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Oxidation state of human serum albumin during regular *kendo* practices

Hajime IMAI[1], Jennifer LUE[1], Kunihide KODA[2], Toru KAMEI[2],
Nanae IMAI[3] and Hajime ARIKAWA[1]

1) Department Health and Physical Education, Faculty of Education, Gifu University, Gifu, 501-1193, Japan;

2) Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, 305-8577, Japan;

3) Kumamoto Prefecture Kendo Federation, Kumamoto, 862-0950, Japan;

4) Heisei College of Health Sciences, Gifu, 500-1131, Japan

Abstract

Human serum albumin (HSA) is a mixture of albumin in reduced form (mercaptalbumin, HMA) and albumin in oxidized form (nonmercaptalbum, HNA). In healthy adults, HSA is usually about 75% HMA and 25% HNA. The oxidation state of HSA can be used as a biomarker for oxidative stress.

In this study, we used the oxidation state of HSA to analyze oxidative stress in 11 members of a university *kendo* team during regular practices. The mean ± standard deviation of recorded fractions of reduced albumin ($\bar{f}$ (HMA)), oxidized albumin ($\bar{f}$ (HNA-1)), and highly oxidized albumin ($\bar{f}$ (HNA-2)) were 76.6±2.7%, 21.3±2.7%, and 2.1±0.5%, respectively, before practice, and 75.1±1.5%, 23.0±1.6%, and 1.9±0.3%, respectively, after practice. There were no significant differences between percentages measured before and after practices.

We therefore conjecture that oxidative stress does not occur during regular practices, and we believe our results indicate the importance of regular, daily, physical activity.

Keywords : *kendo*, regular practice, high-performance liquid chromatography (HPLC), human serum albumin (HSA), human mercaptalbumin (HMA), human nonmercapt-albumin (HNA), oxidative stress

I. Introduction

Human serum albumin (HSA) comprises about 50 - 60% of the volume of all serum proteins. About 40% of this is in plasma, and the other 60% is in interstitial spaces[1]. One of the main structural traits of HSA is the existence of a highly reactive SH group at position 34 from the Nitrogen terminus (cysteine residue; Cys-34). When this group is free, unbound to any other substance, the albumin is said to be in the reduced form (human mercaptalbumin, HMA). On the other hand, when the SH group is covalently bound and shared by the albumin and another substance such as a sulfur-containing amino acid in the blood, the albumin is said to be in the oxidized form (human nonmercaptalbumin, HNA). There are several types of oxidized albumins, including HNA (CYS), in which the SH group is bound to cysteine, and HNA (GLUT), in which it is bound to glutine. Other oxidized albumins (HNA(OXI)) that are further oxidized so that the SH group has formed -SOH (solfenic acid), $-SO_2H$ (solfinic acid), and $-SO_3H$ (solfonic acid) have also been reported[2]. HSA is a mixture of HMA and HNA, and, in healthy adults, the fraction of HMA (f (HMA)) is usually around 75% and the fraction of HNA (f(HNA)) is around 25%[3, 4]. The oxidation state of HSA can be used as a biomarker for oxidative stress[4].

We have previously employed high speed liquid chromatography (HPLC) analysis with a special column able to separate HSA into HMA and HNA, and we have reported results including the following : a) Intense physical training (*kendo* training camp) causes significant decreases in the ratio of HMA in university students[5], and b) this decrease is significantly mitigated by the ingestion of

Brazilian propolis, which has an anti-oxidant effect[6].

Healthy living requires a proper balance of exercise, nutrition, and rest, and the necessity of enforcement of proper exercise has been demonstrated. The question remains: Does oxidative stress occur during regular, daily 2 hour *kendo* practices in the same manner as has been reported for practices during *kendo* training camp? In the present study, we investigated oxidative stress as indicated by the oxidation state of HSA of university students during regular practices.

II. Methods

1. Subjects

Participants were 11 male members of the Gifu University *Kendo* Team who voluntarily consented to all measurements and items under investigation. The mean ± standard deviation age, height, body mass, and body fat percentage were 19.6±4.5 years, 172.5±4.5 cm, 70.9±8.1 kg, and 20.5±3.2%, respectively.

2. Practices

Regular practices were held for two hours from 4 to 6pm. The practice regimen consisted of approximately 20 minutes of basic strike practice, 45 minutes of free bouting, 5 minutes of intense strike practice, and 50 minutes of mock competition practice.

3. Measurements

A. Wet-bulb globe temperature (WBGT), air temperature, and humidity

As a measure of environmental heat, we recorded the WBGT, air temperature, and humidity with a WBGT thermometer (WBGT-101, Kyoto Electronics Manufacturing Co., Ltd.) at 5 minute intervals during practices.

B. Analysis of human serum albumin

We collected blood samples once before and once after practices. The volume of each sample was 3 ml. After collection, we immediately separated plasma constituents with a pressure filter and stored the samples at −80℃.

For analysis of HSA, we followed the methods of Imai et al.[13] and used 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℃
- autosampler: AS-8010 (Tosoh Corporation), injection volume = 2 μl
- 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)

As explained in the Introduction, several forms of HNA have been reported. In this study, we classified HNA into two groups: HNA(CYS) and HNA(GLUT) (hereafter referred to as HNA-1), and HNA(OXI) (referred to as HNA-2).

4. Statistical Analysis

To compare the mean percentages of both reduced albumin and oxidized albumin before and after practices, we used the Wilcoxon signed rank test. We set the level of significance at 5%.

III. Results

1. WBGT, temperature, and humidity

The mean ± standard deviation WBGT, temperature, and humidity were 17.5±0.2℃, 22.3±0.4℃, and 43.5±5.8%, respectively. WBGT during the regular practices in our study was in the relative safety zone (*hobo-anzen*: Replenishment of water is necessary.), according to guidelines for the prevention of heat illnesses[6].

2. Oxidation state of HSA before and after regular practices (Figure 1)

Our measurements of the mean ± standard deviation HSA fractions, as shown in Figure 1, were as follows: Before regular practices, $\bar{f}$ (HMA), $\bar{f}$ (HNA-1), and $\bar{f}$ (HNA-2) were 76.7±2.7%, 21.3±2.7%, and 2.1±0.5%, respectively. After practices, $\bar{f}$ (HMA), $\bar{f}$ (HNA-1), and $\bar{f}$ (HNA-2) were 75.1±1.5%, 23.0 ±1.6%, and 1.9±0.3%, respectively. There were no significant differences between fractions measured before and after practices.

IV. Discussion

The body is furnished with antioxidant mechanisms responsible for dealing with excessive amounts of reactive oxygen and free radicals. Under normal conditions, there is a balance between the forces of oxidation and anti-oxidation, and homeostasis is maintained. Oxidative stress is defined as a condition when this balance of reactions breaks down and oxidation is favored. 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

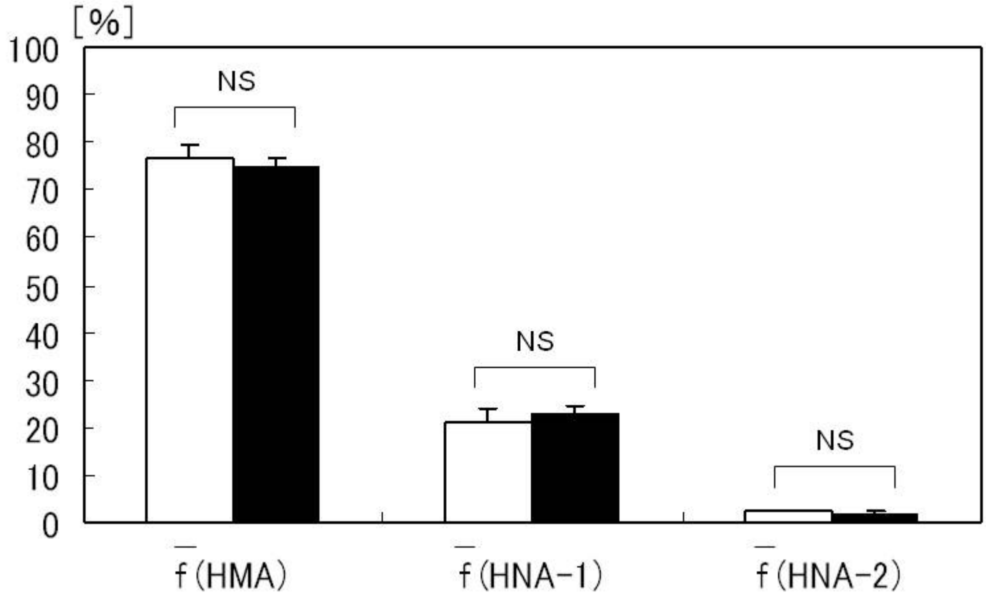

Figure 1 Values (%) for $\bar{f}$ (HMA), $\bar{f}$ (HNA-1), and $\bar{f}$ (HNA-2) of HSA from kendo players (n=11, 19.6 ± 1.2 years old) before (open column) and after (closed column) practices.

Columns represent mean values, and error bars indicate standard deviation. Data from before and after practices were evaluated by the Wilcoxon signed-ranks test to determine significance. NS: not significant.

C, vitamin E, uric acid, bilirubin, and glutathione protects against peroxidation of proteins, lipids, carbohydrates, and nucleic acids (DNA and RNA)[7-10]. HSA is one of these antioxidant substances in the body. The SH group in human blood plasma, which derives mostly from the cysteine residue of albumin can be found in concentrations of 0.5 to 0.8 mM[11]. 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[12,13].

The free SH group (Cys-34) of HSA plays a large part in the body's thiol redox buffer system. Specifically, in the extracellular fluid, it is intimately connected to reactions including the cysteine/cystine system and oxidized/reduced glutathione system, and the oxidized/reduced albumin system shoulders the role of redox buffer for these reactions. There are a variety of reports on the effects which various changes in patients' conditions have on the dynamic redox state[14-16]. As previously stated, in healthy adults, HSA is usually about 75% HMA and 25% HNA[3, 4]. HNA can be further divided into reversibly oxidized albumin, including HNA (CYS) in which the SH group is bound to cystine and HNA (GLUT) in which the SH group is bound to glutine, and irreversibly, highly oxidized albumin termed HNA (OXI), in which the SH group has formed -SOH (solfenic acid), -SO_2H (solfinic acid), or -SO_3H (solfonic acid)[2]. Our HPLC system[3] allowed us to separate and analyze oxidized albumin (HNA-1) and highly oxidized albumin (HNA-2).

We saw no significant changes in fractions of reduced albumin, $\bar{f}$ (HMA), oxidized albumin $\bar{f}$ (HNA-1), and highly oxidized albumin $\bar{f}$ (HNA-2) before and after the regular practices in this study. In other words, we interpret our results to indicate that, unlike kendo training camp during which $\bar{f}$ (HMA) significantly decreases and $\bar{f}$ (HNA) significantly increases[3], oxidative stress does not occur in regular practices. Hot environments contribute to production of reactive oxygen; however, the WBGT during the regular practices in our study was in the relative safety zone (*hobo-anzen*: Replenishment of water is necessary.)[6], and hence the effects environmental heat were comparatively minor.

Concerning the intensity of activity, the regular practices in our study consisted of basic strike practice (*kihon-geiko*), free bouting (*ji-geiko*), intense strike practice (*kakari-geiko*), and mock competition practice (*shiai-geiko*). According to Tatsumi and Hattori[18], the intensity of basic strike practice is approximately 40% of $\dot{V}O_2max$, free bouting is approximatly 55% of $\dot{V}O_2max$, intense strike practice is approximately 70% of $\dot{V}O_2max$, and the peak intensity of mock competition practice is approximately the same as during intense strike practice. In general, anaerobic threshold is distributed around 50-70% of $\dot{V}O_2max$[19], and we surmise that regular practices included a fair amount of intense activity crossing the anaerobic threshold. However, we postulate that, not only the intensity of activity, but also loading of physical activity - i.e. single, regulated exercise loads versus the accumulated loads of repeated training typical of training camp - may effect the redox state of HSA. For example increases of lipid peroxide were not seen in a half marathon study[20], although significant increases were reported in a study on 80km running[21]. The difference we saw in oxidation state of HSA between regular practices and training camp practices can be understood if we conjecture that while repetition and long hours of intense, physical activity to which participants are unaccustomed may yield oxidative stress and cause decreases in reduced albumin and increases in oxidized albumin within the body. In contrast, shorter, regular training periods limited to 2 hours may not result in such significant variations. We believe that the results of our study show that, rather than short spurts of unuseally strenuous physical activity, continuous succession of regular physical activity is useful for healthy strengthening antioxidant functions. Coupled with the results of research in epidemiology which have shown that physical activity may help prevent coronary diseases, high blood pressure, diabetes and other lifestyle-related diseases and even lower the incidence rate of colon cancer and lung cancer[22], we believe this study underscores the necessity of regular, daily physical activity.

V. Conclusion

We used oxidation state of HSA to investigate oxidative stress in members of a university kendo team during regular practices, and we found the mean ± standard deviation fractions of reduced albumin, $\bar{f}$ (HMA), oxidized albumin, $\bar{f}$ (HNA-1), and highly oxidized albumin, $\bar{f}$ (HNA-2) to be 76.7±2.7%, 21.3±2.7%, and 2.1±0.5%, respectively, before practices, and 75.1±1.5%, 23.0±1.6%, and 1.9±0.3%, respectively, after practices.

Since we saw no significant diffrences before and after regular practices, we believe these results indicate that oxidative stress does not occur during regular practices, and also indicate the necessity of regular, daily physical activity.

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