (Dixon & Faulkner, 1971; Holmér, Lundin, & Eriksson, 1974; Kimura, Yeater, & Martin, 1990; Magel, 1971; Magel & Faulkner, 1967). Therefore, by reference only to previous studies that measured \(\text{VO}_{2\text{max}}\) of Japanese male college swimmers in a swimming flume (Hirai, Ogasawara, & Tabata, 1993; Kurokawa, Togashi, Nomura, & Ikegami, 1984; Ogita, Obu, & Tanaka, 2001; Ogita, Onodera, & Tabata 1999; Shimoyama, Kojima, Ichikawa, & Nomura, 2006; Wakayoshi, D’Acquisto, Cappaert, & Troup 1995), \(\text{VO}_{2\text{max}}\) for male subjects was assumed to be 4 L/min in this study. Male swimmers were reported to require 30% more oxygen per minute than female swimmers at a swimming speed of 1.0 m/s (di Prampero, Pendergast, Wilson, & Rennie, 1974; Pendergast, di Prampero, Craig, Wilson, & Rennie 1977). We did not find any study in which \(\text{VO}_{2\text{max}}\) of Japanese female college swimmers was measured; therefore, the \(\text{VO}_{2\text{max}}\) of female subjects in this study was set at 3 L/min. The intensity of each training item was classified into seven categories in the training program. Hirai et al. (1993) and Ogita, Onodera, and Wakayoshi (1998) evaluated intensity in terms of swimming speed, and because swimming speed is proportional to oxygen uptake, they estimated the swimming intensity as \%\(\text{VO}_{2\text{max}}\) on the basis of swimming speed at 100% \(\text{VO}_{2\text{max}}\). From these studies, \%\(\text{VO}_{2\text{max}}\) for the seven categories in this study were set at 50%, 60%, 80%, 100%, 140%, 175%, and 200%.

The swimmers’ estimated energy requirement was calculated using the basal metabolic rate (BMR) multiplied by the physical activity level. To obtain BMR, REE was measured with a computerized, open-circuit, indirect calorimetry system (Aeromonitor [AE300S], Minato Medical Science Co., Ltd., Osaka, Japan) with a mask covering the subject’s nose and mouth. After a fasting period of at least 90 min, the stable oxygen uptake and respiratory quotient were measured after a 15-min rest period. Although 90 min of fasting was not enough to completely eliminate the influence of diet-induced thermogenesis, this was the longest time that the subjects’ training and other schedules allowed. Oxygen and carbon dioxide were measured for 15 min after a 5-min equilibration period with the subject in a sitting position (Hisano et al., 2004; Igawa, Sakamaki, & Miyazaki, 2002). Measurements were obtained between 1:30 and 4 p.m. at a room temperature and a humidity of 21.5–24.6 °C and 35–51%, respectively, during the preparation period and 20.3–25.3 °C and 26–32%, respectively, during the intensive-training period. Data within 2 SDs of the mean were included in the analysis of REE calculated by the equation given by Weir (1949). For calculation of BMR, REE was divided by 1.2 on the basis of the general recognition that REE is 20% more than BMR (Hosoya, 2000). The physical activity level was estimated in a 3-day time study in which the subjects were asked to record the duration of each physical activity, including sleep, with the following formula (Tanaka, 2005):

\[
\text{Physical activity level} = \frac{\sum(\text{activity factor} \times \text{duration (min)})}{1,440 \text{ (min)}}
\]

Activity factor is the intensity of each physical activity expressed as multiples of BMR. In this study, we used activity factors provided by Tanaka (2005), which were calculated from the relative metabolic rate (RMR).

\[
\text{Activity factor} = \frac{\text{RMR} + 1.2 = (\text{energy expenditure during activity} - \text{REE})/\text{BMR} + 1.2}
\]
Because there are no data on the activity factor for intense swim training, the energy requirement during swim training was estimated from the training program of the day on which the time study was conducted.

**Dietary Intake of Energy and Nutrients**

The subjects recorded everything they ate or drank, except water, for any 3 days including 2 training days and 1 day off, during each observational period. Intake of supplements was also recorded and included in nutrient calculation, although these were occasionally taken by only 2 female subjects during each observational period (protein, vitamins, or minerals). The subjects recorded the weight or the portion size of food and how much they left uneaten, if any. They were also asked to photograph the food immediately before and after consumption (when they left food on the plate). A registered dietitian estimated the weight of all food from the records and via analysis of photographs and interviews with the subjects to avoid underreporting. *Standard Tables of Food Composition in Japan*, fifth revised and enlarged edition (Resources Council of the Science and Technology Agency of Japan & Ministry of Education, Culture, Sports, Science and Technology, Japan, 2005), was used to estimate energy, protein, fat, carbohydrates, thiamin, and riboflavin in the food using nutrient-calculation software (Excel Eiyo-kun version 4.0, Kenpakusha, Tokyo, Japan). Cooking loss (Kimura, 2007) was not considered in the estimation of thiamin and riboflavin intake because its percentage varied among the foods, and, moreover, calculation of cooking loss was not necessary to determine the difference over time. Estimated thiamin and riboflavin intakes were expressed as absolute values and relative values per 1,000 kcal of energy intake.

**Thiamin and Riboflavin Concentration in Whole Blood**

Blood samples were taken from an antecubital vein between 8:30 and 9:30 a.m. after overnight fasting to minimize the effect of hypovolemia and food consumption. Blood (1.5 ml) was taken from each of the subjects and placed in a tube with ethylenediaminetetraacetic acid disodium salt (EDTA-2Na), protected from light, and refrigerated until the assay. The thiamin concentration in whole blood was assessed using high-performance liquid chromatography (HPLC) and the thiochrome fluorescence method. The riboflavin concentration in whole blood was determined by HPLC and the lumiflavin fluorescence method. The coefficients of variation of the thiamin assay in October and December 2007 were 13.19% and 9.17%, respectively, and those in the riboflavin assay were 5.51% and 5.35%, respectively.

**Statistical Analysis**

Mean values and standard deviations for the group were obtained from the average values per day of each subject. The results are presented as $M \pm SD$. Mean values of the measurement items in each observational period were compared using a paired $t$ test on the basis of sex.

SPSS 16.0J for Windows (SPSS Japan Inc., Tokyo, Japan) was used for all statistical analyses. The null hypothesis was rejected at the .05 level of probability.

**Results**

**Anthropometry**

No significant change in the subjects’ anthropometrical characteristics was found between observational periods, except for a small increase in the lean body mass of the female subjects (Table 1).

**Training Distance and Energy Expenditure**

For both male and female subjects, training distance significantly increased from the preparation period ($3,528 \pm 0$ m for male subjects and $3,429 \pm 242$ m for female subjects) to the intensive-training period ($6,327 \pm 554$ m and $6,993 \pm 330$ m for male and female subjects, respectively). The estimated energy expenditure during swim training showed a significant change in relation to distance. In comparison with the results in the preparation period, in the intensive-training period both male and female subjects showed an increase of approximately

| Table 1  Subject Characteristics, $M \pm SD$ |
|-----------------|-----------------|-----------------|
|                | Men ($n = 6$)   | Women ($n = 13$) |
|                | Preparation period | Intensive-training period | $p^a$ | Preparation period | Intensive-training period | $p^a$ |
| Age, years    | 19.5 ± 1.0       | 19.5 ± 1.0       | n.s.  | 19.4 ± 1.0       | 19.6 ± 1.0       | n.s.  |
| Height, cm    | 174.3 ± 6.3      | 174.3 ± 6.9      | n.s.  | 164.7 ± 5.3      | 164.8 ± 5.2      | n.s.  |
| Weight, kg    | 66.4 ± 4.5       | 66.8 ± 4.3       | n.s.  | 58.9 ± 7.3       | 59.3 ± 6.8       | n.s.  |
| Body fat, %   | 10.1 ± 1.0       | 10.0 ± 1.5       | n.s.  | 22.8 ± 4.2       | 22.6 ± 4.1       | n.s.  |
| Lean body mass, kg | 59.7 ± 4.1   | 60.1 ± 4.0       | n.s.  | 45.2 ± 4.1       | 45.7 ± 3.9       | .039  |

*Difference between periods was analyzed by paired $t$ test.
500 kcal per training day (496 ± 0 kcal in the preparation period and 995 ± 96 kcal in the intensive-training period for male subjects and 361 ± 27 kcal and 819 ± 48 kcal, respectively, for the same periods for female subjects). No significant change was found in REE and BMR of either the male or female subjects. The physical activity level of the female subjects significantly increased in the intensive-training period, whereas that of male subjects increased but not significantly; however, this appeared to be because of the number of male subjects, considering the significance level (p = .059). The estimated energy requirement significantly increased in the case of both sexes in the intensive-training period. On the other hand, physical activity excluding swim training remained unchanged in the case of both male and female subjects. These results clearly indicate that the increase in the estimated energy requirement was a result of the rise in energy expenditure during swim training (Table 2).

### Dietary Intakes of Energy and Nutrients

Few items related to dietary energy and nutrient intake showed significant changes (Table 3). Although estimated energy expenditure during swim training in the intensive-training period was much higher than that in the preparation period, the daily intake of energy did not change significantly in the case of either male or female subjects (3,158 ± 733 kcal/day in the preparation period and 3,322 ± 378 kcal/day in the intensive-training period for male

### Table 2  Training Distance and Energy Expenditure, M ± SD

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 6)</th>
<th>Women (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preparation period</td>
<td>Intensive-training period</td>
</tr>
<tr>
<td>Training distance, m/day</td>
<td>3,528 ± 0</td>
<td>6,327 ± 554</td>
</tr>
<tr>
<td>Estimated energy expenditure during swim training, kcal/day</td>
<td>496 ± 0</td>
<td>995 ± 96</td>
</tr>
<tr>
<td>Resting energy expenditure, kcal/day</td>
<td>1,876 ± 165</td>
<td>1,923 ± 182</td>
</tr>
<tr>
<td>Basal metabolic rate, kcal/day</td>
<td>1,564 ± 138</td>
<td>1,603 ± 152</td>
</tr>
<tr>
<td>Physical activity level</td>
<td>1.70 ± 0.13</td>
<td>1.83 ± 0.11</td>
</tr>
<tr>
<td>Physical activity level excluding swim training</td>
<td>1.44 ± 0.09</td>
<td>1.40 ± 0.09</td>
</tr>
<tr>
<td>Estimated energy requirement, kcal/day</td>
<td>2,646 ± 146</td>
<td>2,932 ± 335</td>
</tr>
</tbody>
</table>

<sup>a</sup>Difference between periods was analyzed by paired t test.

### Table 3  Dietary Energy and Nutrient Intakes per Day Estimated From 3-Day Food Records, M ± SD

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 6)</th>
<th>Women (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preparation period</td>
<td>Intensive-training period</td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>3,158 ± 733</td>
<td>3,322 ± 378</td>
</tr>
<tr>
<td>Protein, g</td>
<td>102.8 ± 23.2</td>
<td>116.3 ± 21.6</td>
</tr>
<tr>
<td>Protein, g/kg</td>
<td>1.6 ± 0.5</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Protein, % of energy</td>
<td>13.1 ± 0.8</td>
<td>13.9 ± 1.4</td>
</tr>
<tr>
<td>Fat, g</td>
<td>100.1 ± 14.7</td>
<td>98.8 ± 22.5</td>
</tr>
<tr>
<td>Fat, % of energy</td>
<td>29.3 ± 4.8</td>
<td>26.9 ± 6.2</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>442.8 ± 135.1</td>
<td>473.6 ± 76.5</td>
</tr>
<tr>
<td>Carbohydrates, g/kg</td>
<td>6.8 ± 2.5</td>
<td>7.2 ± 1.6</td>
</tr>
<tr>
<td>Carbohydrates, % of energy</td>
<td>57.7 ± 5.3</td>
<td>59.2 ± 5.5</td>
</tr>
<tr>
<td>Thiamin, mg</td>
<td>1.42 ± 0.27</td>
<td>1.44 ± 0.26</td>
</tr>
<tr>
<td>Thiamin, mg/1,000 kcal</td>
<td>0.46 ± 0.06</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td>Riboflavin, mg</td>
<td>1.63 ± 0.45</td>
<td>1.68 ± 0.38</td>
</tr>
<tr>
<td>Riboflavin, mg/1,000 kcal</td>
<td>0.52 ± 0.08</td>
<td>0.51 ± 0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup>Difference between periods was analyzed by paired t test.
subjects and 2,710 ± 431 kcal/day and 2,880 ± 408 kcal/day, respectively, for female subjects). No significant change was observed in dietary thiamin and riboflavin intake except in the relative value of dietary thiamin intake per 1,000 kcal of energy intake of female subjects (0.55 ± 0.22 mg/day in the preparation period and 0.45 ± 0.12 mg/day in the intensive-training period). None of the subjects consumed alcohol during both observational periods, which would have influenced thiamin and riboflavin consumption (Hashizume & Kamishima, 1981; Takaku, Kurokawa, Kasuga, & Kitamura, 2009).

**Thiamin and Riboflavin Concentration in Whole Blood**

Changes in thiamin concentration in the whole blood of male and female subjects showed a similar trend (Table 4); the concentration of this vitamin decreased significantly during the intensive-training period (from 41 ± 6 ng/ml in the preparation period to 36 ± 3 ng/ml in the intensive-training period for male subjects and from 38 ± 10 ng/ml to 31 ± 5 ng/ml for female subjects). In the intensive-training period, the blood thiamin concentrations of 2 female subjects were slightly below the range for a normal person (26–56 ng/ml; proposed by the Japanese Committee for Vitamin Laboratory Standards as the normal clinical range; Itokawa et al., 1999), but they did not show any signs or symptoms of thiamin deficiency, such as anorexia, fatigue, and edema. No significant change was observed in blood riboflavin concentration in either male or female subjects (92.1 ± 14.0 ng/ml in the preparation period and 90.3 ± 13.5 ng/ml in the intensive-training period for male subjects, 94.7 ± 10.5 ng/ml and 93.1 ± 9.0 ng/ml, respectively, for female subjects.

**Discussion and Conclusion**

Although the estimated energy requirements and energy expenditures during swim training were indirectly obtained in the current study, the increase in both variables in the intensive-training period reflected a clear difference in the intensity of physical activity between the two training periods. In addition, the absence of a change in REE, BMR, and physical activity levels, excluding swim training, indicated the large contribution of energy expenditure during swim training in increasing the estimated energy requirement.

Few items related to dietary intake of energy and nutrients, however, showed a significant change. Although it was difficult to evaluate the results of nutrient intake in this study because Japanese dietary reference intakes do not cover athletes, previous studies indicate that the energy and nutrient intake are increased in the case of athletes. Guillard et al. (1989) reported that young male athletes consumed 700 kcal/day more energy than sedentary controls. This was considered equivalent to the additional energy expenditure during training. The vitamin intake of athletes was also more than that of the controls. Fogelholm et al. (1992) and Hasegawa et al. (2000) reported that the dietary intake of energy, thiamin, and riboflavin in athletes increased during the sport season compared with the off-season. Contrary to those studies, in the current study subjects’ energy and nutrient intake did not significantly change despite the increase in energy expenditure.

Whole-blood thiamin concentration showed a significant decline during the intensive-training period, although the change in the case of male subjects was slightly smaller than the coefficient of variation of the thiamin assay in October and it did not cause thiamin deficiency in either sex, whereas the riboflavin concentration showed no significant change in either male or female subjects. If energy expenditure during swim training was increased and dietary intake of thiamin and riboflavin was unchanged, both thiamin and riboflavin concentrations in the whole blood should have decreased because these concentrations immediately reflect dietary intake or deficiency, and in turn the functions of these vitamins in energy metabolism are affected (Itokawa et al., 1991; Itokawa et al., 1995; Itokawa, Takeuchi, Matsuoka, & Hibi, 1989; Itokawa, Takeuchi, Nishino, Matsuoka, & Otsuka, 1989). The following characteristics of thiamin and riboflavin may explain this discrepancy. Thiamin functions in the form of thiamin diphosphate in the pyruvate dehydrogenase complex, which catalyzes production of acetyl-CoA from pyruvic acid (an end product in the glycolytic system), as well as in the alpha-ketoglutaric dehydrogenase complex in the tricarboxylic acid cycle. Riboflavin acts as a flavin adenine dinucleotide and flavin mononucleotide in the electron-transport system in mitochondria. Flavin adenine dinucleotide also plays a role in the beta-oxidation of fat. It accepts hydrogen from

| Table 4 Thiamin and Riboflavin Concentration in Whole Blood, M ± SD |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Preparation period | Intensive-training period |                  | Preparation period | Intensive-training period |                  |
| Thiamin, ng/ml   | 41 ± 6            | 36 ± 3             | .048             | 38 ± 10           | 31 ± 5            | .004             |
| Riboflavin, ng/ml| 92.1 ± 14.0       | 90.3 ± 13.5        | n.s.             | 94.7 ± 10.5       | 93.1 ± 9.0        | n.s.             |

*Difference between periods was analyzed by paired t test.*
fatty acids for the production of acetyl-CoA. Although the requirement for both vitamins increases when energy expenditure increases, thiamin is more sensitive to an increase in carbohydrate metabolism, whereas riboflavin is more sensitive to changes in fat metabolism (Kagawa & Nozawa, 2002; Kimura, 2007).

The percentage of carbohydrates used as an energy resource increases with an increase in the intensity of physical activity (Suzuki & Yanagisawa, 2007). Moreover, riboflavin is reabsorbed by the kidneys when a deficiency occurs (Ohishi, Miura, & Nishino, 1997). Thus, whole-blood thiamin concentration is considered more sensitive than the riboflavin concentration to an increase in energy expenditure brought about by intense training. Belko et al. (1983) reported that 6 weeks of exercise resulted in a deterioration of the biochemical riboflavin status of young female subjects when the dietary riboflavin intake was controlled and the subjects performed mild physical activity (i.e., 20–50 min of jogging, which would increase the use of fat as an energy resource and thus affect the biochemical riboflavin status). In another study, which investigated the effect of a 24-week fitness-type exercise program on the biochemical status of thiamin and riboflavin in female university students, no difference in the biochemical status of riboflavin or thiamin was noted before and during exercise, although dietary intakes of these vitamins remained unchanged. In this case, the author theorized that it was because the subjects’ dietary intake of these vitamins was sufficient or because the exercise was not intense enough to have an effect on the biochemical status of these vitamins (Fogelholm, 1992). Fogelholm et al. (1992) investigated the biochemical status of thiamin, iron, and zinc in elite Nordic skiers and reported that there was no difference in the biochemical thiamin status between Nordic skiers and sedentary controls and that seasonal variations in both groups were also of the same magnitude, despite the large change in the skiers’ physical activity. The dietary thiamin intake of skiers, however, increased with physical activity, and this could have contributed to the maintenance of biochemical thiamin status. In the study by Hasegawa et al. (2000), the dietary thiamin intake of high school speed skaters increased, and their biochemical thiamin status remained unchanged in the sport season; their dietary riboflavin intake and biochemical riboflavin status were also not significantly different in the sport season and off-season. Unlike the situation in the current study, the biochemical thiamin status of the skaters might have been maintained by increased dietary thiamin intake in the sport season; however, the results for biochemical status and dietary intake of riboflavin were similar to those of the current study. For female subjects in the current study, the mean value of thiamin intake per 1,000 kcal of energy intake significantly decreased, which might have been another factor responsible for the decrease in the thiamin concentration in whole blood during the intensive-training period. To confirm the relationships among high physical activity, dietary thiamin intake, and biochemical thiamin status, it is necessary to measure the biochemical thiamin status of a larger number of athletes in a dietary-thiamin-intake-controlled study, that is, a study in which dietary thiamin intake is increased during the intensive-training period.

Possible underreporting in food records also has to be discussed because the result of unchanged energy intake between the preparation and intensive-training periods in this study is inconsistent with the results of previous studies, and absence of a weight change suggests an increased energy intake with an increase in energy expenditure. This phenomenon is referred to as flat-slope syndrome, the tendency of underreporting high intakes (Gersovitz, Madden, & Smiciklas-Wright, 1978; Madden, Goodman, & Guthrie, 1976). Schoeller (1990) stated that individuals tended to report intakes that were closer to perceived norms than to actual intake, and Bandini, Cyr, Must, and Dietz (1997) reported that the accuracy of dietary records decreased as energy expenditure increased. Moreover, the study by Gersovitz et al. suggests that the pattern of dietary records can change in the same subjects. Thus, it is possible that the subjects in the current study underreported their food intake during the intensive-training period, that is, when their energy expenditure and intake actually increased. If there was underreporting, we can assume that the thiamin and riboflavin intake probably increased together with an increase in energy intake in the intensive period. However, in this study, with an increase in intense physical activity, the thiamin concentration in whole blood decreased despite an increased thiamin intake, whereas the riboflavin concentration in whole blood was maintained; thus, the fact that thiamin concentration in whole blood is more sensitive to intense physical activity than is riboflavin concentration still holds true. Because it is difficult to eliminate the possibility of underreporting in food records, a controlled diet will be required to establish the relationships among intense physical activity, dietary thiamin intake, and biochemical thiamin status.

In the case of female subjects, dietary intake is known to increase in the luteal phase of the menstrual cycle (Gong, Garrel, & Calloway, 1989; Tarasuk & Beaton, 1991). In the current study, 3 female subjects were in the luteal phase in the preparation period, and another 4 were in this phase in the intensive-training period. This difference in menstrual-cycle phase may have influenced dietary intake.

In this study, we measured the thiamin and riboflavin concentrations in whole blood as basic indices of the nutritional statuses of thiamin and riboflavin because the concentrations of these vitamins in the whole blood are the primary indicators of biochemical thiamin and riboflavin status in clinical practice in Japan. The subjects were Japanese, and the activity of enzymes, which require thiamin and riboflavin as coenzymes, is rarely used as an index of biochemical thiamin and riboflavin status in this country. Furthermore, the values of these indices reflect the dietary intake of the day and maintain
the level for 5–10 days for thiamin and 2–6 weeks for riboflavin (Takaku et al., 2009). This implies that these indices reflect the dietary intake during the observational periods, which is important in examining the relationships among physical activity, dietary intakes, and biochemical status. Nevertheless, for more profound discussion and accurate comparison with the other studies, activity of enzymes—namely, erythrocyte transketolase activity and thiamin pyrophosphate stimulation effect as indicators of the biochemical status of thiamin and activity of erythrocyte glutathione reductase and its activation by flavin-adenine-dinucleotide as biochemical markers of riboflavin—should also be measured to establish the functional status of thiamin and riboflavin.

The blood thiamin concentrations in 2 female subjects decreased during the intensive-training period to below the range for a normal person; however, there is no reference range for athletes. Thus, it is difficult to evaluate the thiamin concentration in the whole blood of our subjects. However, thiamin and riboflavin requirements would be clearly higher during intensive training because of the increase in energy requirements during the period, and the results of this study also indicate that intense physical activity during intensive training affects the thiamin concentration to a greater extent than the riboflavin concentration in whole blood. This suggests that increased thiamin intake during intensive training is more essential than increased riboflavin intake.

To examine the effect of the decrease in blood thiamin concentration on an athlete’s performance, further research involving measurements of indices representative of athletic ability, such as VO$_{2\max}$ onset of blood lactate accumulation, blood lactate, and anaerobic power, in addition to the variables measured in this study is required.

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References


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