Type of manuscript: Regular Article

Title

Variabilities of salivary human herpesvirus 6/7 and cortisol levels during a three-day training camp in Judo athletes

Names of authors

Shinsuke Tamai¹, Hiroaki Hiraoka², Kazuhiro Shimizu³, Keisuke Miyake⁴, Daisuke Hoshi¹, Kai Aoki⁵,⁶, Koki Yanazawa⁷, Takehito Sugasawa⁵, Kazuhiro Takekoshi⁵ and Koichi Watanabe²*

Affiliations and mailing address

¹Doctoral Program in Sports Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan

²Faculty of Health and Sport Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan

³Department of Sport Research, Japan Institute of Sports Sciences, 3-15-1, Nishigaoka, Kita-ku, Tokyo 115-0056, Japan
A brief running title: Variabilities of salivary HHV-6/7 and cortisol levels

Number of tables: 2

Number of figures: 4

Corresponding author's e-mail address: watanabe.koichi.ga@u.tsukuba.ac.jp
Abstract

Physical fatigue accompanying athletic training has been a problem for ages. While salivary cortisol has traditionally been used to assess physical stressors, salivary human herpesvirus 6 and/or 7 (HHV-6/7) have recently been presented as novel microbiological markers. We examined differences in the short-term variabilities of salivary HHV-6/7 and cortisol levels in athletes. We collected saliva samples from 14 healthy male university Judo athletes who participated in a three-day training camp pre- and post-training each day to measure salivary HHV-6/7 and cortisol levels. Simultaneously, the plasma superoxide dismutase (SOD) inhibition rates and total mood disturbance (TMD) scores in the Profile of Mood States were measured as indicators of physical and psychological stressors, respectively. The plasma SOD inhibition rates significantly increased post-training, but the TMD scores did not change; thus, the physical stressors were relatively higher than the psychological stressors during the training camp. Salivary HHV-6/7 levels increased post-training with a significant main effect of training, but no changes were observed in the daily levels. Only salivary HHV-7 levels showed a significant training × elapsed day interaction. Salivary cortisol levels showed a significant main effect of training, but its levels decreased post-training. These findings suggest that salivary HHV-6/7 are sensitive markers of physical fatigue more than salivary cortisol.
Keywords: fatigue, assessment, saliva, biomarker, virus, hormone
タイトル:
柔道選手における3日間の柔道合宿期間中の唾液中ヒトヘルペスウイルス6・7型およびコルチゾールの変動

著者名:
玉井伸典1、平岡拓晃2、清水和弘3、三宅啓介4、星大輔1、青木海5,6、柳沢亘輝7、菅澤威仁5、竹越一博5、渡部厚一2

所属:
1 筑波大学大学院人間総合科学研究科スポーツ医学専攻，〒115-0056 茨城県つくば市天王台1-1-1
2 筑波大学体育系，〒115-0056 茨城県つくば市天王台1-1-1
3 国立スポーツ科学センタースポーツ科学研究部，〒115-0056 東京都北区西が丘3-15-1
4 中京大学スポーツ科学部，〒470-0393 愛知県豊田市見津町座立101
5 筑波大学医学病院臨床検査医学スポーツ医学研究室，〒115-0056 茨城県つくば市天王台1-1-1
6 日本学術振興会，〒102-0083 東京都千代田区麹町5-3-1
抄録:
アスリートにおいて、トレーニングに伴う身体的疲労は長きに渡って問題となっている。身体的ストレスの評価には唾液中コルチゾールが従来用いられていたが、近年、唾液中ヒトヘルペスウイルス 6 型および 7 型 (HHV-6/7) が新たな微生物学的マーカーとして注目を集めている。そこで我々は、アスリートにおける唾液中 HHV-6/7 やコルチゾールレベルの短期的な変動の違いを調査した。3 日間の合宿に参加した健常男子大学生柔道選手 14 名から、毎日トレーニング前後に唾液を採取し、唾液中 HHV-6/7 およびコルチゾールを測定した。また、身体的および精神的ストレスの指標として、血漿中スーパーオキシドジスムターゼ (SOD) 阻害率と気分プロフィール検査の総合的気分状態 (TMD) スコアをそれぞれ測定した。血漿中 SOD 阻害率はトレーニング後有意に上昇したが、TMD スコアは変化しなかった。このことから、合宿期間中は精神的ストレスよりも身体的ストレスの方が高かったことが示された。唾液中の HHV-6/7 レベルはトレーニング後に上昇し、トレーニングによる主効果が認められたが、日間の変動は認めなかった。また、唾液中 HHV-7 レベル
ルのみでトレーニング・経過日の相互作用が認められた。唾液中コルチゾールについてはトレーニングの主効果が認められたものの、トレーニング後に低下していた。以上の結果から、唾液中 HHV-6/7 は唾液中コルチゾールよりも身体的疲労に対する反応性が高いマーカーであることが示唆された。
Introduction

Physical fatigue is one of the most important problems that athletes face. Overload training is essential to improve athletic performance, but if recovery is insufficient for the training load, physical fatigue will accumulate. This accumulated fatigue leads to "prolonged maladaptation" and results in persistent underperformance, overreaching, and overtraining syndrome (OTS)\(^1\). Therefore, proper assessment of physical fatigue is necessary to adequately maintain the training-recovery balance and to achieve performance improvement.

Liquid biopsies of blood, urine, and saliva are often performed for objective assessments of physical fatigue. In particular, saliva is the most preferable biofluid utilized in the sports field because it can be collected easily, non-invasively, rapidly, and frequently without a medical license\(^2\). One example of salivary biomarkers is cortisol, which is well known as a “stress hormone” for a long time. As training is regarded as a type of stressor, salivary cortisol levels increase after training\(^3,4\). While the hormonal marker has been traditionally used, salivary human herpesvirus 6 and/or 7 (HHV-6/7) have recently attracted attention as novel microbiological markers for assessing physical fatigue\(^5\).

HHV-6/7 belong to the Betaherpesvirinae subfamily and have very similar
characteristics among herpesvirus members. HHV-6/7 are known to infect a high percentage (above 90%) of the general adult population\(^6\), and salivary HHV-6 was detected in 95% of the Judo athletes in the pilot study\(^5\). Furthermore, some previous studies reported that salivary HHV-6/7 levels were increased by physical fatigue accompanied by physical stressors\(^7-9\)). Therefore, salivary HHV-6/7 are expected to be applicable markers for assessing physical fatigue in athletes who experience higher physical stressors.

However, some points remain unknown. Firstly, the short-term variabilities of salivary HHV-6/7 levels are unclear. Despite the fact that the long-term variabilities have been observed in studies lasting several weeks\(^9\), it was unclear whether the levels changed gradually or with rapid fluctuations. Secondly, the differences in variability from other salivary biomarkers are unknown. To our knowledge, salivary HHV-6 levels were reported to have no association with salivary secretory immunoglobulin A (SIgA) levels\(^7\).

Although cortisol is also a representative salivary biomarker\(^3,4\), the difference in variability between salivary HHV-6 and cortisol levels has not been clarified.

To approach these questions, we conducted an observational study on athletes during a training camp. The present study setting was utilized because 1) the training intensity is expected to be sufficient and 2) eating and sleeping are controlled, so the
conditions were ideal for investigating the markers’ variabilities. We aimed to reveal the short-term variabilities of salivary HHV-6/7 levels as novel physical fatigue markers and of salivary cortisol levels as a traditional stress marker.

Materials and Methods

Ethical consideration

This study was approved by the ethical committees of the Faculty of Health and Sport Sciences of the University of Tsukuba (Tai 27-41). In accordance with the Declaration of Helsinki, all participants received explanations, in writing and verbally, about the purpose and method of the study, including its possible risks. Written consent was obtained from the participants. No participants complained of discomfort or adverse physical conditions during the survey.

Participants

We enrolled 14 healthy university male Judo athletes. Their characteristics are shown in Table 1. As there was no competition scheduled after the training camp, the athletes were not in the process of losing weight. We excluded participants with factors that could affect salivary HHV-6 levels, such as smoking, taking medicine, and having labial herpes. We
instructed the participants to refrain from the following before collecting samples: consuming alcohol and caffeine within 12 hours, eating within 1 hour, and brushing their teeth with dental paste.

Study design

The investigation was carried out during a short training camp that took place from September 21st to 23rd, 2019. Sample collection and the Profile of Mood States (POMS) were conducted pre- and post-training. The pre-training investigation was conducted between 7:00 and 8:00, immediately after waking up and before breakfast. The post-training investigation was conducted between 12:00 and 13:00, immediately after morning training and before lunch. This timeframe was set to collect samples at the same time for each day in consideration for diurnal variations in salivary cortisol levels, as we knew that the training program on the third day was only in the morning. The training program during the camp is shown in Table 2.
Sample collection

Saliva and blood samples were used to measure the salivary HHV-6/7 and cortisol levels and the plasma superoxide dismutase (SOD) inhibition rates. Saliva samples were collected as previously reported\(^\text{10}\). The participants were allowed to sit for 5 min, and then they rinsed their oral cavity with distilled water for 30 sec three times. After 5 min of rest, the saliva stored in the oral cavity was swallowed, and a sterile swab (Salivette, Sarstedt AG & Co. KG, Nümbrecht, Germany) was chewed one time/sec for 2 min. The Salivette swabs were then centrifuged at 5000 rpm for 15 min to extract saliva. The extracted saliva was weighed to determine the salivary secretion rate. For blood samples, qualified professionals collected from the antecubital vein into a blood collection tube containing EDTA (Venoject\(^\text{®}\) II, Terumo Corp., Tokyo, Japan). The collection tubes were then centrifuged at 3000 rpm for 15 min, and the supernatant (plasma) was used for the assay. The saliva and blood samples were stored at \(\text{−80°C}\) until the assays.

HHV-6/7 assays

Salivary HHV-6/7 were regarded as novel markers of physical fatigue. Viral DNA was extracted from 200 \(\mu\text{L}\) of saliva using the QIAamp MinElute Virus Spin Kit (Qiagen Inc.,
Hilden, Germany), according to the manufacturer’s protocol. DNA was eluted in 50 µL of elution buffer. HHV-6/7 DNA copies were quantified via the TaqMan quantitative polymerase chain reaction (qPCR) assay with an Applied Biosystems 7500 fast real-time PCR System (Applied Biosystems Inc., CA, USA). The TaqMan qPCR method was performed with reference to previous reports\textsuperscript{11,12}.

Amplifications were performed in triplicate in a total volume of 10 µL containing 5 µL of premix, TaqPath qPCR Master Mix, CG (Applied biosystems Inc.), 0.04 µL of PCR forward primer (50 µM), 0.04 µL of PCR reverse primer (50 µM), 0.25 µL of TaqMan probe (10 µM), 2 µL of the viral DNA, and 2.67 µL of distilled water. The following primers were used for the qPCR: HHV-6 forward primer, 5\textsuperscript{'}-CGC TAG GTT GAG AAT GAT CGA-3\textsuperscript{'}; HHV-6 reverse primer, 5\textsuperscript{'}-CAA AGC CAA ATT ATC CAG AGC G-3\textsuperscript{'}; HHV-6 probe, 5\textsuperscript{'}-FAM-CAC CAG ACG TCA CAC CCG AAG GAA T-MGB-3\textsuperscript{'}; HHV-7 forward primer, 5\textsuperscript{'}-ATG TAC CAA TAC GGT CCC ACT TG-3\textsuperscript{'}; HHV-7 reverse primer, 5\textsuperscript{'}-CAA AGC CAA ATT ATC CAG AGC G-3\textsuperscript{'}; and HHV-7 probe, 5\textsuperscript{'}-FAM-CAC GGC AAT AAC TCT AG-MGB-3\textsuperscript{'}.

The following run method was utilized: 95°C for 20 sec, followed by 45 cycles of 95°C for 3 sec and 58°C (for HHV-6) or 60°C (for HHV-7) for 30 sec. The salivary HHV-6/7 DNA levels were calculated by multiplying the DNA copy number by the amount of secreted saliva; this value was expressed as $\log_{10}$.
Cortisol assay

Salivary cortisol is considered as a traditional stress marker. The concentration was measured using the commercially available enzyme immunosorbent assay kit (Salivary Cortisol ELISA Kit, Salimetrics LLC, PA, USA), in accordance with the manufacturer’s instructions. The salivary cortisol levels were calculated by multiplying the concentration by the amount of secreted saliva; this value was expressed as ng/min.

SOD assay

Plasma SOD is well known as a responsive marker to oxidative stress; thus, we used it as a supplemental indicator of the physical stressor to determine whether the training loads were sufficient. SOD activity was measured as described previously, with several modifications.

Following four solutions were prepared prior to initiating the experiment: 1) Assay buffer as 50 mM sodium phosphate buffer (pH 8.0), containing 0.1 mM diethylenetriamine pentaacetic acid and 0.1 mM hypoxanthine; 2) WST-1 solution, containing 10 mM WST-1 (Dojindo Inc., Kumamoto, Japan) in distilled water; 3)
Catalase solution, containing catalase (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) 2 mg/mL in 50% glycerol; 4) Xanthine oxidase solution, containing xanthine oxidase (Fujifilm Wako Pure Chemical Corp.) 13.8 mg/mL in 50% glycerol. Just before the assay, 20 mL of reaction mixture-1 was prepared by mixing 19.88 mL assay buffer, 100 µL WST-1 solution, and 20 µL catalase solution. Moreover, 2 mL of reaction mixture-2 was prepared by mixing 1.98 mL assay buffer and 20 µL xanthine oxidase solution.

The first step of the assay involved dispensing 200 µL of the reaction mixture-1 on a 96-well microplate. Next, we added 20 µL of plasma samples (×2 diluted with assay buffer) in duplicates and reaction mixture-2 to the wells in order and mixed. There were three different types of blank wells for the assay; Blank 1, only assay buffer was added instead of plasma sample; Blank 2: any plasma sample was added; Blank 3, assay buffer was added instead of reaction mixture-2. Following incubation at 37°C for 20 min, absorbance at 450 nm was measured by a microplate reader (iMark™ Microplate Absorbance Reader, Bio-Rad Laboratories Inc., CA, USA). The SOD inhibition rate was calculated from each absorbance using the following equation: 

\[
\frac{(\text{Blank 1} - \text{Blank 3}) - (\text{sample} - \text{Blank 2})}{(\text{Blank 1} - \text{Blank 3})} \times 100; \text{the values calculated were expressed as a percentage.}
\]
The POMS was used as a supplemental indicator of psychological stressor. Before conducting the POMS questionnaire, the participants were told how to complete the form. The researchers were present during the entire survey to answer questions from participants and to check for missing items on the form. Moreover, we asked coaches not to come in the room because the athletes might not answer honestly if they were present. After administering this survey, we calculated the total mood disturbance (TMD) score by subtracting the vigor score from the total scores for tension-anxiety, depression-dejection, anger-hostility, fatigue, and confusion.

Statistical analysis

All data were expressed as the mean ± SEM. The statistical analysis was performed using SPSS Statistics version 26 (IBM Corp., NY, USA). A two-way repeated ANOVA was used to analyze the main effects of training and elapsed day and to determine $p$-, $F$-, and partial eta squared ($\eta_p^2$) values. If a significant interaction was found, individual differences were examined using the Bonferroni post-hoc test. For all tests, a value of $p < 0.05$ was considered to indicate a statistically significant difference.
Results

Plasma SOD inhibition rates

Fig. 1 shows the data of the plasma SOD inhibition rates. The two-way repeated ANOVA results indicated that the main effect of training was significant ($p = 0.009$, $F = 9.480$, $\eta^2_p = 0.422$). Conversely, the main effect of elapsed day was not significant ($p = 0.068$, $F = 2.982$, $\eta^2_p = 0.187$). Likewise, the training $\times$ elapsed day interaction, which reflects the differences in the increases between the pre- and post-training results for each of the three days, was not significant ($p = 0.390$, $F = 0.977$, $\eta^2_p = 0.070$).

TMD scores

Fig. 2 shows the data of the TMD scores. The two-way repeated ANOVA results indicated that there was no main effect of training ($p = 0.911$, $F = 0.013$, $\eta^2_p = 0.001$) or elapsed day ($p = 0.416$, $F = 0.908$, $\eta^2_p = 0.065$). The interaction was also not significant ($p = 0.984$, $F = 0.017$, $\eta^2_p = 0.001$).
Salivary HHV-6/7 levels

The detection rates of salivary HHV-6/7 were 100%, except for that of HHV-6 at post-training on the first day (detected in 13 of 14 subjects: 92.9%). Fig. 3 shows the data of the salivary HHV-6/7 levels. In both viral markers, the two-way repeated ANOVA results indicated that the main effect of training was significant \( (p = 0.016, F = 7.596, \eta^2_p = 0.369) \) and \( (p = 0.014, F = 8.011, \eta^2_p = 0.381) \), respectively. Conversely, the main effect of elapsed day was not significant \( (p = 0.451, F = 0.755, \eta^2_p = 0.055) \) and \( (p = 0.773, F = 0.260, \eta^2_p = 0.020) \), respectively. The training × elapsed day interaction was significant for only salivary HHV-7 levels \( (p = 0.422, F = 0.751, \eta^2_p = 0.055) \) and \( (p = 0.012, F = 5.304, \eta^2_p = 0.290) \), respectively, and the Bonferroni post-hoc test showed significant differences between pre- and post-training on days 1 \( (p = 0.010) \) and 2 \( (p = 0.002) \).

Salivary cortisol levels

Fig. 4 shows the data of the salivary cortisol levels. The two-way repeated ANOVA results indicated that the main effect of training was significant \( (p = 0.000, F = 21.347, \eta^2_p = 0.055) \) and \( (p = 0.012, F = 5.304, \eta^2_p = 0.290) \), respectively.
Conversely, the results were not significant for the main effect of elapsed day ($p = 0.084, F = 2.729, \eta_p^2 = 0.174$) or the interaction ($p = 0.570, F = 0.574, \eta_p^2 = 0.042$).

Discussion

We conducted an observational study on Judo athletes during a three-day training camp to investigate the short-term variabilities of salivary HHV-6/7 and cortisol levels. Initially, we considered the stressors that the Judo athletes suffered from during the training camp from both physical and psychological aspects. The plasma SOD inhibition rates increased post-training each day, which showed a significant main effect of training. This indicates that the Judo athletes experienced sufficient physical stressors for this experiment to examine the variabilities of the salivary biomarkers. The training load of each day was likely almost the same because there was no main effect of elapsed day or interaction.

Furthermore, there was no main effect or interaction for the TMD scores. These results suggest that although the physical stressors from training were high, the psychological stressors were not as high in this training camp.

From the above discussion, we can further elaborate on the roles of salivary HHV-
The results showed significant main effect of training in both viral markers. The mechanism behind this reactivating HHV-6/7 may be caused by the training-induced oxidative stress. It is well known that training promotes the production of free radicals, which leads to oxidative stress\textsuperscript{13}. Oxidative stress induces endoplasmic reticulum stress in cells\textsuperscript{15}. The process by which exercise induces endoplasmic reticulum stress and reactivates HHV-6 has been summarized previously\textsuperscript{5}. Although the mechanism of how HHV-7 is reactivated by physical stressors is still unclear, its molecular mechanism of latency and reactivation may be basically the same as those of HHV-6\textsuperscript{16}. In the present study, the SOD inhibition rates, a marker of oxidative stress, increased post-training; accordingly, salivary HHV-6/7 levels also increased. Therefore, these viral markers are considered as valid markers of physical fatigue.

Conversely, there was no main effect of elapsed day. A possible reason for this finding was the short duration of the investigation. Previous studies used experimental durations of 9 or 12 weeks to investigate the changes in salivary HHV-6/7 levels\textsuperscript{9,17}. Thus, a relatively long experimental duration of several weeks may be necessary before resting salivary HHV-6/7 levels increase. A significant interaction was observed only for salivary HHV-7 levels, and the post-hoc test showed significant differences between pre- and post-training on days 1 and 2. Although it was unclear why only day 3 did not show a
significant increase, it might have been affected by an increase in pre-training values. If
so, salivary HHV-7 might reflect fatigue that accumulates in a shorter duration than that
reflected by salivary HHV-6. However, this contradicts Kondo’s assertion that salivary
HHV-7 reflects a longer period of fatigue than HHV-6\textsuperscript{18}). There is actually little evidence
available to judge the duration of fatigue reflected by these viral markers; thus, this topic
must be carefully examined in further studies.

Incidentally, Yamauchi et al. used salivary Epstein–Barr virus (EBV), which
causes upper respiratory tract infections (URTI)\textsuperscript{19}). They reported that decreasing salivary
SIgA due to intensive training was associated with reactivating EBV and symptoms of
URTI. Hence, salivary EBV may also be used as a predictive marker for the deteriorating
condition of athletes. However, HHV-6/7 are considered to have higher responsivity than
EBV or cytomegalovirus (both viruses belong to herpesviridae and are known as HHV-4
and HHV-5, respectively) in terms of fatigue markers\textsuperscript{18}).

By contrast, the salivary cortisol levels decreased post-training. This contradicts
the suggestion of a systematic review stating that salivary cortisol increases post-
training\textsuperscript{3}). There are two possible explanations for this finding: the diurnal variation and
the effect of few physical stressors. It is well known that salivary cortisol levels display
diurnal variation due to circadian rhythms in which they peak in the morning and then
gradually decline\(^{20}\). Salivary cortisol levels have also been shown to be affected differently depending on exercise intensity and modality. Referring to the schemes in the previous study\(^{21}\), the training program during the Judo training camp in the present study seemed most similar to the intensity and modality of the power scheme; thus, it did not affect the salivary cortisol levels. Therefore, it is more reasonable to consider that the decrease in the salivary cortisol levels is due to the limited effect of training on diurnal variation rather than the effect of training itself. In addition, salivary cortisol did not show a significant main effect of elapsed day and interaction. Previous studies had investigated elite swimmers and professional cyclists for 3 days and 3 weeks, respectively\(^{22,23}\) and reported no changes in salivary cortisol levels. Collectively, salivary cortisol may be a less responsive marker than salivary HHV-6/7 to the physical stressors.

The study results allow us to reconsider what types of stressors are reflected in salivary cortisol levels. Salivary cortisol levels are known to be affected by psychological stressors\(^{24}\); however, it was previously unclear whether these levels were more strongly influenced by physical or psychological stressors. Now we believe that psychological stressors have a stronger effect on salivary cortisol levels. This idea is supported by some evidence. Salivary cortisol levels can be increased by an experimentally loaded simple psychological stressor\(^{25}\) and by sports situations with a small physical stressor and a large
psychological stressor, such as a golf competition\(^{26}\). Although some reports that loaded
the physical stressors showed that salivary cortisol levels were only increased by high-
intensity exercise\(^{21,27}\), it can be inferred that such an intensive exercise is accompanied
with the psychological stressor. Additionally, the results of the present study in which
salivary cortisol levels did not change even though physical stressors were loaded may be
due to the lower psychological stressors.

Further studies should address two issues due to the limitation of this study. One
is that the specificity must be examined. In this study, the effect of physical stressors on
salivary HHV-6/7 levels was examined, but the effect of psychological stressors was not.
To confirm that salivary HHV-6/7 are specific markers for physical stressors, it must be
clarified psychological stressors have no effect on salivary HHV-6/7 levels. The other
issue is related to performance. The relationship between salivary HHV-6/7 levels and
cognitive performance has already been examined\(^{28}\), but the relationship with physical
performance is unknown. Due to the difficulty in evaluating Judo performance, it was
unable to describe to what extent performance was actually reduced in this study. Hence,
the correlation between salivary HHV-6/7 levels and athletic performance in sports such
as track and field and swimming, in which fatigue is more simply reflected in performance,
must be investigated.
In conclusion, we investigated the short-term variabilities of salivary HHV-6/7 and cortisol levels during a three-day training camp in Judo athletes. As a result, salivary HHV-6/7 levels increased after training, whereas salivary cortisol levels decreased. These findings suggest that the microbiological markers may have a relatively high sensitivity to physical stressors. In particular, salivary HHV-7 might reflect more short-term accumulated physical fatigue than that reflected by salivary HHV-6. Future studies on salivary HHV-6/7 should examine its relationship with psychological stressors and performance.

Acknowledgments

We are grateful to the Japan Sport Council for supporting this research. We would like to thank Enago (www.enago.jp) for the English language review.

Conflict of Interest

The authors declare that they have no potential conflict of interest.

Author Contributions

ST and HH conceived and designed the research. ST, HH, KS, KM, and DH conducted
the investigation. ST, KA, KY, and TS performed the assays and analyzed the data. ST, HH, KS, KM, and DH interpreted the results of the experiments. ST drafted the manuscript. HH, KS, KM, and DH edited and revised the manuscript. KT and KW have carefully supervised the manuscript preparation and any revisions. All authors approved the final version of the manuscript.

References


Table 1. Characteristics of the participants.

Table 2. Training program.

Fig. 1. Variability of the plasma SOD inhibition rates.

Fig. 2. Variability of the TMD scores.

Fig. 3. Variabilities of the salivary HHV-6/7 levels. The results of the post-hoc test showed

\[ *p < 0.05 \]

in the comparison between pre- and post-training.

Fig. 4. Variability of the salivary cortisol levels.
Table 1. Characteristics of the participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>19.9 ± 1.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.4 ± 7.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.1 ± 18.7</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.3 ± 4.4</td>
</tr>
<tr>
<td>Rank in Judo (Dan)</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>15 min</td>
<td>Warm up</td>
</tr>
<tr>
<td>8 min</td>
<td>Standing techniques</td>
</tr>
<tr>
<td>20 sec × 5</td>
<td>Quick driving drill</td>
</tr>
<tr>
<td>5 min</td>
<td>Free practice</td>
</tr>
<tr>
<td>10 min</td>
<td>Throwing techniques</td>
</tr>
<tr>
<td></td>
<td>Lunch break</td>
</tr>
<tr>
<td>15 min</td>
<td>Warm up</td>
</tr>
<tr>
<td>10 min</td>
<td>Standing techniques</td>
</tr>
<tr>
<td>5 min</td>
<td>Free practice</td>
</tr>
<tr>
<td>3 min</td>
<td>Rest</td>
</tr>
<tr>
<td>30 min</td>
<td>Taking Ippon practice</td>
</tr>
<tr>
<td>3 min</td>
<td>Rest</td>
</tr>
<tr>
<td>Free time</td>
<td>Ground techniques</td>
</tr>
</tbody>
</table>
Training: $p = 0.009$
Elapsed day: $p = 0.068$
Interaction: $p = 0.390$
Training: $p = 0.911$
Elapsed day: $p = 0.416$
Interaction: $p = 0.984$
Training: $p = 0.000$
Elapsed day: $p = 0.083$
Interaction: $p = 0.570$