Vitamin C Supplementation and Salivary Immune Function Following Exercise-Heat Stress

Andres E. Carrillo, René J. L. Murphy, and Stephen S. Cheung

Purpose: Prolonged physical exertion and environmental heat stress may elicit postexercise depression of immune cell function, increasing upper respiratory tract infection (URTI) susceptibility. We investigated the effects of acute and short-term vitamin C (VC) compared with placebo (PL) supplementation on URTI susceptibility, salivary immunoglobulin A (s-IgA), and cortisol responses in healthy individuals following prolonged exercise-heat stress. Methods: Twelve participants were randomized into the VC or PL group in a double-blind design. For 12 days, participants consumed 3 × 500 mg tablets of VC or PL per day, with testing completed at baseline, then following acute (1 d) and short-term (8 d) supplementation. Participants performed 120.1 ± 49.6 min of cycling at 54 ± 6% \( \dot{V}_{O2\text{max}} \) in a hot (34.8 ± 1.0°C and 13 ± 3% relative humidity) environment, with saliva samples collected at pre-, post-, and 72 h postexercise. Health logs specifying URTI symptoms were completed for 7 days postexercise. Results: A 2 × 3 × 3 mixed ANOVA with a post hoc Bonferroni correction factor revealed a significant linear trend in postexercise cortisol attenuation in the VC group, 21.7 ± 15.1 nmol/L (mean ± SD) at baseline, to 13.5 ± 10.0 at acute, to 7.6 ± 4.2 after short term (\( P = .032 \)). No differences were detected in ratio of s-IgA to protein or URTI symptoms between groups. Conclusions: These data suggest that vitamin C supplementation can decrease postexercise cortisol in individuals performing exercise similar to that of a half-marathon or marathon in hot conditions. However, no changes in s-IgA and URTI were evident, possibly due to previous moderate training and reduced physical and psychological stress compared with athletes participating in ultramarathons.

Keywords: immunity, immunoglobulin A, cortisol, infection, exercise

Physical exertion combined with environmental heat stress could exaggerate the exercise-induced immune response, thus potentially hindering protection.

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against common illnesses.\textsuperscript{1,2} Host protection, immune function, and upper respiratory tract infection (URTI) susceptibility are improved by moderate/low-intensity activity,\textsuperscript{3,4} whereas prolonged/high-intensity activity causes numerous negative changes in immunity,\textsuperscript{5} particularly during the “open window” (72 h postexercise).\textsuperscript{5} Following a 160-km running race, 24% of the 155 ultramarathoners experienced reduced immunity and an episode of URTI.\textsuperscript{6} When considering the prevalence of increased environmental and internal body temperatures during endurance events,\textsuperscript{7} it is surprising that only a few studies have examined the mechanisms and consequences underlying URTI during the open-window period following prolonged exercise-heat stress.

Heat stress, whether as a consequence of increased environmental temperatures or physical exertion, negates the immune and endocrine system response.\textsuperscript{8,9} The limited amount of research investigating the effects of prolonged exercise in hot conditions on salivary immunoglobulin A (s-IgA) reveals no changes across various environmental temperatures.\textsuperscript{10} Postexercise increases in cortisol reduces T-lymphocyte count, limiting the potential for B-lymphocyte activation and therefore reducing antibody production.\textsuperscript{11} When considering the importance of immunoglobulin A (IgA) as the predominant humoral factor of the local immune system within the oral cavity, and as the primary defense mechanism against URTI, the speculation is that an exaggerated URTI response will result following exercise-heat stress.

Vitamin C is a water-soluble vitamin that positively influences the immune system by providing antioxidant protection and by attenuating the stress-induced cortisol response.\textsuperscript{12} Acute vitamin C supplementation increases plasma antioxidant capacity during prolonged exercise, but has little to no effect on the immunoendocrine response.\textsuperscript{13} Further, short-term (7 days; 1500 mg) vitamin C supplementation attenuates cortisol secretion\textsuperscript{14} and has revealed inconsistent effects on s-IgA concentration.\textsuperscript{15} Some potential mechanisms causing the vitamin C–induced reduction in cortisol secretion include the inhibition of specific enzymes involved in cortisol synthesis\textsuperscript{16} and the concurrent release of vitamin C and cortisol from the adrenal glands during oxidative stress.\textsuperscript{13} Considering the notion that high cortisol concentrations reduce s-IgA synthesis\textsuperscript{17} and transepithelial transport of s-IgA,\textsuperscript{18} this reduction in cortisol is important in potentially lowering URTI risk among athletes. However, no data are currently available investigating the acute and short-term influence of vitamin C on changes in cortisol, s-IgA, and URTI risk following prolonged exercise-heat stress.

The majority of studies investigating cortisol, s-IgA, and URTI often record changes in these parameters following the completion of an ultra-endurance event, such as an ultramarathon.\textsuperscript{6,19,20} The information available for nonelite, active individuals among the general population is limited. Therefore, the purpose of the current study was to examine the effects of acute and short-term vitamin C supplementation on cortisol, s-IgA, and URTI susceptibility following prolonged physical activity (simulating a half-marathon or marathon) in a hot environment. We hypothesized that acute (to a lesser extent) and short-term vitamin C supplementation would attenuate the cortisol response, reduce disturbances in s-IgA, and lower URTI symptoms following prolonged exercise-heat stress.
Methods

Participants
This study was conducted following the Canadian Tri-Council Policy for the ethical treatment of human participants, with approval obtained from the appropriate institutional research ethics board. Twelve healthy, aerobically fit individuals (males: 8; females: 4; age: 23.4 ± 4.6 y; height 173.2 ± 7.6 cm; body mass 69.6 ± 8.4 kg; body fat percent 12 ± 6%; and VO2max 54.3 ± 6.5 mL·kg⁻¹·min⁻¹) participated in this randomized double-blind placebo-controlled study. Vitamin/mineral supplementation greater than 100% recommended nutrient intake on a regular basis within 2 weeks of testing was an exclusion criterion to ensure normative resting s-IgA levels. The data collected by Thompson et al²¹ were used to estimate that 6 participants per group has a 93% power to detect between-group (vitamin C and placebo) differences in cortisol concentrations at 24 hours postexercise. When considering the dose implemented in Thompson et al²¹ (400 mg), it was expected that the higher dose (1500 mg) in our study would maintain this difference at 72 h postexercise (open window). Each participant was randomly assigned to either the vitamin C (VC; males: 3; females: 3) or placebo (PL; males: 5; females: 1) group. Female participants performed all measurements during the early follicular phase, when estradiol levels are decreased, and (with the exception of one female in the VC group) were not oral contraceptive users. Salivary IgA and cortisol measurements from the oral contraceptive user did not reveal substantially different values when compared with the remaining subjects.

Preliminary Measurements
Participants were instructed to complete a 3-day diet record (2 weekdays, 1 weekend) and the data were analyzed using nutritional software (Fuel 2.3B, LogiForm International Inc., Quebec, Canada). Beginning 2 weeks before the experiment, participants were provided with a food guide and instructed to follow a diet high in carbohydrates (~60% of daily calories), moderate in VC (60 to 90 mg/day), and to maintain the same caloric intake as analyzed using the 3-day diet record.

In an initial familiarization session, anthropometric and skinfold measures were taken to calculate body composition. Participants then performed an incremental cycle ergometer (Monark Ergomedic 818 E, Sweden) test to exhaustion for the determination of maximal oxygen uptake (VO2max) using a metabolic cart (True One 2400, ParvoMedics, UT). A submaximal ride on a stationary ergometer for 12 minutes at 40, 50, 60, and 70% (3 minutes at each intensity) of VO2max was used to calculate the workload equivalent to 55% VO2max.

Experimental Protocol
During the 24 hours before each exercise trial, participants were asked to refrain from engaging in any intense prolonged physical activity. On three occasions separated by at least 7 days, participants reported to the laboratory at approximately 1400 following a 4-h fast. Baseline exercise trials were performed at pre-
supplementation, with acute and short-term measurements collected on day 2 and day 9 of the supplementation period, respectively. Exercise trials were performed at 1400 when s-IgA and cortisol concentrations appear to have stabilized after normal variation during the 6 hours after awakening. Participants were asked to empty their bladder and baseline body mass (rectal probe inserted and dressed for exercise) was recorded. Thereafter, skin thermistors were securely placed at specific sites on the participant.

For transferability of findings to the fit-but-nonelite general population, the exercise task simulated a prolonged exercise bout (eg, half-marathon) but was deliberately shorter than the ultramarathons often used in testing immune function postexercise. Participants performed a 3-h [or to fatigue, or a rectal temperature ($T_{re}$) of 39.5°C] exercise trial on a stationary cycle ergometer at 55% $V_{O2max}$ in a hot environment (34.8 ± 1.0°C, 13 ± 3% humidity). The 3-h exercise protocol has been previously shown to elicit significant increases in cortisol secretion and diminution of innate immune function. In addition, prolonged physical activity in a hot environment (30°C, 76% humidity) has been shown to reveal elevated amounts of circulating cortisol when compared with exercise alone. In all exercise trials, female participants were dressed in a T-shirt, shorts, shoes, and socks, whereas the males were dressed in shorts, shoes and socks. Pre-, post-, and 72 h postexercise saliva samples were collected for each condition. Measurements of heart rate (HR), blood pressure, thermal comfort vote (TCV), and thermal sensation (TS) were collected during exercise.

**Thermal Measurements**

Core temperature was measured using a rectal ($T_{re}$) temperature probe (Mon-A-Therm Core, Mallinkrodt Medical, St. Louis, MO) inserted 15 cm beyond the anal sphincter. Skin temperature ($T_{sk}$) was measured using thermistors (Mon-A-Therm Skin, Mallinkrodt Medical) at six sites (forehead, chest, upper arm, upper back, thigh, and calf) on the left side of the body to calculate the area-weighted mean skin temperature (mean $T_{sk}$). Mean body temperature (mean $T_{body}$) was calculated using a core and skin temperature weighting value of mean $T_{body} = 0.65(T_{re}) + 0.35(mean T_{sk})$. Three thermal values (preexercise, immediately following 5-min warm-up, and immediately before exercise cessation) were recorded using a portable data unit (SmartReader 8 Plus, ACR Systems, Surrey, BC, Canada) and stored on a computer with accompanying software.

**Physiological Measurements**

Metabolic rate was calculated during exercise from expired gases using a metabolic cart. Data were collected for 3 minutes at 30-min intervals starting after 10 min of exercise to ensure the participants maintained the desired intensity (55% $V_{O2max}$). For statistical purposes, only two metabolic values were used for analysis (after 10 min of exercise, and the final reading before exercise cessation). Heart rate was measured telemetrically (Vantage XL, Polar Electro Oy, Finland) and recorded at pre, following the 5-min warm-up, and immediately before exercise cessation.
Subjective Measurements

Subjective ratings of thermal comfort vote (TCV; from 5 [extremely uncomfortable] to 1 [comfortable], and thermal sensation (TS; from 0 [unbearably cold] to 9 [very hot], with 5 as thermoneutral) were recorded using scales modified from Gagge et al.\textsuperscript{25} Values were recorded preexercise, immediately following 5-min warm-up, and immediately before exercise cessation.

Antioxidant Supplementation

Participants were randomized into VC or PL groups. For 12 days, participants consumed three 500-mg tablets of VC or PL each day (one at each meal) in a double-blind fashion. The PL tablets contained microcrystalline cellulose—a common substance used in the formation of tablets and capsules. High dosage of VC supplementation for 7 days has been previously shown to attenuate cortisol secretion and reduce URTI frequency.\textsuperscript{12,26} Vitamin C and PL tablets were ingested for 3 days following the final exercise trial to provide supplementation during the open window period. The investigators maintained regular contact with each participant during the study to ensure the supplements were consumed. Furthermore, each participant was asked to return the pill containers at the end of the supplementation period to ensure and verify compliance with the dosage schedule. Participants were asked to refrain from consuming alcohol and caffeine during the supplementation period, while normal exercise duration/frequency was maintained.

Infection Logs

Participants recorded daily infection logs using the URTI symptoms questionnaire as described by Nieman et al\textsuperscript{3} assessing URTI frequency and intensity for 7 days before and subsequent to each experimental trial. Using the symptom scale (0—not present, 1—mild, 2—moderate, or 3—severe), each of the following health statements were coded: 1) No health problems today; 2) Cold symptoms (runny, stuffy nose, sore throat, coughing, sneezing, colored discharge); 3) Flu symptoms (fever, headache, general aches and pains, fatigue and weakness, chest discomfort, cough); 4) Allergy (itchy eyes, stuffy nose, clear discharge); 5) Nausea, vomiting, diarrhea; 6) Muscle, joint, or bone problems/injury; and 7) Other health problems (to describe in comment blank). A participant was considered to have an URTI if either a cold or flu was coded with a 1, 2, or 3 symptom rating. In addition, an URTI episode was defined as having started when participants coded for a cold or flu with a 1, 2, or 3 symptom rating, for more than 2 days and separated by at least 1 week from the previous episode.\textsuperscript{3}

Hydration Status

The hydration program was designed to prevent dehydration and to simulate events with periodic aid stations (eg, half-marathons, bike tours). Participants were weighed at 1400 for 3 days before participating in any of the experimental
sessions, and participants were asked to consume 30 mL·kg⁻¹ body mass of water between 0900 and 2100 the day before each exercise trial. On trial days, participants were required to be ±1% from baseline body mass before commencing the exercise bout. Participants were required to drink 250 mL of Gatorade every 15 minutes while cycling. Carbohydrate ingestion has been found to reduce cortisol levels during prolonged activity. Therefore, the previously mentioned Gatorade ingestion strategy was implemented in both groups and in all conditions to control carbohydrate consumption.

Saliva Collection and Analysis

At each experimental session, subjects were asked to swallow and rinse out the mouth with water ~10 minutes before providing a saliva sample. Rested, seated participants provided samples of approximately 1.5 mL of unstimulated saliva into a paper cup at pre, post, and 72 h postexercise. Thereafter, the saliva was extracted using a transfer pipette and placed into a 1.5-mL Eppendorf tube, and stored using standard procedures at −80°C until removed for analysis.

Cortisol and salivary IgA measures were analyzed using an Expanded Range High Sensitivity Salivary Cortisol and a Salivary Secretory IgA Indirect Enzyme Immunoassay Kit, respectively (Salimetrics, State College, PA). Total salivary protein was measured using the Bradford Protein Assay (Amresco Inc., Solon, OH). Standards, bovine serum albumin (BSA), and unknown samples were incorporated into each well of a 96-well plate, and were measured in duplicate. The coefficients of variation for the analytical methods were 5%, 6%, and 4% for cortisol, IgA, and total salivary protein, respectively.

Statistical Analysis

Statistical significance was set at \( P < .05 \) level, and values were expressed as group means ± SD. VC and PL groups were compared for participant characteristics (eg, height, body mass, \( V_{O2\text{max}} \), BF %) and nutritional (eg, energy, carbohydrate, fat, protein, vitamin C) measures using independent \( t \) test. Thermal (\( T_r \), \( T_{\text{skin}} \), and \( T_{\text{body}} \)), salivary (cortisol, s-IgA, and total salivary protein), and participant data (eg, HR, BP, TS, and TCV) were analyzed by using a 2 (VC and PL groups) × 3 (times of measurement) mixed ANOVA. If Mauchly’s test for sphericity was not met, a Huynh–Feldt correction was applied to the \( F \)-ratio. Post hoc analysis was carried out by a paired \( t \) test with a Bonferroni correction factor. If no statistical pairwise comparisons were detected, trend analysis data were examined to observe the changes seen over the entire range of supplementation conditions. Because the URTI data were not normally distributed, a nonparametric Mann–Whitney \( U \) test of two independent samples was completed to determine the URTI symptom differences between the VC and PL groups. In addition, the Friedman test of \( k \) related samples comparison was used to determine the within group differences in URTI. Statistical analysis was performed using SPSS 12.0 software (SPSS, Chicago, IL).
Results

Table 1 provides demographic, anthropometric characteristics, fitness, and nutritional data for the subjects who participated in this study. No significant differences in age, height, body mass, body fatness, and \( V_{\text{O}_2\text{max}} \) were present between the VC and PL groups. The dietary analysis measured from 3-d food records did not differ significantly between groups.

Exercise Responses

Conditions within the environmental chamber did not differ significantly between the VC and PL groups, with a mean temperature of \( 34.8 \pm 1.0^{\circ}\text{C} \) and \( 13 \pm 3\% \) relative humidity. Table 2 outlines the physiological and subjective responses to the exercise session. Mean exercise duration in the chamber across both groups and all conditions was \( 120.1 \pm 49.6 \text{ min} \), with \( 109.5 \pm 52.6 \text{ min} \) for the VC group, and \( 130.7 \pm 45.3 \text{ min} \) for the PL group. Mean exercise time between conditions was \( 116.7 \pm 51.1, 122.3 \pm 53.2, \) and \( 121.3 \pm 48.5 \text{ min} \) for the baseline, acute, and short-term condition, respectively. In both groups and in all conditions, mean exercise intensity was consistently maintained at \( 54 \pm 6\% \) of \( V_{\text{O}_2\text{max}} \). Participants lost \( 0.3 \pm 0.6 \text{ kg} \) (calculated using post- and preexercise body mass) during the experimental protocol.

Thermal Data

This study was successful at inducing exercise-heat stress for the purpose of evaluating the relationship between vitamin C and immunity. During all trials, \( T_{re} \)

<table>
<thead>
<tr>
<th>Table 1 Analysis of Participant Characteristics and Energy Intake of Vitamin C and Placebo Supplemented Individuals</th>
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<tbody>
<tr>
<td>Vitamin C (n = 6)</td>
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<td>--------------------------------------</td>
</tr>
<tr>
<td><strong>Anthropometry and Fitness</strong></td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Body Mass (kg)</td>
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<tr>
<td>Body Fat (%)</td>
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<tr>
<td>( V_{\text{O}_2\text{max}} ) (mL·kg(^{-1})·min(^{-1}))</td>
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<tr>
<td><strong>Nutritional Data</strong></td>
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<tr>
<td>Energy (kcal)</td>
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<tr>
<td>Carbohydrate (% energy)</td>
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<tr>
<td>Protein (% energy)</td>
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<tr>
<td>Fat (% energy)</td>
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<tr>
<td>Vitamin C (mg)</td>
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<td></td>
</tr>
</tbody>
</table>

Note. \( V_{\text{O}_2\text{max}} \) maximal oxygen consumption. Data are presented as group means ± SD.
Table 2  Physiological, Subjective, and Thermal Responses Before Exercise (Pre-Ex; Except for Oxygen Uptake Taken at 10 min into Exercise) and at the Point of Completion (Post-Ex) of Exercise at 55% $V_{O2max}$ in the Heat (35°C, 13% Relative Humidity)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Acute</th>
<th>Short-term</th>
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<tbody>
<tr>
<td></td>
<td>Pre-Ex</td>
<td>Post-Ex</td>
<td>Pre-Ex</td>
</tr>
<tr>
<td>Heart rate (b·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC</td>
<td>64.8 ± 7.5</td>
<td>153.5 ± 16.3*</td>
<td>67.5 ± 10.0</td>
</tr>
<tr>
<td>PL</td>
<td>70.8 ± 15.8</td>
<td>167.5 ± 8.2*</td>
<td>70.0 ± 14.6</td>
</tr>
<tr>
<td>Oxygen uptake (% of $V_{O2max}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC</td>
<td>53.5 ± 6.4</td>
<td>55.9 ± 5.1</td>
<td>52.3 ± 4.8</td>
</tr>
<tr>
<td>PL</td>
<td>52.4 ± 7.3</td>
<td>55.8 ± 8.9</td>
<td>47.2 ± 5.1</td>
</tr>
<tr>
<td>Thermal Sensation [TS, scale 0 (unbearably cold) to 9 (very hot)]</td>
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<td></td>
</tr>
<tr>
<td>VC</td>
<td>5.2 ± 0.8</td>
<td>7.3 ± 0.8*</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>PL</td>
<td>5.2 ± 0.4</td>
<td>7.2 ± 2.6</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Thermal Comfort Vote [TCV, scale 1 (comfortable) to 5 (uncomfortable)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC</td>
<td>1.2 ± 0.3</td>
<td>3.3 ± 0.7*</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>PL</td>
<td>1.0 ± 0.0</td>
<td>3.9 ± 1.3*</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
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<td></td>
</tr>
<tr>
<td>VC</td>
<td>37.2 ± 0.3</td>
<td>38.6 ± 0.7*</td>
<td>37.3 ± 0.4</td>
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<tr>
<td>PL</td>
<td>37.4 ± 0.2</td>
<td>39.1 ± 0.1*</td>
<td>37.3 ± 0.3</td>
</tr>
<tr>
<td>Mean skin temperature (°C)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>VC</td>
<td>35.4 ± 0.5</td>
<td>37.7 ± 0.7*</td>
<td>35.5 ± 0.5</td>
</tr>
<tr>
<td>PL</td>
<td>35.7 ± 0.7</td>
<td>37.7 ± 1.0*</td>
<td>35.8 ± 0.5</td>
</tr>
<tr>
<td>Mean body temperature (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC</td>
<td>36.5 ± 0.3</td>
<td>38.3 ± 0.6*</td>
<td>36.7 ± 0.2</td>
</tr>
<tr>
<td>PL</td>
<td>36.8 ± 0.3</td>
<td>38.6 ± 0.4*</td>
<td>36.8 ± 0.2</td>
</tr>
</tbody>
</table>

Note. Data presented for the baseline (no supplementation), acute (1 day supplementation) and short-term (7 days supplementation) sessions. *Indicates significant difference between Pre-Ex and Post-Ex within each session ($P < .05$). Data are presented as group means ± SD.
increased significantly from a preexercise mean value of 37.3 ± 0.3°C to an end-exercise (final $T_{re}$ measure before exercise cessation) mean value of 38.9 ± 0.6°C ($P < .05$). Mean $T_{sk}$ increased for both groups, in all conditions ($P < .05$) during exercise from its initial temperature of 35.7 ± 0.5°C to 38.3 ± 0.9°C. There were no differences detected in $T_{re}$, mean $T_{sk}$, or thermal sensation and comfort between the VC and PL groups in all time cycles during each condition.

**URTI and Saliva Analysis**

Although not significant ($P = .35$), during the 14-day period following day 1 of the supplementation period, fewer reported symptoms of URTI were recorded in the VC group compared with PL (21 days and 31 days, respectively). Further, non-parametric test of two independent samples comparison revealed no significant differences in mean URTI frequency (participant-days with URTI) for each condition (baseline, acute, and short-term) between the VC (14, 9, and 12 days) and PL (18, 16, and 15 days) groups during the 7 days following the prolonged exercise trial (Figure 1). In addition, nonparametric Friedman test of $k$ related samples comparison detected no significant differences in repeated measures of mean URTI frequency within each group (VC and PL) between each condition (baseline, acute, and short-term) (Figure 1).

Mean salivary cortisol concentration for both groups increased significantly ($P < .05$) in all conditions from a preexercise value of 4.4 ± 3.5 nmol/L to a postexercise value of 14.0 ± 12.6 nmol/L and significantly decreased ($P < .05$) to 5.6 ± 6.2 nmol/L at 72 h postexercise. A main effect of trial (baseline, acute, and short term) was observed for postexercise cortisol ($P = 0.027$) in the VC group,

![Figure 1](image_url)

**Figure 1** — Total participant days with URTI during the 7 days following the prolonged exercise trial in the vitamin C (black bars) and placebo (gray bars) groups for each condition (baseline, acute, and short term).
but post hoc analysis found no difference between trials. However, post hoc trend analysis revealed a significant linear trend ($P = 0.032$) within the VC group in postexercise cortisol between each condition (Figure 2). No significant differences were detected in postexercise mean cortisol concentrations between conditions in the PL group. Between-group differences in cortisol concentrations were detected at preexercise during the baseline condition, and at postexercise during the short-term condition ($P < .05$). No other between-group differences was observed. A secondary analysis of cortisol with baseline vitamin C intake incorporated as a covariate did not substantially change the current results.

Mean salivary IgA-to-protein ratio increased during exercise in all conditions for both groups; however, these differences and comparisons between groups

![Figure 2](image-url)  
**Figure 2** — Mean (SD) cortisol for the vitamin C (Figure 2 Vitamin C) and placebo (Figure 2 Placebo) group (preexercise, postexercise, and 72 h postexercise) for each condition; baseline (●), acute (■), and short term (▲). Linear trend analysis revealed a significant attenuation of cortisol increase with acute compared with baseline, and short-term compared with acute and baseline.
were not significant \((P > .05); \) Figure 3). Further, significant alterations were detected in s-IgA concentrations, increasing from a preexercise value of \(59.6 \pm 7.6 \text{ mg·L}^{-1}\) to a postexercise value of \(104.3 \pm 13.3 \text{ mg·L}^{-1}\), and significantly decreasing to \(64.7 \pm 7.1 \text{ mg·L}^{-1}\) at 72 h postexercise \((P < .05)\). No differences were detected in postexercise and at 72 h postexercise s-IgA concentration and s-IgA-to-protein ratio between the baseline, acute, and short-term trials for the VC group. Furthermore, no differences in s-IgA-to-protein ratio (Figure 3) or s-IgA concentration were found between the VC and PL groups in all time cycles (pre, post, 72 h postexercise) for each condition \((P > .05)\). As described above, whether

**Figure 3** — Mean (SD) salivary IgA-to-protein ratio for the vitamin C (Figure 3 Vitamin C) and placebo (Figure 3 Placebo) group (preexercise, postexercise, and 72 h postexercise) for each condition; baseline (●), acute (■), and short term (▲).
s-IgA was expressed relative to total protein or total s-IgA concentration, between-
and within-group comparisons revealed similar results.

Discussion

We observed a significant linear trend of postexercise attenuations in cortisol con-
centrations following acute and short-term supplementation within the VC group,
whereas no attenuations were observed within the PL group. However, no differ-
ences were detected in s-IgA concentration and s-IgA-to-protein ratio, nor were
the prevalence of URTI symptoms significantly reduced or different between
groups. As suggested by Nieman et al,14 the present results imply that vitamin C
supplementation may not directly affect immunity until extreme distances are
achieved, or when the result of a possible summation effect occurs from acute
negative changes (possibly due to overtraining), after each exercise bout.

The acute response to vitamin C supplementation is in agreement with a pre-
vious study by Davison et al,13 where the combination of acute vitamin C and
carbohydrate supplementation significantly blunted postexercise cortisol. It is
possible that carbohydrate ingestion caused a reduced cortisol response, as inges-
tion has also been found to blunt hypothalamic-pituitary axis activation in response
to prolonged exercise, reducing cortisol levels.13 However, considering that exer-
cise duration means were not significantly different between conditions, carbohy-
drate ingestion was similar between trials for both groups in the current study.

Short-term vitamin C supplementation provoked an even greater attenuation
in postexercise cortisol when compared with acute supplementation, with placebo
levels unchanged. These findings support previous work by Peters et al,12 who
revealed post-ultramarathon cortisol attenuation following 7 days of 2 × 500 mg
tablets of vitamin C. Therefore, despite the fact that the duration of the activity in
the current study was substantially reduced when compared with an ultramar-
athon, it appears that variations in cortisol secretion are rather sensitive to vitamin
C ingestion. The biochemical mechanism by which vitamin C supplementation
blunts the postexercise heat stress cortisol response remains somewhat unclear.
Some investigators have proposed that high concentrations of vitamin C within
the adrenal glands can act as a pro-oxidant by reducing ferric iron (Fe³⁺) to the
ferrous state (Fe²⁺), which acts as a free radical catalyst.16 Specifically, it is pro-
posed that the formation of these oxidants could potentially impair cortisol syn-
thesis by negatively influencing key enzymes.13,16 Nonetheless, the relationship
between cortisol and vitamin C seems to exist regardless of the supplementation
time frame, exercise duration, and environmental temperatures.

In contrast to the cortisol response, postexercise s-IgA concentration and
s-IgA-to-protein ratio remained unchanged immediately following exercise in all
conditions within both groups, supporting previous reports during exercise27 and
when comparing exercise in thermoneutral versus hot conditions.10 However, sali-
vary flow rate was significantly reduced in both studies following exercise, pos-
sibly as a result of dehydration and potentially influencing s-IgA secretion rate.28
In the current study, saliva flow rate was not measured. However, in contrast to
previous studies that either provided water ad libitum\textsuperscript{10} or failed to mention any hydration procedures,\textsuperscript{12} hydration was controlled throughout each exercise session, resulting in only a 0.3-kg reduction in body mass. Taken as a whole, because the findings in the current study are similar to the aforementioned research, it is logical to suggest that, regardless of hydration status or saliva flow rate, the s-IgA concentration and s-IgA to protein ratio will remain unchanged when using the exercise protocol implemented in this study. Therefore, this study supports the assertion of Blannin et al.\textsuperscript{27} that although the quantity of saliva may be influenced by prolonged exercise and hydration status, there appears to be no detrimental effects on the quality of saliva produced.

Some reports indicate a significant reduction in postexercise s-IgA in elite endurance athletes often correlated with URTI susceptibility following ultramarathon performance.\textsuperscript{5,6,15} Moreover, vitamin C has been shown to reduce the severity of ultramarathon-induced URTI, with no confirmation of any s-IgA variations.\textsuperscript{14} The present results do not directly contradict or support the aforementioned study since there were no significant attenuations in URTI prevalence, nor were there any postexercise s-IgA alterations within both groups following vitamin C supplementation. The exercise protocol was intended to simulate a prolonged bout of exercise (similar to a half-marathon or marathon) in hot conditions for healthy, aerobically fit individuals. In addition, despite being in excellent health relative to their age groups, the participants in this study were not considered elite endurance athletes nor did they experience the same prolonged training volumes/intensities as marathoners and ultramarathon athletes. Therefore, the participants may have benefited from enhanced immune response from moderate activity\textsuperscript{29} but did not experience extensive prior training or any prerace stress, both of which occur before ultramarathon participation and have been shown to negatively influence immunity.\textsuperscript{4,30}

Salivary IgA concentration was expressed using a standard method (relative to total protein) and revealed similar results when analyzed as total s-IgA content. A recent study by Koch et al.\textsuperscript{31} showed that changes in s-IgA expressed relative to total protein was correlated with s-IgA relative to secretion rate, osmolality, and to total s-IgA concentration. The findings from Koch et al.\textsuperscript{31} suggest the possibility that our s-IgA data expressed relative to total protein may have revealed similar results if expressed relative to secretion rate or osmolality. This study further reiterates the need to establish a universal standard for expressing s-IgA.

This study has some limitations. It was our intention to have the participant’s work to exhaustion, until 3 hours had elapsed, or until reaching a rectal temperature of 39.5°C. The vitamin C and placebo groups exercised for a mean duration of 109.5 ± 52.6 min and 130.7 ± 45.3 min respectively, and postexercise rectal temperature values do not significantly differ. Therefore, the placebo group may have experienced more stress during the exercise bout, which may provide some explanation as to why postexercise cortisol concentrations were elevated during the acute and short-term trials when compared with the steady attenuation observed in the vitamin C group. In addition, mean time to exhaustion for the placebo group was lower during the baseline trial (122.5 ± 50.6 min) when compared with the acute and short-term trials (137.5 ± 48.8 min and 132.0 ± 43.7 min, respectively), which may partly explain why no postexercise increase in cortisol was evident during the baseline trial.
Practical Implications

Despite increased environmental temperatures, vitamin C supplementation tends to blunt the postexercise cortisol response following both acute and short-term supplementation. Considering high cortisol concentrations has often revealed an immunosuppressive response, the potential vitamin C-induced reduction in cortisol may preserve immune function following endurance performance similar to that of a half-marathon or marathon in hot conditions. The lack of influence on s-IgA and URTI may potentially be due to the more moderate previous training experience and amount of physical and psychological stress placed on the participants compared with previous reports on elite endurance athletes participating in highly competitive ultramarathons.

Acknowledgments

The authors would like to thank all the participants who volunteered for this study. Phil Campagna and David Westwood from the School of Health and Human Performance at Dalhousie University provided technical and statistical assistance, respectively. The study was supported by Dalhousie University Faculty of Graduate Studies.

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