The Effects of High Element the Pamper Pole Jump on Anxiety State and Oral Immunity

Tzai-Li Li1,* , Wen-Yu Chan1, Benjamin Rush1

1 Department of Sports and Leisure Studies, National Dong Hwa University, Hualien, Taiwan, 905, R.O.C.

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Abstract

The Pamper Pole jump has long been recognized as stressful challenge and appears to easily induce fear and anxiety, which may activate sympathetic nervous system and hypothalamic-pituitary-adrenal axis, and may subsequently influence oral immunity. The aim of this study was to determine the effects of anxiety induced by the Pamper Pole jump on salivary IgA and cortisol responses. With the approval of the local Ethics Committee, 9 healthy men and 6 women (age 21.8 ± 0.4 years, height 1.66 ± 0.02 m, body mass 63.9 ± 2.3 kg; means ± SEM ), who were recreationally active and never experienced the Pamper Pole jump before, participated in this study. Subjects performed the Pamper Pole jump in each trial, separated by at least 6 days. For avoidance of circadian variation, the two experimental trials were conducted at same time of day. No food and sleep was allowed after 11:30 am. No water was allowed to consume 30 min before each trial until the trials finished. Subjects were encouraged to climb up to the top of the Pamper Pole after wearing safety equipments and then jumping out off the pillar. Saliva samples were collected at 10 min before filling in Beck Anxiety Inventory sheet (Pre-EX), arrival to the top of the Pamper Pole (Pre-Jump), and 30 min (P-30-EX) and 60 min (P-60-EX) after jumping out. Heart rate was recorded at Pre-EX, just before climbing (Pre-Climb), immediately arrival to the top of the Pamper Pole (Top), Pre-Jump, and immediately after jumping out (Post-Jump) by radiotelemetry. The main findings of this study were: (1) experiences of the Pamper Pole jump appeared not to influence responses of blood pressure, the scores of Beck Anxiety Inventory, saliva flow rate, salivary IgA concentration and secretion rate, heart rate, and salivary cortisol; (2) the situation of the Pamper Pole jump significantly decreased saliva flow rate; (3) the situation of the Pamper Pole jump significantly increased heart rate. In conclusion, the findings of this study suggested that the Pamper Pole jump did not appear to induce substantial anxiety enough to alter oral immunity and the experience of conducting the Pamper Pole jump may not affect the feeling of anxiety.

Keywords: Pamper Pole jump, Saliva flow rate, Salivary IgA, Anxiety, Cortisol

Introduction

Saliva is a colourless liquid with a density ranging from 1.002 to 1.012 g·mL-1, comprising organic and inorganic constituents with more than 99% water. An average saliva volume secreted each day through the salivary glands approaches 750 mL, which represents a rate of approximately 0.5 mL·min-1 arising from the submandibular glands (65%), parotid glands (23%), minor mucous glands (8%) and sublingual glands (4%) [1].

Immunity against microorganisms at cavities and tracts is mainly due to secretory immunoglobulin A (IgA), which has been also considered as the first line of defence to infection in the lumen of the respiratory tract and gut [2]. Therefore, salivary IgA (sIgA) has been adopted to be a key indicator in determining the effect of different forms of stress on mucosal immunity. The low sIgA levels and/or chronic sIgA deficiency appeared to facilitate the adherence and entrance of pathogens through the epithelial surface [3, 4], increasing frequency of upper respiratory tract infection (URTI) episodes [5], recurrent URTI [6], or reduced protection against certain epithelial infections [7]. In a meta-analysis, Jemmott and McClelland [8] indicated that the low local levels of sIgA could compromise immune resistance to respiratory infections.

Mucosal immunity and susceptibility to URTI appeared to associate with exercise stress since various aspects of immune function are temporarily altered following exercise [9]. Epidemiological studies have suggested that intensive prolonged training or competition is related to an elevated incidence of URTI, placing athletes at a higher risk compared with controlled counterparts during and after competition or training [10-13]. Peters and Bateman [13] reported that the runners who completed a 35-mile ultramarathon had more than 2-fold incidence of URTI within 2 weeks post race compared with the matched controls. The yearly running mileage seemed to be a determinant factor of developing URTI according to the study of Heath et al. [11] which showed that individuals who ran more than 3.8 miles per day, on average, had a 2-fold
higher incidence of URTI than those who ran less than 1.3 miles per day. Nieman et al. [12] also reported that the risk of an infectious episode was 5-fold higher in the marathon runners within a week post race compared with runners who trained but did not compete. Subsequently, Nieman [14] hypothesised the relationship between URTI susceptibility and exercise workload as a J-shaped curve. This J-shaped curve model predicts that the individuals who exercise moderately are at less risk of infection, whereas those who exercise heavily are more at risk compared with sedentary counterparts.

The salivary glands are innervated by both sympathetic nerve and parasympathetic nerves [15, 16]. Parasympathetic stimulation induces a noticeable elevation in regional blood flow to salivary glands by vasodilation, resulting in a higher saliva flow rate with a relatively low protein concentration; whereas the sympathetic stimulation causes vasoconstriction, resulting a lower saliva flow rate but rich in protein [16-18]. Cortisol has been suggested to play an important role in inhibiting sIgA mobilization [19]. Wira et al. [20] reported a decline in sIgA level at 24 h after a single injection of dexamethasone. A subsequent study [3] showed a fall of 77% in IgA concentration, an augmentation in bacterial adherence (2.4-fold), and an increased incidence of bacterial translocation to the mesenteric lymph nodes (60% vs 0%) observed after 2 days in dexamethasone-treated rats. The levels of polymeric IgA and antigen-specific IgA antibody in serum were also reported to be increased after dexamethasone treatment [21]; however, the sIgA level and antigen-specific IgA production after oral antigenic challenge was markedly inhibited. These data suggested that glucocorticoids might impair mucosal IgA synthesis, secretion and function and facilitate bacterial translocation [22].

Adventure education originated in 1912 by a German scholar Kurt Hahn, who thought that school education was too emphasis on intellectual development and did not provide complete and perfect growing opportunities to students [23]. Therefore, Hahn decided to develop a series of plans to improve ill health, confidence deficiency, and thoughtlessness, which were generally existed in most students. One of the most effective methods in the plan was to provide the challenge experiences to students, which then progressively developed into adventure education within the past decades.

High elements, which manipulate in the air, and low elements, which operate on the ground are the main activities of adventure education. The Pamper Pole jump (Figure 1), a single pillar with about 8 meters high, is one of the high elements. Participants are encouraged to climb up to the top of the Pamper Pole after wearing safety equipments, then jumping out off the pillar, and trying to reach a swing (about 1 meter in front of the Pamper Pole). This challenge activity appears to easily induce fear and anxiety, which may subsequently activate sympathetic nervous system and hypothalamic-pituitary-adrenal axis. However, no study has been carried out to date to investigate the possible alterations of humoral immunity under such stressed conditions. Hence, the aim of this study was to examine the effects of anxiety induced by the Pamper Pole jump on salivary IgA and cortisol responses.

Methods

Subjects

9 healthy men and 6 women (age 21.8 ± 0.4 years, height 1.66 ± 0.02 m, body mass 63.9 ± 2.3 kg; means ± SEM), who were recreationally active and never experienced the Pamper Pole jump before, participated in this study. After receiving written information and passing a Health Questionnaire screening, subjects signed an informed consent. Subjects were requested to complete the dietary record sheet on the day prior to the First trial and then repeated the same diets according to the dietary record sheet before the Second trial. Subjects were also asked not to perform any strenuous exercise or consume alcohol or medication for 1 day before each trial. The protocol was approved by the local Ethics Committee.

Experimental Procedures

For investigating the effect of the Pamper Pole jump experiences on anxiety (the familiarization effect), two trials was designed in this experiment. Subjects performed the Pamper Pole jump in each trial, separated by at least 6 days. For avoidance of circadian variation, the two experimental trials were conducted at the same time of the day as Figure 2. No food and sleep was allowed after 11:30 am. No water was allowed to consume 30 min before each trial until the trials finished. Five main steps in this study were: (1) subjects arrived at the adventure education court at 13:50 and then their heart rate and blood pressure were measured (Omron HEM-722C, Japan), and saliva samples were taken after 10 min rest; (2) subjects were asked to complete Beck Anxiety Inventory (BAI) sheet; (3) subjects wore a Polar heart rate radiotelemetry and safety equipments; (4) subjects were asked to climb up to the top of the Pamper Pole and then jumped out after completing the procedures; (5) subjects were rested for further 60 min.

Saliva samples were collected at 10 min before filling in BAI sheet (Pre-EX), arrival to the top of the Pamper Pole (Pre-Jump), and 30 min (P-30-EX) and 60 min (P-60-EX) after jumping out.

Heart rate was recorded at Pre-EX, just before climbing (Pre-Climb), immediately arrival to the top of the Pamper Pole (Top), Pre-Jump, and immediately after jumping out.
(Post-Jump) by radiotelemetry.

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 at −20°C until analysis.

Saliva flow rate (mL·min⁻¹) was determined by weighing. The density of saliva was assumed to be 1.0 g·mL⁻¹ [16]. The concentration of salivary IgA (mg·L⁻¹) was determined by a sandwich-ELISA method similar to that described by Gomez et al. [24]. 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, Poole, UK), 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 g·L⁻¹ bovine serum albumin in PBS). Sample analysis was performed in quadruplicate using saliva samples diluted 1 in 500 with deionised water. A range of standards (Human colostrum IgA, I-2636, Sigma) up to 600 µg·L⁻¹ was used for calibration. Standards were incorporated into each micro-well plate, and all samples from a single subject were analysed on a single plate. The plates were incubated for 90 min at room temperature. Following another washing step, the substrate, ABTS (Boehringer Mannheim, Lewes, UK), was added and after 30 min the absorbance was measured at 405 nm.

The sIgA secretion rate (µg·min⁻¹) was calculated by multiplying the sIgA concentration by the saliva flow rate.

Salivary cortisol (DRG Instruments GmbH, Germany) was determined using enzyme-linked immunosorbant assay (ELISA) kits. The intra-assay coefficient of variation was 6.9%.

Statistical Analysis

All results are presented as mean values and standard errors of the mean (± SEM). Data were checked for normality, homogeneity of variation and sphericity before statistical analysis, and the Huynh-Feldt method was applied for adjustment of degree of freedom for the F-tests. Data were analyzed using a two-factor (trial × time) repeated measures ANOVA with post hoc Tukey tests and paired t tests applied where appropriate. Blood pressure and BAI scores were

![Figure 2. Experimental procedures](image-url)
examined using paired t-test. P, t, and adjusted F values are presented and P < 0.05 was accepted significant.

Results

Blood Pressure and the Scores of Beck Anxiety Inventory
There were no significant differences between the First trial and the Second trial in blood pressure and the BAI scores (Table 1).

Table 1 Changes in blood pressure and the score of Beck Anxiety Inventory

<table>
<thead>
<tr>
<th></th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Scores of Beck Anxiety Inventory*</th>
</tr>
</thead>
<tbody>
<tr>
<td>The First trial</td>
<td>112.7 ± 4.2</td>
<td>71.9 ± 2.8</td>
<td>5.9 ± 1.0</td>
</tr>
<tr>
<td>The Second trial</td>
<td>113.2 ± 3.6</td>
<td>72.8 ± 2.6</td>
<td>5.3 ± 0.9</td>
</tr>
</tbody>
</table>

* 0-7: minimal level of anxiety, 8-15: mild anxiety, 16-25: moderate anxiety, and 26-63: severe anxiety

Saliva Flow Rate
For the saliva flow rate, there was only a significant main effect of time (F3, 42 = 4.554; P = 0.015), which was significantly decreased at Pre-Jump compared with other timepoints in both trials (Figure 3).

Salivary IgA Concentration
There were no significant main effects of trial (F1, 14 = 1.482; P = 0.244), time (F3, 42 = 3.092; P = 0.181), and interaction between trial and time (F3, 42 = 1.265; P = 0.299) for the salivary IgA concentration (Figure 4).

Salivary IgA Secretion Rate
No significant main effects of trial (F1, 14 = 0.086; P = 0.774), time (F3, 42 = 3.040; P = 0.083), and interaction between trial and time (F3, 42 = 0.302; P = 0.676) were found in salivary IgA secretion rate (Figure 5).

Salivary Cortisol Concentration
There were no significant differences for the salivary cortisol concentration (Figure 6).

Heart Rate
For the heart rate, there were significant main effect of time
Discussion

The main findings of this study were: (1) experiences of the Pamper Pole jump appeared to influence responses of blood pressure, the BAI scores, saliva flow rate, salivary IgA concentration and secretion rate, heart rate, and salivary cortisol; (2) the situation of the Pamper Pole jump significantly decreased saliva flow rate; (3) the situation of the Pamper Pole jump significantly increased heart rate.

The Pamper Pole jump has long been recognized as stressful challenge and appears to easily induce fear and anxiety, which may subsequently activate sympathetic nervous system and hypothalamic-pituitary-adrenal axis. However, the results of the present study demonstrated that the Pamper Pole jump may only evoke mild stress because only a moderate increase in heart rate was observed with a steady level in salivary cortisol and may not induce large anxiety as previous prediction since the BAI scores showed minimal level of anxiety. The results of this study also showed that the experience of conducting the Pamper Pole jump has no significant familiarization effect.

The results of this study showed a significant decrease in saliva flow rate immediately arrival to the top of the Pamper Pole compared with other timepoints. Salivary glands are innervated by both parasympathetic cholinergic nerves and sympathetic adrenergic nerves. During stressed situation, the sympathetic stimulation is increased and induces vasoconstriction, which limits saliva secretion rate [16]. Heart rate was the highest at the same timepoint and therefore it is not surprising to find a significant decrease in saliva flow rate in the present study.

Regulation of slgA secretion is mainly via a long-term (days) modification of slgA synthesis and/or a short-term (minutes) mobilization (transcytosis) stimulated by sympathetic nerves [25]. Proctor et al. indicated that the acute sympathetic stimulation increased slgA secretion via elevated transcytosis from the glandular IgA pool in a dose-independent manner above a certain threshold [26]. In this study, we did not find significant elevations in salivary IgA concentration and secretion rate between trials and during experimental protocol. The reason for this observation may be attributable to insufficient sympathetic stimulation under the challenge of the Pamper Pole jump since salivary cortisol concentration remained unchanged.

In conclusion, the findings of this study suggested that the Pamper Pole jump does not appear to induce substantial anxiety enough to alter oral immunity and the experience of conducting the Pamper Pole jump may not affect the feeling of anxiety.

Reference


AUTHORS BIOGRAPHY

Tzai-Li Li
Employment
Associate professor and director of PE Center, National Dong Hwa University, Taiwan
Degree
PhD
Research interests
Exercise Immunology
E-mail: leej@mail.ndhu.edu.tw

Benjamin Rush
Employment
Contract teacher of PE Center, National Dong Hwa University, Taiwan
Degree
PhD student
Research interests
Adventure studies
E-mail: banjyamn@mail.ndhu.edu.tw

Wen-Yu Chan
Employment
Counseling Center, National Hualien Agriculture Vocational High School, Taiwan
Degree
Master
Research interests
Sports medicine and psychology
E-mail: aac1025@yahoo.com.tw