Hirosaki Med. J. 65：1—11，2014

ORIGINAL ARTICLE

EVALUATION OF 6-MONTH PERIODISATION IN MALE EKIDEN RUNNERS BY ASSESSMENT OF MUSCLE FATIGUE AND IMMUNE FUNCTION

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Abstract In order to assess 6-month periodisation of top university ekiden runner, the changes of muscle fatigue and immune functions of 17 male university ekiden runners were investigated in each training period. The investigation was carried out during the normal training period (6 months before the race), before and after the intensive training camp period (3 or 4 months before the race), at the start of the conditioning period (one month before the race) and at the end of the conditioning period (10 days before the race). Investigation parameters were myogenic enzymes, serum opsonic activity (SOA) and serum superoxide dismutase (SOD). Neither levels of myogenic enzymes nor immunoglobulins and complements showed any significant changes in each period. The level of SOA significantly decreased after the training camp compared to before, and tended to recover towards the race. SOD levels were significantly higher after the training camp and at the conditioning period compared to before, which might be a reaction against the oxidative stress induced by training. Conclusion, the subjects had good physical management during the training periods prior to the race from the standpoint of muscle fatigue and immune functions. Furthermore, the parameters used in this study may be useful to assess runners' physical condition during a long season of training.

Hirosaki Med. J. 65：1—11，2014

Key words: Neutrophil function; myogenic enzyme; long-distance runner; periodization.

原　著

男子大学駅伝選手における6か月のピリオダイゼーションの筋疲労と免疫機能による評価

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抄録　東洋大学駅伝男子選手17名(2009年箱根駅伝で優勝)を対象に，トレーニング期毎に筋疲労，免疫機能を調査し，彼らが行っているピリオダイゼーションの良否を評価した．調査は通常トレーニング期，強化期，調整期に実施した．調査項目は，筋逸脱酵素，免疫関連項目(免疫グロブリン，補体，血清オプソニン化活性)であった．筋逸脱酵素，免疫グロブリンおよび補体はシーズン中有意に変化しなかった．血清オプソニン活性は通常トレーニング期に比して強化期に有意に低下したが，試合期に向け上昇した．また，キャンプ直後からシーズン後半にかけて，血清スーパーオキシドディスムターゼ(SOD)活性の上昇がみられ，酸化ストレスの亢進と対応した抗酸化機能の亢進が示唆された．以上より，本対象者は，大略良好なシーズン中のピリオダイゼーションを行ったと結論された．また本調査項目が長期のシーズンの身体コンディションの評価に有用と考えられた．

弘前医学　65：1—11，2014

キーワード：好中球機能；筋逸脱酵素；長距離ランナー；ピリオダイゼーション．

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Received for publication, December 26, 2012
Accepted for publication, January 7, 2013

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別刷請求先：中路重之
平成24年12月26日受付
平成25年1月7日受理

Introduction

Ekiden is a term referring to a long-distance relay running race, typically on roads. The lengths of ekiden in Japan can vary greatly, as can the number of runners on a team. For example, the national high school championship involves 5 girls in a 21 kilometer race and 7 boys in 42.195 kilometer race. In the national inter-prefecture championships, 9 women run 42.195 kilometers and 7 men run 48 kilometers. For the collegiate Hakone Ekiden, a 2-day event, 10 male athletes for each team run 219 kilometers.

Ekiden (Road relay) races are traditionally held in various places in Japan throughout the year, especially over the autumn and winter seasons. Among all the marathon races, many of them set the Hakone Ekiden as the final goal.

Each runner is required to compete within the team to represent the university, and thus, they often carry out intensive training, aiming to beat their own records whenever they participate in the race. As mentioned above, official races are held throughout the year, and athletes must be able to maintain their best conditions for winning the race, although it is difficult to improve their skills while maintaining good conditions over a long period of time.

In other words, it is extremely important for them to be able to adjust the amount of training and its contents so that they can be in perfect condition for the race. Thus, many runners often use the method of periodization, where the training period is divided into several periods (training period, tapering period etc), involving different training goals and contents[1, 2].

It has been reported that long-term, long-hour and highly intensive training by athletes causes an accumulation of physical and mental fatigue, leading to a reduction in physical function[3-5]. Many researchers also reported that chronic sports disorders are often caused by inadequate/insufficient recovery from fatigue and/or reduced function after exercise sessions[3-5].

Moreover, in previous studies on the relationship between physical exercise and immune function, athletes were found to be prone to infections such as upper respiratory tract infection due to immunosuppression caused by highly-intensive, highly-frequent and long-term physical exercise[6, 7].

Our research group also investigated the changes in muscle fatigue and immune function in a variety of athletes by assessing their myogenic enzymes and neutrophil functions, and found that highly intensive, acute exercise caused muscle fatigue and immunosuppression[8-14]. We have also suggested that monitoring the changes in athletes' myogenic enzymes and neutrophil functions over a long period of time is one effective method to manage their health and could also be useful as a conditioning index during training[8-18].

In the present study, in order to assess the seasonal conditioning of top university ekiden runners, the changes of muscle fatigue and immune functions of male university ekiden runners were investigated in each periodization.

Subjects and method

1. Subjects and investigation period

The subjects were 17 male ekiden club members (including 7 reserve runners) at Toyo University. The average age, height, body weight, relative body fat and fat-free mass were 19.9 ± 0.9 years, 169.4 ± 5.4cm, 54.5 ± 4.7kg, 6.1 ± 2.3% and 51.2 ± 4.0 kg, respectively (Table 1).

The investigation was carried out at 5 points between June 2008 and December 2008. On the first investigation day (June 14th 2008), we assessed their health and conditions during the normal training period (6 months before the

race). The same parameters were assessed for the second and third investigation days on September 5th 2008 (4 months before the race before the training camp) and September 30th 2008 (3 months before the race after the training camp) during the intensive training period. Finally, in order to assess the runners' physical conditions, the investigation was carried out before the Hakone Ekiden race, on November 22nd 2008 (one month before the race: at the start of the conditioning period) and December 24th 2008 (10 days before the race: at the end of the conditioning period)

The study was approved by the Ethics Committee of Hirosaki University School of Medicine. The study protocol and purpose were thoroughly explained and written consent was obtained from all subjects prior to the investigation.

2. Body composition and blood parameters

After subjects' heights were measured, their body weight, relative body fat and fat-free mass were measured by the impedance method using the multi-frequency body composition meter (MC-190, TANITA Corp, Tokyo, Japan).

Each subject was asked to record the contents of their training regimen over the week before each assessment day including the running distance and training time. According to the information provided, the training time and training distance were calculated.

Blood samples (15ml) were taken early morning on each investigation day when subjects were under fasting condition. Five milliliters of the sample was used to analyze the blood cell components, and the remaining 10ml was centrifuged at 3,000 rpm for 10 minutes to extract blood serum for further analysis.

For investigation of immune-related functions, leukocyte, neutrophil and lymphocyte counts were determined. Several myogenic enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine kinase (CK) as well as immunoglobulins (IgG, IgA and IgM) and complements (C3 and C4) were measured. In order to determine the anti-oxidative function of blood serum, the activity of superoxide dismutase (SOD) and thiobarbituric acid reactive substance (TBARS) was also measured.

All blood cell components were measured using an Automated Blood Cell Analyzer (System XE-2100 and SE-9000 Kobe, Japan), and levels of AST, ALT, LDH and CK were measured using the JSCC standardized method. For measurements of immunoglobulins and complements, the turbidimetric immunoassay (TIA) was used. The SOD activity was measured with the NBT reduction method. All measurements of blood biochemical items were consigned to an independent laboratory (Mitsubishi Chemical Medience).

4. Measuring method for serum opsonic activity (SOA)

In this study, SOA was examined with lucigenin (bis-N-methylacridinium nitrate (Sigma Chemical Co., St. Louis, MO, USA)) and luminol (5-amino-2,3-dihydro-1,4-phthalazinedion (Sigma Chemical Co., St. Louis, MO, USA)). Chemiluminescence detected with lucigenin (lucigenin-dependent chemiluminescence response, LgCL) and that detected with luminol (luminol--dependent chemiluminescence response, LmCL) were used for assessment.

Serum opsonic activity was measured with the luminol chemiluminescence assay based on the ROS produced when standard neutrophils phagocytized opsonized zymosan in serum from the subjects[19, 20]. Luminol, used as a chemiluminigenic probe, was dissolved with 1 mol / L NaOH and the solution was adjusted to an isotonic state of 12.5 mmol / L at pH 7.4 by adding HCl, ultrapure water and NaCl. The

luminol solution was diluted with HBSS to 0.5 mmol / L before use.

ZymosanA (Sigma, USA) was suspended in Hank's balanced salt solution (HBSS) at a concentration of 5 mg/ml and then opsonization was performed by adding the solution to the serum samples (final concentration of 20%) and incubating at 37 °C for 30 min. The particles were then washed twice with HBSS and resuspended in HBSS at a concentration of 5 mg/ml.

Standard neutrophils were obtained from the peripheral blood of a healthy adult male volunteer, wherefrom whole blood was modified by centrifugation through Mono-Poly resolving medium. The neutrophils were suspended to 3×10^6 cell/ml using an automatic blood cell counter (Coulter MD II, Coulter Co. Ltd., Tokyo, Japan).

Opsonized zymosan (OZ) suspension and chemiluminigenic probes prepared as described above were added to each well of black flat-bottom microplates (Greiner Japan, Tokyo, Japan), and 50 µl of standard neutrophils was added. The plates were automatically measured on the Auto Luminescence Analyzer, Alfa system (Tokken, Funabashi, Japan)[21]. All measurements were performed at 37 °C. The results were evaluated using the maximum light emission (peak height: PH) of chemiluminescence response[21, 22].

5. Statistical analysis

All values were presented as means ± standard deviation, and a one-way ANOVA and the Bonferroni method were used for statistical analyses to test the differences among the average values of all groups. All values at $p<0.05$ were considered to be statistically significant.

Results

Subjects' body weights after the training camp, at the start and the end of conditioning periods increased significantly compared to before the training camp ($p<0.05$, $p<0.01$ and $p<0.05$, respectively, Table 1). Their relative body fat in those periods also increased significantly compared to before the training camp ($p<0.01$ for all). Fat-free mass before and after the training camp / at the start and the end of conditioning periods decreased significantly compared to the normal training period ($p<0.05$, $p<0.05$, $p<0.05$ and $p<0.01$ respectively).

The training hours per week after the training camp were significantly longer compared to before the training camp ($p<0.01$ for both, Table 1). The training hours per week at the start and the end of condition periods were significantly shorter compared to after the training camp ($p<0.01$ for all). Training hours and running distance per week were the longest after the training camp, and were shorter at the end of the conditioning period.

No significant differences were observed in changes of myogenic enzymes over each periodization in this investigation (Table 2).

Although there were changes in white blood cell and neutrophil counts in each periodization, no significant differences were observed (Table 2).

The level of IgA at the start of conditioning period increased significantly compare to the normal training period and before the training camp ($p<0.05$ for both, Table 2).The level of IgM at the end of conditioning period increased significantly compared to before and after the training camp ($p<0.05$ and $p<0.01$). No other values were shown to have significant changes.

The level of LgCL・PH after the training camp was significantly low ($p<0.05$) compared to before the training camp (Table 3). The

Table 1 Changes in anthropometric parameters and contents of training during the 6-month periodization

	Normal training period (6 months before the race)	Intensive training period (training camp)		At the start of the conditioning period (one months before the race)	At the end of the conditioning period (10 days before the race)
		Before (4 months before the race)	After (3 months before the race)		
Weight (kg)	54.5 ± 4.7	53.9 ± 4.1	54.6 ± 4.2 †	55.1 ± 4.2 ††	55.1 ± 4.3 †
Relative body fat (%)	6.1 ± 2.3	7.1 ± 2.3	8.1 ± 2.2 **††	9.3 ± 2.1 **††‡‡	9.5 ± 2.3 **††‡‡
Fat-free mass (kg)	51.2 ± 4.0	50.0 ± 3.4 *	50.1 ± 3.6 *	49.9 ± 3.4 *	49.8 ± 3.6 **
Total training hours one week before investigation (hours)	14.8 ± 5.5	13.7 ± 3.4	17.9 ± 4.3 ††	13.2 ± 2.6 ‡‡	12.5 ± 3.4 ‡‡
Total running distance one week before investigation (km)	159.7 ± 78.1	141.1 ± 66.6	185.5 ± 90.3	143.8 ± 33.1	120.1 ± 51.3
Average training hours one week before investigation (hours/day)	2.1 ± 0.8	2.0 ± 0.5	2.6 ± 0.6 ††	1.9 ± 0.4 ‡‡	1.8 ± 0.5 ‡‡

Subjects were 17 male runners
Values are shown as the mean ± standard deviation.
*: p<0.05, **: p<0.01; Significant difference from normal training period
†: p<0.05, ††: p<0.01; Significant difference from the value before the training camp
‡‡: p<0.01; Significant difference from the value after the training camp

Table 2 Changes in blood parameters in the 6-month periodization

	Normal training period (6 months before the race)	Intensive training period (training camp)		At the start of the conditioning period (one months before the race)	At the end of the conditioning period (10 days before the race)
		Before (4 months before the race)	After (3 months before the race)		
AST (IU/l)	27.4 ± 9.6	26.4 ± 10.6	30.8 ± 10.1	37.4 ± 16.4	31.5 ± 13.3
ALT (IU/l)	22.6 ± 10.6	20.5 ± 7.5	24.1 ± 8.5	25.2 ± 9.3	21.1 ± 9.4
LDH (IU/l)	243.8 ± 61.8	239.0 ± 64.6	241.8 ± 72.5	248.2 ± 75.2	226.7 ± 70.8
CK (IU/l)	344.4 ± 314.9	316.1 ± 249.4	373.6 ± 240.6	526.6 ± 336.5	449.7 ± 314.0
Leukocyte cell counts (/μl)	5294 ± 1246	5835 ± 1408	5382 ± 1123	5865 ± 1302	5688 ± 1388
Neutrophil cell counts (/μl)	2479 ± 851	2922 ± 1404	2523 ± 900	2894 ± 1115	2811 ± 1054
IgA (mg/dl)	188.6 ± 64.9	189.7 ± 62.2	190.6 ± 60.1	201.6 ± 66.1 *†	200.2 ± 63.4
IgG (mg/dl)	1069 ± 243	1040 ± 221	1033 ± 193	1073 ± 204	1088 ± 201
IgM (mg/dl)	94.3 ± 39.5	92.9 ± 41.5	91.3 ± 40.3	99.1 ± 43.7	101.4 ± 44.8 ††‡
C3 (mg/dl)	90.8 ± 10.2	87.5 ± 11.4	93.1 ± 15.1	90.6 ± 9.3	92.2 ± 12.2
C4 (mg/dl)	20.3 ± 8.0	18.6 ± 7.1	19.9 ± 6.7	20.4 ± 7.2	19.4 ± 8.1

Subjects were 17 male runners
Values are shown as the mean ± standard deviation.
*: p<0.05; Significant difference from normal training period
†: p<0.05; Significant difference from the value before the training camp
‡‡: p<0.01; Significant difference from the value after the training camp

Table 3 Changes in oxidative and antioxidative functions in the 6-month periodization

	Normal training period (6 months before the race)	Intensive training period (training camp)		At the start of the conditioning period (one months before the race)	At the end of the conditioning period (10 days before the race)
		Before (4 months before the race)	After (3 months before the race)		
LgCL・PH (cpm)	118.9 ± 39.4	112.5 ± 21.2	100.0 ± 15.0 †	103.2 ± 19.5	104.0 ± 26.3
LmCL・PH (cpm)	972 ± 204.8	1029 ± 215	963 ± 206.4 †	941 ± 224 ††	986 ± 258
SOD (%)	1.7 ± 0.5	2.4 ± 1.4	4.1 ± 1.4 **††	3.1 ± 1.1 **‡‡	4.0 ± 1.1 **††
TBARS (nmol/mL)	2.9 ± 0.8	3.0 ± 0.8	3.2 ± 1.3	3.0 ± 0.9	3.6 ± 1.9

Subjects were 17 male runners
Values are shown as the mean ± standard deviation.
LgCL: Lucigenin-dependent chemiluminescence response
LmCL: Luminol-dependent chemiluminescence response
PH: Peak height, SOD: Superoxide dismutase, TBARS: Thiobarbituric acid reactive substance
**: p<0.01; Significant difference from normal training period
†: p<0.05, ††: p<0.01; Significant difference from the value before the training camp
‡‡: p<0.01; Significant difference from the value after the training camp

level of LmCL・PH was also significantly low after the training camp compared to before the training camp (p<0.05). The level of LmCL・PH at the start of the conditioning period was also significantly low compared to before the training camp (p<0.01), and was the lowest during the research periods.

SOD levels after the training camp, at the start and end of the conditioning period were significantly high compared to the normal

training period ($p<0.01$ for all). The levels of SOD after the training camp and at the end of conditioning were high significantly compared to before the training camp ($p<0.01$ for both). However, the level of SOD at the start of the conditioning period was significantly low compared to after the training camp ($p<0.01$).

Discussion

For many athletes, peaking and tapering are extremely important to enable them to exploit their maximum skills and achieve their optimal performance in a competition[23, 24]. Periodization involves a combination of peaking, tapering and well-planned training. An appropriate periodization is not only useful for athletes to exploit their skills in a competition, but also important when preparing to achieve their best conditions for the competition[2]. In terms of the periodization in this study, subjects carried out training for 6 months before the Hakone Ekiden race which was divided into a normal training period, intensive training period including a training camp and conditioning period including tapering period. Subjects performed training in each training period aiming for different goals. In other words, they were trying to plan adequate training hours and running distance between the normal training period and intensive training period so that they would have reached their peaks by the end of the training camp.

In terms of the changes of body composition values, the level of relative body fat during the normal training period was the lowest among all other periods. It suggested that hot and humid summer in Japan accelerated energy metabolism[25], resulting in reduced body weight and relative body fat. In the present study, body weight and relative body fat were found to increase towards the race day. This was probably due to the change in seasons from summer to fall, where temperature and humidity became lower, providing a more suitable environment for athletes to conduct their training. The changes in relative body fat obtained in this study (6-9%) were considered adequate for conditioning of athletes, because previous reports showed that appropriate relative body fat is within such a range[26, 27].

Highly intensive physical exercise causes excessive contraction of muscle tissues and has other impacts on the overall physical condition of the athlete, as well as changes in and damage to muscle tissues. These changes accelerate the membrane permeability of muscle cells allowing the excellulation of myogenic enzymes and leading to increased blood concentrations[28-30]. Thus, assessment of serum myogenic enzymes was reported as a useful index for determining long-term chronic muscle fatigue[31]. In this study, levels of myogenic enzymes increased after the training camp till the start of the conditioning period, and tended to decrease afterwards. In other words, our subjects' muscle fatigue reached its peak during the training camp period and conditioning period, when training hours and running distance were the longest. However, throughout the investigation this change was not significant. Thus, subjects were able to have an adequate periodization including peaking and conditioning (including tapering) for Hakone Ekiden race.

Many previous researchers have reported that leukocyte count increases after acute physical exercise, and its extent depends on the intensity of exercise[32]. Similarly, fractions of leukocytes such as lymphocytes and neutrophils have also been reported to increase after physical exercise[32]. These increases are considered as an inflammatory reaction to muscle damage and changes in tissue morphology and have been reported to be associated with inflammatory cytokines[33]. Moreover, other previous studies suggested

that increases in the leukocyte count were not only led by an inflammatory reaction caused by physical exercise, but also from stimulation of certain growth hormones, adrenaline, noradrenaline and cortisol[34]. Other researchers also reported that leukocyte and neutrophil counts decreased after long-term physical exercise[35, 36]. These decreases were also suggested to be due to reduced catecholamine levels caused by the overtraining syndrome[35].

In the present study, we did not observe any significant increases in leukocyte or neutrophil counts. Thus, subjects were unlikely to have suffered from the overtraining syndrome. In terms of the conditioning, subjects in this study were able to maintain the appropriate blood concentrations of leukocytes, i.e. maintain immune function throughout the investigation period. This therefore suggested that subjects' nutrient intake and their amount of rest had been considered to match the amount of training they performed.

In previous studies that investigated the relationship between immunoglobulins / complements and physical exercise, it was reported that immunoglobulins and complements tended to either increase or decrease or remained unchanged after exercise[37-39]. In the present study, levels of IgA and IgM increased significantly at the start of the conditioning period and at the end of conditioning period, respectively. However, such changes may be activated by a reaction to stress and changes in or damage to muscle tissues during exercise.

There are two types of defense mechanism against infection in a human body, i.e. specific and non-specific.There are two types of defense mechanism against infection in a human body, e.g. specific and non-specific.

One of the non-specific defense mechanisms depends on phagocytosis, and neutrophils in particular are considered the most essential of this group of cells. Immunoglobulins and complements are important in opsonizing foreign bodies to increase the efficiency of phagocytosis by neutrophils[40]. Neutrophils engulf opsonized foreign bodies and sterilize them by reactive oxygen species (ROS). Although ROS has an important role in the sterilization of foreign substances, excess ROS production can be problematic as it can damage normal cells resulting in oxidative tissue damage[41, 42].

The SOA level as measured in the present study has been recognized as an index for ROS levels as it reflects the extent of ROS production[43, 44]. Also, the level of LgCL corresponds to production of superoxide (O_2^-), which is a substance produced at the primary stage of ROS metabolism, and is considered to have low toxicity in the body 45-48). Moreover, the level of LmCL reflects the production of hypochlorous acid (HOCl / OCl-), which is highly toxic and is produced when superoxide is metabolized by myeloperoxidase. Thus, it has also been used as an index for ROS production[45-48]. We therefore used levels of SOA as an index for ROS production and neutrophil functions in this study.

In previous studies, PA was reportedly reduced following transient highly intensive exercise[48]. In terms of the relationship between transient exercise and ROS production capability, it was found to be either accelerated[49, 50] or suppressed[51, 52] by transient physical exercise. In our previous studies, accelerated ROS production capability and reduced PA were observed after the transient judo training, which caused reduced immune function and oxidative damage to muscle tissues. We have also reported that elevated SOA levels were observed after transient exercise performed by sumo wrestler and long distance runners[11, 53]. On the other hand, when athletes perform intensive exercise for a long period of time, their neutrophil function at rest and reaction against exercise burden was lower compared to normal adults at

rest[54-57]. Also, one of our studies on university female judoists showed that intensive training performed by judoists during the training camp caused the decrease of neutrophil function and immunosuppression[16].

In the present study, SOA was found to be decreased significantly after the training camp, when training hours and running distance were the longest. In other words, long period of long distance running resulted in the exacerbation of physical fatigue and suppression of immune function, and these conditions persisted for 2 months even after the training camp.

According to the changes in SOA over the investigation period, the LmCL-PH and LgCL of SOA were found to decrease during the training camp, and further low levels of LmCL-PH continued until the start of the conditioning period, but LmCL-PH levels recovered at the end of conditioning period (10 days before the race). From the peaking and tapering point of view, the condition of the subjects' immune function was not ready for the Hakone Ekiden race.

This result also suggested that athletes require at least a two-month conditioning period to recover from reduced immune function.

The anti-oxidative function of SOD that is available in the body is stress that damages normal tissues in the body[58]. Also, transient physical exercise is known to accelerate ROS production, and a corresponding increase in serum SOD activity is recognized as an anti-oxidative reaction[59, 60]. Ohishi et al reported that long-term training accelerated ROS production and resulted in activation of SOD[61].

In the present study, SOD levels were significantly high after the training camp and at the start and end of conditioning period compared to before the training camp or normal training period. SOD activity increased in response to an increase in ROS production, and continued at high levels thereafter. The reason for this might be habituation.

This study investigated the training situation and changes in conditions of university male ekiden runners over 6 months before the Hakone Ekiden race. The study was conducted by assessing muscle fatigue and immune-related functions (immunoglobulins, complements, and oxidative/anti-oxidative functions). As a result, subjects were well-conditioned in terms of recovery from muscle fatigue and were able to maintain desirable muscle functions throughout the year. Although slight muscle fatigue and partially reduced immune function were observed during the intensive training period and one month after that, the subjects were able to recover from such condition by 1 month of tapering during the conditioning period. Also, accelerated oxidative stress that occurs daily was kept low by SOD, leading to high anti-oxidative function. Moreover, the training program planned by the team was considered to consist of adequate peaking and tapering which resulted in their winning the Hakone Ekiden race.

Further, the parameters used in this study may be useful to assess the physical condition for a long season.

As this study investigated one ekiden team only, further investigation is required for other ekiden runners to clarify the associations between their training, immune function and muscle fatigue.

Acknowledgement

The authors would like to thank the members of the track club at Toyo University as well as many other researchers for their generous support.

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