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Improving water supply to participants in *kendo* training camp in hot summer conditions : an investigation from the angle of serum albumin oxidation state

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Abstract

This study focused on 10 participants of a university kendo team's training camp held in hot, summer conditions. We divided the participants into one group (the high-intake group) of five who were given extra opportunities to drink water during practices, and another group (the standard-intake group) of five who were given only the usual opportunities to drink. We recorded data including the amount of water intake and perspiration during practices and conducted analysis with special attention to the oxidation state of participants' serum albumin. We achieved the following conclusions:

The high-intake group drank significantly more water than the standard-intake group ($p=0.0163$). However, this additional water was not average to suppress increases in body temperature significantly. Before the training camp, the fraction of serum albumin in the reduced state ($\bar{f}(\mathrm{HMA})$) ± standard error was 67.4±0.7% for the high-intake group and 65.2±1.4% for the standard-intake group. After the camp, $\bar{f}(\mathrm{HMA})$ was 53.7±2.2% for the high-intake group and 51.2±1.2% for the standard-intake group. $\bar{f}(\mathrm{HMA})$ after the camp was significantly less than $\bar{f}(\mathrm{HMA})$ before the camp for both groups ($p=0.0431$ for both groups), but there was neither a significant difference between groups before the camp nor between groups after the camp. A comparison of the present study with a previous study leads us to conjecture that participants in the present study underwent more internal oxidative stress due to more intense heat and environmental conditions at the training camp in this study, and we believe that further investigation into water supply methods is necessary.

Keywords : kendo training camp, intense summer conditions, water intake, serum albumin (human), oxidation state

I. Introduction

Although kendo is an indoor activity the incidence of heat illnesses is high. One possible explanation is that, during practice, participants must wear hot, stuffy protective equipment regardless of the season. Thus, athletes may sweat more readily in kendo than in other sports. Furthermore, during midsummer and midwinter training sessions, traditional disciplining of the body and spirit is commonly practiced in intense environmental conditions without air conditioning. Even in the heat of summer, in many places it is customary — perhaps stemming from the pattern of discipline — to limit water intake to once during practice, or even completely forgo intake of water during practice. Accordingly, maintenance of bodily fluids and regulation of internal temperature is especially difficult, and we cannot

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ignore the possibility that adequate precautions to protect against heat illnesses and allow athletes to sustain optimal performance are not always enforced in such cases.

In addition, we find reports that exercise in excess of moderate levels causes oxygen consumption to increase and accelerates the creation of reactive oxygen species (ROS) during exercise. Not only exercise, but also other factors such as extreme-temperatures and low-pressure, low-oxygen environments may accelerate the creation of reactive oxygen species. Researchers have suggested that this contributes to oxidative stress, which occurs when reactive oxygen species surpass the scope of anti-oxidant mechanisms[17]. However, the existence of antioxidant enzymes such as the superoxide dismutase (SOD), catalase (CAT), and other antioxidant substances such as glutathione peroxidase (GSH-Px), unbound sulfhydryl groups (SH), vitamin C, vitamin E, uric acid, bilirubin, and glutathione protects against excessive oxidation of nucleic acids (DNA and RNA), sugars, lipids, and proteins when reactive oxygen species are generated in the body[3, 12].

One of the body's antioxidants is the SH group in human blood plasma, which derives mostly from the cysteine residue of albumin and can be found in concentrations of 0.5 to 0.8 mM[15]. This is several to 10 times the concentration of other antioxidants, and it has been reported that plasma SH carries a crucial role as an antioxidant in the bloodstream[8,18]. There are few reports on the relationship between exercise and the SH of plasma proteins, but Inayama et al.[7] and Saito et al.[16] have investigated the fluctuation of SH of plasma proteins before and after a marathon and before and after athletic training camp. In both cases, researchers found the concentration of plasma protein SH decreases significantly.

We find also related reports on human serum albumin (HSA). Each molecule of HSA contains one SH group[2,15]. When this SH is free, unbound to any other substances, the albumin is in a reduced state and is called human mercaptalbumin (HMA). When, on the other hand, the SH forms an intermolecular covalent bond with, say, a sulfer-containing amino acid or other molecule, the albumin is in an oxidized state and is called human nonmercaptalbumin (HNA). The oxidation state of HSA can be used as a biomarker indicative of oxidative stress within the body[5]. Reports on the relationship between exercise and the oxidation state of human serum albumin are limited outside of the authors' own studies[1,4-6]. In these studies, we reported[1,5,6] that the average fraction of albumin in the reduced form ($\bar{f}$(HMA)) after summer training camp is significantly less than before, and during summer training camp, there is repetition and accumulation of exercise loads yielding oxidative stress. We conjecture that during exercise in hot summer conditions, heat stress and dehydration causing increase in body temperature and decrease in bodily fluids may generate reactive oxygen species and free radicals and thereby lead to oxidative stress; however, the effect of water intake on the oxidation state of HSA has yet to be reported.

In the current study, we investigated the method of supplying water to athletes during summer kendo training camp and studied the effects of water intake and environmental conditions on the oxidation state of HSA.

II. Methods

1. Subjects

Subjects consisted of ten male members of the Gifu University Kendo Team who volunteered and gave their informed consent to the items under investigation. Five participants (hereafter referred to as the high-intake group) were given extra opportunities to drink water during practices, while the other five participants (the standard-intake group) were given only the usual opportunities. Physical attributes of the subjects were as listed in

Table 1.

Table 1. Physical Attributes of Subjects

High-Intake Group (n=5)	Standard-Intake Group (n=5)	p
19.8 ± 0.7	20.0 ± 0.5	*ns*
171.0 ± 1.3	170.6 ± 1.5	*ns*
64.7 ± 1.4	64.2 ± 3.1	*ns*
13.2 ± 1.5	12.6 ± 1.0	*ns*

average ± standard error *ns* : nonsignificant (Mann-Whitney U-test)

2. Content of training camp practices

Practices at the training camp were held once each morning from 08:00 until about 10:30 (about 2 1/2 hours) and once each afternoon from 14:00 until about 18:00 (about 3 hours). On the first day of camp, only the afternoon practice was held, yielding a total of seven practices in four days. Each full day of practice included approximately 50 minutes of basic strike practice (*kihon-geiko*), 110 minutes of free bouting (ji-geiko), 20 minutes of intense strike practice (*kakari-geiko*), and 120 minutes of mock competition practice (*shiai-geiko*).

3. Method of supplying water

During practices, participants drank exclusively tap water that we purified through a filter and maintained at about 7°C. They drank from a container with an attached straw which allowed them to drink without removing their protective heard gear (*men*).

Ingestion of water by the standard-intake group was limited, as customary, to the set rest periods in each practice — once during the morning practice and twice during the afternoon practice. The high-intake group was given the same opportunities plus additional opportunities to drink water during short breaks in practice for a total of three times during each morning practice and three to five times (mode=4) during each afternoon practice.

4. Measurements

a. Wet-bulb globe temperature (WBGT)

We measured the WBGT of the practice hall with a WBGT thermometer (WBGT-101, Kyoto Electronics Manufacturing Co., Ltd.) at five minute intervals during practices.

b. Mass of participants (kg) and amount of water intake (kg)

In order to investigate the amount of water intake and the amount of water lost in each practice, we recorded the amount of water intake during each session and measured the weight of each participant to the nearest 0.001 kg with a high-precision scale (D-7470, Mettler Corporation) before and after each practice. We calculated perspiration (kg), ratio of water intake to perspiration (%), and percent decrease in body weight as follows:

perspiration[kg] = (weight before practice [kg] + water intake [kg])－weight after practice [kg]
ratio of water intake to perspiration [%] = water intake [kg]－perspiration [kg] ×100
percent decrease in body weight [%] = (body weight before practice [kg]－body weight after practice [kg]) ÷ body weight before practice × 100

c. Body temperature

To investigate the effect of water intake on suppression of rising body temperatures, we measured each participant's eardrum temperature (MC-505, Omron Corporation) and axillary (underarm) temperature (C-202, Terumo Corporation) before and after each practice.

d. Analysis of human serum albumin (HSA)

In order to investigate how the oxidation state of HSA changed over the duration of the summer training camp, we collected blood samples from subjects once before the first practice of the camp and once after completion of the last practice. After collecting the samples, we separated plasma constituents on the spot with a pressure filter and stored the samples at -80°C.

We further separated the components according to the modified methods of Imai et al.[4] with a high-performance liquid chromatography (HPLC) system consisting of the following:

- HPLC column: Shodex Asahipak ES-502N 7C (Showa Denko K.K.), column temperature = 35.0±0.5°C
- autosampler: AS-8010 (Tosoh Corporation), injection volume = 2 μm
- pump: CCPM (Tosoh Corporation), flow rate = 1 ml/min
- fluorescence detector: ES-8000 (Tosoh Corporation), excitation wavelength = 280 nm, detection wavelength = 340 nm
- super-system controller: SC-8020 (Tosoh Corporation)
- eluation: A, B two-component gradient method (ethanol concentration gradient, 0→5%)
  - solvent A: 0.05 M sodium acetate, 0.40 M sodium sulfate
  - solvent B: 0.05 M sodium acetate, 0.40 M sodium sulfate, 10% ethanol (pH = 4.85)

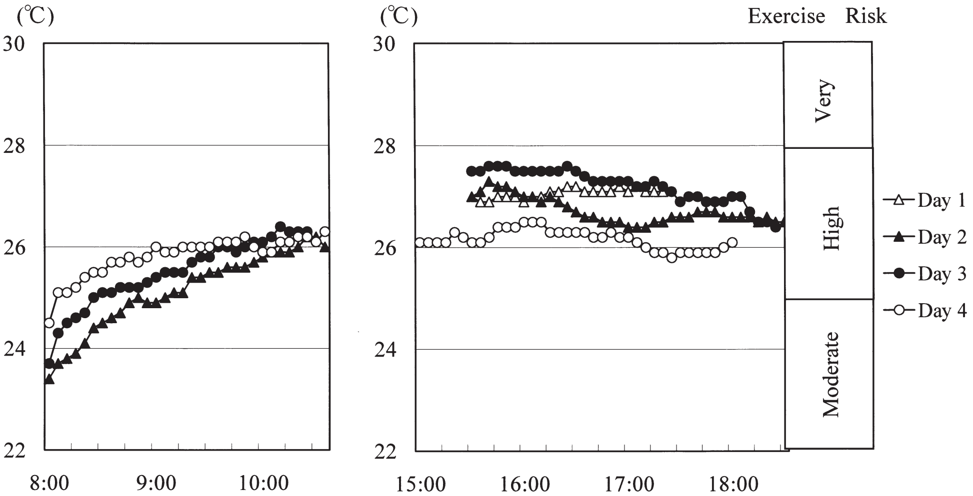
5. Statistical Analysis

We used the Mann-Whitney U test to compare differences between the two groups in change in body temperature before and after practice, amount of perspiration, amount of water intake, ratio of water intake to perspiration, percent decrease in body weight during practice, and average fraction of albumin in the reduced form ($\bar{f}$(HMA)). We used the Wilcoxon signed rank test to compare ($\bar{f}$(HMA)) before and after the training camp. We set the level of significance at 5%.

III. Results

1. WBGT during practices (Figure 1)

The average value ± standard deviation of the WBGT during practice was 27.11±0.1°C the first afternoon, 25.1±0.8°C the morning of the second day, 26.7±0.3°C the afternoon of the second day, 22.5±0.7°C the morning of the third day, 25.5±0.7°C the afternoon of the third day, 25.8±0.4°C the morning of the fourth day, and 26.2±0.2°C the afternoon of the fourth day. According to certain exercise guidelines for prevention of heat illnesses[10], these readings range from moderate risk (Water intake should be enforced.) to high risk (Rest pe-



a. Morning Practices
b. Afternoon Practices

Figure 1. Fluctuations in WBGT during Summer Training Camp

riods should be enforced.), and readings stayed within the high-risk range throughout afternoon practices.

2. Amount of water intake and perspiration during practices (Table 2)

a. Amount of water intake (kg)

The standard-intake group drank an average ± standard error of 0.7±0.1 kg of water per practice, while the high-intake group drank an average of 1.3±0.2 kg of water per practice, significantly more than the standard-intake group (p=0.0163).

b. Amount of perspiration (kg)

The standard-intake group lost an average ± standard error of 2.7±0.1 kg of water through perspiration, while the high-intake group also lost an average of 2.8±0.1 kg of water through perspiration, resulting in no measured significant difference.

c. Ratio of water intake to perspiration (%)

For the standard-intake group, the average± standard error of the ratio of water intake to perspiration was 28.0±2.0%, while, for the high-intake group, the average ratio was 52.2±9.1%, significantly higher than the standard-intake group (p=0.0090).

d. Percent decrease in body weight (%)

The standard-intake group exhibited an average± standard error decrease in body weight of 3.0±0.2%, while the high-intake group exhibited an average decrease of 2.0±0.4%, significantly less than the standard-intake group (p=0.0472).

Table 2. Amount of water intake and perspiration during practice

	High-Intake Group (n=5)			Standard-Intake Group (n=5)			p
Amount of Water Intake (kg)	1.3	±	0.2	0.7	±	0.1	0.0163
Amount of Perspiration (kg)	2.8	±	0.1	2.7	±	0.1	*ns*
Ratio of Water Intake to Perspiration (%)	52.2	±	9.1	28.0	±	2.0	0.009
Percent Decrease in Body Weight (%)	2.0	±	0.4	3.0	±	0.2	0.0472

average ± standard error *ns* : nonsignificant (Mann-Whitney U-test)

3. Difference in body temperature (eardrum and axillary temperature) before and after practice (Table 3)

a. Eardrum temperature (°C)

Eardrum temperature after practice was an average ± standard error 2.1±0.2°C higher than before practice for the standard-intake group, and 2.2±0.1°C higher than before practice for the high-intake group. There was no significant difference between groups.

b. Axillary temperature (°C)

Axillary temperature after practice was an average ± standard error 1.4±0.2°C higher than before practice for the standard-intake group, and 1.3±0.1°C higher than before practice for the high-intake group. There was no significant difference between groups.

Table 3. Difference in Body Temperature Measured Before and After Practice

	High-Intake Group (n=5)			Standard-Intake Group (n=5)			p
Change in Eardrum Temperature (°C)	2.2	±	0.1	2.1	±	0.2	*ns*
Change in Axillary Temperature (°C)	1.3	±	0.1	1.4	±	0.2	*ns*

average ± standard error *ns* : nonsignificant (Mann-Whitney U-test)

4. Average fraction of albumin in reduced form ($\bar{f}$(HMA)) (%) (Figure 2)

Before the training camp, $\bar{f}$(HMA) ± standard error of $\bar{f}$(HMA) was 67.2±1.4% in the standard-intake group and 67.4±0.7% in the high-intake group, revealing no significant difference between groups. After the training camp, $\bar{f}$(HMA) was 51.2±1.2% in the standard-intake group and 53.7±2.2% in the high-intake group, and again there was no significance between groups. However, by comparing values before and after the camp, we find that, in both groups, $\bar{f}$(HMA) decreased significantly (p=0.431).

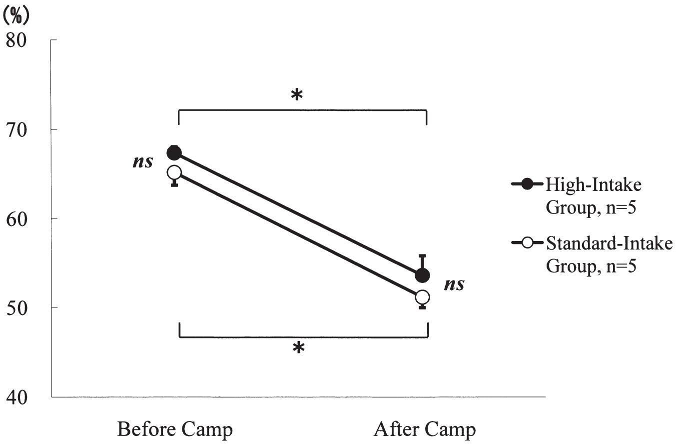


Figure 2. $\bar{f}$(HMA) Before and After Summer Training Camp

Errorbars show standard error

* : $p<0.05$ (Wilcoxon signed rank test)

ns : no significant difference (beteen the high-intake group and the standard-intake group) (Mann-Whitney U-test)

5. Comparison with $\bar{f}$(HMA) recorded at a training camp in an air-conditioned facility[1] (Figure 3)

A previous study[1] conducted by our group at a kendo training camp in the summer of 1998 also included measurements of $\bar{f}$(HMA) before and after the camp. The contents of one day's worth of practice were similar to the present study, although the 1998 study spanned

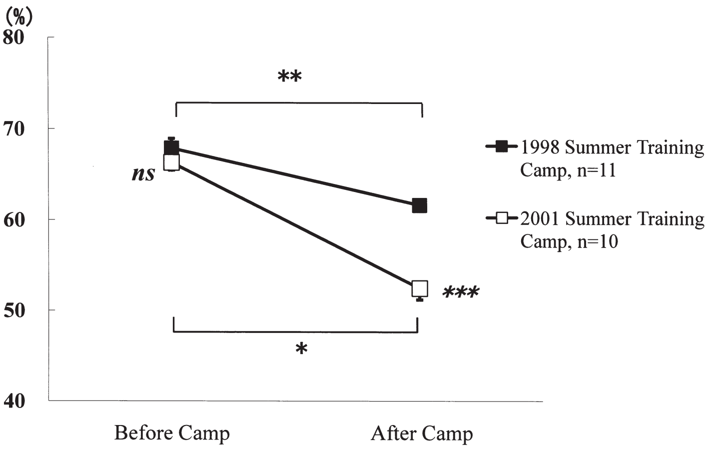


Figure 3. $\bar{f}$(HMA) Before and After Training Camp: Comparison of 1998† and 2001

Errorbars show standard error †1998 data is adopted from Arikawa et al.(1)

** : $p<0.01$, * : $p<0.05$ (Wilcoxon Signed Rank Test)

*** : *p<0.001*, **ns** : no significant difference (between 1998 and 2001data) (Mann-Whitney U-test)

1998 Summer Training camp consisted of 9 practices with average WBGT = 22.0±0.31°C (moderate risk range)

2001 Training Camp consisted of 7 practices with average WBGT = 26.2±0.31°C (high risk range) a total of nine practices and the average ± standard deviation WBGT was 22.0±0.3°C (caution range). Before the camp, $\bar{f}$(HMA) ± standard error was 67.9±1.1% in the 1998 study, compared to 66.2±0.8% for all participants in the present study; there was no significant difference across studies. However, after completion of the training camp, $\bar{f}$(HMA) dropped to 61.6±0.4% in the 1998 study, compared to 52.4±1.2% in the present study, a significant difference between studies (p=0.0001). The decrease in $\bar{f}$(HMA) during the training camp of the 1998 study was significant (p=0.0033), as was the decrease in $\bar{f}$(HMA) in the present study (p=0.0051).

IV. Discussion

The WBGT was in the moderate- to high-risk range during the morning practices and stayed within the high-risk range throughout afternoon practices, and we therefore infer that the possibility of developing heat illnesses existed during the training camp in this study. When the WBGT falls in the moderate-risk range, it is important that water intake be enforced, and when the WBGT falls in the high-risk range, it is important that rest periods be enforced[10]. We feel that our scheme of supplying water (in containers with attached straws during breaks in practice) was better suited for summer's intense environmental conditions than conventional methods.

The high-intake group ingested significantly more water than the standard-intake group (Table 2). However, although the amount of water lost through perspiration tended to be somewhat greater and the before-and-after changes in eardrum temperature and axillary temperature tended to be somewhat less for the high-intake group, differences between groups were not significant for these measurements (Table 3). According to a previous study[14], the amount of water intake has no effect on the amount of perspiration when the ingested water is chilled. We therefore believe that one possible reason we did not see a significant difference in the amount of perspiration in the present study is that we supplied the participants with water chilled to about 7°C. Studies[13,14] have also reported that the ingestion of chilled water can have a direct cooling effect on the body and thereby suppress increases in body temperature; the more water is ingested, the greater this effect is. However, we believe that at the training camp in the present study, the difference in the amounts of water ingested by the two groups was not great enough to create a significant difference in either eardrum or axillary temperature.

Concerning the maintenance of body fluid levels, the ratio of water intake to perspiration was 52.2±9.1% for the high-intake group compared to 28.0±2.0% for the standard-intake group. While this percentage was significantly larger for the high-intake group, neither group approached the 80% mark which is said to be effective for prevention of heat illnesses[11] (Table 2). We therefore believe that even the high-intake group was not ingesting sufficient amounts of water. As a result, during some practices, the high-intake group lost an average body weight of more than 2%, which has been given as the limit for sustained performance[19]. Some member of the high-intake group lost more than 3%, the point at which the body begins to lose the ability of thermoregulation[11]. The standard-intake group lost an average body weight in excess of 3% during every afternoon practice, while some members lost more than 3% in every practice. Accordingly, we believe that bodily fluid levels were not being sufficiently maintained by the standard-intake group, and that its members were not only at risk of losing performance ability but also at high risk of developing heat illnesses. It cannot be said that even the high-intake group — which displayed a significant loss in body weight — maintained body fluid levels sufficiently, and we believe that they too were at risk of developing heat illnesses. We saw large individual variations in loss of body weight, and so, as previous studies have also reported[9] we believe that in order to maintain performance abilities and prevent occurrences of heat illnesses, it is important to consider not only group averages, but also the changes in body weight of individuals.

Reports on the relationship between exercise and the oxidation state of HSA are limited outside of our work[1, 4-6], although we do find related reports[7,16] concerning exercise and sulfhydryl groups of plasma proteins. In our earlier studies[1,5,6] conducted at midsummer training camps, $\bar{f}$(HMA) after the training camp was significantly less than $\bar{f}$(HMA) before the training camp. Saito et al.[16] has found that plasma protein sulfhydryl levels are also significantly lower after training camp than before. Similarly, in the present study, $\bar{f}$(HMA) of both groups was significantly lower after the training camp than before (Figure 2). There was neither a significant difference in $\bar{f}$(HMA) between the high-intake group and the standard-intake group before the training camp nor a significant difference between groups after the training camp (Figure 2). We conjecture that perhaps neither group ingested enough water to significantly aid thermoregulation, and while there was some benefit for maintenance of body fluid levels, perhaps the effect was not enough to create a significant difference in $\bar{f}$(HMA) between the groups.

We have cited values of $\bar{f}$(HMA) from our previous study[1] of G-University Kendo Team members before and after summer training camp in 1998 in an air-conditioned facility and compared them with $\bar{f}$(HMA) measured before and after the summer training camp in the present study (Figure 3). In both studies $\bar{f}$(HMA) was significantly less after the training camp than before the training camp; $\bar{f}$(HMA) decreased 6.2±1.14% in the 1998 study and 13.9±1.20% in the present study. There was no significant difference between the two studies in $\bar{f}$(HMA) before training camp, but $\bar{f}$(HMA) after training camp was significantly less in the present study than in the 1998 study. Previous research[5-7] has indicated the possibility of oxidative stress due to repetition and accumulation of exercise loads. However, the contents of the training camp in the present study were similar to those of the 1998 study, and there were also fewer practices in the present study, so we believe that the significantly lower $\bar{f}$(HMA) which we found after training camp indicate, in addition to exercise load, the presence of another factor contributing to oxidative stress. We conjecture that this factor was hot environmental conditions, since the WBGT in the 1998 study was in the moderate-risk range, while the WBGT in the present study rose to the high-risk range. In other words, we conjecture that in the summer training camp of the present study, excessive oxidative stress occurred in participants due to the hot summer conditions which translated into WBGT readings in the high-risk range.

Regarding the effect of our scheme for water distribution, we saw no significant difference between groups in suppression of rises in eardrum and axillary temperatures. Although the high-intake group lost significantly less body weight, we do not believe they retained enough to sustain performance and protect against heat illnesses. Accordingly — because the concept of disciplining the body and spirit is so strongly engrained in kendo practice and because the prevalence of this way of thinking may lead to difficulties in increasing water intake — we believe that further improvement of water supply methods is necessary in order to prevent heat illnesses and facilitate effective practices. Again, the water supply method investigated in the present study was insufficient to create a difference between groups in the change in $\bar{f}$(HMA),and we therefore believe that further investigation is needed.

In addition to the effect of repetition and accumulation of exercise loads, a comparison to the 1998 data[1] leads us to conjecture that oxidative stress may also occur due to summer's intense environmental conditions. We believe that, for this reason, if the body cannot cope with the heat, an athlete will have difficulty practicing effectively. Accordingly, these results indicate the necessity of preventative measures such as conducting practices in air-conditioned facilities with good air circulation to keep temperatures from building up as a means of preventing heat illnesses and facilitating effective practices.

V. Conclusions

In the present study, we investigated the method of supplying water to participants at a summer kendo training camp and studied the effect of water intake and environmental conditions on the oxidation state of HSA, and we reached the following conclusions:

1. The WBGT readings in the high-risk range indicated that the summer kendo training camp was conducted in an environment associated with a high risk of developing of heat illnesses.
2. During the summer kendo training camp, increase in the number of opportunities for participants to drink water led to a significant increase in the amount of water ingested, but even this was not sufficient to significantly aid on the body's thermoregulation. While there was some benefit for maintenance of body fluid levels, it cannot be said that this was sufficient precaution against heat illnesses. We believe that there is further need to investigate the means of supplying water to athletes.
3. Regarding the oxidation state of HSA before and after the summer training camp, we saw no significant difference in $\bar{f}$(HMA) between the high-intake group and the standard-intake group. Comparison of $\bar{f}$(HMA) in our study to $\bar{f}$(HMA) taken before and after a summer training camp in 1998 in a previous study led us to conjecture that excessive oxidative stress occurred in participants in our study due to severe hot conditions.

We believe our results indicate the need for improving the means of water supply beyond conventional methods. We believe that, since exercise in summer's intense environmental conditions involves the possibility of oxidative stress due to environmental heat as well as repetition and accumulation of exercise loads, if the body cannot cope with the heat, an athlete will have difficulty practicing effectively. Accordingly, we believe our results indicate the necessity of preventative measures such as conducting practices in air-conditioned facilities with good air circulation to keep temperatures from building up as a means of preventing heat illnesses and facilitating effective practices.

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