Effects of exercise, age and gender on salivary secretory immunoglobulin A in elderly individuals

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ABSTRACT

The influence of age and gender on salivary secretory immunoglobulin A (SIgA) in response to moderate exercise training was studied in 158 elderly subjects. Subjects were assigned to an exercise training group (EXC: 51 males, 74 females) or a non-exercise control group (CON: 11 males, 22 females). The subjects in each group were separated into four age-gender subgroups (60–69-yr-old males, over 70-yr-old males, 60–69-yr-old females, over 70-yr-old females) and compared by age and gender. Subjects in EXC participated in exercise sessions 5-days a week for 6 months. Saliva samples were collected both before and after the study period. The SIgA secretion rates were significantly increased after training ($p < 0.05$) in all the age-gender subgroups of EXC (60–69 males: 41%, over 70 males: 55%, 60–69 females: 40%, over 70 females: 38%); no age- or gender-

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related differences were observed. On the other hand, none of the age-gender subgroups of CON showed significant changes in the SIgA secretion rate; also, there were no age- or gender-related differences. In conclusion, enhancement of mucosal immune function following regular moderate exercise training occurs in elderlies in their 60s and over 70 years, and in both, males and females.

INTRODUCTION

Respiratory infections such as pneumonia and influenza virus infection, including upper respiratory tract infection (URTI), are particularly common and serious illnesses among elderly people (11). Mangtani et al. (16) showed that people in their 70s were more susceptible to influenza virus infections than those in their 60s. Gutiérrez et al. (10) showed that the incidence of pneumonia and influenza virus infection increased dramatically with aging and it was higher in males, in particular very elderly males were at greatest risk for URTI. In fact, the mortality from pneumonia among elderly were higher in the 70s group than in the 60s group, and higher in males than in females (30). There is a study that shows males are generally less resistant to infections caused by bacteria and viruses than females are (2). These reports suggest that age- and gender-related differences of the mucosal immune function might lead to differences in susceptibility to URTI.

Salivary secretory immunoglobulin A (SIgA) plays a crucial role in mucosal immune function and is the first line of defense for the human body against pathogenic microbial invasion (15). Specifically, SIgA inhibits attachment and replication of certain pathogens to prevent colonization on mucosal surfaces. Therefore, low levels of SIgA are linked to the incidence of URTI (8, 14). In fact, salivary SIgA secretion decreases with aging (19). This age-related decline in SIgA might invite pathogenic microbial invasion and, therefore, engender a higher frequency of URTI in elderly people. The influence of gender on SIgA output in the elderly remains to be elucidated.

In recent years, the influence of physical activity on the immune function has received considerable attention (24). Previous studies demonstrated that moderate physical activity reduced the incidence of URTI and increased resting concentration of SIgA (14, 17, 22). However, there were only a few studies in relation to elderly subjects. Previous work from our laboratory showed that elderly people who accumulated moderate daily physical activity had higher salivary SIgA secretions than their predominantly sedentary peers (27). In other work, we tested the effect of long-term moderate exercise training on the mucosal immune function in elderly subjects and demonstrated that salivary SIgA levels gradually increased with time through the training period (1). However, no information is available regarding the effects of age and gender on mucosal immunologic change in response to exercise training among the elderly.

It clearly is of clinical importance to understand the influence of exercise, age and gender on the mucosal immune function in elderly people. A safe prescription of exercise for elderly people must include the distinction between normal and abnormal exercise-induced responses, so that impairment of their immune systems may be avoided. Based on the needs discussed above, we designed a 6-month clinical study (which included a large number of elderly subjects) to examine if there were age-related (in the 60s vs. over 70 years) and/or gender-related differences of the mucosal immune function (salivary SIgA secretion) in response to moderate exercise training.

METHODS

Subjects

From among independently living, sedentary elderly people in Japan, we recruited 125 healthy elderly volunteers (51 males, 74 females) aged 60 to 82 as the exercise training group (EXC) and 33 healthy elderly volunteers (11 males, 22 females) aged 62 to 81 as the non-exercise control group (CON). Potential subjects were given a detailed explanation of the risks, stress, and potential benefits of the study before they signed an informed consent form. Based on the results of medical examinations within 6 months prior to the study and self-reported medical histories, the following exclusion criteria were determined for all subjects: hormone replacements, acute illness from infection within the preceding 3 months, metabolic disorders, and major surgery during the preceding 6 months. In addition, all subjects have passed a complete medical examination within the past year and received written permission from a sports doctor to be included in the study. Also, all participants did not experience a catarrh they perceived during 6 months of study period. EXC subjects participated in an exercise program for a period of 6 months. We asked CON subjects not to participate in any other formal exercise outside of that provided during the study. All participants completed an exercise program or a control program for 6 months. To investigate whether differences of age and gender on the mucosal immune function existed among elderly subjects, we separated both the EXC and CON subjects by age and gender into four subgroups: 60–69-yr-old males, over 70-yr-old males, 60–69-yr-old females, and over 70-yr-old females. This study was approved by the Ethical Committees of the Institute of Health and Sport Sciences and the Institute of Clinical Medicine of University of Tsukuba, and that it conformed to the principles outlined in the Declaration of Helsinki.

Measurement of double-product break-point

A double-product break-point (DPBP) which is the point of accelerating double product (DP, heart rate, HR x systolic blood pressure, SBP) has been shown to have strong positive correlations with the lactate and ventilatory threshold (23). Because the method to measure DPBP is non-invasive and involves no excessive strain, it is thought to be a useful index to monitor endurance exercise intensity among elderly people. In this study, the DPBP was measured before (PRE) and after (POST) the 6 months study period, according to the procedures of a previous study (23). Subjects sat and rested for at least 5 min, and then they took a bicycle ramp loading exercise test. This test consisted of 4 min of cycling at 20 W followed by a ramp slope at 10 W every 1 min. The test was stopped when the subject reached a 75% of estimated HR max (220 – age). Their DP with HR and brachial arterial SBP were measured and recorded every 15 s via an automated sphygmomanometer (CM-4001, Kyokko, Tokyo, Japan) during the test. The DP was calculated from the mean HR and SBP and plotted against the work rate. The DPBP was determined visually as the point at which a clear and sustained increase of the DP slope occurred.

Physical fitness tests

Subjects performed physical fitness tests which comprised six tests (isometric grip strength using a handgrip dynamometer, muscle endurance using sit-up test during 30 s, body balance using one leg balance with eyes open, flexibility of the body using the sit-and-reach test, agility using 10 m obstacle walking time, and endurance using 6-min walking distance) at PRE and POST, as described "Physical Fitness Test" by Japan Ministry of Education, Culture, Sports, Science and Technology (29).

Measurement of daily physical activity

In order to assess the physical activity, we used an electrical pedometer (Kenz Lifecorder; Suzuken Co. Ltd., Nagoya, Japan). With regard to this pedometer, a previous study showed accuracy for the assessment of counting steps (26). Participants were instructed to wear an electrical pedometer for 14 consecutive days during all waking hours, except during bathing at PRE and POST. Participants were instructed to go about their normal lives unrestricted and were asked not to look at the electrical pedometer to see how many steps they had taken each day. Electrical pedometer placement was standardized on the belt or waistband, according to the manufacturer's recommendation.

Saliva analysis

Before and after 6-month exercise training, at 8:30–9:30 a.m. after an overnight fast, saliva samples were obtained, as described in previous studies (1, 27). Subjects sat and rinsed out their mouths with sterilized water (30 s x 3 times), and then rested for at least 5 min. Saliva production was stimulated by the chewing of sterilized cotton (Salivette; Saersted, Vümbrecht, Germany) for one minute at a frequency of 1 chew/s. The amount of saliva in grams was converted to milliliters assuming a saliva density of 1 g/ml, as described in a previous study (19). We measured salivary SIgA concentrations by using an enzyme-linked immunosorbent assay (ELISA) according to the procedures of previous studies (1, 27). For analysis of the SIgA levels, data were expressed as the SIgA secretion rate (μg/min). This rate was calculated by multiplying the absolute SIgA concentration μg/ml) by the saliva flow rate (ml/min), which was calculated by dividing the total volume of saliva obtained in each sample (ml) by the time taken to produce the sample (min).

Exercise program

Subjects in the EXC group conducted an exercise program 5-days a week for 6 months. They were supervised and conducted by experienced instructors. The training program involved stretching for warm-up, endurance training, resistance training, and stretching for cool-down. The endurance training was a cycle-ergometer exercise (30 min) at 80% work rate of DPBP from beginning to end of the training period. The resistance training comprised seven exercises (push-up squat, sit-up, back-extension, leg-extension, hip-extension, and leg-curl) without using any weights. The number of sets of exercises was gradually increased as well, starting at 1 set in the first week and gradually progressing to 3 sets. Resistance exercises were performed slowly, requiring 3 s for the concentric phase and 3 s for the eccentric phase for each repetition (10 repetitions). Subjects in the CON group simply maintained their normal physical activity levels during the study.

Statistical analysis

All data were represented as means (SE). For all analyses, $p < 0.05$ was considered statistically significant. All variables in EXC and CON groups were analyzed using 2 (EXC and CON groups) x 2 (PRE and POST) repeated measures ANOVA. If a significant interaction was revealed, between EXC and CON, and between PRE and POST, differences in descriptive variables were tested using each unpaired- and paired-Student *t* test. In each EXC and CON, differences in descriptive variables were tested using 2 (males and females) x 2 (PRE and POST) or 2 (60–69-yr-old and over 70-yr-old) x 2 (PRE and POST) repeated-measures ANOVA. Moreover, among the age-gender subgroups, differences in descriptive pre-intervention variables were tested using 2 (60–69-yr-old and over 70-yr-old) x 2 (males and females) x 2 (EXC and CON) unrepeated-measures ANOVA. Among the four age-gender subgroups in each EXC and CON, differences in descriptive variables were tested using 2 (60–69-yr-old and over 70-yr-old) x 2 (males and females) x 2 (PRE and POST) repeated-measures ANOVA. A Fisher's PLSD post-hoc test was performed whenever there were significant effects in ANOVA. A post hoc power analysis was conducted by using actual values obtained from this study to verify of the null hypothesis.

RESULTS

The calculated mean SIgA secretion rates at PRE and POST were 38.3 (2.0) and 54.8 (2.1) μg/min in the total EXC group, and 36.3 (3.3) and 35.2 (2.8) μg/min in the total CON group, respectively. A significant group x time interaction in SIgA secretion rate was revealed ($p < 0.01$). Although the SIgA secretion rate at PRE did not show any inter-group differences between EXC and CON, the rate at POST was significantly higher in the EXC group compared with the CON group ($p < 0.01$). The secretion rate in the EXC group was significantly increased after training ($p < 0.01$), while there was no significant change in the CON group.

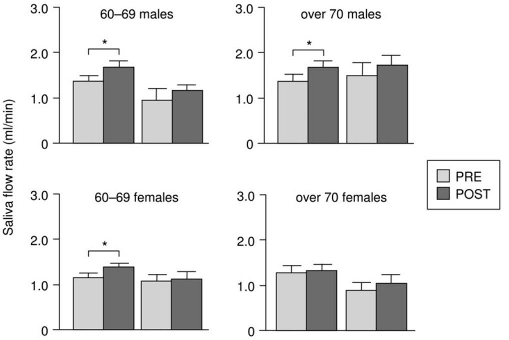

Figure 1. Changes of saliva flow rate in EXC and CON classified by age and gender. Age-gender subgroups involved 60–69 males (n = 26), over 70 males (n = 25), 60–69 females (n = 55), and over 70 females (n = 19) in EXC, and 60–69 males (n = 6), over 70 males (n = 5), 60–69 females (n = 13), and over 70 females (n = 9) in CON. Values are mean (SE).
* Significant difference from PRE value: $p < 0.05$.

The mean value of the work rate at DPBP before and after the study period were 1.00 (0.02) and 1.01 (0.02) W/kg in the EXC group. The work rate at DPBP did not change significantly following exercise training. With regard to physical fitness tests in EXC, grip strength at PRE and POST were 29.9 (0.8) and 30.2 (0.8) kg, frequency of sit-up during 30 s were 12.4 (0.7) and 15.0 (0.7), one leg balance with eyes open were

Table 1. Physical characteristics in EXC and CON classified by age and gender. Values are means (SE); BMI, body mass index. * Within-subgroups time effect, $p < 0.05$. § Main age effect, $p < 0.05$. ¶ Main gender effect, $p < 0.05$.

	60–69 males		over 70 males		60–69 females		over 70 females	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
EXC								
Number	26		25		55		19	
Age (yr)	67 ± 0.2		73 ± 0.6		65.3 ± 0.3		73.2 ± 0.5	
Height (cm) ¶	160.8 ± 1.1		161.7 ± 1.2		151.3 ± 0.7		148.0 ± 1.3	
Weight (kg) ¶	62 ± 1.4	62.3 ± 1.4	62.1 ± 1.7	62 ± 1.5	56 ± 1.0	55.2 ± 1.0	53 ± 1.5	51.4 ± 1.5 *
BMI (kg/m^2)	23.8 ± 0.5	24.1 ± 0.5	24 ± 0.6	24 ± 0.5	24.4 ± 0.5	24.1 ± 0.4	24.0 ± 0.5	23.4 ± 0.5 *
CON								
Number	6		5		13		9	
Age (yr)	67.2 ± 1.1		75.4 ± 2.0		66.0 ± 0.6		74 ± 0.8	
Height (cm) §, ¶	166.1 ± 2.8		161.4 ± 2.6		150.5 ± 1.5		143.7 ± 1.1	
Weight (kg) ¶	63.6 ± 3.4	64.4 ± 3.0	62.4 ± 4.7	63.1 ± 3.5	56 ± 2.7	56.1 ± 2.7	53 ± 2.6	54.6 ± 2.3
BMI (kg/m^2)	23.1 ± 1.2	23.4 ± 1.0	23.8 ± 1.2	24.2 ± 0.7	25 ± 1.0	24.6 ± 1.0	26 ± 1.6	26.4 ± 1.0

74.6 (4.4) and 78.9 (4.3) s, sit-and-reach were 35.9 (0.8) and 41.1 (0.8) cm, 10 m obstacle walking time were 7.30 (0.16) and 6.51 (0.14) s, and 6-min walking distance were 518.7 (6.5) and 569.7 (6.7) m. The records in frequency of sit-up, sit-and-reach, 10 m obstacle walking and 6-min walking distance showed significant improvement following exercise training ($p < 0.01$).

Mean step count per day at PRE and POST were 7,094 and 8,135 in EXC, and 5,934 and 6,136 step/day in CON. The group x time interaction was not statistically significant. CON did not show significant changes in mean step count per day during 6 months, suggesting that CON subjects could keep their normal physical activity for a period of 6 months.

Physical characteristics for the four age-gender subgroups in both the EXC group and the CON group are shown in Table 1. It can be seen that the EXC group and the CON group were of similar body composition before the study period. Typical gender differences ($p < 0.05$) were noted for height and body mass, with higher values among the males. Body mass and body mass index (BMI) did not change significantly after the study period in either EXC or CON, except for a significant decrease in females over 70 years old in the EXC group ($p < 0.05$).

Age-related differences were not revealed for saliva flow rate, SIgA concentration and SIgA secretion rate in both EXC and CON. Gender-related differences ($p < 0.05$) were noted for both the saliva flow rate and the SIgA concentration in EXC. Saliva flow rate in males was significantly higher than in females ($p < 0.05$), while SIgA concentration was significantly lower in males compared with females ($p < 0.05$). The SIgA secretion rate did not show any gender-related differences between EXC and CON. In both EXC and CON, a significant time x gender interaction or time x age interaction was not revealed in saliva flow rate, SIgA concentration and SIgA secretion rate. These saliva-related parameters of EXC were significantly increased following exercise training in both males and females, and in both 60s and over 70 years ($p < 0.05$), while CON did not show significant changes.

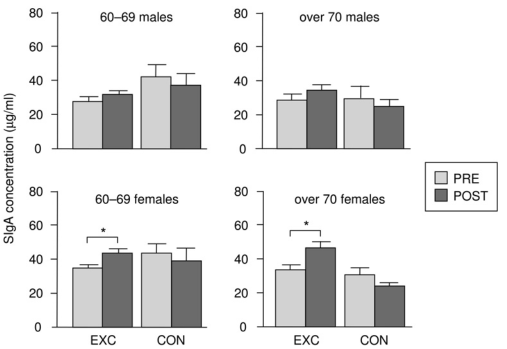

Figure 2. Changes of SIgA concentration in EXC and CON classified by age and gender. Age-gender subgroups involved 60–69 males (n = 26), over 70 males (n = 25), 60–69 females (n = 55), and over 70 females (n = 19) in EXC, and 60–69 males (n = 6), over 70 males (n = 5), 60–69 females (n = 13), and over 70 females (n = 9) in CON. Values are mean (SE).

* Significant difference from PRE value: $p < 0.05$.

Four age-gender subgroups in both the EXC and CON group had similar saliva flow rates, SIgA concentrations, and SIgA secretion rates before the study period. The saliva flow rates in EXC were significantly increased after exercise training ($p < 0.05$), except that there was no significant change in females over the age of 70 (Figure 1). In addition, the SIgA concentration in females was significantly increased after training ($p < 0.01$), whereas there was no change in the SIgA concentration of males (Figure 2). In CON, saliva flow and SIgA concentration did not significantly change during the study period (Figure 1 and 2). Based on age or gender, all four subgroups of EXC experienced a significant enhancement of SIgA secretion rate after training (60–69 males: 41%, over 70 males: 55%, 60–69 females: 40%, over 70 females: 38%) ($p < 0.05$), while all four subgroups of CON did not experience any significant change (Figure 3).

DISCUSSION

We designed this study to provide a multifactor analysis of salivary SIgA change in the elderly in response to exercise training. Our primary findings were that moderate exercise training apparently enhances the salivary SIgA level in elderly subjects and that age and gender do not make a difference – that is, enhancement of the mucosal immune function following moderate exercise training could occur in both age groups (60s and over 70 years) and in both elderly males and females.

Our previous study revealed that salivary SIgA secretions in 45 elderly subjects were significantly increased after exercise training, but this study did not include a non-exercise control group (1). On the other hand, since the present study included a larger number of elderly subjects (n = 125) and a control group, it supports the idea that long-term exercise training enhances the SIgA level among the elderly.

Indeed, the susceptibility to and mortality from viral infections among the elderly were higher in males than in females (2, 10, 16, 30). In addition to URTI, the prevalence of *Helicobacter pylori* infection, which is strongly associated with peptic ulcer and gastric cancers, also occurs predominantly in males, and in elderly people (5). SIgA in saliva and gastric juice correlate with protection against *Helicobacter pylori* infection (9, 32). Thus, age- and gender-related differences of the mucosal immune function might lead to differences in susceptibility to infections. Our results showed that saliva flow rate in males was higher than in females, whereas SIgA concentration was lower in males compared with females. These results were in agreement with previous findings (6, 20). Eliasson et al. (6) indicated that there is a negative correlation between SIgA concentration and saliva flow rate. Therefore, males had higher saliva volume and lower SIgA concentration compared with females. Our

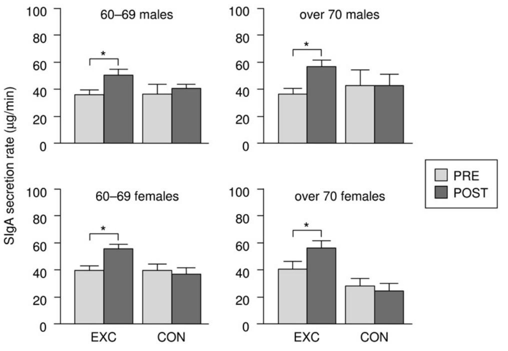

Figure 3. Changes of SIgA secretion rate in EXC and CON classified by age and gender. Age-gender subgroups involved 60–69 males (n = 26), over 70 males (n = 25), 60–69 females (n = 55), and over 70 females (n = 19) in EXC, and 60–69 males (n = 6), over 70 males (n = 5), 60–69 females (n = 13), and over 70 females (n = 9) in CON. Values are mean (SE).
* Significant difference from PRE value: $p < 0.05$.

results showed that males and females had similar values of SIgA secretion rate. This result was consistent with a previous result (25). A previous study had suggested that upper respiratory tract surface areas (URTSA) were linked to body size (18). In our present study, both height and body weight were higher in males than in females, suggesting that males might have larger URTSA. In this regard, there is less SIgA on the mucosal surface in males than in females, leading to less resistance to infections in males. Moreover, Klein et al. (13) reported that the expression of genes which encoded for pro-inflammatory and antiviral cytokines (e.g., TNF-α, IL-1, and IFN-γ), T cell (e.g., CD3 and TCR), and immunoglobulin super-family (e.g., IgM, IgG, and MHC class I and II) proteins reactive to Seoul virus infection in lung tissue was lower in male rats than in female. These findings suggested that T cell-mediated immune response and immunoglobulin super-family activity as well as SIgA-mediated barrier function could be important issues, especially for elderly males. Moderate exercise training could increase SIgA secretion, leading to enhancement of SIgA-mediated barrier function in elderly people, especially in elderly males.

Our study is the first to examine the influences of age- and gender-related differences on SIgA response to exercise training in the elderly. To our knowledge, only one study has directly investigated the effects of age on SIgA response to exercise training, and there has been no study on the effects of gender. Tharp reported that chronic basketball exercise could increase the resting levels of SIgA, but no age-related difference was observed between the pre- and post-pubescent boys studied (28). In this study, exercise-induced increase of SIgA secretions was observed both in males and females, and in those over 70 years as well as those in their 60s. So, for the elderly over 70 years, especially for males, moderate exercise training could be a suitable strategy to bolster resistance to infections.

The reduction of salivary flow and oral dryness are widespread in elderly people. Saliva has roles not only in direct antibacterial, antiviral, and antifungal activity, but also in protecting and maintaining the integrity of the oral mucous membrane by lubrication and soft tissue repair. Decreased saliva flow and altered composition of saliva may increase susceptibility to caries, periodontal disease, and oral mucosal lesions (3, 21). Moreover, Fox et al. (7) reported that individuals who suffered from dry mouth syndrome had an increased incidence of URTI. Our results showed that saliva flow rates were increased by moderate exercise training in elderly subjects. Moderate exercise training might be a strategy to alleviate oral dryness and bolster improvement of saliva flow in elderly subjects.

So far, the mechanisms underlying enhanced SIgA levels through exercise training in the elderly are not clear. IgA is secreted by plasma cells residing beneath the acinar epithelium in the salivary glands. Transport of IgA across the epithelial cell barrier requires polymeric Ig receptor (pIgR) (31). A recent study done in our laboratory showed that acute intense exercise reduced the level of pIgR mRNA expression in the submandibular gland along with a decrease in salivary SIgA concentration, suggesting that expression of pIgR could respond to exercise (12). Salivary glands are stimulated by both parasympathetic cholinergic nerves and sympathetic adrenergic nerves, and salivary flow and composition, along with nervous system stimulation and change in hormone concentrations, may be modified by exercise (4). It is therefore likely that moderate exercise training may stimulate and up-regulate pIgR production, leading to the increase in

SIgA secretion. Further studies should elucidate the mechanism of salivary SIgA in response to moderate exercise training. If this mechanism was clearly understood, more effective health-related programming could be established to enhance the mucosal immune function in elderly people.

The present study has a certain limitation. There was a smaller sample size of non-exercise control subjects compared with exercise-training subjects that limited our power to do subgroup analyses on characteristics such as age and gender. It is related to the stringent inclusion criteria and the difficulty of finding healthy, non-frail and sedentary elderly subjects who are willing and unable to enroll in any other formal exercise program during 6 months. Further studies need to use proper numbers of subjects (the same number as in the exercise group) for control groups which engage in sham-training such as mild flexibility and calisthenics under low-intensity and low-frequency. Although the CON subjects were lower than EXC subjects, a power analysis test revealed that 33 subjects in CON and 125 subjects in EXC would respectively yield 84.5% and 100% power for detecting a change.

In conclusion, 6 months of moderate exercise training increased salivary SIgA secretion in elderly subjects, irrespective of their gender and age. Exercise is a suitable strategy to improve the mucosal immune function in both males and females in their 60s and even over 70 years.

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