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ORIGINAL ARTICLE

ACCUMULATION OF THE FATIGUE AFTER THE MATCH IN UNIVERSITY EKIDEN RUNNERS

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Abstract An intensive training performed by athletes causes accumulation of physical fatigues, which leads to immunity depression and overtraining syndrome. Especially in endurance sports, physical fatigue tends to accumulate as it takes time to recover from physical conditions after the race or intensive training. In this study, we have investigated the conditions of marathon runners after one week of Ekiden (relay road race) according to the levels of muscle enzymes and serum opsonic activity (SOA). Subjects were 28 top male runners in Japanese University Ekiden. Out of all 28 subjects, 11 of them who had a race before a week of investigation were considered as the race group, and the rest were considered as the control group. Although no significant difference were found in running distance and duration of training among the two groups, muscular enzyme levels were significantly high ($p<0.05$), and SOA levels of LgCL, PH and AUC tended to be higher in the race group than the control group. This showed the higher damage of the muscle, and a possibility that it may have reflected the increase in ROS production in the race group compared to the control group. In conclusion, there was a possibility that the endurance of athletes were not fully recovered from the muscle fatigue causing increase of ROS production even after a week of the race. Therefore, long-distance runners were suggested to carry out a reduced amount of less-intense, less-frequent training with sufficient rests at least for a week after a race competition.

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Key words: endurance sport; muscle damage; immune function; reactive oxygen species; overtraining.

原　著

大学駅伝選手における試合後の疲労の蓄積

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抄録　高強度・長時間のトレーニングは身体的疲労の蓄積を来たし，免疫力低下やオーバートレーニング症候群を招くことが知られている．特に持久系競技は身体的疲労の回復に時間がかかり蓄積しやすいことが報告されている．本研究は大学駅伝選手の試合一週間後のコンディションを筋逸脱酵素および血清オプソニン化活性の観点から調査した．大学駅伝選手28名のうち，調査一週間前の試合に出場した11名を試合群とし，残り17名を対照群とした．試合を含む調査前一週間の走行距離・時間は2群間に差がなかったが，対象群に比べて試合群は筋逸脱酵素値が有意に高く($p<0.05$)，オプソニン化活性が高かった．結果より，試合群では試合から一週間経過しても筋損傷が完全に回復しておらず，活性酸素種産生が高まった状態であった可能性が示唆された．それゆえ，長距離選手は少なくとも試合後一週間の練習強度・頻度を軽減させ，十分な休養をとることが必要であると考えられた．

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Introduction

In order to achieve athletes' personal goals and to win the tournament/competition, many of them perform a long period of highly intensive training everyday. By performing such intensive training continuously, it is known to cause physical fatigue which needs to be adequately removed. However, previous researches had reported that it has not been removed adequately and thus, accumulation of such fatigue has been occurring, leading to the overtraining syndrome as well as various other injuries in many cases.[1-3] Especially in endurance sports including marathon, the risk of upper respiratory infection is high, and such risk has been reported to last up to 2 weeks after the race or intensive training.[4] Also, the recovery of muscle strength and/or power has been reported to take approximately a week to recover after the marathon races.[5] Therefore, total of 1 to 2 weeks of rest were suggested for athletes to recover from their physical conditions after the intensive training or races. A number of researches have been reported on athletes' physical conditions immediately after the marathon races, although only a few had followed up their conditions after a week of the races. Thus, the immunological changes as well as durations required by marathon runners to recover from physical fatigue and muscle damages caused during marathon races are still unknown.

In terms of the defense mechanisms against infection, it can be classified into specific and/or non-specific defense mechanisms. The phagocytosis by neutrophil is especially important in non-specific defense mechanism in a human body. During phagocytosis, foreign bodies are opsonized and efficiently ingested by phagocytes, and they are then destroyed by reactive oxygen species (ROS) produced by neutrophils.[6] Therefore, serum opsonic activity (SOA) is commonly used as a marker of neutrophil functions. Other than immunoglobulins and complements, some substances such as collectin, pentraxin and anti-coagulant proteins are also considered as opsonins.[7] On the other hand, excessive production of ROS is known to damage normal tissues.[8] Moreover, increased amount of ROS is considered to produce when SOA is enhanced, making it more prone to oxidative stress.[9]

In previous studies, SOA remained unchanged during conditioning period of the training. It was also found to be unchanged or increased during the normal training period, and found to be increased after a competition or 30km of marathon.[10-13] Therefore, it was suggested that as the physical load increases, SOA is elevated and induce oxidative stress.[14] The oxidative stress is the exacerbation factor of muscle damage, and continuous oxidative stress was reported to cause overtraining.[15] In the present study, we have investigated the conditions of marathon runners after one week of race of Ekiden race (relay road race) according to their muscular enzymes and SOA.

Subjects and Method

Subjects were top 28 male runners (age of 20.4 ± 0.9 years old, height of 169.2 ± 4.6 cm and weight of 53.6 ± 3.8 kg) who participated in University Ekiden. Out of 28 subjects, 11 of them had run 9.7km in University Ekiden competition on July 5th, 2008. Those 11 subjects were considered as the race group, and the rest were considered as the control group. Both groups had continued performing the normal training right after the day of competition, and according to their record of training, running distance and time for one week before the investigation was not different between both groups, because the control group also had voluntary training on the day of Ekiden

competition. The investigation was carried out one week after the race, in the early morning of July 12th. The purpose and method of the present research were thoroughly explained prior to the investigation, and consent was obtained from each subject. The present investigation was also approved by the Ethics Committee at the Hirosaki University Graduate School of Medicine.

Medical interview / questionnaire: The questionnaire was given out to obtain the information on subjects' total running distance, duration of training and training schedule for 7 days including the investigation day.

Anthropometry: Anthropometric parameters, body weight and body fat percentage (%fat) were measured by electrical impedance using the MC-190 system (TANITA, Tokyo, Japan).

Blood biochemistry: Blood samples were taken from forearm vein of subjects, and aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), γ-glutamyltransferase (γ-GTP), immunoglobulins (IgG, IgA, IgM), and complements (C3, C4) were measured using the biochemical auto analyzer H7700 (Hitachi High-Technologies, Tokyo, Japan). Leukocytes and neutrophils were measured by the Sysmex XE-2100 (Olympus, Tokyo, Japan). In the present study, all measurements were consigned to the Mitsubishi Chemical Medience Corporation (Aomori, Japan).

Serum opsonic activity: In the present study, the Luminol-dependent chemiluminescence (LmCL) and Lucigenin-dependent chemiluminescence (LgCL) were used to measure SOA, which was the same method as the previous studies.[10, 11, 16, 17] The blood samples were left untouched for 30 minutes after collection and centrifuged to extract blood serum samples. They were then froze using dry ice and kept at the laboratory. For opsonization, zymosan (Zymosan A, Sigma, St-Louis, CA, USA) of 5mg/ml and Hank's balanced salt solution (HBSS) were added to defrosted blood serum samples, and they were incubated at 37 degrees C for 30 minutes. After neutrophils were isolated from blood samples by using the Mono-Poly resolving medium (Dainippon Sumitomo Pharma, Osaka, Japan), neutrophil suspensions were prepared by adjusting the neutrophil number to 3200/μL through dilution with HBSS. The chemiluminogenic probes used were bis-N-methylacridinium nitrate (lucigenin, Sigma) and 5-amino-2,3-dihydro-1,4- phthalazinedione (luminol, Sigma), and they were adjusted with HBSS to the concentration of 0.5 mmol/L and pH 7.4. These were then dissolved with 1mol/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 NaOH. Before using the samples, the solutions were diluted with HBSS to obtain the concentration of 0.5 mmol/L.

LmCL and LgCL responses of each sample were analysed in a 96-well microplate at the same time. 50 ul of neutrophil suspension, 50 uL of opsonized zymosan (OZ), 50 uL of lucigenin or luminol solution and 100 uL of HBSS were added into the each well of microplate. The final concentration of lucigenin and luminol were 0.1 mmol/L.

Chemiluminescense was measured continuously for 45 minutes at 37 degrees C using the Alpha system auto luminescence analyzer (Tokken, Chiba, Japan). The results were evaluated using the maximum light emission (peak height: PH), and the time to reach PH (peak time: PT) according to the curve of the chemiluminescence response and the area under the curve (AUC).

Table 1 Physical characteristics of study subjects, training distance and duration a week before the investigation.

	Race Group (n = 11)	Control Group (n = 17)	P value
Age (year)	20.1 ± 0.8	20.6 ± 0.9	0.526
Height (cm)	170.1 ± 5.6	168.6 ± 4.1	0.472
Weight (kg)	54.5 ± 3.6	53.0 ± 4.0	0.398
Body fat percentage (%)	5.8 ± 1.6	6.3 ± 1.8	0.523
BMI (kg/m^2)	18.8 ± 0.8	18.6 ± 1.1	0.677
TT (min/week)	765.5 ± 185.2	901.3 ± 220.3	0.103
TD (km/week)	151.5 ± 31.3	148.7 ± 47.6	0.525

Mean ± S.D. BMI: body mass index. TT: time of training. TD: training distance.

Table 2 Differences of muscular enzyme levels and liver functions between the race group and the control group.

	Race Group (n = 11)	Control Group (n = 17)	P value
CK (IU/L)	444.4 ± 243.2	242.2 ± 87.3	0.022
AST (IU/L)	29.7 ± 8.6	23.6 ± 6.2	0.040
ALT (IU/L)	24.7 ± 8.2	19.1 ± 6.2	0.047
LDH (IU/L)	266.8 ± 46.1	222.0 ± 27.9	0.003
γ-GTP (IU/L)	19.0 ± 2.9	19.8 ± 5.6	0.962

Mean ± S.D.

Statistical analysis: All results were presented as mean ± SD, and the differences between two groups were analyzed using the two sample t-test or Mann-Whitney U test (SPSS ver.12J). In the present study, all values at $p < 0.05$ were considered statistically significant.

Results

As shown in the Table 1, the physical characteristics of subjects (such as age (20.4 ± 0.9 years), height (169.2 ± 4.6cm), body weight (53.6 ± 3.8kg) and body fat percentage (6.1 ± 0.7%)) between the two groups were not found to be statistically significant. In terms of the muscle enzyme levels and liver function, CK, AST, ALT and LDH were found to be significantly higher in the race group ($p<0.05$), whereas no difference in the γGTP level was observed between the two groups (Table 2).

No significant changes were observed in levels of leukocyte, neutrophil, immunoglobulins or complements (Table 3). In terms of SOA, significant changes were not observed between the two groups, though levels of LgCL, PH and AUC tended to be higher in the race group (Table 4).

Discussion

In terms of age, running distance, duration of training and physical characteristics, no significant differences were found between the two groups. Moreover, subjects in both groups were considered to be under the same physical stress after one week of the race. The average running distance in the race group was 151.5 ± 31.3km, and 148.7 ± 47.6km in the control group, which was not significantly different. For both groups, subjects ran the average of greater than 20km a day as a training, which was considered as a sufficient training for an endurance

Table 3 Differences of leukocyte, neutrophil, immunoglobulins and complements between the race group and the control group.

	Race Group (n = 11)	Control Group (n = 17)	P value
Leukocyte (/μL)	5290.9 ± 974.1	5617.6 ± 1327.2	0.489
Neutrophil (/μL)	2563.4 ± 571.0	2814.6 ± 1061.4	0.760
IgG (mg/dL)	1031.2 ± 184.8	1075.1 ± 269.1	0.640
IgA (mg/dL)	191.2 ± 65.0	206.5 ± 60.8	0.532
IgM (mg/dL)	92.5 ± 53.7	108.0 ± 29.7	0.090
C3 (mg/dL)	83.8 ± 10.0	87.4 ± 9.2	0.346
C4 (mg/dL)	17.3 ± 5.0	20.4 ± 7.5	0.311

Mean ± S.D.

Table 4 Differences of serum opsonic activity between the race group and the control group.

		Race Group (n = 11)	Control Group (n = 17)	P value
LgCL	PH(cpm)	8824.4 ± 754.9	8128.7 ± 1039.4	0.067
	PT(sec)	1462.9 ± 202.6	1408.9 ± 175.1	0.460
	AUC(cpm*s)	261507.9 ± 24140.1	238000.6 ± 33448.4	0.055
LmCL	PH(cpm)	147441.9 ± 12378.7	147905.1 ± 12034.2	0.922
	PT(sec)	1306.9 ± 71.5	1298.8 ± 92.0	0.853
	AUC(cpm*s)	3379359.5 ± 235091.1	3441185.7 ± 273382.1	0.543

Mean±S.D. LgCL: lucigenin-dependent chemiluminescence, LmCL: luminol-dependent chemiluminescence, PH: peak height, PT: peak time, AUC: area under the curve.

runner.[18]

CK, AST, ALT and LDH are commonly used as the markers of muscular damage.[19] After prolonged exercise, serum CK activity markedly elevates, and lasts for 24-48 hours.[20] With more intensive exercise, increase in serum CK level was observed and reached peak on the fourth day and it decreased until the tenth day.[20] Although the amount of exercise performed in a week was not significantly different between the two groups, muscular enzyme level was significantly high in the race group compared to the control group. Therefore, it was suggested that the race group did not have enough time to recover to the pre-race condition even after one week of the race; though it was reported that half-life of muscle CK after marathon race was 49h.[21] This showed that denaturation and damage of the muscle were greater in the race group than the control group, suggesting that muscle fatigue occurred in the race had persisted for one week. Therefore, it was considered that the intensity of the race was high, even there were no significant changes in running distance and training time between the groups. During the match, all athletes have to face the unfamiliar running environment such as slopes, running speed, as well as the competitive mentality during the race. Thus, athletes were required to adjust themselves to combat the greater amount of stress during the race in order for them to win the competition.

Typically, acute exercise leads to temporal changes in the ratio of leukocytes, and these changes recover within 24 hours after the exercise, and this is also the case for the athletes performing the daily training.[22, 23] It was also reported that long-term training may decrease

blood leukocyte count, although a constant results have not been found up until now.[22, 24] In the present study, no significant differences in numbers of leukocytes, immunoglobulins or complements were observed between the race group and the control group. Thus, their immunological indices were considered to be recovered back to the normal state after a week of the race.

Also, oxidative stress is the exacerbating factor of muscular damage and fatigue, and it is known to be related to overtraining.[15] In the present study, lucigenin and luminol were used as sensitizer for measuring SOA. Because LgCL mainly reacts with superoxide (O_2-) which is produced at the beginning of ROS metabolism, it is considered to reflect the activation of ROS production system.[25] On the other hand, O_2- is metabolized to highly reactive substances such as hypochlorous acid (HOCL) and hydroxyl radical (・OH) by myeloperoxidase (MPO). LmCL is known to reflect the amount of HOCL and ・OH productions and thus, it was suggested that increased amount of LmCL was suggested to cause oxidative stress.[26-29] In the previous studies, it was reported that LgCL and LmCL had increased after intensive exercise, and possibilities of normal tissue damage by increase of ROS production and oxidative stress had been suggested. In this study, LgCL was significantly higher in race group than control group, so ROS production was considered to be increased even one week after the race. On the other hand, there was no significant difference in LmCL between the two groups, so it was considered that there was no difference in ROS production which led to oxidative stress between the two groups. In other words, it was considered that ROS production of subjects in race group was accelerated, however, oxidative stress was inhibited after one week of the race.

Recently, it was reported that IL-6 secreted from damaged muscle was found to accelerate the ROS production in neutrophils.[30] Therefore, ROS was suggested to be involved in recovery and regeneration of muscle tissue.[31] As mentioned above, muscle damage in the race group was higher than control group in the present research. SOA in the race group tended to be higher than control group, due to their higher degree of muscle damage, and thus, the increase of SOA in the present study was considered to be a reaction caused by muscle damage. At the same time, LgCL reflected activity of ROS production, so there was a possibility that it may have reflected the increase in ROS production in the race group. However, there was no difference in LmCL between the two groups, which was considered to reflect oxidative stress. It seemed that the metabolic pathway metabolized O_2- and suppressed the production of HOCL and ・OH, which recovered the oxidative stress.

In the case when athletes kept performing the exercise without the full recovery from previous physical stress, physical fatigue as well as biochemical and immunological fatigues were found to accumulate on top of what they already had. In the present study, the same training was performed in both groups for a week after the race, and thus, subjects in the race group had not fully recovered from the physical fatigue they had from the race. Therefore, long-distance runners were suggested to carry out a reduced amount of less-intense, less-frequent training with sufficient breaks at least for a week after they participated in a race competition under unusual conditions. Also, a periodical medical check-ups mentioned in the present article is necessary in order to maintain an adequate health care of long-distance runners.

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