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Abdominal Breathing Increases Salivary IgA Secretion

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ABSTRACT

Inagawa H, Iwase T, Nagai K, Watanabe S, Komiyama K. Abdominal Breathing Increases Salivary IgA Secretion. **JEPonline** 2012;15(5):57-67. Current evidence indicates that the number of elderly people and those with disabilities who exercise or engage in sports for health preservation have increased. Effective exercise reduces susceptibility to illness, particularly to upper respiratory tract infections. Concentration of SIgA and cortisol in saliva reflect changes in immune function. The aim of this study was to determine the effects two types of exercise on IgA and cortisol secretion. Fifty-four healthy adult subjects in their 40s (16 male and 16 female) or 60s (11 male and 11 female) volunteered to participate in this study. The subjects consisted of two groups: those who exercised on treadmill (TM group, 16 subjects in their 40s and 10 subjects in their 60s) and those who performed abdominal breathing supervised by an instructor (AB group, 16 subjects in their 40s and 12 subjects in their 60s). The TM group exercised for 30 min at 6 km·h⁻¹ while the AB group exercised for 30 min supervised by an instructor. The SIgA, free SC, and cortisol levels were measured in all subjects before and after exercise. Significant differences were observed between the TM and AB groups in the level of the SIgA and SC secretion in saliva. The SIgA and FSC concentration were observed to increase by abdominal breathing. The findings indicate that the mucosal immune activity is increased by abdominal breathing in elderly and disabled subjects, which suggests that future prevention programs for older people should be developed.

Key Words: Abdominal Breathing, SIgA, SC, Cortisol

INTRODUCTION

Today, the average life expectancy is over 80 yrs of age in many countries. Although a major cause of death is malignancy, pneumonia is also a major cause of deaths in the elderly. Pneumonia ranks as the fourth leading cause of death in Japan in people 60 yrs of age or older, and the percentage of elderly deaths due to pneumonia is increasing. In order to prevent the development of pneumonia, it is necessary to improve mucosal immunity in the upper respiratory tract.

Regular exercise has received considerable attention for its positive influence on health preservation in the elderly and those with disabilities. While many studies have investigated the role of treadmill exercise on health and well-being, these studies have primarily focused on the professional athlete (10,17). Relatively speaking, only a few reports have focused on the effects of exercise in the general population and chronically ill older adults (14,27).

Secretory immunoglobulin A (SIgA) is the dominant immunoglobulin isotype found on mucosal surfaces where it acts as a first line of defense against microbial invasion. It is composed of two molecules of IgA, joining (J) chain and a polymeric immunoglobulin receptor (pIgR) (16). IgA and J chain are synthesized by plasma cells in the submucosa and secreted as dimeric (d) IgA. dIgA bind to pIgR on the basement membrane of mucosal epithelial cells. The dIgA-pIgR complex is transported to the apical membrane and released to mucosal surface as SIgA. SIgA has been shown to inhibit the attachment of viruses and bacteria, to neutralize toxins, and to show antibody-dependent cytotoxicity (12,30,34). Recent investigations (7,8) suggest that the SIgA concentrations vary throughout the day due to a range of variables including dietary factors, daily mood, and exercise.

Cortisol is a steroid hormone. It is an important member of the glucocorticoid family. Cortisol is secreted from the adrenal cortex via the hypothalamic-pituitary-adrenal axis in response to stressors (including physical exertion). Increased cortisol levels are associated with anxiety, depression, and intensive physical exercise (23). Cortisol is involved catabolic processes as it reduces protein synthesis, increases protein degradation and inhibits inflammatory processes and immunity. Measuring cortisol concentration in saliva provides an important reference for blood cortisol levels. Cortisol monitoring during sports detects the stress response to physical exertion in exercise or training (24,32,33).

The present study investigated the effects of two different simple exercises, treadmill and abdominal breathing, on the concentration of SIgA and cortisol in saliva. Two generations of subjects were examined (40-yr olds vs. 60-yr olds) in order to compare age differences. We hypothesized that the data would support the development of future prevention program for older individuals.

METHODS

Subjects

The study protocol received ethical approval in accordance with the committee of Nihon University School of Dentistry. Fifty-four healthy adult subjects in their 40s (16 male and 16 female) or 60s (11 male and 11 female) volunteered to participate in this study (Table 1). The subjects consisted of two groups: those who exercised on treadmill (TM group, 16 subjects in their 40s and 10 subjects in their 60s) and those who performed abdominal breathing supervised by an instructor (AB group, 16 subjects in their 40s and 12 subjects in their 60s). The TM group exercised for 30 min at $6 \text{ km}\cdot\text{h}^{-1}$ while the AB group performed exercises for 30 min.

Table1. Number of Subjects and Average of Age in Each Group.

| | 40-yr olds | 60-yr olds |
|----------|---------------|--------------|
| AB group | 16 (44.5±2.6) | 12(66.6±1.6) |
| TM group | 16 (46.5±2.4) | 10(64.6±2.7) |

Assay Procedures: Saliva Collection

All subjects rinsed their mouths with 50 ml of water 20 min before starting the exercises. During the exercises, the subjects were prohibited from taking any food or drink. The saliva was collected from each subject into 50 ml plastic test tubes before and after exercise. All samples were centrifuged at 14,000 rpm for 5 min, and supernatant was maintained at -20° C until examination.

Total Protein

The concentration of total protein in each saliva sample was measured using the *DC* protein assay kit (BIO RAD). Each saliva sample was diluted 5 times with PBS 0.05% Tween20 (PBS-T). Five μ l of saliva and standard solution was mixed with 25 μ l of reagent A and 200 μ l of reagent B in a microtiter plate. The reaction mixture was maintained at room temperature (RT) for 15 min. The amount of absorption of each mixture was measured with a 595 nm plate reader (BIO RAD). Bovine serum albumin was used as standard protein.

SIgA

The SIgA concentration in the saliva was measured with a EIA-SIgA test kit (Medical & Biological Laboratories). Each saliva sample was diluted 40 times with a reaction buffer and 10 μ l of the diluted sample and standard solution was added to 400 μ l of reaction buffer. Styrene beads coated with anti-human SC antibody were incubated with each sample and standard solution for 1 hr at RT. After being adequately washed with PBS, the beads were reacted with HRP conjugated anti-human IgA antibody. The beads were rewashed with PBS and, then, in an incubated o-phenylene diamine solution containing H₂O₂ (OPD) for 20 min to colorization. H₂SO₄ solution was added to the reaction mixture to stop the reaction. The amount of absorption of each reaction mixture was detected with a spectrophotometer (Beckman).

FSC

The FSC concentration in each saliva sample was measured using ELISA. Fifty μ l of anti-free human SC antibody (Nordic) diluted 1000 times with 1% BSA-PBS were applied to each well of a micro-titer plate. The wells were washed three times with PBS-T and, then, 200 μ l of 1% BSA-PBA were applied for 1 hr to block nonspecific binding. Then, the solution was discarded. Fifty μ l of the samples or standard solution were applied to each well and incubated for 1 hr at RT. After washing was completed, 50 μ l of Hrp-anti human SC antibody (DAKO) were added to the wells and incubated for 1 hr at RT. One hundred μ l of OPD solution were added to each well for colorization of HRP-conjugated antibody (Hrp) and incubated for 20 min. Twenty-five μ l of 2M H₂SO₄ were added to stop the reaction. The amount of absorption of each reaction mixture was measured on the plate reader. The FSC used as standard solution was purified from human colostrums previously prepared in our laboratory (4).

Cortisol

The concentration of cortisol in each saliva sample was measured using the SALIVARY CORTISOL ENZYME IMMUNOASSAY KIT (Salimetrics). Twenty-five μl of samples and standard was applied to each well of the microtiter plate. Fifty-five μl of Hrp conjugated cortisol were applied to each well and incubated for 1 hr at RT. After washing was completed, 200 μl of OPD solution were added to each well for colorization and incubated for 25 min. Twenty-five μl of 2M H_2SO_4 were added to stop the reaction. The amount of absorption of each reaction mixture was measured on the plate reader.

Statistics

The values are expressed as the mean \pm SD for all the subjects. All statistical comparisons of data obtained before and after exercise were performed using the Student's paired t-test. A alpha level of $P=0.05$ was considered to be statistically significant.

RESULTS

Concentration of Total Protein

The total protein concentration of each sample is shown in Figure 1. The average concentration of total protein before exercise in the AB group of the subjects in their 40s was 2.5 ± 0.5 mg/ml that of the subjects in their 60s was 2.5 ± 1.2 mg/ml. In the TM group, the average concentration of total protein before exercise in the subjects in their 40s was 2.9 ± 0.7 mg/ml and that of the subjects in their 60s was 2.5 ± 3.1 mg/ml. The concentration of total protein in saliva measured after exercise was not statistically different ($P > 0.05$) from before exercise.

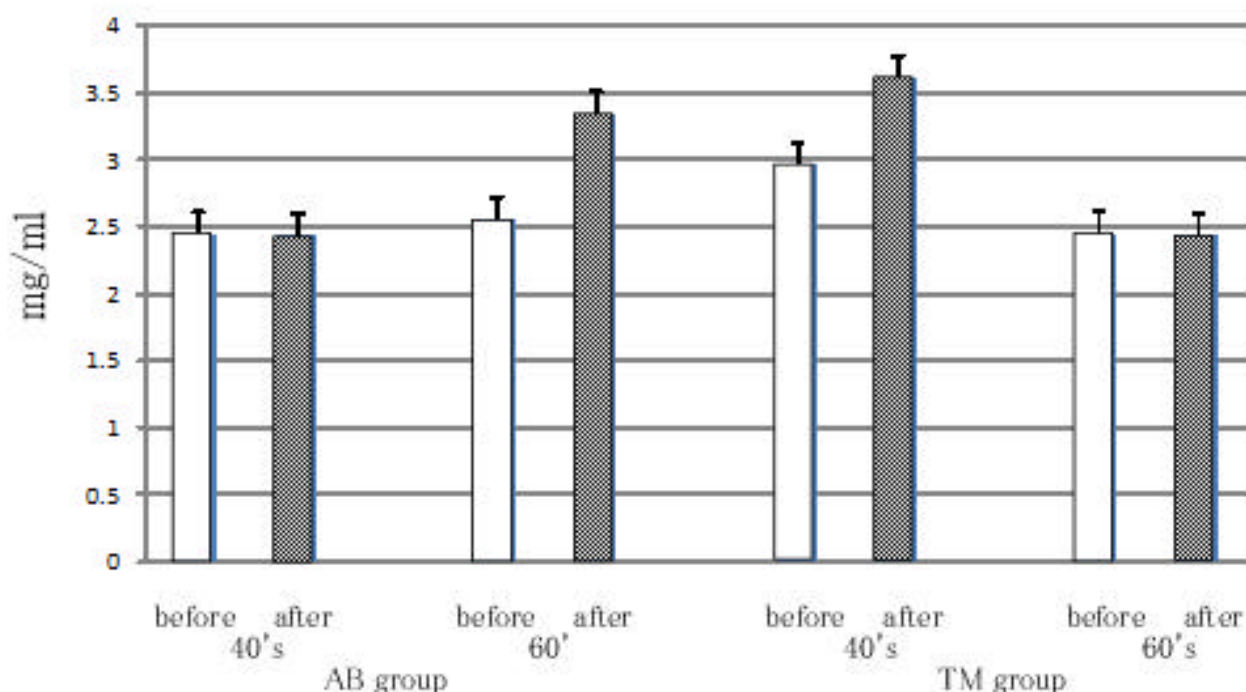


Figure 1. The concentration of total protein in saliva was not changed after exercise in both the AB group and the TM group in the 40s and 60s generation.

Concentration of SIgA

The concentration of SIgA in saliva showed some differences with respect to age in the AB group (Figure 2). In the AB group, the average concentration of SIgA before exercise in the subjects in their 40s was 234 ± 45 mg/ml and that of the subjects in their 60s was 367 ± 76 mg/ml. In the TM group, the average concentration of SIgA before exercise in the subjects in their 40s was 365 ± 66 mg/ml and that of the subjects in their 60s was 463 ± 115 mg/ml. The concentrations of SIgA in the TM group were higher than those in the AB group. In the AB group, the average concentration of SIgA measured after exercise in the subjects in their 40s was 384 ± 52 mg/ml and that of the subjects in their 60s was 595 ± 172 mg/ml. The concentration of SIgA significantly increased after exercise in the AB group. However, no differences were observed in SIgA concentration measured before and after exercise in the TM group.

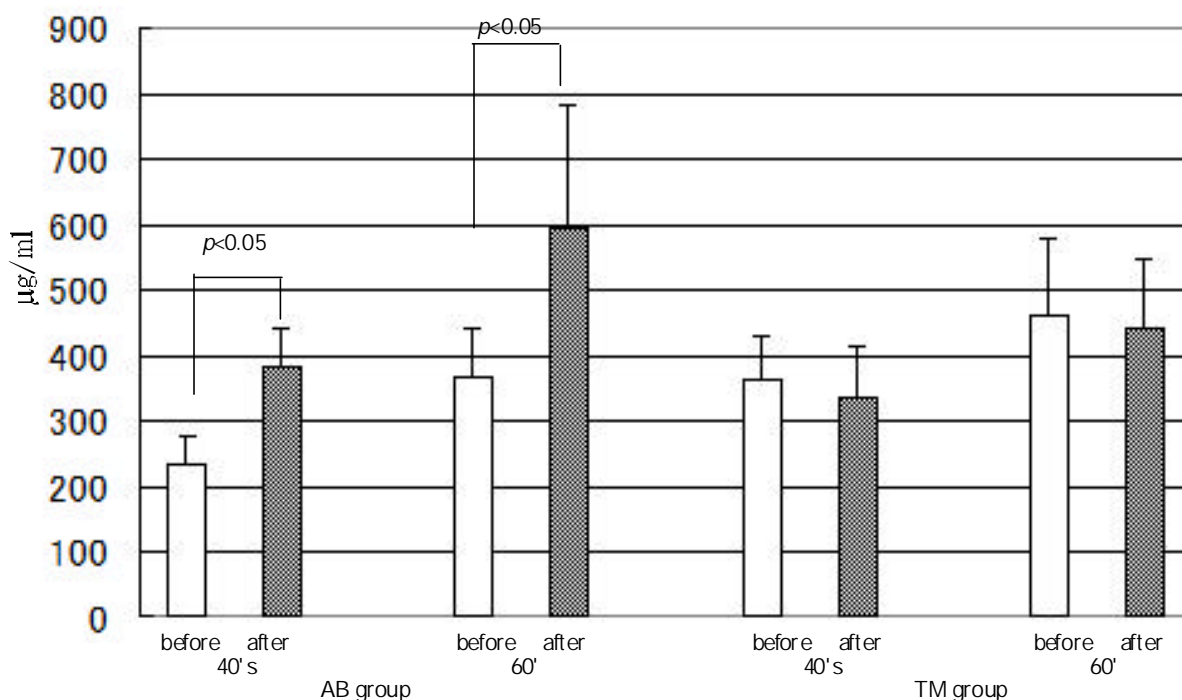


Figure 2. The concentration of SIgA was significant increased after exercise in the AB group in the 40s and 60s generation. But, SIgA concentration was not changed after exercise in the 40s and 60s generation in the TM group.

Concentration of FSC

In the AB group, the average FSC concentration in saliva measured before exercise in the subjects in their 40's was 2.9 ± 0.4 mg/ml and that in the subjects in their 60's was 2.0 ± 0.3 mg/ml (Figure 3). In the TM group, the average FSC concentration in saliva measured before exercise in the subjects in their 40's was 5.6 ± 1.2 mg/ml and that in the subjects in their 60's was 6.0 ± 1.8 mg/ml. The FSC concentration in the subjects in their 60's in the AB group were significantly increased. The concentrations of FSC in saliva in the TM group were higher than those in the AB group. However, no significant differences were found in the ratio of concentrations measured before and after exercise.

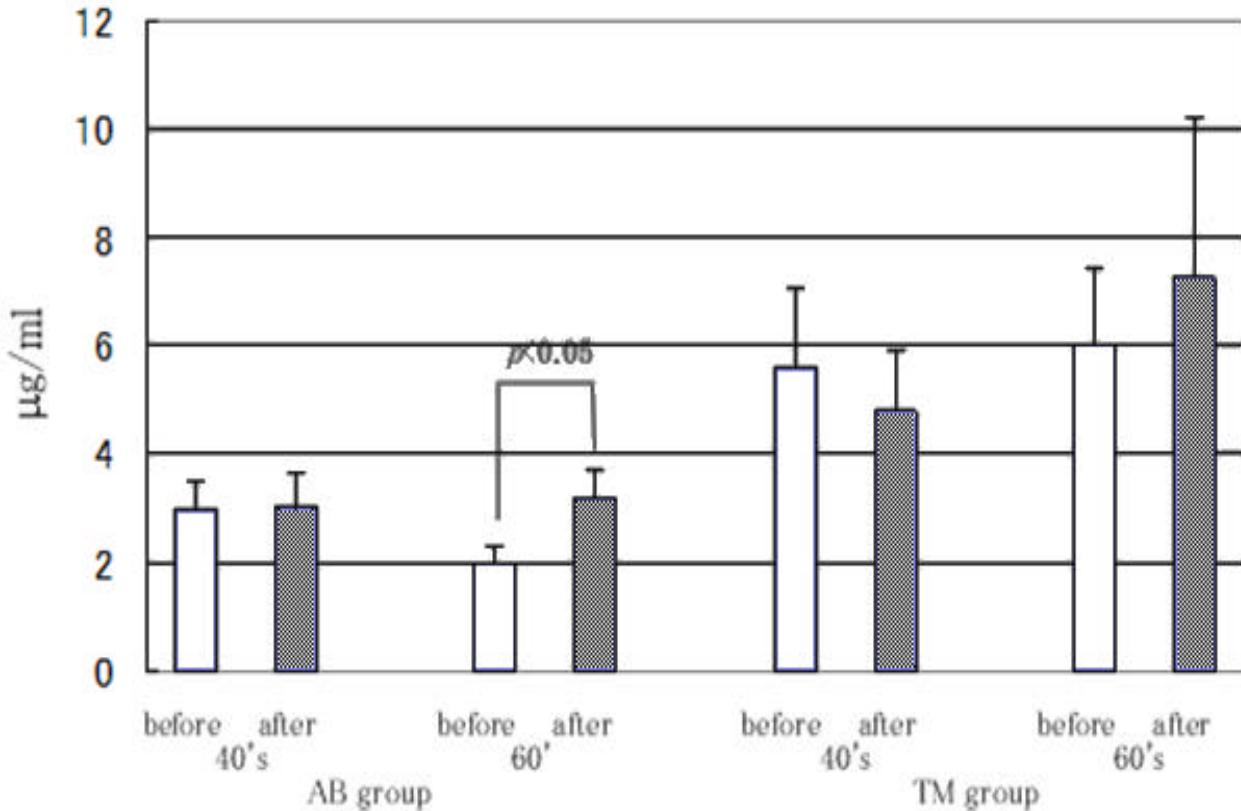


Figure 3. The concentration of free SC was increased only in the 60s generation of the AB group.

Concentration of Cortisol

The concentrations of cortisol in saliva measured after exercise were decreased in all subjects (Figure 4). In the AB group, the average cortisol concentration measured before exercise in the subjects in their 40s was $0.121 \pm 0.02 \mu\text{g/dl}$ and that in the subjects in their 60s was $0.104 \pm 0.01 \mu\text{g/dl}$. In the TM group, the average cortisol concentration measured before exercise in the subjects in their 40s was $0.118 \pm 0.01 \mu\text{g/dl}$ and that the subjects in their 60s was $0.122 \pm 0.01 \mu\text{g/dl}$. In the AB group, the average cortisol concentration measured after exercise in the subjects in their 40's was $0.100 \pm 0.01 \mu\text{g/dl}$ and the the subjects in their 60s was $0.080 \pm 0.01 \mu\text{g/dl}$. In the TM group, the average cortisol concentration measured after exercise in their 40s was $0.082 \pm 0.02 \mu\text{g/dl}$ and that in the subjects in their 60s was $0.086 \pm 0.0 \mu\text{g/dl}$.

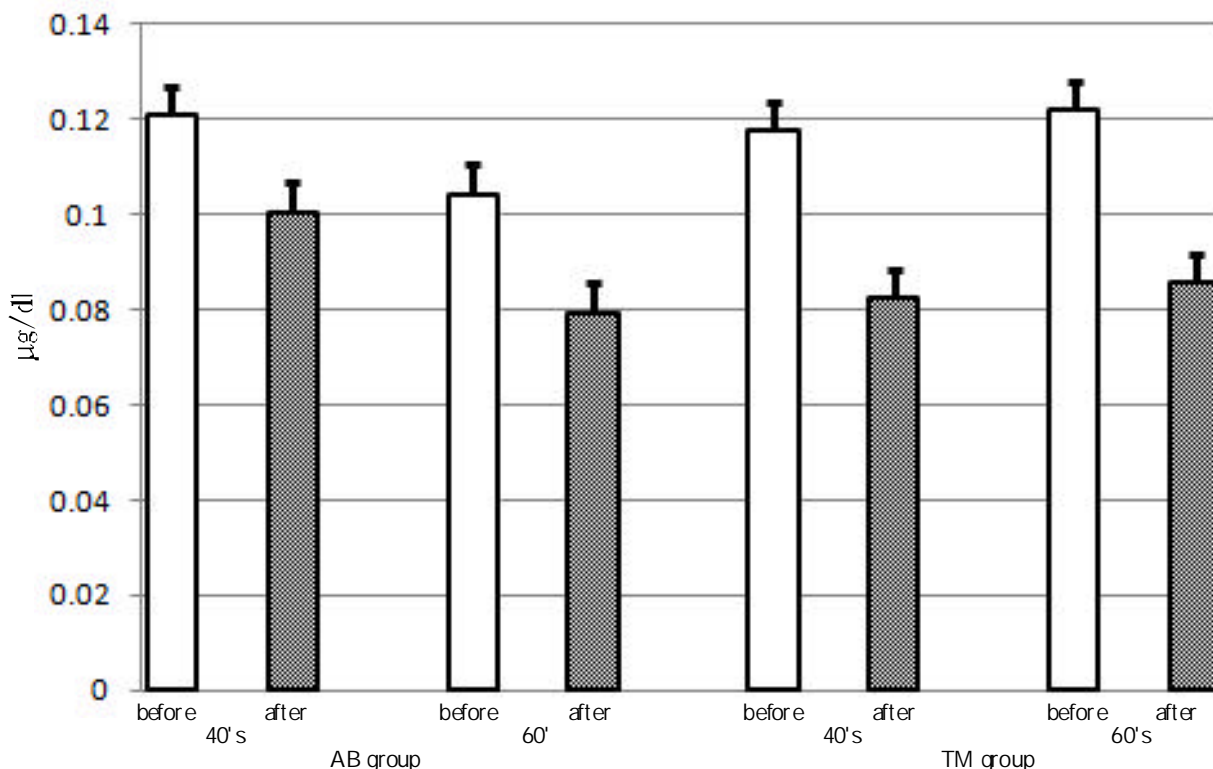


Figure 4. The concentration of cortisol was increased after exercise in the AB group and the TM group in the 40s and 60s generation.

DISCUSSION

Saliva encourages soft tissue repair by reducing clotting time and accelerating wound contraction. The protective function of saliva results from the salivary role in the maintenance of ecological balance in the oral cavity. This is carried out by debridement/lavage, aggregation and the reduction of adherence by both immunological and non-immunological means, and direct antibacterial activity. Saliva also possesses anti-fungal and anti-viral properties. Saliva effectively maintains pH in the oral cavity, thus contributing to the regulation of plaque pH. It also helps to neutralize reflux acids in the esophagus (11). SIgA is the most abundant antimicrobial protein in mucus secretions, including saliva in the oral cavity. Salivary IgA functions as part of innate immunity. It is considered the best indicator of mucosal immunity since it act as the first line of defense by neutralizing and preventing viral pathogens from entering the body via mucosal surfaces (28).

In the present study, we demonstrated that the SIgA secretion is significantly increased following abdominal breathing (AB) supervised by an instructor in subjects in their 40s and 60s. On the other hand, 30 min of treadmill exercise did not significantly alter the SIgA level in saliva. This is an interesting finding since many studies have indicated that physical activities and susceptibility to upper respiratory tract infections are associated with decreased SIgA secretion in saliva in a "J-shaped" relationship (20). Decreases in the SIgA levels have been shown to be associated with increased incidences of upper respiratory tract infections in elite athletes and college football players (3,5,6,9,21,22). This discrepancy may indicate subtle differences in the subject characteristics and

the quality and type of their exercise programs. Our subjects exercised at recreational levels while others studies have focused on elite athletes.

Polymeric Ig receptor (pIgR) present on mucosal epithelial cells can bind to dimeric IgA and pentameric IgM. The pIgR-dimeric IgA complex is transported from the basal membrane to the apical membrane of epithelial cells and released on the mucosal surface as SIgA. The extra cellular domain of pIgR cleaved from the cell membrane is combined with plgs. However, 60% of the total pIgR in mucosal secretions is present as pIgR without dimeric IgA or IgM as the so-called free secretory component (FSC) (1). There exist few reports regarding FSC concentrations in secretions such as saliva because of technical difficulties associated with measuring methods. We developed a specific antibody against polyclonal and monoclonal anti-human SC. In addition, the functions of FSC have not been made clear. The inhibition of adhesions of *E coli* and *Candida* to epithelial cell lines has recently been reported (2,31). Additionally, the FSC inhibits the chemotactic activity of IL-8 by using the carbohydrate of FSC. Interestingly, since FSC is thought to possess anti-inflammatory and anti-infective properties (13,19), it is important to point out that FSC increased in the 60-yr-old subjects in the AB group (while the levels of FSC measured in the TM group were higher than those measured in the AB group).

The cortisol present in saliva originates from passive diffusion of cortisol from the blood. Thus, the concentration of cortisol found in saliva closely reflects the levels of unbound cortisol in blood (32,33). Monitoring the salivary cortisol level is good method of evaluating health or disease under stressful condition. Both the noninvasiveness and ease of sampling and storage of saliva for testing are considered to be important. The salivary cortisol level is an inexpensive, yet valid and reliable measure of the level of bioactive cortisol in the body. It represents responses to short, high intensity bouts of exercise, and hormonal changes to exercise show greater sensitivity in saliva than corresponding blood measurements (29). That is why salivary cortisol concentration quickly increases following sporting events such as rugby matches, competitive kickboxing matches, wrestling competitions and Bruce treadmill test (15,18,25,26).

CONCLUSIONS

The findings in the present study indicate that the cortisol level is decreased after exercise both abdominal breathing and treadmill exercises. Additionally, no difference in cortisol levels was observed between subjects in their 40s and those in their 60s. The results suggest that the abdominal breathing and treadmill exercise performed in this study were not significantly stressful for the subjects. That is, the intensity of the recreational exercises used in the present were not stressful, but rather had a relaxing effect on the subjects. It is noteworthy that the level of SIgA increased with mild exercise such as the abdominal breathing. It may be possible to apply this finding to elite athletes who engage in heavy physical exercise in order to prevent decrease in SIgA secretion in saliva. Hence, these results should be useful in preventing upper respiratory infection in the elderly and those with any disabilities.

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