Effects of physical activity, body fat, and salivary cortisol on mucosal immunity in children

Thomas J. Cieslak, Gail Frost, and Panagiota Klentrou
Faculty of Applied Health Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1
Submitted 22 April 2003; accepted in final form 5 August 2003

Cieslak, Thomas J., Gail Frost, and Panagiota Klentrou. Effects of physical activity, body fat, and salivary cortisol on mucosal immunity in children. J Appl Physiol 95: 2315–2320, 2003. First published August 8, 2003; 10.1152/japplphysiol.00400.2003.—This study examined relationships among physical activity, body composition, and stress- and immunity-related variables in fifth grade children (10–11 yr) in Southern Ontario. The 29 boys and 32 girls, who participated in the study, performed a 20-m shuttle run for prediction of aerobic fitness. Bioelectrical impedance was used to assess relative body fat. 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 48 h to estimate their average total distance traveled per day. SIgA was significantly correlated with reported URTIs but was 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), whereas long-term SIgA reproducibility was nonsignificant for repeated measurements taken after 6 wk. 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.

aerobic fitness; secretory immunoglobulin A; upper respiratory tract infection

THERE IS EVIDENCE THAT EXERCISE influences natural immunity, T- and B-cell functions, and cytokine responses through hemodynamic changes and hormonal secretion in adults (7, 33). 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, whereas intense exercise evokes a negative response (22, 23, 34, 37). Moderate exercise has been shown to enhance cell-mediated immunity 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. (15) 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, SIgA also increases (6, 49). Nieman et al. (31, 33) did not find any relationship between URTI and body mass index 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 (34). 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 (34).

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 5 yr, decrease slightly until the age of 7, and then remain relatively stable to age 9 (9). Other studies comparing children to adults found that children did not achieve adult levels of immunoregulation until they were almost 11 yr old (35). However, these studies did not control for other factors, such as diet, climate, season, or amount of exposure and/or 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. (3) did not find any significant differences in leukocyte and lymphocyte levels, natural killer cells, and natural

Address for reprint requests and other correspondence: P. Klentrou, Faculty of Applied Health Sciences, Brock Univ., St. Catharines, Ontario, Canada L2S 3A1 (E-mail: nota.klentrou@brocku.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
killer cell activity between trained and untrained children, 9–17 yr of age. A study of 8- to 10-yr-old children found no relationship between peak O₂ consumption (V̇O₂) and immune function, but body mass was found to be significantly correlated with SIgA concentration, serum leukocyte counts, monocytes, and granulocyte phagocytosis (32). 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-yr-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 6 wk 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 risk when performing the experimental tests, and a disease, or any other condition that would put the subject at risk. Of the initially recruited subject cohort, 80% returned a signed parental consent form. Exclusion criteria were the risk when performing the experimental tests, and a disease, or any other condition that would put the subject at risk. Of the initially recruited subject cohort, 80% returned a signed parental consent form. Exclusion criteria were the risk when performing the experimental tests, and a disease, or any other condition that would put the subject at risk.

Physical activity and fitness assessments. Predicted peak aerobic power was estimated by using the 20-m 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 V̇O₂ was determined by multiplying the metabolic equivalent (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, SE of the estimate = 0.14), and the reproducibility has been reported (r = 0.975) for measurements taken on the same subject within a 1-wk period (19).

In addition, this measurement has been shown to be a valid test in a school setting for children 6–17 yr 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). Total activity time has been proposed as a more appropriate measurement for children than the combined energy cost of physical activities (13). The validity of the HAES has been evaluated by Hay (13), and the test-retest reliability was found to be >0.80 (13, 14).

The Participation Questionnaire was used to estimate both the amount of physical activity and the nature of the participation by using three categories: free-time activity, organized activity time, and total time spent in activities (12). 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 (12). The Participation Questionnaire 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 (12).

Total distance traveled per day was measured by using a Digi-Walker pedometer (New Lifestyles). The device recorded the child’s physical activity in steps by 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 2 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 and Wood (42) examined the Digi-Walker to determine its effectiveness as a tool for assessing physical activity patterns. The pedometers tended to underpredict 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 was used to estimate the percent body fat by using the input variables of physical activity level, body frame size, height, mass, and gender, as previously described (21). The skin was cleaned with 70% alcohol, and four surface electrodes were applied: two on the right hand at the second metacarpal and the wrist between the styloid processes of the radius and ulna, and two on the right foot at the second metatarsal and the heel between the second metatarsal and lateral malleolus. With the subject 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). Short- and long-term reproducibility of this technique has been reported as r = 0.999 for measurements made on the same subject within 1 day.
Table 2. Salivary IgA, SIgA-to-albumin ratio, cortisol, body fat, aerobic power, and physical activity levels in male and female children

<table>
<thead>
<tr>
<th>Variables</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>SlgA, ml/l</td>
<td>133.4 ± 17.4</td>
<td>134.8 ± 20.7</td>
</tr>
<tr>
<td>SlgA/albumin</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Salivary cortisol, mmol/l</td>
<td>3.0 ± 0.5</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>V02max, ml·kg⁻¹·min⁻¹</td>
<td>45.2 ± 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/day</td>
<td>4.9 ± 0.4</td>
<td>5.4 ± 0.9</td>
</tr>
<tr>
<td>Free 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>22.5 ± 1.2</td>
</tr>
<tr>
<td>Locomotion, m/day</td>
<td>120.5 ± 13.7*</td>
<td>95.4 ± 6.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. SIgA, secretory IgA; SlgA/albumin, ratio of SlgA to albumin; V02max, maximum O2 consumption; HPA, habitual physical activity. *P < 0.01 between genders.
shown in Table 3, the hypoactive children had significantly lower predicted peak VO$_2$ 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 >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 (Fig. 1).

As shown in Table 4, organized activity and free-time activity were significantly related to peak VO$_2$. The total activity score was significantly correlated with peak VO$_2$, distance traveled per day, and resting salivary cortisol levels. Distance traveled per day was also significantly correlated with peak VO$_2$, as well as with time spent in organized sport activities. Salivary cortisol was significantly correlated with body fat and time spent in organized sport. SIgA and SIgA/Alb demonstrated a significant relationship only with incidence of URTI (Table 4). The incidence of URTI was also correlated with total activity score, weekly habitual activity, and resting salivary cortisol (Table 4).

The intraclass correlation coefficient for initial and post-6-wk measures of salivary cortisol was $r = 0.66$. The intraclass correlation coefficients for SIgA and SIgA/Alb were $r = 0.23$ and $r = 0.20$, respectively. When the means were compared, initial and post-6-wk 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/Alb 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 also been shown that sedentary adults are more susceptible to infectious disease, compared with active adults (16, 33). The results of the present study suggest that this

---

**Table 4. Correlation coefficients among total sickness days (URTI), organized activity time, free-time activity, total activity, SIgA, body fat, aerobic fitness (VO$_2$ max), salivary cortisol, distance traveled per day, SIgA/Albumin, and weekly habitual activity**

<table>
<thead>
<tr>
<th>URTI, days</th>
<th>TA Score</th>
<th>OAT Score</th>
<th>FTA Score</th>
<th>SIgA, ml/l</th>
<th>%BF</th>
<th>VO$_2$ max, ml kg$^{-1}$ min$^{-1}$</th>
<th>sC, nmol/l</th>
<th>Distance Traveled, m/day</th>
<th>SIgA/Albumin</th>
<th>HA, days/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>URTI, days</td>
<td>-0.42‡</td>
<td>-0.30*</td>
<td>-0.27a</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>0.86†</td>
<td>0.67†</td>
<td>-0.003</td>
<td>0.15</td>
<td>0.34†</td>
<td>0.29†</td>
<td>0.45†</td>
<td>0.04</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>OAT score</td>
<td>0.20</td>
<td>0.002</td>
<td>0.10</td>
<td>0.27a</td>
<td>0.30a</td>
<td>0.29†</td>
<td>0.45†</td>
<td>0.04</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>FTA score</td>
<td>-0.003</td>
<td>0.13</td>
<td>0.25a</td>
<td>0.12</td>
<td>0.28</td>
<td>-0.04</td>
<td>0.32</td>
<td>0.12</td>
<td>-0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>SIgA, ml/l</td>
<td>0.08</td>
<td>-0.006</td>
<td>0.003</td>
<td>0.002</td>
<td>0.88†</td>
<td>0.11</td>
<td>0.06</td>
<td>0.14</td>
<td>0.14</td>
<td>0.23</td>
</tr>
<tr>
<td>%BF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.55‡</td>
<td>0.120</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>VO$_2$ max, ml kg$^{-1}$ min$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>sC, nmol/l</td>
<td>-0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance traveled, m/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.27</td>
<td>-0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIgA/albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA, days/wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TA, total activity; OAT, organized activity time; FTA, free-time activity; %BF, percent body fat; sC, salivary cortisol; HA, weekly habitual activity. Significant difference: *P < 0.05; †P < 0.01; ‡P < 0.001.
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 levels and physical activity markers (3, 32). It is possible that SIgA would 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 they 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. (32), 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 >25% did report a higher frequency of flu and cold symptoms than their counterparts. Salivary IgA levels and SIgA/Alb 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 SIgA levels in adults. Their subjects, however, were in job situations in which they were not necessarily exposed to increased infection levels as children are, and not all of 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 whether 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 SIgA (27) and salivary IgA are reached between 1 and 7 yr 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 6 wk. The interclass correlation coefficient was high (r = 0.66) for cortisol and low for both SIgA (r = 0.23) and SIgA/Alb (r = 0.20). This indicates that the initial and post-6-wk values for SIgA and SIgA/Alb were not significantly related. In contrast, it is interesting to note that initial and post-6-wk values of both of these variables were not significantly different, as shown by ANOVA. Gleson et al. (8) also found variability in SIgA levels in the children’s saliva samples from the ages of 1 to 5, but concluded that, because there seemed to be a plateau from 5 to 7 yr of age, SIgA would remain relatively stable from that point on. Because 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 be 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, whereas 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 affect 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-yr-old children. Salivary IgA levels seem to vary in this age group. This variability needs to be investigated further so that a definitive statement can be made 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, whereas children with relative body fat exceeding 25% reported significantly more sick days than the rest of the cohort.

REFERENCES


