

The Effect of Repeated Intensive Pulls of Tug-of-War on Immunoendocrine Responses

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Abstract

The aims of this study were to determine the effect of repeated intensive pulls of tug-of-war on the same day on leukocyte mobilization, neutrophil functions, stress hormones, Th1/Th2 cytokine balance, and salivary responses. Eight elite tug-of-war pullers (age 16.5 ± 0.3 years, height 173.6 ± 1.4 cm, body mass 69.2 ± 2.3 kg, VO_{2max} 46.4 ± 0.7 mL·kg⁻¹·min⁻¹) were voluntarily recruited from a senior high school. After passing a health questionnaire screen, subjects signed an informed consent. Visit 1 required the subject to come for measurement of physiological variables. Four days later, subjects participated in either exercise trial (EX) or control trial (REST) by a counterbalanced order. EX required the subject to pull 6 sets consisting of 2 pulls with 5 min and 1.5 min rest in between, respectively. The pulling weight was 1.5 folds of body mass. Blood sampled at pre-exercise and immediate post-exercise, whereas saliva sampled at pre-exercise, post-set 2, post-set 4, and immediate post-exercise. REST required the subject to have a resting day in the gymnasium and offering blood and saliva samples at the same timepoints as EX. Water ingestion was allowed ad libitum during trial except for 5 min before saliva sampling. The main findings of this study were: (1) Pulling endurance was significantly decreased in the second pull compared with the first pull at Set 1, Set 2 and Set 3; furthermore, the pulling endurance was also significantly declined after Set 2 in both pulls compared with Set 1. (2) Repeated intensive pulls (RIP) significantly activated hypothalamic-pituitary-adrenal (HPA)-axis with higher concentrations of both plasma and salivary cortisol and lower saliva flow rate in exercise trial compared with the values in rest trial. (3) RIP significantly increased plasma lactate concentrations. (4) RIP did not appear to affect hematocrit, hemoglobin, plasma glucose, leukocyte mobilization, neutrophil functions, plasma Th1/Th2 cytokine balance, and salivary immunoglobulin A (IgA) and IgA1 concentration and their secretion rates. In conclusion, the findings of the present study suggest that the repeated intensive pulls of tug-of-war may induce activations of HPA-axis and sympathetic nervous system. However the extents of activations appear not strong enough to extensively disturb homeostasis of immunoendocrine. Furthermore, the heart rate may not be an appropriate indicator for assessing exercise intensity in relatively static exercise of tug-of-war.

Keywords: Tug-of-war, Stress hormones, Neutrophil function, Th1/Th2 cytokines, IgA

Introduction

For recent decades, exercise has been considered as a stressor manipulated to investigate immune responses via different duration, intensity, and frequency. [1]. Regular moderate intensity exercise has been linked with the reduced susceptibility to infection, reduced severity of infection, and improved antibody responses to vaccine [2-4]. However, prolonged strenuous exercise has been shown to affect the circulating numbers and functions of immune cells [5, 6] and

immune function [7-11]. These effects are thought to be largely mediated by the actions of elevated levels of circulating stress hormones (e.g. adrenaline, cortisol and growth hormone) and altered regulatory cytokines [12].

Immunity against microorganisms at remote sites, such as the nasal cavity, oral cavity, respiratory tract, digestive tract and gut, is primarily due to secretory immunoglobulin A (IgA), which has been considered as the first line of defence to infection in the lumen of the respiratory tract and gut [13]. Secretory IgA is produced by local plasma cells and function as a multi-layered mucosal defense. For example, IgA prevents antigens and microbes from adhering to and penetrating the epithelium (immune exclusion), interrupts replication of intracellular pathogens during transcytosis through epithelial cells (intracellular neutralization), and binds antigens in the

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with increased susceptibility to infection and reduced

lamina propria facilitating their excretion through the epithelium back into the lumen (immune excretion) (Lamm, 1998). Hence, salivary IgA (sIgA) has been used to be a key indicator in determining the effect of different forms of stress on mucosal immunity.

Neutrophils, which represent 50-60% of the total blood leukocytes, serves as a last line of defence to block the "open window" during the period of immunodepression after intensive prolonged exercise [14]. The neutrophil is an effective phagocyte and is considered to be an important part of innate immunity, playing a critical role in the host defence against various bacterial infections [3]. Following the activation and recognition, neutrophils kill invading pathogens through both oxygen-dependent (release of reactive oxygen species, ROS) and oxygen-independent (release of proteases) mechanisms [15, 16]. An impaired neutrophil microbicidal capacity may increase the susceptibility to infection in stressed athletes [15].

Lymphocytes are continuously recruited into the circulation from the onset of exercise and are subsequently exiled from circulation after about 90 minutes during exercise [7]. Therefore, the circulating lymphocytes is declined below pre-exercise values in the post-exercise period after prolonged strenuous exercise [5, 6]. However, it has not been well established whether all T lymphocyte subsets are affected to the same extent. Strenuous exercise seems to temporarily suppress antiviral defense through the declines in antigen-specific Th1 cytokine production (interleukin-2 and interferon- γ), T cell proliferation to antigen, natural killer cell function, and the alveolar/peritoneal cytokine production that may result in a decreased resistance to respiratory viral infection [17-19].

The CD4+ T helper cells can be divided into Th1 and Th2 according to their cytokine profile. Th1 cells mainly secrete interleukin-2 (IL-2), interferon- γ (IFN- γ), and tumor necrosis factor β (TNF- β), which promote cellular immunity; Th2 cells primarily secrete IL-4, IL-5, IL-10, and IL-13, which promote humoral immunity [20]. The results of previous studies have strongly suggested that stress hormones differentially regulate Th1/Th2 patterns and type 1/type 2 cytokine secretion [21]. Glucocorticoids has been proven to inhibit the production of IL-12, IFN- γ , IFN- α , and TNF- α by antigen-presenting cells and Th1 cell [21], but up-regulate the production of IL-4, IL-10 and IL-13 by Th2 cells, which may systemically cause a selective suppression of the Th1-cellular immunity axis and a shift toward Th2-mediated humoral immunity [21, 22]. In contrast, The specific cellular response to catecholamines is dependent on the type of adrenergic receptor expressed [23]. In general, adrenaline inhibits T cells producing IFN- γ and IL-2 [21, 24].

The increase of plasma adrenaline in a 2.5-hr run at 75% $\dot{V}O_{2\max}$ appeared to suppress IL-2-producing T cells, but did not seem to affect IL-4-producing T cells in the circulation [25]. Therefore, exercise may shift immunity toward a relative Th2 dominance. A recent study showed that a 2.5 h of cycling exercise at 65% $\dot{V}O_{2\max}$ resulted in a decrease in the number and percentage of IFN- γ positive

CD4+ and CD8+ T lymphocytes [26]. Kohut et al. [11] indicated that cytokine production appeared to be suppressed in exercising mice compared with control mice 2 days post-infection and post-exercise, and these alterations would be recovered at 7 days post-infection. The reduction of cytokines following exhaustive exercise appears due to decreased antigen-specific cell expansion since a 6- to 10-fold decrease in the number of antigen-specific cells producing IFN- γ at 8 days post-exercise was found [27]. Although the Th1 cytokine production was demonstrated to decrease immediately post-exercise and return to baseline within 20 hours [28], the exercise-induced alteration in antigen-specific cells would persist for up to 3 days [17].

Few studies have been done to examine the effects of tug-of-war sport on physiological responses. Previous study has investigated the injury patterns among elite tug of war athletes through a survey during World Tug of War Championships in 1998, which reported that strains and sprains consisted over half of all injuries, and the back (42%), should-upper limb (23%), and knee (17%) were most commonly injured and injury patterns were similar among males and females [29]. Another study tried to determine the aerobic power ($VO_{2\max}$), body composition, strength, muscular power, flexibility, and biochemical profile of an elite international squad of tug of war athletes, which indicated that international level tug of war participants have excellent strength and above average endurance relative to body size, but have relatively low explosive leg power and back flexibility [30].

As part of their routine training regimen, elite athletes may train several times each day. Repeated bouts of strenuous exercise with short recovery intervals on the same day has been shown to induce greater hypothalamic-pituitary-adrenal (HPA) activation and larger perturbation of circulating leukocyte counts and function [5, 6]. However, there is no study has been investigated how the repeated intensive exercise on the same day affects the immune function and balance of Th1 and Th2 cytokines. Because a major factor governing the outcome of infectious diseases is the selection of Th1- versus Th2-predominant adaptive responses during and after the initial invasion of the host, strenuous exercise-induced stress and the consequent stress-induced Th2 shift might affect the susceptibility of infection and/or the course of an infection, which is primarily through cellular immune mechanisms [21]. Therefore, the aims of this study are to determine the effect of repeated intensive pulling of tug-of-war on the same day on leukocyte mobilization, neutrophil functions, stress hormones, Th1 and Th2 cytokine balance, and salivary responses.

Materials & Methods

Subjects

Eight elite tug-of-war pullers (age 16.5 ± 0.3 years, height 173.6 ± 1.4 cm, body mass 69.2 ± 2.3 , $VO_{2\max}$ 46.4 ± 0.7 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) were recruited from Keelung Municipal Nuan-Nuan Senior High School. All of the subjects were given a study information sheet explaining the aims and protocol of

the study. After passing a health questionnaire screen, subjects signed an informed consent. All subjects were non-smokers and were asked to refrain from alcohol and medication at least 48 hours before sampling.

Experimental Design

All visits were in the gymnasium of Keelung Municipal Nuan-Nuan Senior High School. *Visit 1* required the subject to come for measurement of physiological variables. Four days later, subjects participated in either exercise trial (EX) or control trial (REST). The order of the trial was randomized in a counterbalance design. EX required the subject to pull 6 sets, which consist of 2 pulls each apart with 90 seconds rest, on the weight of 1.5 folds of body mass starting at 15:00. REST required the subject to have a resting day in the gymnasium (no exercise but have blood and saliva sampling). Water ingestion was allowed *ad libitum* during trial except for 5 min before saliva sampling. The experimental protocol was as demonstrated as Figure 1.

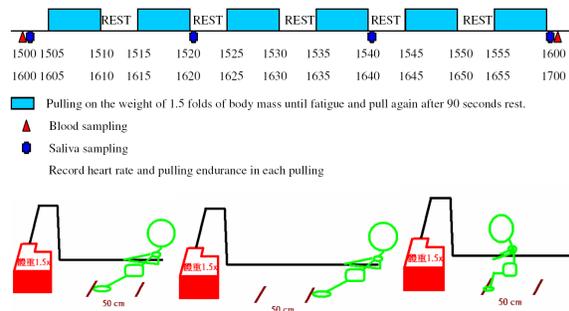


Figure 1. Experimental protocol

Saliva Collection and Analysis

Participants were seated during all saliva collections. With an initial swallow to empty the mouth, unstimulated whole saliva was collected by expectoration into a pre-weighed vial (7ml-capacity plastic Bijou tubes with screw top) for 2 min with eyes open, head tilted slightly forward and making minimal orofacial movement. All saliva samples were stored immediately at -20°C until analysis.

Saliva flow rate ($\text{mL}\cdot\text{min}^{-1}$) was determined by weighing. The density of saliva was assumed to be $1.0\text{ g}\cdot\text{mL}^{-1}$ [31]. The concentration of salivary IgA, IgA1 ($\text{mg}\cdot\text{L}^{-1}$) were determined by a sandwich-ELISA method similar to that described by Gomez et al. [32]. Briefly, flat-bottomed microtitration plates (Costar EIA/RIA plate, Sigma, Poole, UK) were coated with the primary antibody, rabbit anti-human IgA (I-8760, Sigma) or IgA1 (I-7262, Sigma), at a dilution of 1 in 800 in carbonate buffer, pH 9.6, and kept at 4°C over night. After washing with phosphate buffered saline (PBS, pH 7.2) the plates were coated with blocking protein solution ($2\text{ g}\cdot\text{L}^{-1}$ bovine serum albumin in PBS). Sample analysis was performed in triplicate using saliva samples diluted 1 in 500 with deionized water. A range of standards (Human colostrum IgA (I-2636) and IgA1 (I-4123), Sigma) up to $600\text{ }\mu\text{g}\cdot\text{L}^{-1}$ is used for calibration. Standards were incorporated into each micro-well plate, and all samples from a single subject were analyzed on a single plate. The plates were incubated for 90 min at room temperature.

Following a washing step, peroxidase-conjugated goat anti-human IgA (A-4165, Sigma) is added and the plate incubated for a further 90 min at room temperature. Following another washing step, the substrate, ABTS (Boehringer Mannheim, Lewes, UK), is added and after 30 min the absorbance was measured at 405 nm. The secretion rate ($\mu\text{g}\cdot\text{min}^{-1}$) of sIgA and sIgA1 was calculated by multiplying the concentrations of sIgA and sIgA1, respectively, by the saliva flow rate.

Blood Collection and Analysis

Venous blood samples were taken from an antecubital vein by venepuncture for 10 ml, and were collected into three Vacutainer tubes (Becton Dickinson, Oxford, UK), which were immediately stored at 4°C for later treatment and measurement within 8 hours. Blood samples in two K3EDTA vacutinners (4 mL) were used for haematological analysis including haemoglobin, haematocrit, and total and differential leukocyte counts by an automated haematology analyser (Sysmex SE-9000, Sysmex Corporation, Japan). Plasma volume changes were calculated according to Dill and Costill [33]. From blood taken into a lithium heparin vacutainer (7 mL), 1 mL was immediately added to an eppendorf tube (1.5 mL capacity) containing 50 μL of bacterial stimulant solution (Stimulant, Sigma, Poole, UK). Blood and bacterial stimulant were mixed by gentle inversion and then incubated for 1 h at 37°C , with gentle mixing every 20 min. After incubation, the mixture was centrifuged for 2 min at 15000 g . The supernatant was immediately stored at -20°C prior to analysis of elastase concentration within 2 months. The amount of elastase released per neutrophil in response to bacterial stimulation was calculated according to Robson et al. [34].

A microplate luminometer cell activation kit (Knight Scientific Limited, Plymouth, UK) was used to measure the neutrophil oxidative burst activity. Sample analysis was performed in duplicate as follows: 20 μL of K₃EDTA whole blood sample was added into a dilution tube with 2 mL of blood dilution buffer (HBSS without calcium and magnesium but with 20 mM HEPES, pH 7.4). A 20 μL aliquot of each diluted sample was then added into an opaque white microplate well. 90 μL reconstitution and assay buffer (HBSS with 20 mM HEPES, pH 7.4) was then added into each well followed by the addition of 20 μL reconstituted Adjuvant-KTM and 50 μL Pholasin® ($10\text{ }\mu\text{g}\cdot\text{mL}^{-1}$). The microplate was placed into a luminometer (Triad Multimode plate readers, Dynex Technologies Limited, UK) after adding 20 μL PMA (phorbol-12-myristate-13-acetate) into each well. After 20 sec shaking and incubation at 37°C , Pholasin®-enhanced chemiluminescence (CL) was recorded as relative light units (RLU) at 20 sec intervals for 10 min, and the incremental area under the curve (IAUC) was calculated. The oxidative burst activity per cell was calculated by dividing the IAUC by the neutrophil count in each sample.

Th1/Th2 cytokines were determined by Human Th1/Th2 Cytokine Kit II (BDTM Cytometric Bead Array, BD Biosciences, USA). Sample analysis was performed as follows: 10 μL /test of each Human Th1/Th2 Cytokine Capture Bead suspension was mixed in a polypropylene tube and vortex

briefly. After centrifuged at 200 g for 5 min at 20°C, the supernatant of Capture Bead mixture was carefully aspirated and discarded. The mixed Capture Beads pellet was then resuspended with Serum Enhancement Buffer (equal volume to amount removed) and vortex thoroughly and was incubated for 30 min at room temperature protecting from direct exposure to light. Human Th1/Th2 Cytokine Standards were reconstituted by serial dilutions using the Assay Diluent. The mixed Capture Beads (50 µL), Human Th1/Th2-II PE Detection Reagent (50 µL), Human Th1/Th2 Cytokine Standard dilutions (50 µL), and samples were in turn added into the appropriate assay tubes. The assay tubes were then incubated for 3 h at room temperature protecting from direct exposure to light. After incubation, 1 mL of Wash Buffer was added into each assay tube. After centrifuged at 200 g for 5 min at 20°C, the supernatant of each assay tube was carefully aspirated and discarded. 300µL of Wash Buffer was added to each assay tube to resuspend the bead pellet. The concentrations of plasma Th1/Th2 cytokines were analyzed on a flow cytometer (Cytomics™ FC500 Flow Cytometry, Beckman Coulter, USA).

The remaining K₃EDTA and heparinized whole blood was spun at 3000 g for 10 min in a refrigerated centrifuge at 4 °C. The plasma obtained was immediately stored at -80 °C prior to analysis (analyzed within 2 months). Plasma concentrations of glucose and lactate were analyzed using Glucose (HK) Assay

Kit (GAHK-20, Sigma, USA) and Lactate Assay Kit (Trinity Biotech, USA). Plasma cortisol (DRG Instruments GmbH, Germany) and elastase (Merck, Lutterworth, UK) were determined using enzyme-linked immunosorbant assay (ELISA) kits. The intra-assay coefficient of variation was 6.9% and 3.9% for cortisol and elastase, respectively.

Statistical Analysis

All results are presented as mean values and standard errors of the mean (\pm SEM). Data were checked for normality, homogeneity of variance and sphericity before statistical analysis, and where appropriate the Huynh-Feldt method was applied for adjustment of degrees of freedom for the *F*-tests. Data were analysed using a two-factor (trial \times time) repeated measures ANOVA with *post hoc* Tukey and paired *t*-tests, where appropriate. Statistical significance was accepted at *P* < .05.

Results

The Physiological Variables and Response

The physiological variables such as grip strength, back strength, body fat percentage, the performance of 2.4 km run, and pulling weight are showed in Table 1. Heart rate responses during pulling are showed in Figure 2, which demonstrated that there is no significant difference between trials and among timepoints.

Table 1. The Physiological Variables of the Subjects (n=8)

| | Hand Grip (kgf) | | Back Strength | Body Fat (%) | 2.4 km Run | Pulling weight (kg) |
|------|-----------------|-------|---------------|--------------|------------|---------------------|
| | Left | Right | (kgf) | | (min) | |
| Mean | 38.9 | 42.6 | 134.6 | 8.7 | 11.1 | 102.5 |
| SEM | 2.8 | 2.3 | 11.7 | 1.4 | 0.2 | 3.1 |

Table 2. Hematological Alteration

| | | Hematocrit (%) | Hemoglobin (g·dL ⁻¹) | Plasma Glucose (mM) |
|------|------|----------------|----------------------------------|---------------------|
| EX | Pre | 44.6 \pm 0.6 | 14.91 \pm 0.19 | 5.18 \pm 0.27 |
| | Post | 45.8 \pm 0.8 | 14.80 \pm 0.21 | 5.28 \pm 0.15 |
| REST | Pre | 45.3 \pm 1.0 | 14.87 \pm 0.19 | 5.22 \pm 0.36 |
| | Post | 44.1 \pm 0.7 | 14.92 \pm 0.19 | 5.02 \pm 0.19 |

Values are mean \pm SEM (n=8). No significant differences between trials and among timepoints in all parameters.

Table 3. Leukocyte Mobilization

| | | Leukocytes | Neutrophils | Lymphocytes | Monocytes |
|------|------|-----------------|-----------------|-----------------|-----------------|
| EX | Pre | 3.99 \pm 0.36 | 3.96 \pm 0.32 | 2.32 \pm 0.13 | 0.46 \pm 0.03 |
| | Post | 8.60 \pm 0.60 | 5.20 \pm 0.56 | 2.61 \pm 0.17 | 0.52 \pm 0.03 |
| REST | Pre | 7.55 \pm 0.61 | 4.60 \pm 0.43 | 2.18 \pm 0.16 | 0.51 \pm 0.07 |
| | Post | 8.14 \pm 0.72 | 5.18 \pm 0.55 | 2.26 \pm 0.14 | 0.52 \pm 0.07 |

Concentration unit is 10⁹·L⁻¹. Values are mean \pm SEM (n=8). No significant differences between trials and among timepoints in all parameters.

Pulling performance

Significant differences in main effect of trials, main effect of timepoints, and interaction of trials and timepoints were found in pulling endurance (Figure 3). The pulling endurance in the first pull was significantly longer than that in the second pull at Set 1, Set 2, and Set 3. The pulling endurance was significantly declined at Set 3 and thereafter both in the first pull and the second pull, which may indicate that muscular strength and

endurance may need to be enhanced for better performance in real type of tug-of-war competitions.

Hematological Parameters

Hematological Alteration

There were no significant alterations in hematological parameters including hematocrit, hemoglobin, and plasma glucose concentration between trials and timepoints (Table 2).

Leukocyte Mobilization

No significant alterations in leukocyte mobilization including leukocyte, neutrophil, lymphocyte, and monocyte counts were observed between trials and timepoints (Table 3)

Neutrophil Functions

The neutrophil functions of both oxygen-dependent (release of reactive oxygen species measured by oxidative burst) and oxygen-independent (release of proteases measured by elastase levels) pathways did not found to be altered (Table 4).

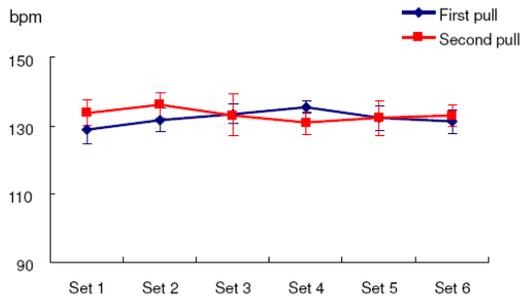


Figure 2. Heart Rate Responses. Values are mean ± SEM (n=8). No significant differences between trials and among timepoints.

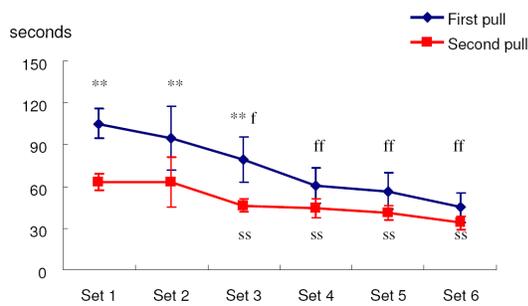


Figure 3. Pulling Endurance. Values are mean ± SEM (n=8). Significantly different between trials (** P<0.01); significantly different from Set 1 in the first pull († P<0.05; †† P<0.01); significantly different from Set 1 in the second pull (ss P<0.01)

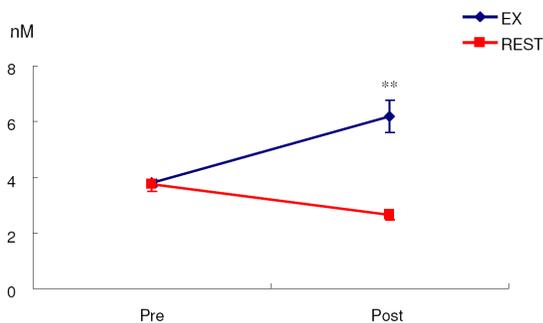


Figure 4. Plasma Lactate Concentrations. Values are mean ± SEM (n=8). Significantly different between trials (** P<0.01).

Plasma Lactate

There were significant differences in main effect of trials, main effect of timepoints, and interaction of trials and timepoints

(Figure 4). The plasma lactate concentrations in exercise trail were significantly higher than those in resting trial at post-exercise, which indicated that tug-of-war pulling largely increased anaerobic metabolism to supply energy for performing this form of short-term intensive exercise.

Plasma Cortisol and Cytokines

Plasma Cortisol

There were significant differences in main effect of trials, main effect of timepoints, and interaction of trials and timepoints in plasma cortisol concentrations (Figure 5). The plasma cortisol concentrations in exercise trail were significantly higher than those in resting trial at post-exercise, which indicated that the HPA-axis was massively activated by tug-of-war pulling.

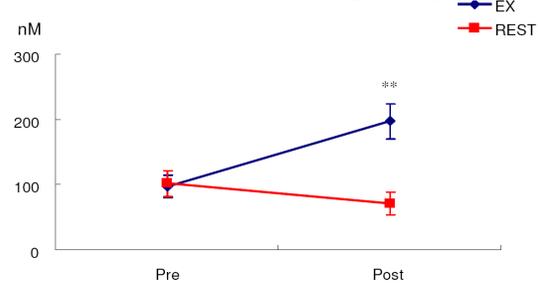


Figure 5. Plasma Cortisol Concentrations. Values are mean ± SEM (n=8). Significantly different between trials (** P<0.01).

Plasma Cytokines

No significant alterations in plasma cytokines (IL-10 and TNF) and LPS-stimulated plasma cytokines (IL-2, IL-4, IL-6, IL-10, TNF, and IFN-γ) were found (Table 5 and Table 6). The results suggested that the Th1/Th2 balance did not be altered during exercise manipulation of tug-of-war sport.

Salivary Parameters

Salivary IgA

There were no significant differences in main effect of trials, main effect of timepoints, and interaction of trials and timepoints in IgA level, IgA secretion rate, IgA1 secretion rate and ratio of IgA1/IgA (Table 7). However, a significant difference in the main effect of timepoints was observed in IgA1 levels, which showed significantly increased IgA1 levels at P-Set 2, P-Set 4, and P-Set 6 compared to those at Pre.

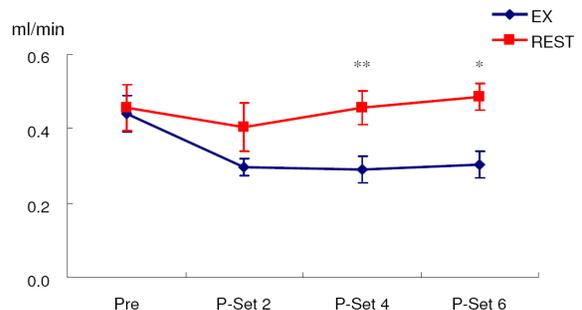


Figure 6. Saliva Secretion Rate. Values are mean ± SEM (n=8). Significantly different between trials († P<0.05; ** P<0.01).

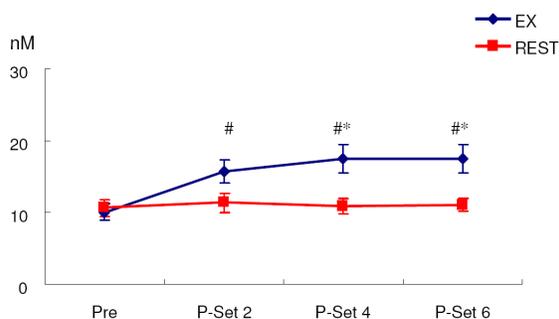


Figure 7. Salivary Cortisol Concentrations. Values are mean \pm SEM (n=8). Significantly different between trials ([#] P<0.05); significantly different from Pre in EX trial ([#] P<0.05).

Saliva Secretion Rate

The saliva secretion rates at P-Set 4 and P-Set 6 were significant lower in exercise trial than in resting trial (Figure 6), which implied that the tug-of-war pulling may induce the sympathetic activation and subsequently elicit vasoconstriction.

Salivary Cortisol

The salivary cortisol concentrations at P-Set 4 and P-Set 6 were significant higher in exercise trial compared with those in resting trial and the levels at P-Set 2, P-Set 4 and Set 6 were significant higher than the levels at Pre in exercise trial (Figure 7), which implied that the tug-of-war pulling may induce the activation of HPA-axis.

Table 4. Neutrophil Functions

| | | Total Elastase ($\mu\text{g}\cdot\text{L}^{-1}$) | Elastase per Cell ($\text{fg}\cdot\text{cell}^{-1}$) | Total OB (folds of Pre) | OB per Cell (folds of Pre) |
|------|------|--|--|-------------------------|----------------------------|
| EX | Pre | 1107 \pm 120 | 278 \pm 20 | 1.00 \pm 0.00 | 1.00 \pm 0.00 |
| | Post | 1208 \pm 93 | 243 \pm 21 | 1.26 \pm 0.11 | 1.00 \pm 0.12 |
| REST | Pre | 1168 \pm 96 | 265 \pm 19 | 1.00 \pm 0.00 | 1.00 \pm 0.00 |
| | Post | 1334 \pm 86 | 272 \pm 28 | 1.00 \pm 0.03 | 0.87 \pm 0.07 |

OB: oxidative burst. Values are mean \pm SEM (n=8). No significant differences between trials and among timepoints in all parameters.

Table 5. Plasma Cytokines

| | | Interleukin-10 ($\text{ng}\cdot\text{L}^{-1}$) | Tumor Necrosis Factor ($\text{ng}\cdot\text{L}^{-1}$) |
|------|------|--|---|
| EX | Pre | 32.99 \pm 3.45 | 26.51 \pm 4.99 |
| | Post | 24.34 \pm 2.76 | 22.35 \pm 4.19 |
| REST | Pre | 19.55 \pm 2.10 | 20.38 \pm 2.14 |
| | Post | 20.65 \pm 2.08 | 22.78 \pm 1.86 |

Values are mean \pm SEM (n=8). No significant differences between trials and among timepoints in all parameters.

Table 6. Lipopolysaccharide (LPS)-stimulated Plasma Cytokines

| | | IL-2 ($\text{ng}\cdot\text{L}^{-1}$) | IL-4 ($\text{ng}\cdot\text{L}^{-1}$) | IL-6 ($\text{ng}\cdot\text{L}^{-1}$) | IL-10 ($\text{ng}\cdot\text{L}^{-1}$) | TNF ($\text{ng}\cdot\text{L}^{-1}$) | IFN- γ ($\text{ng}\cdot\text{L}^{-1}$) |
|------|------|--|--|--|---|---------------------------------------|---|
| EX | Pre | 15.96 \pm 4.72 | 5.44 \pm 1.74 | 31.02 \pm 9.99 | 10.28 \pm 2.86 | 1066 \pm 331 | 43.5 \pm 11.7 |
| | Post | 9.60 \pm 3.20 | 6.09 \pm 1.03 | 20.36 \pm 5.46 | 5.91 \pm 1.89 | 860 \pm 252 | 27.6 \pm 11.4 |
| REST | Pre | 16.85 \pm 7.07 | 9.17 \pm 2.47 | 22.18 \pm 7.42 | 12.63 \pm 5.37 | 1085 \pm 519 | 42.9 \pm 18.8 |
| | Post | 14.81 \pm 5.11 | 6.35 \pm 1.85 | 18.79 \pm 5.70 | 9.61 \pm 3.31 | 565 \pm 184 | 44.4 \pm 16.0 |

IL: interleukin; TNF: tumor necrosis factor; IFN- γ : interferon- γ . No significant differences between trials and among timepoints in all parameters. Values are mean \pm SEM (n=8).

Table 7. Salivary IgA Concentrations

| | | IgA Level ($\text{mg}\cdot\text{L}^{-1}$) | IgA1 Level ($\text{mg}\cdot\text{L}^{-1}$) | IgA Secretion rate ($\mu\text{g}\cdot\text{min}^{-1}$) | IgA1 Secretion rate ($\mu\text{g}\cdot\text{min}^{-1}$) | Ratio of IgA1/IgA |
|------|---------|---|--|--|---|-------------------|
| EX | Pre | 144 \pm 26 | 108 \pm 16 | 67 \pm 16 | 49 \pm 10 | 0.82 \pm 0.07 |
| | P-Set 2 | 212 \pm 38 | 148 \pm 22 [*] | 65 \pm 14 | 45 \pm 9 | 0.74 \pm 0.04 |
| | P-Set 4 | 241 \pm 45 | 164 \pm 26 [*] | 69 \pm 12 | 47 \pm 7 | 0.73 \pm 0.05 |
| | P-Set 6 | 204 \pm 33 | 150 \pm 20 [*] | 63 \pm 12 | 45 \pm 7 | 0.79 \pm 0.06 |
| REST | Pre | 135 \pm 26 | 103 \pm 16 | 65 \pm 17 | 49 \pm 11 | 0.78 \pm 0.02 |
| | P-Set 2 | 158 \pm 26 | 115 \pm 15 | 64 \pm 15 | 46 \pm 9 | 0.75 \pm 0.04 |
| | P-Set 4 | 148 \pm 22 | 110 \pm 14 | 67 \pm 11 | 50 \pm 7 | 0.76 \pm 0.04 |
| | P-Set 6 | 156 \pm 23 | 127 \pm 15 | 76 \pm 14 | 62 \pm 9 | 0.85 \pm 0.04 |

Values are mean \pm SEM (n=8). Significantly different from Pre in the same parameter (^{*} P<0.01).

Discussion

The main findings of this study were: (1) Pulling endurance was significantly decreased in the second pull compared with the first pull at Set1, Set 2 and Set 3; furthermore, the pulling endurance was also significantly declined after Set 2 in both pulls compared with Set 1. (2) Repeated intensive pulls (RIP) significantly activated HPA-axis with higher concentrations of both plasma and salivary cortisol and lower saliva flow rate in exercise trial compared with the values in rest trial. (3) RIP significantly increased plasma lactate concentrations. (4) RIP did not appear to affect hematocrit, hemoglobin, plasma glucose, leukocyte mobilization, neutrophil functions, plasma Th1/Th2 cytokine balance, and salivary IgA and IgA1 concentration and their secretion rates.

The present study is the first one to inspect the effects of repeated intensive pulls of tug-of-war training on immunoendocrine responses. Previously, only few studies have been done to examine the effects of tug-of-war sport on physiological responses. Warrington et al [30] tried to determine the aerobic power (VO₂max), body composition, strength, muscular power, flexibility, and biochemical profile of an elite international squad of tug of war athletes, which indicated that international level tug of war participants have excellent strength and above average endurance relative to body size, but have relatively low explosive leg power and back flexibility. Another study was to investigate the injury patterns among elite tug of war athletes through a survey during World Tug of War Championships in 1998, which reported that strains and sprains consisted over half of all injuries, and the back (42%), should-upper limb (23%), and knee (17%) were most commonly injured and injury patterns were similar among males and females [29]. In the present study, we found that the pulling endurance was significantly declined after Set 2 in both pulls, which may indicate the shortage of muscular strength and endurance. In the future, if pullers want to enhance their performance, they may need to pay more attentions to resistance training which mocks the real tug-of-war competitions.

The heart rate responses in this study was similar to recent studies, which showed that the heart rate in static pull was about 130 beats·min⁻¹ [35]. The results of this study indicated that the tug-of-war competition might not induce strong impact on hematological and immunological steady state of internal milieu since the protocol of this study was mimicked to the situation of real competition. However, the increases of plasma lactate post exercise indicated that tug-of-war pulling was a short-term intensive exercise since it largely depended on anaerobic metabolism for energy supply. Therefore, it was hypothesized here that the heart rate during pulling may not be able to respond the real intensity since the muscular contraction of whole body may retard the blood circulation. Repeated intensive pulls appeared to activate HPA-axis since the higher concentrations of both plasma and salivary cortisol were observed in this study. However the extent of HPA activation appeared not strong enough to influence hematological parameters and cytokine alterations since we did not find significant alterations in the mobilization of

leukocyte and its subsets, neutrophil functions, and Th1/Th1 cytokines balance.

In conclusion, the findings of the present study suggest that the repeated intensive pulls of tug-of-war may induce activations of HPA-axis and sympathetic nervous system. However the extents of activations appear not strong enough to extensively disturb homeostasis of immunoendocrine. Furthermore, the heart rate may not be an appropriate indicator for assessing exercise intensity in relatively static exercise of tug-of-war.

References

- [1] Hoffman-Goets, L. and B.K. Pedersen. (1994). Exercise and the immune system: a model of the stress response? *Immunology Today*, 15, 382-387.
- [2] Kohut, M.L., B.A. Arntson, W. Lee, K. Rozeboom, K.J. Yoon, J.E. Cunnick, & J. McElhaney. (2004). Moderate exercise improves antibody response to influenza immunization in older adults. *Vaccine*, 22, 2298-2306.
- [3] Nieman, D.C. (1994). Exercise, infection, and immunity. *International Journal of Sports Medicine*, 15, S131-S141.
- [4] Matthews, C.E., I.S. Ockene, P.S. Freedson, M.C. Rosal, P.A. Merriam, & J.R. Hebert. (2002). Moderate to vigorous physical activity and risk of upper-respiratory tract infection. *Medicine & Sciences in Sports & Exercise*, 34, 1242-1248.
- [5] Li, T.L. and M. Gleeson. (2005). The effects of carbohydrate supplementation during the second of two prolonged cycling bouts on immunoendocrine responses. *European Journal of Applied Physiology*, 95, 391-399.
- [6] Li, T.L. and M. Gleeson. (2004). The effect of single and repeated bouts of prolonged cycling on leukocyte redistribution, neutrophil degranulation, IL-6 and plasma stress hormone responses. *International Journal of Sport Nutrition and Exercise Metabolism*, 14, 501-516.
- [7] Nieman, D.C. (1997). Immune response to heavy exertion. *Journal of Applied Physiology*, 82, 1385-1394.
- [8] Nieman, D.C., L.M. Johanssen, J.W. Lee, & K. Arabatzis. (1990). Infectious episodes in runners before and after the Los Angeles marathon. *Journal of Sports Medicine and Physical Fitness*, 30, 316-328.
- [9] Peters, E.M. and E.D. Bateman. (1983). Ultramarathon running and upper respiratory tract infections. An epidemiological survey. *South African Medical Journal*, 64, 582-584.
- [10] Davis, J.M., M.L. Kohut, L.H. Colbert, D.A. Jacson, A. Ghaffar, & E.P. Mayer. (1997). Exercise, alveolar macrophage function, and susceptibility to respiratory infection. *Journal of Applied Physiology*, 83, 1461-1466.
- [11] Kohut, K.L., G.W. Boehm, & J.A. Moynihan. (2001). Prolonged exercise suppresses antigen-specific cytokine response to upper respiratory infection. *Journal of Applied Physiology*, 90, 678-684.
- [12] Mackinnon, L.T. (1999). *Advances in Exercise Immunology*. Champaign IL: Human Kinetics.

- [13] Quan, C.P., A. Berneman, R. Pires, S. Avrameas, & J. Bouvet. (1997). Natural polyreactive secretory immunoglobulin A autoantibodies as a possible barrier to infection in human. *Infection and Immunity*, 65, 3997-4004.
- [14] Pedersen, B.K. (1999). Exercise and Immune Function, In M. Schedlowski and U. Tewes, Editors. *Psychoneuroimmunology: An interdisciplinary introduction*, Kluwer Academic/Plenum Publishers: New York. p. 341-358.
- [15] Fukatsu, K., N. Sato, & H. Shimizu. (1996). 50-mile walking race suppresses neutrophil bactericidal function by inducing increases in cortisol and ketone bodies. *Life Sciences*, 58, 2337-2343.
- [16] Johnson, J.L., E.E. Moore, D.Y. Tamura, G. Zallen, W.L. Biffi, & C.C. Silliman. (1998). Interleukin-6 augments neutrophil cytotoxic potential via selective enhancement of elastase release. *Journal of Surgical Research*, 76, 91-94.
- [17] Kohut, M.L., A.E. Martin, D.S. Senchina, & W. Lee. (2005). Glucocorticoids produced during exercise may be necessary for optimal virus-induced IL-2 and cell proliferation whereas both catecholamines and glucocorticoids may be required for adequate immune defense to viral infection. *Brain, Behavior and Immunity*, 19, 423-435.
- [18] Hoffman-Goetz, L., R. Keir, R. Thorne, M.E. Houston, & C. Young. (1986). Chronic exercise stress in mice depresses splenic T lymphocyte mitogenesis in vitro. *Clinical and Experimental Immunology*, 66, 551-557.
- [19] Hoffman-Goetz, L., R. Thorne, & M.E. Houston. (1988). Splenic immune responses following treadmill exercise in mice. *Canadian Journal of Physiology and Pharmacology*, 66, 1415-1419.
- [20] Morel, P.A. and T.B. Oriss. (1998). Crossregulation between Th1 and Th2 cells. *Critical Review of Immunology*, 18, 275-303.
- [21] Elenkov, I.J. and G.P. Chrousos. (1999). Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends in Endocrinology and Metabolism*, 10, 359-368.
- [22] Elenkov, I.J. (2004). Glucocorticoids and the Th1/Th2 balance. *Annals of the New York Academy of Sciences*, 1024, 138-146.
- [23] Gajewski, T.F., S.R. Schell, & F.W. Fitch. (1990). Evidence implicating utilization of different T cell receptor-associated signaling pathways by Th1 and Th2 clones. *Journal of Immunology*, 144, 4110-4115.
- [24] Moynihan, J.A., T.A. Callahan, S.P. Kelley, & L.M. Campbell. (1998). Adrenal hormone modulation of type 1 and type 2 cytokine production by spleen cells: dexamethasone and dehydroepiandrosterone suppress interleukin-2, interleukin-4, and interferon-gamma production in vitro. *Cell Immunology*, 185, 58-64.
- [25] Steensberg, A., A.D. Toft, H. Bruunsgaard, M. Sandmand, J. Halkjer-Kristensen, & B.K. Pedersen. (2001). Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *Journal of Applied Physiology*, 91, 1708-1712.
- [26] Lancaster, G.I., Q. Khan, P.T. Drysdale, F. Wallace, A.E. Jeukendrup, M.T. Drayson, & M. Gleeson. (2005). Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. *Journal of Applied Physiology*, 98, 565-571.
- [27] Kapasi, Z.F., M.L. McRae, & R. Ahmed. (2005). Suppression of viral specific primary T-cell response following intense physical exercise in young but not old mice. *Journal of Applied Physiology*, 98, 663-671.
- [28] Weinstock, C., D. Konig, R. Harnischmacher, J. Keul, A. Berg, & H. Northoff. (1997). Effect of exhaustive exercise stress on the cytokine response. *Medicine & Sciences in Sports & Exercise*, 29, 345-354.
- [29] Smith, J. and B. Krabak. (2002). Tug of war: introduction to the sport and an epidemiological injury study among elite pullers. *Scandinavian Journal of Medicine & Science in Sports*, 12, 117-124.
- [30] Warrington, G., C. Ryan, F. Murray, P. Duffy, & J.P. Kirwan. (2001). Physiological and metabolic characteristics of elite tug of war athletes. *British Journal of Sports Medicine*, 35, 396-401.
- [31] Chicharro, J.L., A. Lucia, M. Perez, A.F. Vaquero, & R. Urena. (1998). Saliva composition and exercise. *Sports Medicine*, 26, 17-27.
- [32] Gomez, F.E., J. Villegas, & H. Bourges. (1991). An enzyme-linked immunosorbent assay for human secretory immunoglobulin A in parotid saliva. *La Revista de Investigacion Clinica*, 43, 351-358.
- [33] Dill, D.B. and D.L. Costill. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, 37, 247-248.
- [34] Robson, P.J., A.K. Blannin, N.P. Walsh, L.M. Castell, & M. Gleeson. (1999). Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. *International Journal of Sports Medicine*, 20, 128-135.
- [35] Van Heerden, H.J., J.R. Clark, & B. Malga. (2002). *Physiological Responses*, in *Research Report - Project 4*, Tug of War International Federation. p. 1-11.

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