

# Effects of different periods of rapid weight loss on dehydration and oxidative stress

## Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Manuscript Preparation
- E** Funds Collection

**Mio Nishimaki**<sup>1ABCDE</sup>, **Hiroki Tabata**<sup>2ABCD</sup>, **Masayuki Konishi**<sup>3ABCD</sup>, **Stefan Pettersson**<sup>4CD</sup>, **Shizuo Sakamoto**<sup>3ABD</sup>

<sup>1</sup> Department of Sport Science, Japan Institute of Sport Sciences, Tokyo, Japan

<sup>2</sup> Graduate School of Sport Sciences, Waseda University, Saitama, Japan

<sup>3</sup> Faculty of Sport Sciences, Waseda University, Saitama, Japan

<sup>4</sup> Department of Food and Nutrition, and Sport Science, University of Gothenburg, Gothenburg, Sweden

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## Abstract

### Background and Study Aim:

Many athletes will lose weight 5% or more within 7 days. Many reports have been published on the negative health effects of rapid weight loss (RWL) in wrestlers. This study aim was the effects of different periods of RWL on dehydration state and oxidative stress.

### Materials and Methods:

Participants were nine male collegiate wrestlers who reduce their body mass by 5% within short period in randomized order using the same methods. They have experienced 1-day, 3-days and 7-days) weight loss separated by more than 4 weeks. All participants reduced 5% of their body mass in all trials. Following the weight loss, they tried to regain all of their lost weight with an ad libitum diet for 14 h. Body composition and biochemical variables were measured at baseline and immediately after weight loss and weight regain.

### Results:

There were no statistically significant differences in hematocrit, serum sodium, chloride, potassium, calcium, osmotic pressure, and antidiuretic hormone. For plasma aldosterone concentrations and plasma d-ROMs concentrations, two-way analysis of variance revealed the main effect of time ( $p < 0.05$ ). RWL (loss of 5% of body weight within 7 days) is surmised to have increased oxidative stress via dehydration and elevated levels of aldosterone.

### Conclusions:

Although different weight loss periods did not yield any changes, RWL of 5% of body weight was suggested to increase oxidative stress. It is necessary to study the influence of weight loss cycling on athlete's disease risk in the future.

### Keywords:

aldosterone • dehydration • body composition • oxidative stress • rapid weight loss

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### Conflict of interest:

Authors have declared that no competing interest exists

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The study was approved by the ethics committee of Waseda University (2013-272)

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### Author's address:

Mio Nishimaki, Department of Sports Science, Japan Institute of Sports Sciences, Tokyo, 3-15-1 Nishigaoka, Kita-ku, Tokyo 115-0056, Japan; e-mail: mionishimaki@gmail.com

**Wrestling** – *noun* a sport in which two contestants fight by gripping each other using special holds, each trying to force the other's shoulders onto a mat [31].

**Weight loss** – *noun* the fact of losing weight or of becoming thinner [31].

**Weight-loss plan** – *noun* a scheme to reduce body weight, usually by reducing calorie intake, increasing physical activity or a combination of both [31].

**Weight reduction** – *noun* same as **weight loss** [31].

**Fluids** – *plural noun* all liquids that rehydrate the body, including water, cordials, fruit juice, tea and coffee [31].

**Oxidative stress** – *noun* damage to cells caused by free radicals produced in aerobic metabolism [31].

**Dehydration** – *noun* a dangerous lack of water in the body resulting from inadequate intake of fluids or excessive loss through sweating, vomiting or diarrhoea [31].

**Antioxidant** – *noun* a substance that makes oxygen less damaging, e.g. In the body or in foods or plastics [31].

**Aldosterone** – *noun* a hormone, secreted by the adrenal gland, that regulates the balance of sodium and potassium in the body and the amount of body fluid [31].

**Ad libitum** – *adjective* used for referring to food intake that is not controlled by a strict nutritional plan [31].

**Body composition** – athlete's predisposition in terms of the proportion of active mass and inactive mass in the body.

**Adherence** – *noun* the act of sticking to a routine or programme [31].

**dROMs** – test have a unimodal distribution that picks between 250 and 300 CARR U (i. e. between 20 and 24 mg/dL H<sub>2</sub>O<sub>2</sub>). Units of measurement are indicated with the initials "CARR U", i. e. Carratelli Units, by the name of the Italian research chemist Mauro Carratelli. Carratelli Units (CARR U): 1

## INTRODUCTION

To create an equal playing level regarding physical strength and body mass combat sports athletes such as wrestlers or judokas must qualify for competition by weighing-in at a designated weight category the day before competition. However, in an attempt to alter this power to weight ratio, athletes often engage in pre-competition rapid weight loss (RWL) where the majority of weight loss usually takes place 1 to 7 days prior to official weigh-in [1]. To reach their weight target, athletes use different passive, e.g., reduced food and fluid intake and/or fluid deprivation by heat exposure [2, 3], or active, e.g., increased exercise [4-6], strategies. Most of these RWL strategies affect body water and electrolyte stores, which have been reported to alter a number of physiological functions crucial to athletic performance and health including thermoregulation [7], cardiovascular functions and metabolism [8].

Oxidative stress, defined as an imbalance between production of reactive oxygen species (ROS) and antioxidant defense, increases acutely during exercise and periods of intensified training [9]. Although acute exercise-induced ROS production yields positive adaptive responses for athletes, excessive oxidative stress is also known to initiate damage on endogenous proteins, lipids, and DNA, and oxidative stress is possibly involved in the development of diabetes, cancer, and neurological disorders [10, 11].

Furthermore, Paik et al. [12] observed an increase in oxidative stress following moderate dehydration (3% BM loss via thermal stress), and this effect was further augmented by subsequent exercise. Other investigators reported that aldosterone, which is involved in the regulation of body sodium and water homeostasis, causes expression of nicotinamide adenine dinucleotide + hydrogen oxidase, leading to increased production of ROS causing oxidative stress [13-15]. Collectively, considering that weight category athletes often combine active and passive dehydration strategies suggests that RWL practices might amplify ROS production. Yanagawa et al. [16] supports this contention, demonstrating increased oxidative stress in wrestlers following a 12-day weight loss. However, to the extent to which oxidative stress is influenced by the dynamics of the RWL is currently unknown.

This study aims to investigate the effects of different periods of RWL on dehydration state and oxidative stress.

## MATERIAL AND METHODS

### Participants

Nine competitive male collegiate wrestlers aged 20 ± 2 years; height: 167.1 ± 4.3 cm; body mass (BM) 69.6 ± 7.5 kg; and body mass index (BMI) 24.9 ± 2.0 kg/m<sup>2</sup> with previous experience of RWL (>5% of BM loss at least 5 times/season over the last 3 years) was recruited. All participants provided written informed consent and undertook a medical examination for cardiovascular and metabolic diseases before commencing the first experimental trial. The study was performed in accordance with the Declaration of Helsinki 2008 and was approved by the ethics committee of WASEDA University (2013-272).

### Experimental design

The experimental design is illustrated in Figure 1. Each participant completed three experimental trials where they were asked to reduce their BM by 5% within 25 h (1 day), 73 h (3 days), or 169 h (7 days) as they would in an actual competition. Respective trial consisted of three visits to the laboratory where assessment of body composition and blood sample collection was performed in a fed and euhydrated state (baseline, T1), after the 1-, 3-, and 7-day rapid weight loss regimen (T2) and following 16 h of recovery (T3) with ad libitum food and fluid consumption. Each trial was separated by 4 weeks in a randomized cross-over design.

Twenty-four hours before T1, the participants were instructed to refrain from alcohol, caffeine, and high-fat foods and not to perform vigorous exercise. Before arrival to the laboratory, participants consumed a standardized lunch consisting of 840 kcal, and 126, 31.5, and 23.3 g of carbohydrates, protein, and fat, respectively. Following T1, participants received instructions to reduce BM by 5, 0.7, and 1.7% BM per day in the 1-, 3-, and 7-days weight loss trial, respectively, and the first author monitored adherence to the weight loss plan on a daily basis. One week before the first baseline measurement (T1) throughout the whole study period, participants were instructed not to consume any drugs or dietary supplements that could influence metabolism, hydration, or vitamin/mineral status.

### Anthropometric and body composition assessment

At T1, T2, and T3, BM was measured using an electronic scale (InnerScan, BC-520, TANITA, Japan). After height and BM measurements, lean

body mass (LBM), intracellular fluid (ICF), extracellular fluid (ECF), and total body water (TBW) were estimated by bioelectrical impedance method (InBody 710, Biospace Co, Japan). BIA measurements were performed according to the manufacturer's recommendation.

**Biochemical variables**

Venous blood samples were collected before (baseline, T1) and after rapid weight loss (RWL, T2) and following the 16-h weight regain period (recovery 16-h, T3). Samples were allowed to clot for 30 min at room temperature and then centrifuged at 3500 rpm for 10 min to measure serum and plasma blood markers. Obtained serum was dispensed into plain micro tubes and stored at -80°C until the assay. To measure plasma blood markers, blood samples collected in tubes containing ethylenediaminetetraacetic acid (EDTA) were immediately centrifuged and stored at -80°C until the assay.

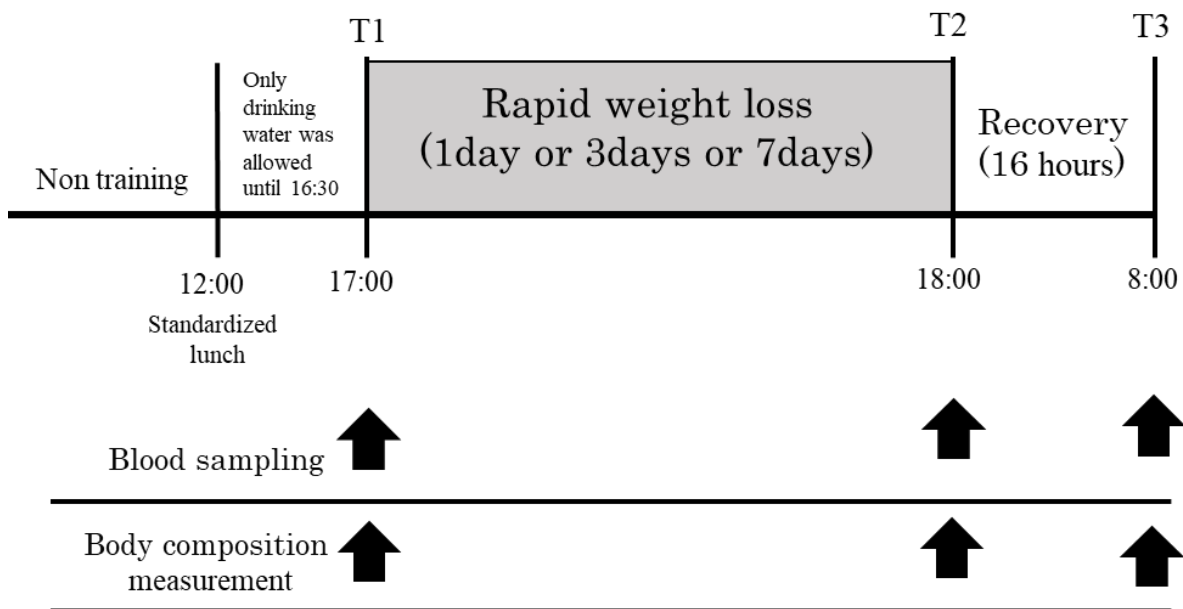
Hematocrit (HCT) was determined in EDTA-treated venous blood using an automatic blood cell counter (pocH-100i; Sysmex, Kobe, Japan). Serum concentrations of derivatives of reactive oxygen metabolites (reactive oxygen metabolite-derived compound [dROMs; see glossary] test) and the biological antioxidant potential (BAP test) were measured using assay kits

from Diacron (Milan, Italy). Serum osmotic pressure (Osm), sodium (Na), chloride (Cl), potassium (K), calcium (Ca), plasma antidiuretic hormone (ADH), and aldosterone (ALD) were analyzed by SRL, Inc. (Tokyo, Japan). Plasma concentrations of thiobarbituric acid-reactive substances (TBARS) and activities of superoxide dismutase (SOD) were measured using assay kits from Cayman Chemicals. Changes in serum and plasma volumes during the acute bout of exercise were calculated using a previously outlined method [17] and were used for the collection of blood markers.

**Statistical analysis**

First, the normal distribution of all data was checked using the Kolmogorov-Smirnov test. All data are presented as a mean and standard deviation (± or SD). Two-way (time × trials) analysis of variance (ANOVA) and post hoc analyses with Bonferroni's test were used to compare dependent variables at T1, T2, and T3. P values <0.05 were taken to indicate statistical significance. Additionally, Cohen d effect sizes (ES) were reported when appropriate. Magnitudes of ES were classified as: <sup>a</sup>trivial (0–0.19), <sup>b</sup>small (0.20–0.49), <sup>c</sup>medium (0.50–0.79) and <sup>d</sup>large (≥0.80) [18]. Data analysis was performed using IBM SPSS Statistics 24 software (SPSS Japan Inc.).

CARR U corresponds to 0.08 mg/100 ml H<sub>2</sub>O<sub>2</sub>. Oxidative stress level (CARR U): 300-320 borderline range; 321-340 low level; 341-400 mid-level; 401-500 high level; >500 very high level. Normal (average) range: 250-300 CARR U [32].



**Figure 1.** Study design.

## RESULTS

### Pretrial measurements

The participants successfully reached their relative RWL target (5%) following the 1, 3, and 7 trials ( $5.06 \pm 0.51$ ,  $4.95 \pm 0.52$ , and  $4.91 \pm 0.34\%$ , respectively) and BM, LBM, ICF, ECF, and TBW were significantly reduced at T2 compared to baseline values across trials (Table 1). Moreover, BM, LBM, TBW, ICF, and ECF values at T3 were significantly higher than those T2 all trials. There were no statistically significant differences in HCT, Osm, and levels of Na, Cl, K, Ca, ADH, and ALD. Two-way ANOVA revealed the main effect of time ( $p < 0.05$ ) (Table 2) for HCT, Na, Cl, K, Osm, and plasma ALD concentrations. Moreover, Na, Osm, and ALD concentrations at T2 were significantly higher than those at T1 during all trials. The Na and ALD concentrations at T3 were significantly higher than those at T1 during all trials. The HCT level, Cl, and K concentrations at T3 were significantly lower than those at T2 during all trials (Table 2).

### Plasma concentrations of markers of oxidative stress and antioxidant capacity

No statistically significant differences in plasma concentrations of d-ROM, BAP (Figures 2 and 3),

and TBARS (Table 3) were found. Two-way ANOVA revealed a main effect of time ( $p < 0.05$ ) (Figure 2) for plasma d-ROMs concentrations. There were significant differences in activities of SOD: in the 1-day trial were also increased in at T1 compared to those at T2 and T2 to T3 ( $p < 0.05$ ). Activities of SOD in the 7-days trial were decreased at T1 compared to that at T2 ( $p < 0.05$ ) (Table 3).

## DISCUSSION

A 5% loss of body weight did not affect the blood electrolyte concentrations but led to a significant increase in the Osm levels and Na and ALD concentrations. In addition, although a 5% loss of body weight increased oxidative stress, there was no difference in oxidative stress between the different periods of RWL. These results demonstrate that differences in the RWL periods do not affect the electrolyte concentrations or oxidative stress of wrestlers.

No significant changes in the blood concentrations of K, Cl, and Ca before, during, and after weight loss were observed. However, there was

**Table 1.** Changes of body composition in time function (baseline, T1; RWL, T2; recovery 16-h, T3) during experiment RWL in wrestlers (n = 9).

Variable (kg)	Trial (day/days)	T1	T2	T3	P			Effect size
		Mean SD	Mean SD	Mean SD	T1 vs T2	T1 vs T3	T2 vs T3	
BM	1	68.5 ± 7.9	65.1 ± 7.5	67.5 ± 7.4	0.000	0.000	0.000	<sup>d</sup> 0.969
	3	69.0 ± 8.2	65.6 ± 7.7	67.5 ± 7.7				
	7	68.3 ± 8.2	65.0 ± 7.7	67.2 ± 8.0				
LBM	1	59.9 ± 6.9	57.6 ± 6.4	59.1 ± 6.6	0.000	0.000	0.000	<sup>d</sup> 0.960
	3	59.8 ± 6.7	57.6 ± 6.6	58.9 ± 6.0				
	7	59.9 ± 6.8	57.5 ± 6.4	59.0 ± 6.9				
TBW	1	45.2 ± 4.1	43.3 ± 3.8	44.4 ± 4.1	0.000	0.000	0.001	<sup>d</sup> 0.958
	3	44.9 ± 3.8	43.2 ± 4.1	44.0 ± 3.7				
	7	45.0 ± 4.1	43.1 ± 3.8	44.3 ± 4.3				
ICF	1	28.4 ± 2.6	27.5 ± 2.6	28.0 ± 2.8	0.000	0.000	0.000	<sup>d</sup> 0.933
	3	28.2 ± 2.4	27.3 ± 2.6	27.8 ± 2.4				
	7	28.3 ± 2.7	27.3 ± 2.6	27.9 ± 2.8				
ECF	1	16.8 ± 1.5	15.9 ± 1.2	16.4 ± 1.4	0.000	0.000	0.004	<sup>d</sup> 0.962
	3	16.7 ± 1.5	15.9 ± 1.5	16.2 ± 1.4				
	7	16.7 ± 1.4	15.8 ± 1.2	16.4 ± 1.5				

**BM** body mass; **LBM** lean body mass; **TBW** total body water; **ICF** intracellular fluids; **ECF** extracellular fluids. Magnitudes of ES <sup>d</sup>large ( $\geq 0.80$ ) effect size between time.

**Table 2.** Changes of concentrations of hematological indicators during experiment RWL in wrestlers (n = 9).

Variable (indicator)	Trial (day/days)	T1	T2	T3	P			Effect size	Reference range
		Mean SD	Mean SD	Mean SD	T1 vs T2	T1 vs T3	T2 vs T3		
HCT (%)	1	45.9 ± 2.6	47.0 ± 2.6	44.6 ± 2.2					
	3	46.9 ± 2.4	47.0 ± 2.5	45.5 ± 1.4			0.010	<sup>b</sup> 0.497	39.8 – 51.8
	7	47.1 ± 2.3	48.3 ± 2.7	45.0 ± 2.6					
Na (mEq/L)	1	141.8 ± 1.6	142.7 ± 1.6	141.1 ± 1.7					
	3	140.7 ± 1.6	142.4 ± 1.4	141.2 ± 1.5	0.005	0.010		<sup>c</sup> 0.605	136 – 147
	7	140.9 ± 1.8	141.7 ± 1.1	141.3 ± 0.9					
Cl (mEq/L)	1	105.3 ± 1.6	98.7 ± 12.1	113.1 ± 17.0					
	3	103.1 ± 1.5	103.5 ± 12.1	107.8 ± 14.1			0.025	<sup>b</sup> 0.382	98 – 109
	7	103.8 ± 1.6	101.0 ± 17.0	116.2 ± 22.2					
K (mEq/L)	1	4.1 ± 0.2	4.1 ± 0.5	4.6 ± 0.6					
	3	4.2 ± 0.3	4.3 ± 0.5	4.3 ± 0.6			0.043	<sup>b</sup> 0.373	3.6 – 5.0
	7	4.2 ± 0.3	4.2 ± 0.7	4.7 ± 0.9					
Ca (mg/dL)	1	9.3 ± 0.3	9.2 ± 1.2	10.2 ± 1.3					
	3	9.4 ± 0.4	9.8 ± 1.0	9.8 ± 1.2				<sup>b</sup> 0.291	8.5 – 10.2
	7	9.4 ± 0.3	9.4 ± 1.4	10.2 ± 1.9					
Osm (mOSM/Kg)	1	290.2 ± 8.2	294.0 ± 9.2	291.4 ± 8.9					
	3	287.6 ± 5.3	291.0 ± 7.0	288.0 ± 6.3	0.020			<sup>c</sup> 0.706	276 – 292
	7	286.8 ± 6.6	289.9 ± 6.5	288.2 ± 7.8					
ADH (Pg/mL)	1	3.4 ± 2.5	3.5 ± 1.5	4.5 ± 3.0					
	3	4.2 ± 2.7	5.9 ± 4.3	5.5 ± 3.5				<sup>b</sup> 0.207	<3.8
	7	3.6 ± 2.5	4.0 ± 2.1	4.5 ± 3.8					
ALD (Pg/mL)	1	152.5 ± 74.4	219.6 ± 107.0	214.3 ± 89.1					
	3	142.9 ± 34.6	233.3 ± 61.9	265.6 ± 89.0	0.001	0.036		<sup>c</sup> 0.605	35.7 – 240
	7	155.8 ± 72.5	296.3 ± 119.1	258.9 ± 164.1					

**HCT** hematocrit; **Na** sodium; **Cl** chloride; **K** potassium; **Ca** calcium; **Osm** osmotic pressure; **ADH** antidiuretic hormone; **ALD** aldosterone. Magnitudes of ES: <sup>b</sup>small (0.20–0.49), <sup>c</sup>medium (0.50–0.79) effect size between time.

a significant reduction in the Na and ALD concentrations, Osm level, and TBW after weight loss, suggesting that RWL resulted in dehydration. A previous study found that weight loss before a real competition significantly increased blood sodium concentrations and reduced blood potassium concentrations[19]; the results of this study are the same as those of previous studies. The level of HCT showed no alteration although the body water was significantly decreased after RWL [19], but several studies confirmed that the HCT does not necessarily increase during weight reduction.

In the present study, no changes in electrolyte levels were observed. The Na and ALD concentrations and Osm level after weight regain were significantly higher than those immediately after

weight loss in all trials. Therefore, the TBW decreased significantly because of the RWL of 5% of the body weight. The amounts of ICF and ECF were comparable. ALD promoted reabsorption of Na in the kidneys, possibly increasing the amount of Na in the blood. These results indicated that ALD produced by the adrenal cortex regulated electrolyte and body fluid volumes, thereby maintaining homeostasis. Differences attributed to the weight loss period were not recognized.

In the present study, RWL of 5% of body weight was suggested to increase oxidative stress. A previous study involving male collegiate wrestlers examined the effects of weight loss on oxidative stress 12 days prior to a competition. In that study, urinary 8-OHdG and urinary biopyrin levels, both of which are oxidative stress markers,

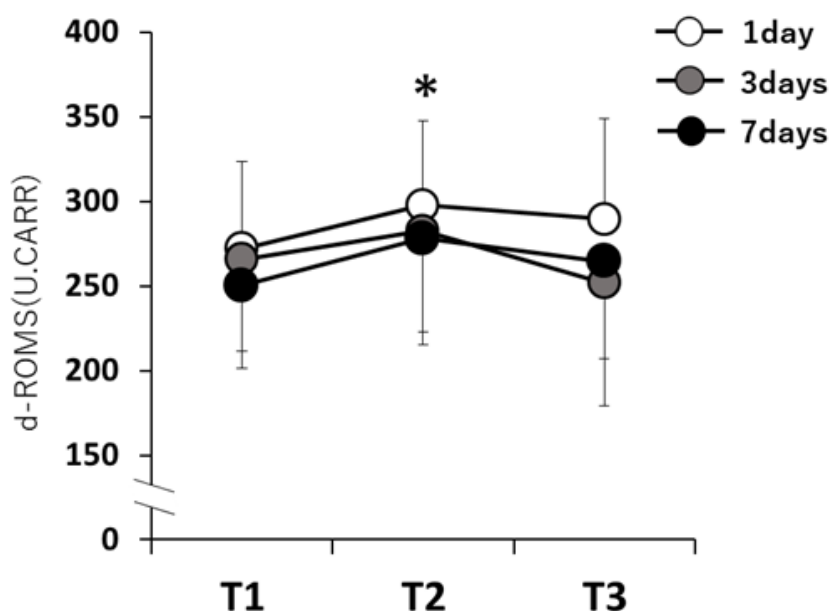
**Table 3.** Changes of oxidative stress and antioxidant capacity during experiment RWL in wrestlers (n = 9).

Variable (indicator)	Trial (day/days)	T1	T2	T3	P				Effect size	Reference range
		Mean±SD	Mean±SD	Mean±SD	T1 vs T2	T1 vs T3	T2 vs T3	Interaction		
<b>SOD (%)</b>	1	12.1±1.6	13.0±1.8	11.9±1.4	0.003		0.007	0.002	<sup>b</sup> 0.403	6.4 – 12.8
	3	12.0±2.2	12.1±1.8	11.8±1.6						
	7	12.9±0.7	11.5±1.1	12.1±0.6	0.038					
<b>TBARS (µM)</b>	1	0.6±0.5	1.3±1.0	0.9±0.6					<sup>b</sup> 0.255	<2.5
	3	0.7±0.4	1.4±0.9	1.3±1.2						
	7	0.7±0.5	1.1±1.0	1.3±1.5						

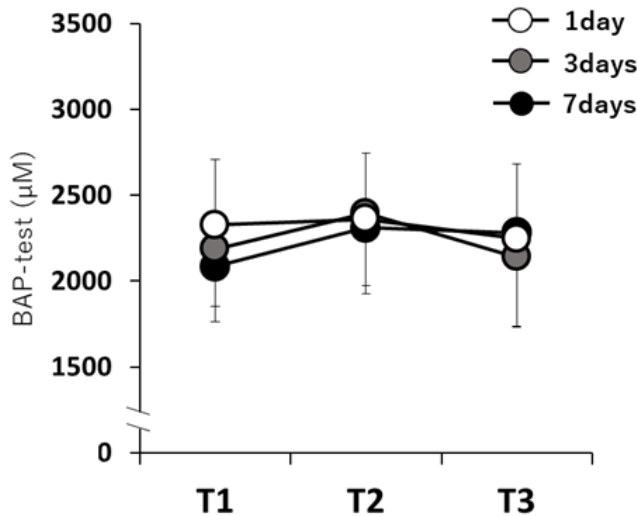
SOD superoxide dismutase; TBARS malondialdehyde. Magnitudes of ES <sup>b</sup>small (0.20–0.49) effect size between time.

were significantly higher on weigh-in day than they were 12 days before weigh-in [16]. Similarly, in this study, oxidative stress marker levels in the blood increased after weight loss. Although the 12-day weight loss period reported in the previous study was longer than the weight loss period in the present study, the point when the wrestlers

in the previous study actually began losing weight is unclear. In addition, because the wrestlers in the previous study were weighed before an actual competition, the amounts of weight loss were inconsistent. In the present study, all participants lost 5% of their body weight; by standardizing the amount of weight the participants lost



**Figure 2.** Serum d-ROMs (U.CARR) of serum samples taken at T1, T2, T3 during 1day, 3days, 7days (mean values and SD; \*significantly different from T1).



**Figure 3.** Serum BAP-test ( $\mu\text{M}$ ) of serum samples taken at T1, T2, T3 during 1day, 3days, 7days (mean values and SD).

per day, we were able to compare the oxidative stress response and dehydration based on the weight loss period. In the present study, although RWL of 5% of the body weight increased oxidative stress, dynamics of the RWL had no effect on weight loss periods. The results of the present study suggested that a 5% loss of body weight within 7 days increased oxidative stress regardless of the weight loss period. There was no significant difference in T3 compared to T2. It was suggested that increased oxidative stress does not return by 16 h recovery by free eating after weight loss.

Environmental factors such as hyperthermia and dehydration increase ROS production [20]. The increase in oxidative stress after weight loss in the present study may have been achieved because the weight was lost via dehydration. Although the present study did not designate a weight loss method, most participants lost 5% of their body weight by fasting, using saunas, and exercising while wearing a rubber/plastic suit. These weight loss methods were found to cause dehydration and significantly reduce TBW. Reduced TBW leads to reduced total body Na and Cl levels, resulting in the secretion of aldosterone from the adrenal cortex [21]. ALD promotes renal Na reabsorption and K excretion [22], and it acts on

the myocardium and vascular smooth muscle, thereby increasing oxidative stress [23, 24]. In the present study, blood ALD levels were significantly higher after weight loss. RWL (5% loss of the body weight within 7 days) was surmised to have increased oxidative stress via dehydration and elevated ALD levels.

Although oxidative stress markers increased following 5% RWL, there were no significant changes in levels of antioxidant markers such as BAP. However, there were significant differences in activities of SOD. Moderate oxidative stress is known to induce antioxidant enzymes and activate metabolism of endogenous antioxidants [25, 26]. SOD is the first antioxidant barrier among antioxidant enzymes [27]; however, in the present study, the antioxidant marker BAP did not demonstrate any significant changes. These results signify that RWL involving dehydration, even if the weight loss is less than 5% of body weight, may have negative effects (hyponatremia, decreased thermoregulatory function, muscle spasm) on the body. Furthermore, many wrestlers rapidly lose much more than 5% of their body weight prior to competitions; therefore, the results of the present study suggest that at weigh-in, these wrestlers are experiencing high levels of oxidative stress as well as reduced



antioxidant activity. In the future, it will be necessary to compare the effects of different rates of weight loss on wrestlers' bodies.

There were several limitations to the present study. First, the number of participants was small and the weight class was limited. Future examinations of the effects of RWL on oxidative stress will need to include participants in other weight classes, such as heavyweight wrestlers. Second, the rate of weight loss was low. This study involved consistent weight loss of 5%; however, for real-world competitions, many wrestlers reduce their body weight by more than 5% [28, 29]. Therefore, it is necessary to study the effects of RWL of  $\geq 5\%$  on oxidative stress and antioxidant activity. Third, this study did not examine the participants' diets during RWL. Because of excessive caloric restriction, the intake of nutrients during RWL is likely extremely low. However, vitamins and other micronutrients used by wrestlers may affect the antioxidant markers [30]. Therefore, future studies should include diet surveys.

This study compared the effects of different periods of RWL of 5% of body weight on oxidative stress and dehydration for male collegiate

wrestlers. Although different weight loss periods did not yield any changes, RWL of 5% of body weight was suggested to increase oxidative stress.

## CONCLUSIONS

This study results suggested that a RWL of 5% of body weight increases oxidative stress regardless of difference in time. As for the electrolyte concentration, no significant change due to weight loss was observed. Athletes perform this weight loss program several times a year, why repeated excessive oxidative stress conditions increase the risk of various diseases. For these reasons, it is necessary to study the influence of weight loss cycling on athlete's disease risk in the future. This study will help to prepare effective weight reduction guidelines for conditioning and improving performance of combat sports athletes.

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