Effects of 12 months of exercise training on salivary secretory IgA levels in elderly subjects

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Background: The immune system declines in efficiency with advancing age, making the elderly less resistant to pathogenic microorganisms. Upper respiratory tract infection (URTI) is a common illness. Recent studies have shown that suppression of secretory immunoglobulin A (SIgA) is associated with increased incidence of URTI.

Objective: To assess the effect of exercise on salivary SIgA in elderly subjects.

Methods: Forty-five elderly subjects (18 men, 27 women; mean (SD) age 64.9 (8.4) years) performed both 60 minute resistance and 60 minute moderate endurance training a week for 12 months. Saliva samples were obtained before training, and at four and 12 months during the training period. Salivary SIgA concentrations were measured by enzyme linked immunosorbent assay, and the SIgA secretion rate was calculated.

Results: SIgA concentrations before training, and at four and 12 months during training were 24.7 (14.4), 27.2 (14.2), and 33.8 (18.5) µg/ml respectively. SIgA secretion rates were 29.5 (26.0), 33.8 (27.2) and 46.5 (35.1) µg/min respectively. The results indicate that both the concentration and secretion rate of SIgA significantly (p<0.01) increased during 12 months of exercise in these elderly subjects.

Conclusion: Regular moderate exercise seems to enhance mucosal immune function in elderly subjects.

Methods

Subjects
Forty-five sedentary, but healthy elderly subjects (18 men, mean (SD) age 64.9 (8.4) years; 27 women, mean (SD) age 64.0 (8.9) years; 63.7 (6.9) years) volunteered to participate in a 12 month clinical study to measure the effects of single 60 minute resistance and 60 minute moderate endurance training sessions a week on salivary SIgA levels. The aim was to determine whether 12 months of moderate combined exercise training could enhance salivary SIgA in elderly subjects.

Methods

Subjects

Abbreviations: URTI, upper respiratory tract infection; SIgA, secretory immunoglobulin A; VO2max, maximal oxygen uptake; NK, natural killer; HR, heart rate
training programme. Subjects were recruited by the corporation of Taiyo village officer. Potential subjects were given a detailed explanation of the risks, stress, and potential benefits of the study before they signed an informed consent form. The study protocol was in accordance with the policy statement of the institutional review board of the University of Tokyo. All subjects had passed a complete medical examination within the past year and received written permission from a specialist sports doctor to be included in the study. In addition, subjects that met the exclusion criteria of the American College of Sports Medicine were not allowed to participate. No subjects were receiving treatment known to affect immune function. There were no patients with allergies or acute infections.

**Study design**

The study was designed to examine the combined effects of resistance exercise and endurance exercise. Saliva samples were collected from subjects on three occasions (November 1997, March 1998, November 1998) during a 12 month moderate exercise training programme. Saliva samples were collected at 0830–0930 hours after the subjects had fasted for at least nine hours, had avoided exercise for 12 hours, and had rested in a seated position for at least 15 minutes.

**Exercise**

The subjects trained twice a week at a fitness club, once in a group session for resistance training and once for endurance training. About 10 minutes of stretching preceded each training session. Both training sessions were supervised and conducted by an experienced instructor who was also responsible for the heart rate (HR) measurement.

The resistance training session comprised two sets of inner thigh, rowing, squat, trunk curl, chest press, and back extension exercises (8–15 repetitions), using a combination of free weights and an exercise machine and 20–30 minutes on a cycle ergometer at 60% maximal HR. Upper and lower body exercises were alternated to minimize fatigue, and a sufficient rest interval between the two sets was allowed.

The endurance training programme comprised flexibility and aerobic exercises, such as step exercise with music and ball games. The target HR was calculated using the HR reserve and aerobic exercises, such as step exercise with music and a sufficient rest interval between the two sets was allowed.

The resistance training session comprised two sets of inner thigh, rowing, squat, trunk curl, chest press, and back extension exercises (8–15 repetitions), using a combination of free weights and an exercise machine and 20–30 minutes on a cycle ergometer at 60% maximal HR. Upper and lower body exercises were alternated to minimize fatigue, and a sufficient rest interval between the two sets was allowed.

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Estimation of individual VO$_{2\text{MAX}}$

Individual VO$_{2\text{MAX}}$ was estimated by the method of Åstrand with some modification.$^{22}$ Each subject sat on a cycle ergometer and performed a three-stage incremental ergometer exercise for 12 minutes. Exercise intensity was determined according to age and sex. During the exercise, mean HR was measured before the end of each stage.

**Saliva collection**

Saliva samples were collected as described previously.$^{23}$ Briefly, timed whole mixed saliva samples were collected after the mouth had been rinsed thoroughly with distilled water. Saliva production was stimulated by chewing a sterile cotton swab (Salivette; Sersted, Vümbrecht, Germany) at a frequency of 60/60 seconds, and saliva was separated from the cotton by centrifugation at 3000 rpm. After measurement of the sample volume, saliva samples were frozen at -80°C and stored until the end of the study period.

**Assays**

Salivary SIgA concentrations were measured by enzyme linked immunosorbent assay (ELISA) as described previously.$^{23}$ Briefly, a 96 well microtitre plate (Immulon II; Dynex Technologies, Chantilly, Virginia, USA) was coated with rabbit anti-(human secretory component) IgG fraction (MBL, Nagoya, Japan) overnight at 4°C. After the addition of 250 µl phosphate buffered saline containing 1% bovine serum albumin (Sigma, St Louis, Missouri, USA), wells were blocked for two hours. Saliva samples were thawed, centrifuged at 10 000 rpm for five minutes, and diluted (1:20) with phosphate buffered saline containing 1% bovine serum albumin; 100 µl of each was added and the mixture incubated for one hour. Using purified human SIgA (Organon Teknika, Durham, North Carolina, USA), known concentrations of SIgA were also plated to establish standard values. After the plate had been washed with phosphate buffered saline/Tween, goat Fab’ anti-IgA conjugated with horseradish peroxidase (MBL) was added to the plate and the mixture incubated for one hour. After washing, substrate solution was added and the colour intensity produced after 15 minutes was measured by a microplate reader (Bio-Rad Laboratories, Hercules, California, USA) at 490 nm. All samples were assayed in duplicate, and the average of absorbance values was used as the representative value. Regression analysis using the relation of standard SIgA concentrations and amount of absorbance (nm) was used to interpolate the concentration of SIgA in the samples. Figure 1 shows a typical standard curve. To avoid interassay variability, all samples from each subject were assayed on the same plate. The interassay coefficient of variation of the method, based on analysis of 82 duplicate samples, was 6.2%. Concentrations of total protein in the saliva were measured by using the method of Bradford (Bio-Rad Laboratories).

**Statistical analysis**

For analysis of SIgA levels, data were expressed in two forms: (a) absolute concentration of SIgA (µg/ml); (b) SIgA secretion rate (µg/min), or the total amount of SIgA appearing on the mucosal surface per unit time. SIgA secretion rate was calculated by multiplying absolute SIgA concentration (µg/ml) by 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 saliva sample (minutes). Salivary SIgA concentration, saliva flow rate, and SIgA secretion rate were analysed separately by one way analysis of variance with repeated measures to determine the effect of training. When one way analysis of variance showed significant effects, a Fisher’s post hoc test was performed. For all analyses, p<0.05 was accepted as significant.

**RESULTS**

**Estimated VO$_{2\text{MAX}}$**

The estimated VO$_{2\text{MAX}}$ had significantly increased after 12 months of moderate exercise training (31.4 (5.5) v 32.5 (6.8) ml/min/kg; mean (SD); p<0.05).

**Saliva concentration of total proteins**

Mean concentrations of total proteins before training, and after four and 12 months of training were 0.72 (0.16), 0.73
DISCUSSION

Our study provides evidence that regular moderate exercise increases salivary SIgA levels in elderly subjects. This result suggests that moderate exercise may improve the mucosal immunity of the elderly. To our knowledge, the present data are the first to show that mucosal immune function (salivary SIgA levels) is enhanced in elderly subjects after 12 months of moderate exercise. However, our study did not include a control group, and the exact contribution of each exercise type (resistance training and endurance training) could not be separated; rather the results should be viewed as the effect of moderate exercise on mucosal immune function in the elderly.

Mackinnon and Jenkins reported that resting salivary SIgA levels in athletes had not changed after eight weeks of interval training. One explanation for the difference from our results may be differences in baseline characteristics of the participating subjects—for example, age and endurance capacity. Reviews of reports by several groups have suggested that the effects of exercise on the immune system depend on the level of fitness of the participating subjects and the intensity and duration of the exercise, such that exercise in sedentary subjects generally induces improvement in immune parameters. Our study may support this view. It is possible that the time period used by Mackinnon and Jenkins was too short to influence resting salivary SIgA levels.

Saliva is a complex mixture of secretions from the parotid, submandibular, and sublingual glands and also from many other smaller glands. These glands are the most important source of SIgA in the upper respiratory tract. SIgA plays an important part in immune protection at the mucosal surface by providing specific antibodies in response to pathogens, and forms an exclusion barrier at the mucosal surface to prevent antigen entry. The lack of non-specific SIgA at the mucosal surface or the inability to produce specific SIgA can lead to increased risk of infection, as in cases of IgA deficiency.

Gleeson et al provided evidence that reduced levels of salivary SIgA are associated with increased frequency of episodes of URTI. Mackinnon and Jenkins suggested that at least part of the increased susceptibility to URTI in athletes may be due to reduction of SIgA output resulting from both decreased SIgA secretion and inhibition of salivary flow that occurs briefly after intense exercise. Miletic et al reported that elderly subjects had significantly reduced saliva flow compared with young subjects. SIgA secretion rate is a function of absolute immunoglobulin concentration and saliva flow rate, and reflects the total amount of immunoglobulin available on the mucosal surface. Most of the enhancement in SIgA concentration in this study. Levels of SIgA in the elderly were significantly lower than in young healthy subjects (data not shown). Miletic et al also reported that SIgA secretion rates at rest were lower in the elderly than in young subjects. This reduced rate of SIgA secretion may explain the higher susceptibility of the elderly to infections.

In this study, we show that moderate exercise increased salivary SIgA levels. In contrast, it has been found that intense exercise training decreases salivary SIgA levels in young adults. These phenomena may be related to the “J” curve hypothesis modelled for the relation between exercise and URTI.

At present, the underlying mechanism of enhanced salivary SIgA levels after 12 months of training in elderly subjects is not clear. It is generally accepted that the Th1 cell subset becomes dominant relative to that of Th2 during aging. Exercise may result in more balanced Th1 and Th2 levels, whereby more immunoglobulins are being produced in local tissues. Although it is known that plasma concentrations of IgA and IgG increase with age, this may reflect decreased plasma volume.
Take home message

SlgA levels in 45 elderly subjects had significantly increased after 12 months of exercise, indicating that regular moderate exercise enhances mucosal immune function in the elderly.

Our society is characterised by the growing number of elderly persons, who have a high risk of illness and infection such as URI. Thus, there is a need for a simple method to evaluate immune function in order to design new strategies to improve such function. Saliva is an easily retrievable sample material, which can be collected non-invasively. We emphasise that this study presents important results about only one aspect of the beneficial effects of exercise in the elderly, although we were unable to use a proper control group (untrained group or sham-trained group). Finally, we have shown here that exercise is a suitable strategy for improvement of mucosal immune function in the elderly.

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