Effects of Physical Activity, Body Fat and Salivary Cortisol on Mucosal Immunity in Children

Thomas J. Cieslak, Gail Frost, Panagiota Klentrou
Faculty of Applied Health Sciences, Brock University

Abbreviated title: Determinants of Mucosal Immunity in Children

Corresponding Author
Panagiota Klentrou
Faculty of Applied Health Sciences
Brock University
St. Catharines, Ontario L2S 3A1
Tel: (905) 688-5550 ext.4538
Fax: (905) 688-8364
E-mail: nota.klentrou@brocku.ca
Abstract

This study examined relationships among physical activity, body composition, and stress- and immunity-related variables in fifth grade children (10-11 yrs) in Southern Ontario. The 29 boys and 32 girls, who participated in the study, performed a 20m shuttle run for prediction of aerobic fitness. Bioelectrical impedance was used to assess relative body fat (%BF). Standardized questionnaires were used to determine physical activity related variables and frequency of Upper Respiratory Tract Infection (URTI). Resting saliva samples were collected and tested for resting cortisol and resting secretory Immunoglobulin A (SIgA). Subjects wore a pedometer for 48h to estimate their average total distance traveled per day. Secretory IgA was significantly correlated with reported URTIs but not related to salivary cortisol, physical activity, fitness level or relative body fat. Children who spent more time in sport activities and had higher aerobic fitness reported fewer "sick" days. Children with body fat higher than 25% reported significantly (p<0.05) more sick days than the rest of the cohort. There were no gender differences in SIgA, URTI frequency and cortisol levels. The test-retest reproducibility for salivary cortisol was 0.66 (p<0.01), while long term SIgA reproducibility was non-significant for repeated measurements taken after six weeks. Resting secretory immunity was not strongly related to fitness and physical activity, but there was evidence that reduced physical activity and excess body fat can result in higher URTI incidence.

**Key Words:** aerobic fitness, secretory Immunoglobulin A, upper respiratory tract infection
INTRODUCTION

There is evidence that exercise influences natural immunity, T- and B-cell functions and cytokine responses, through hemodynamic changes and hormonal secretion in adults (30). The magnitude of the effect on the immune system depends on the intensity, duration, and chronicity of exercise. Moderate exercise is believed to have a positive effect on the immune system while intense exercise evokes a negative response (22, 23, 33, 37). Moderate exercise has been shown to enhance cell-mediated immunity (CMI) and increase secretory IgA (SIgA), leading to improved immunity against infection (24). Recent studies have also demonstrated that moderate physical activity reduces the incidence of upper respiratory tract infections (URTI) by as much as 30% (16, 26). Resting concentration of SIgA is also increased with moderate activity (16, 26). Intense training in elite athletes has been linked to a weakened immune system, and increased risk of infection at the mucosal levels (22, 23).

In addition to intense exercise, cortisol levels and body composition have been associated with immunosuppression. Hucklebridge et al. (12) have shown that increased psychological stress resulting in increased cortisol secretion caused a decreased rate of salivary IgA secretion. Other studies have shown that as cortisol levels increase during stressful situations, secretory IgA also increases (6, 43). Nieman et al. (30, 32) did not find any relationship between URTI and Body Mass Index (BMI) in elite marathon runners. However, in a study on obese women, a significant relationship between obesity and elevated levels of leukocytes and lymphocytes was seen (33). Obesity was also related to suppressed levels of monocyte and mitogen-stimulated lymphocyte proliferation as well as other immune markers, supporting the concept that obesity is
associated with alterations in, and even suppression of, immunity (33).

It has long been suspected that the younger the individual, the less effective the immune defense. When examining the expression of IgA in children, Gleeson et al. (9) found significant fluctuation in salivary IgA levels. Salivary IgA was found to peak at the age of five years, decrease slightly until the age of seven, and then remain relatively stable to age nine (9). Other studies comparing children to adults found that children did not achieve adult levels of immunoregulation until they were almost 11 years old (35). However, these studies did not control for other factors such as diet, climate, season or amount of exposure/contact to densely populated areas, all of which could have significantly affected the immune system. Very few studies have tried to link immunity to physical activity in youth. There are scant data comparing SIgA levels and the incidence of URTI in moderately active children to those of sedentary children. Tharp (41) found that resting SIgA levels increased over time in children who trained for, and played, basketball. Boas et al. (4) did not find any significant differences in leukocyte and lymphocyte levels, natural killer (NK) cells and NK cell activity between trained and untrained children, 9 to 17 years of age. A study of 8 to 10 year old children found no relationship between peak VO$_2$ and immune function, but body mass was found to be significantly correlated with SIgA concentration, serum leukocyte counts, monocytes, and granulocyte phagocytosis (34). Recently, adolescents who spent less time in sport activities have also reported significantly higher URTI frequency (17).

The goal of this study was to examine relationships between mucosal immunity, physical fitness levels, stress levels, and relative body fat in 10 to 11 year old children attending public schools in Southern Ontario, Canada. It was hypothesized that low levels
of physical activity, and elevated levels of resting salivary cortisol and body fat would result in decreased resting SIgA, and higher frequency of URTI. A secondary objective of this work was to test the long term reproducibility of both resting salivary cortisol and IgA for repeated measures taken six weeks apart.

METHODS

Subjects

The project and all protocols were approved by the Brock University Human Ethics Review Board. Sixty one fifth grade students (29 boys, 32 girls) participated in the study. Subject characteristics are presented in Table 1. Subjects were recruited from three schools in Southwestern Ontario. Subjects came from classes of students from randomly selected schools that agreed to participate. All fifth grade students enrolled in the selected schools were provided with a project package containing a study description and a parental consent form. Permission was obtained from school officials, and the purpose and potential risks of the study were explained carefully to parents, before obtaining consent. Of the initially recruited subject cohort, 80% returned a signed parental consent form. Exclusion criteria were the presence of chronic medical conditions such as asthma, heart disease or any other condition that would put the subject at risk when performing the experimental tests, and a flu vaccination in the past 12 months. The study was conducted in May and June, during the Northern hemisphere spring-summer. Any medication taken for treatment of illness was recorded.
Physical Activity and Fitness Assessments

Predicted peak aerobic power was estimated using the 20m shuttle run of Léger and Lambert (19). Subjects continued through the stages until they could no longer keep pace with the cadence of the tape and the last completed stage was recorded. An estimate of each individual’s peak \( \dot{V}O_2 \) was determined by multiplying the MET value associated with the final completed level of activity by 4.6 ml kg\(^{-1}\) min\(^{-1}\) for 1 MET as suggested by Allor and Pivarnik (1). This test has been validated against a direct laboratory protocol (r=0.91, SEE=4.16), and the reproducibility has been reported (r = 0.975) for measurements taken on the same subject within a one-week period (19). In addition, this measurement has been shown to be a valid test in a school setting for children 6 to 17 years of age (20).

The Habitual Activity Estimation Scale (HAES) was used to estimate the time spent in all forms of habitual activity, i.e. the number of hours of habitual physical activity per day (13). The questionnaire divides a day into four periods and activities are ranked according to intensity. Total duration of daily activity was then used to calculate the total weekly habitual activity (h wk\(^{-1}\)). Total activity time has been proposed as a more appropriate measurement for children than the combined energy cost of physical activities (12). The validity of the HAES has been evaluated by Hay (15), and the test-retest reliability was found to be >0.80 (14, 15).

The Participation Questionnaire (PQ) was used to estimate both the amount of physical activity and the nature of the participation, using three categories: free time activity (FTA), organized activity time (OAT), and total time spent in activities (TA) (13). Participation scores are referred to as activity units. Each unit refers to participation...
in one activity on a regular or seasonal basis. An activity unit in the Organized Sport section refers to participation on a single sport team (either school or community), playing on an intramural team, or participating in a series of lessons. An activity unit in the Free Choice section refers to any active leisure pursuit as a preferred choice after school, on weekends, or with family and friends (13). The PQ has been validated against the Teachers' Evaluation of Physical Activity (r=0.62) and test-retest reliability was reported to be 0.81 for grades 4-8 and 0.89 for grades 9-12 (13).

Total distance traveled per day (TD) was measured using a Digi-Walker© pedometer (New Lifestyles Inc, Missouri, USA). The device recorded the child’s physical activity in steps using a step counter. Each individual’s step was measured to the nearest centimeter. The steps counted by the pedometer were then multiplied by the individual’s stride length to determine total distance traveled in meters. All subjects were required to use the Digi-Walker© for two consecutive days. Each monitor was calibrated to accurately record the movements of the subject. A 2-day activity log accompanied the pedometer. The subjects recorded daily physical activities other than general locomotion. The log ensured that data recorded by the pedometer were valid, accurate, and reliably recorded. The Digi-Walker© pedometer was chosen because of stability and reliability in addition to cost effectiveness and ease of operation. Welk et al. (42) examined the Digi-Walker© to determine its effectiveness as a tool for assessing physical activity patterns. The pedometers tended to under-predict high intensity activities simply because fewer steps were needed to complete the activity. As a result, distinguishing between differing levels of physical activity may not be possible. However, the authors do support use of the pedometer as an indicator of daily activity (42).
**Body Fat Measurements**

Bioelectrical impedance analysis (BIA) was used to estimate the percent body fat (%BF) using the input variables of physical activity level, body frame size, height, mass, and sex previously described (21). The skin was cleaned with 70% alcohol and four surface electrodes were applied: two on the right hand at the 2nd metacarpal and the wrist between the styloid processes of the radius and ulna, and two on the right foot at the 2nd metatarsal and the ankle between the medial and lateral malleoli. With the subject in supine, an electrical current of 50 kHz and 0.8 mA was applied through the electrodes, to determine whole body resistance (Quantum II, RJL Systems, USA). Short and long term reproducibility of this technique has been reported as $r = 0.999$ for measurements made on the same subject within one week, and 0.977 for repeat measurements up to one month apart, resulting in a coefficient of variance of 2.5% (18). All participants were provided written information to standardize the procedure, and BIA measurements were made prior to the shuttle run to avoid problems associated with dehydration and changes in skin temperature, electrolyte concentration and glycogen stores (18). The validity and reliability of this method has been demonstrated successfully in children and adolescents (36, 40).

**Saliva Testing**

Following body composition assessment subjects submitted to collection of two saliva samples. For each of these samples, one milliliter of unstimulated, whole mixed saliva was collected using cylinder-shape swabs (SARSTEDT Inc., Quebec, Canada) placed in the mouth. Subjects were instructed to moisten/chew lightly on the swab for one minute. After a two minute interval a second sample was collected. Since there is
evidence that acute maximal exercise results in a delayed onset of cortisol secretion, care was taken to ensure that the saliva samples were collected with sufficient time after the aerobic power test to avoid this effect. Following collection of the first sample, temperature of the subject was taken using an auditory thermometer (FIRSTTEMP GENIUS Model 3000A Tympanic Thermometer, Mansfield, MA) to ensure cortisol contained in the second saliva sample was not affected by acute stress. After sampling, the swabs were placed directly into plastic tubes, and then stored using standard procedures at -20°C (8) until the samples were centrifuged to extract the saliva from the swab and assayed. The subjects were instructed not to consume any food or drink for at least one hour prior to saliva collection, and the mouth was not rinsed with water prior to sampling, to avoid altering resting SIgA levels.

Secretory IgA in saliva was measured by radial immunodiffusion using the BINARID™ kit (Binding Site Limited, UK) (6, 25). Radial immunodiffusion developed by Mancini et al. (25) involves a quantitative gel diffusion technique with antibody incorporated into the agar. More specifically, an agar plate is prepared by incorporating antibody throughout the agar. The test sample is put into a small antigen well, and on diffusion into the agar, forms a ring of antibody-antigen precipitate around the well. The diameter of the precipitate ring reflects the concentration of the antigen. As the protein concentration in saliva is dependent upon a number of factors, including vascular permeability and mouth dryness, it is recommended that the concentration of secretory IgA be related to the level of salivary albumin present in the sample (6, 25). Results were, therefore, expressed as SIgA/albumin ratio (SIgA:Alb). Since the literature suggests that salivary concentration is influenced by the time of the day and the saliva collection method (2), saliva samples were collected at the same time during the day, in
the morning, and using the same collection method. The reliability for SIgA:Alb was \( r = 0.82 \).

Cortisol levels were assessed using a DPC coat-a-count Cortisol Kit. Total plasma concentrations of cortisol were measured in duplicate by commercial solid-phase \(^{125}\text{I}\) radio-immunoassay kits. \(^{125}\text{I}\)-labeled cortisol competes for antibody sites for cortisol within the sample. The antibody is bound to the wall of the polypropylene tube, so when the supernatant is decanted the antibody-bound fraction of the radiolabeled cortisol is still present. The amount of cortisol present in the sample is measured by a gamma counter. Reference ranges are from 3.5 - 27.0 nmol/L at 8am and < 6.0 nmol/L at 10pm for both sexes and all ages, including children.

**Frequency of URTI**

A one-month Health Log (33) was used to record the incidence and duration (number of days) of URTI’s. Subjects recorded cold and flu symptoms each day of the month using a set of codes provided with the log. The severity of the symptoms was rated by each subject as mild, moderate, or severe. Parental supervision was required to ensure accurate recording of the symptoms. This method was chosen to eliminate participant bias when recording from memory. All logs were also completed during the Northern hemispheres’ spring-summer (April to June), which is a moderate to high infection season for Canada. The total number of days with URTI symptoms was calculated for each subject, with days being counted only if two or more consecutive days of cold or flu symptoms were reported (33).

A randomly selected subgroup (n = 15) was assessed a second time, six weeks after initial testing. Follow-up testing was conducted to examine the
reproducibility of salivary measures. During this follow-up visit, all the tests were repeated.

**Statistical Analysis**

One-way ANOVA was used to compare males and females on physical activity, %BF, aerobic power, SIgA and salivary cortisol. Pearson correlation analysis was used to detect relationships among all the variables and intraclass correlation analysis was used to test the reproducibility of resting salivary cortisol and IgA measures (pre and six weeks post). All data analyses were conducted using SPSS 11 for Windows. A minimum value of \( p<0.05 \) was accepted to indicate a statistically significant result. All data were checked for normality and equality of distribution, prior to any analysis being performed.

**RESULTS**

There were no statistically significant differences between genders found in physical characteristics (Table 1). Physical activity levels, salivary cortisol, relative body fat, predicted aerobic power, SIgA as well as SIgA expressed as SIgA/albumin ratio (SIgA:Alb) are presented in Table 2. Significant differences were found between genders in predicted peak \( \dot{V}O_2 \) and in distance traveled per day (Table 2). No significant difference was evident between genders in reported levels of physical activity and relative body fat. There was no significant difference between genders in either SIgA or SIgA:Alb (Table 2).

Based on the HAES questionnaire, children who were active less than 3 h·day\(^{-1}\)
were considered hypoactive, while those who recorded activity levels above 3 h·day\(^{-1}\) were considered active. Twenty two percent of boys reported less than 3 h·day\(^{-1}\) of habitual physical activity while 31.8% of girls did not achieve this level. As shown in Table 3, the hypoactive children had significantly lower predicted peak \(\dot{\text{V}}\text{O}_2\) and SIgA:Alb, as well as significantly higher relative body fat and frequency of URTI. Moreover, body fat values revealed that 40% of the children (50% of boys and 42% of girls) had relative body fat above 25%. Children with relative body fat higher than 25% reported significantly more days with cold and flu symptoms and total sick days than the rest of the cohort (Figure 1).

As shown in Table 4, organized activity and free time activity were significantly related to peak \(\dot{\text{V}}\text{O}_2\). The total activity score (TA) was significantly correlated with peak \(\dot{\text{V}}\text{O}_2\), distance traveled per day and resting salivary cortisol levels. Distance traveled per day was also significantly correlated with peak \(\dot{\text{V}}\text{O}_2\) as well as with time spent in organized sport activities. Salivary cortisol was significantly correlated with body fat and time spent in organized sport. Secretory IgA and SIgA/albumin ratio demonstrated significant relationship only with incidence of URTI (Table 4). The incidence of URTI was also correlated with total activity score, weekly habitual activity, resting salivary cortisol (Table 4).

The intraclass correlation coefficient for initial and 6-week post measures of salivary cortisol was \(r=0.66\). The intraclass correlation coefficients for SLgA and SIgA:Alb were \(r=0.23\) and \(r=0.20\) respectively. When comparing the means, initial and 6-
week post measurements of SIgA and SIgA:Alb were not significantly different.

**DISCUSSION**

Results of the present study suggest that, when classified by level of habitual physical activity, more active children have a higher SIgA and SIgA/albumin ratio, and reduced frequency of URTI than those who are less active (Table 3). Reduced frequency of URTI has been recently reported in active adolescents (17). It has been also shown that sedentary adults are more susceptible to infectious disease, when compared with active adults (16, 30). The results of the present study suggest that this may also be true in children. Another interesting finding is that despite the correlation found between lower incidence of URTI and higher activity levels as well as between lower incidence of URTI and higher SIgA, SIgA did not demonstrate a significant relationship with the physical activity variables. This lack of relationship may be due to the homogeneity of the SIgA levels among subjects or to the low level of physical activity that may not have been adequate to show the same associations as for elite athletes. Salivary IgA is believed to be the first line of defense for the human body against pathogenic microbial invasion and several studies have suggested a direct association between SIgA levels and exercise in adults (9, 10, 16). However, other studies in children have also shown no significant relationship between SIgA and physical activity markers (3, 34). It is possible that SIgA was not be a good indicator of the children’s resting mucosal immunity as they have had greater exposure to infectious agents at the school, where were surrounded by large numbers of other children, which may have induced chronic elevations in SIgA.

When examining the total cohort, there was no significant relationship between
SIgA and body fat. This is in contrast to the results of Nieman et al. (34), who found that there was a correlation between body fat levels and SIgA in children. In addition, when categorized by relative body fat values, children in the present study with body fat higher than 25\% did report higher frequency of flu and cold symptoms than their counterparts. Salivary IgA levels and SIgA/albumin ratios were not significantly related to cortisol levels either. Cortisol has been shown to be an indicator of stress (4). Our results would seem to suggest that stress may not have any more of an effect on secretory immunity in children than physical activity does. Nevertheless, a correlation between higher salivary cortisol and lower incidence of URTI was found (Table 4). Resting levels of salivary cortisol as well as incidence of URTI and SIgA were not correlated with aerobic fitness (Table 4). Other studies have also demonstrated that immune function indicators and cortisol levels are independent of aerobic fitness level (27).

No gender differences were found in levels of SIgA and SIgA:Alb among the children in the present study. Schouten et al. (38) observed gender differences in secretory IgA levels in adults. Their subjects, however, were in job situations where they were not necessarily exposed to increased infection levels as children are, and not all the subjects were exposed to the same work environment.

Salivary analysis is a practical way to measure biochemical markers in children. One of the objectives of the present study was to determine if salivary cortisol and SIgA concentrations were reliable tools for assessing resting stress and immunity levels in younger individuals. Several studies report that adult levels of secretory IgA (27) and salivary IgA are reached between one and seven years of age (8, 39) but there are very few published reports on short or long-term reproducibility of SIgA especially in children. In the present study, SIgA, SIgA:Alb and salivary cortisol values
were reexamined after six weeks. The interclass correlation coefficient was high ($r=0.66$) for cortisol and low for both SIgA ($r=0.23$) and SIgA/albumin ratio ($r=0.20$). This indicates that the initial and six week post-values for SIgA and SIgA/albumin ratio were not significantly related. In contrast, it is interesting to note that initial and 6 week post-values of both these variables were not significantly different, as shown by ANOVA. Gleeson et al. (8) also found variability in SIgA levels in the children’s saliva samples from the age of one to five, but concluded that since there seemed to be a plateau from five to seven years of age SIgA would remain relatively stable from that point on. Since SIgA was not significantly related to any of the other variables, it is reasonable to believe that SIgA, although very practical as a tool for assessing state of immunity in children, may not a reliable measure when used alone. However, given the small sample size in this study, it would be premature to conclude that it does not provide useful information when screening secretory immunity in children. Salivary IgA levels change in a yearly circadian rhythm in adults (29). Circadian rhythm in this case refers to a predictable change in SIgA and serum IgA that is determined by the seasons. Winter or colder temperatures cause an increase in concentration of SIgA and serum IgA, while warmer temperatures or summer are linked to a decrease in SIgA and serum IgA. The rhythm in children may be more complex. It is possible that the time of day or year, or the type of environmental exposure (i.e. in school or not) could all be affecting the values.

In summary, this study showed that SIgA is not significantly correlated with salivary cortisol levels, physical activity, body fat and cardiorespiratory fitness in 10 to 11 year old children. Salivary IgA levels seem to vary in this age group. This variability needs to be investigated further in order to be able to make a definitive statement about the reproducibility of secretory immunity measures. This study supports the
importance of physical activity for children’s resistance to infection. Children who spent more time in sport activities and had higher aerobic fitness reported fewer sick days while children with relative body fat exceeding 25% reported significantly more sick days than the rest of the cohort.
REFERENCES


42. Welk GJ, and Wood K. Physical activity assessment in physical education: A

Figure Legends

**Figure 1:** Reported days with cold and flu symptoms and total sick days for children with relative body fat >25% and children with relative body fat <25% (*p<0.05; **p<0.01).
Table 1: Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Males (n=29)</th>
<th>Females (n=32)</th>
<th>Total Cohort (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean ± SE)</td>
<td>(mean ± SE)</td>
<td>(mean ± SE)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>10.5 ± 0.4</td>
<td>10.4 ± 0.5</td>
<td>10.4 ± 0.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>142.6 ± 1.4</td>
<td>142.2 ± 1.5</td>
<td>142.4 ± 1.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.6 ± 3.9</td>
<td>87.7 ± 3.5</td>
<td>87.6 ± 2.5</td>
</tr>
</tbody>
</table>
**Table 2:** Salivary IgA, S IgA/albumin ratio, cortisol, body fat, aerobic power and physical activity levels in male and female children

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males (n=29) (mean ± SE)</th>
<th>Females (n=32) (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIgA (ml L⁻¹)</td>
<td>133.4 ± 17.4</td>
<td>134.8 ± 20.7</td>
</tr>
<tr>
<td>SIgA/albumin Ratio</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Salivary Cortisol (nmol L⁻¹)</td>
<td>3.0 ± 0.5</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>$\dot{VO}_2$ max (ml·kg⁻¹·min⁻¹)</td>
<td>48.2 ± 0.9**</td>
<td>45.3 ± 0.7**</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>25.3 ± 1.3</td>
<td>21.5 ± 1.8</td>
</tr>
<tr>
<td>HPA (h·d⁻¹)</td>
<td>4.9 ± 0.4</td>
<td>5.4 ± 0.9</td>
</tr>
<tr>
<td>Free Time Activity (score)</td>
<td>13.0 ± 0.7</td>
<td>13.2 ± 0.6</td>
</tr>
<tr>
<td>Organized Activity Time (score)</td>
<td>10.0 ± 1.1</td>
<td>9.6 ± 0.9</td>
</tr>
<tr>
<td>Total Activity (score)</td>
<td>23.0 ± 1.4</td>
<td>22.8 ± 1.2</td>
</tr>
<tr>
<td>Locomotion (m·day⁻¹)</td>
<td>120.5 ± 13.7**</td>
<td>95.4 ± 6.5**</td>
</tr>
</tbody>
</table>

**p<0.01 between genders**
Table 3: Health-related characteristics of active and hypoactive children grouped on the basis of the habitual physical activity (HPA) value from the Habitual Activity Estimation Scale (Hay, 1997).

<table>
<thead>
<tr>
<th></th>
<th>Total Cohort (mean ± SE)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active (HPA&gt;3h d⁻¹)</td>
<td></td>
<td>Hypoactive (HPA&lt;3h d⁻¹)</td>
</tr>
<tr>
<td></td>
<td>n=28</td>
<td></td>
<td>n=33</td>
</tr>
<tr>
<td>SIgA/albumin ratio</td>
<td>3.2 ± 0.1**</td>
<td>2.4 ± 0.2**</td>
<td></td>
</tr>
<tr>
<td>Relative body fat (%)</td>
<td>21.2 ± 1.4*</td>
<td>25.2 ± 2.0*</td>
<td></td>
</tr>
<tr>
<td>Predicted peak VO₂</td>
<td>47.8 ± 0.6*</td>
<td>44.9 ± 1.0*</td>
<td></td>
</tr>
<tr>
<td>(ml min⁻¹·kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>URTI frequency (days)</td>
<td>3.3 ± 0.5*</td>
<td>9.3 ± 1.7*</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05    ** p<0.01
Table 4. Correlation Coefficients among total sickness days (URTI), organized activity time (OAT), free time activity (FTA), total activity (TA), secretory IgA (SIgA), body fat (%BF), aerobic fitness (VO2max), salivary cortisol (sC), distance traveled per day (m d⁻¹), SIgA/albumin ratio (SIgA:Alb) and weekly habitual activity (HA).

<table>
<thead>
<tr>
<th></th>
<th>URTI (days)</th>
<th>TA (score)</th>
<th>OAT (score)</th>
<th>FTA (score)</th>
<th>SIgA (ml L⁻¹)</th>
<th>% BF</th>
<th>VO2max (ml kg⁻¹ min⁻¹)</th>
<th>sC (nmol L⁻¹)</th>
<th>m d⁻¹</th>
<th>SIgA:Alb (ratio)</th>
<th>HA (d wk⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>URTI (days)</td>
<td>-</td>
<td>-0.42**</td>
<td>-0.30*</td>
<td>-0.27*</td>
<td>-0.55***</td>
<td>-0.16</td>
<td>-0.19</td>
<td>-0.32**</td>
<td>-0.29*</td>
<td>-0.49**</td>
<td>-0.42**</td>
</tr>
<tr>
<td>TA (score)</td>
<td>-</td>
<td></td>
<td>0.86**</td>
<td>0.67**</td>
<td>-0.003</td>
<td>0.15</td>
<td>0.34**</td>
<td>0.29*</td>
<td>0.48**</td>
<td>0.009</td>
<td>-0.01</td>
</tr>
<tr>
<td>OAT (score)</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
<td>0.002</td>
<td>0.10</td>
<td>0.27*</td>
<td>0.30*</td>
<td>0.45**</td>
<td>0.04</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>FTA (score)</td>
<td>-</td>
<td>-0.003</td>
<td>-0.003</td>
<td>0.13</td>
<td>0.26*</td>
<td>0.12</td>
<td>0.28</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.22</td>
<td></td>
</tr>
<tr>
<td>SIgA (ml L⁻¹)</td>
<td>-</td>
<td>-0.08</td>
<td>-0.006</td>
<td>-0.006</td>
<td>0.003</td>
<td>0.002</td>
<td>0.88***</td>
<td>-0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% BF</td>
<td>-</td>
<td>0.11</td>
<td>-</td>
<td>0.11</td>
<td>0.06</td>
<td>0.18</td>
<td>0.14</td>
<td>-0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO2max (ml kg⁻¹ min⁻¹)</td>
<td>-</td>
<td>-0.01</td>
<td>-</td>
<td>-0.03</td>
<td>0.03</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sC (mmol L⁻¹)</td>
<td>-</td>
<td>-</td>
<td>-0.3</td>
<td>-</td>
<td>0.03</td>
<td>0.3</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m d⁻¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.27</td>
<td>-0.02</td>
<td>-1.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIgA:Alb (ratio)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA (d wk⁻¹)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05  **p<0.01  ***p<0.001