The Effects of Walking Exercise Training on Immune Response in Elderly Subjects

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The immune function declines in efficiency with advancing age, making the elderly less resistant to pathogenic microorganisms. The effects of walking exercise training (five 30-min walking sessions/week at 80% VT) on salivary secretory IgA (SIgA) and plasma lymphocyte subpopulations were studied in elderly subjects. Thirty sedentary, elderly subjects (8 men, 22 women; age 66.7 ± 7.4 years) performed walking exercise for 3 months. Aerobic power, body composition, and immune function were examined before (Pre) and after training (3 months). Salivary SIgA flow rate were measured by enzyme linked immunosorbent assay (ELISA), while lymphocyte subpopulations were measured by flow cytometry. SIgA flow rate significantly increased at 3 months, especially in 64-year-olds and under (U-64), 65–85-year-olds (65-85), and female elderly subjects. Number of total lymphocytes, NK cell, and memory-Th cell significantly decreased at 3 months. We conclude that 3 months of walking provides enhancement of mucosal immune function in elderly subjects, although it is not associated with an improvement in lymphocytes.

Keywords: walking, immunology, elderly

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after such transient intense exercise as a marathon (Akimoto, T, et al., 1998). Strong continuous exercise training brings about a decline of immune function, which is represented by over-training syndrome. When intense exercise training is continuously performed, natural killer (NK) cell activation or salivary SIgA secretion declines sharply (Gleeson, M, et al., 1995; Akimoto, T, et al., 1998). Immune functional depression after intense exercise causes defenseless against infection at one stage (open window). As a result, incidence of URTI may increase (Pedersen, B. K, et al., 1998).

Nowadays, walking is promoted for health. A survey by the Prime Minister’s Office ranked ‘walking’ as the top answer (34%) to the question of "What exercise or sport did you perform for the past one year?" Walking is most popular especially among middle-aged and elderly people. Anyone can perform walking at any time alone or with friends as an easy exercise. It is thought to be useful for preventing life-style diseases by improving insulin resistance, increasing muscle and blood flow, and decreasing adipose tissue (Izumi, T., 2003).

Exercise may stimulate the immune system and activate immune function of the elderly as well. If continuous exercise training enables maintenance and enhancement of their immune function, it can greatly contribute to promoting health. There are, however, few research findings from this perspective. It does not necessarily mean that exercise always favorably affects the immune system. On the contrary, excessive exercise may hurt immune function. We have reported that 12-month exercise training can enhance salivary SIgA level and increase cell population of lymphocyte subset of the elderly (Akimoto, T, et al., 2003; Koizumi, K, et al., 2003).

The purpose of this study is to examine how walking as exercise training affects salivary SIgA and lymphocyte subset of the elderly.

2. Method

2.1. Subjects

Thirty healthy elderly without any exercise habits (8 male, 22 female, mean age: 67.5 ± 7.3 yrs) were recruited for this study. Table 1 shows the physical characteristics of the subjects. All subjects were given a complete explanation of the study and provided written informed consent before training. The present study was approved by the Life and Environmental Science Ethics Committee of the Graduate School of Tokyo University.

2.2. Content of Training

The subjects performed five days weekly training for three months starting from July, 2003. Using a pedometer (Omron), they walked for thirty minutes per day. The walking pace and speed were adjusted to make the heart rate reach 80% VT (ventilation threshold) at exercise. Data from the pedometer were taken two days a week at the TARA Center of Tsukuba University and the number of steps were checked VO2 peak, VT, and basal metabolism (BM) were measured through analysis of exhaled gas at exercise before (Pre) and after (3 months) training using a bicycle ergometer.

2.3. Salivary and Blood Sampling

Saliva and blood were collected before and after
training. After thoroughly rinsing their mouths with distilled water three times and resting for five minutes in a locus position, whole saliva was collected by masticating cotton wool (Salivette, Assist) sixty times a minute. The saliva obtained from Salivette received centrifugation for ten minutes by 3000 rpm was preserved at -40 degrees centigrade. Blood samples were collected from the elbow vein at rest and preserved in two EDTA-2K sampling blood vessels.

2.4. Measuring Items

Salivary SIgA concentration was measured by Enzyme-Linked Immunosorbent Assay (ELISA) using the antihuman secretory component (SC) antibody (DAKO) and antihuman IgA antibody (MBL). Microplates (Immulon-2: Dynatech) were precoated with the anti-SC antibody diluted at X1000 in carbonate buffer added by 100 μl/well. The plates were blocked by 1% bovine serum albumin (BSA) in phosphate buffer saline (PBS), and salivary samples diluted at X41 were added to each well and allowed to incubate at room temperature for 60 min. After washing, anti-IgA antibody conjugated to horseradish peroxidase (HRP) diluted at X1000 were added to each well and incubated at room temperature for 60 min. The plates were washed, and then reaction solution containing o-phenylene diamine was added to each well. Absorbance was measured by a microplate reader (Biorad) to calculate SIgA concentration. By the product of SIgA concentration (μg/ml) and saliva flow rate (ml/min), SIgA flow rate (μg/min) was calculated.

One of the blood-collecting vessels was used to measure leukocyte population (White blood cell: WBC) and leukocyte demarcation using a multi-item hemocyte analyzer (SE-9000, Sysmex) provided by SRL Co., Ltd. The lymphocyte population was calculated by the product of the leukocyte population and leukocyte demarcation. The other vessel was used for analyzing lymphocyte demarcation by flow cytometry (FACS Calibur, Becton Dickinson). Three color flow cytometry was performed by incubating whole blood cells with three directly conjugated monoclonal antibodies (fluorescein isothiocyanate: FITC, allophycocyanin: APC, and phycoerythrin: PE). The antibodies used were antihuman CD3 (FITC, DAKO), CD4 (APC, Coulter), CD8 (PE, DAKO), CD28 (FITC, Becton Dickinson), CD45RO (FITC, Becton Dickinson), CD56 (APC, Coulter), and IFN-γ (FITC, Becton Dickinson). The cell population of each lymphocyte was calculated by the product of the lymphocyte population and lymphocyte demarcation. The measured items were CD3+/CD56+ (NK), CD3+ (T), CD4+/CD8+ (T-helper, Th), CD4+/CD8+ (T-cytotoxic, Tc), CD4+/CD45RO+ (memory-Th, mTh), CD4+/IFN-γ+ (Th-type 1, Th1) cell population, and Th/Tc cells rate.

2.5. Statistics

Values were expressed as mean ± SD of all the subjects. All the statistical comparisons between before and after training were made using Student’s paired t-test. P values of <0.05 were considered significant (StatView, version 5.0).

3. Result

3.1. Physical Characteristics, Training Performance, and Physical Strength Test

Table 1 shows age, height and weight of all the subjects. There was no big change between before and after training in weight. Frequency of training was 24.7 ± 8.0 times per month. VO2peak was 1143.4 ± 236.9 and 1176.2 ± 264.2 before and after training, respectively. VT/BM was 13.4 ± 2.9 and 13.2 ± 3.7, respectively. Neither recognized any significant change between before and after training.

3.2. Salivary SIgA flow rate

All 30 subjects’ salivary SIgA flow rates were 43.4 ± 28.4 μg/min and 66.8 ± 45.0 μg/min before and after training, respectively. It shows significant increase after training compared with before (p = 0.003, Table 2, Figure 1). Change of the salivary SIgA flow rates by training was checked by dividing the subjects by age and sex. The age was divided into the elderly (65-85yrs) and under 64 yrs (U-64). Both the elderly and U-64 showed a significant increase flow rates after the start compared with before (p < 0.05, Figure 2). Analyzed by sex, females showed a significant increase after training compared with before (p < 0.05, Figure 3). Males did not recognize any significant change but had a tendency to increase (p = 0.104, Table 2).
3.3. Number of leukocytes and lymphocytes

The leukocyte population (WBC) was $5130.0 \pm 1320.7$ cells/μl and $4873.3 \pm 1126.5$ cells/μl before and after training, respectively, with no significant change between them (Table 3). The number of lymphocytes was $1999.2 \pm 524.0$ cells/μl and $1751.4 \pm 521.8$ cells/μl before and after training, respectively, and showed a significant decrease after training compared with before ($p = 0.017$, Table 3). Both leukocyte and lymphocyte populations were within the range of standard values.

In lymphocyte subsets, NK cell population was $362.2 \pm 267.3$ cells/μl and $264.9 \pm 161.6$ cells/μl while mTh was $392.3 \pm 300.4$ cells/μl and $235.6 \pm 127.4$ cells/μl before and after training, respectively, both of which showed a significant decrease after training compared with before ($p < 0.05$, Table 3).
cell population was 1083.3 ± 481.9 cells/μl, 1139.0 ± 417.6 cells/μl, Th was 651.5 ± 375.0 cells/μl and 639.4 ± 321.4 cells/μl, Tc was 365.0 ± 257.8 cells/μl and 401.0 ± 136.9 cells/μl, Th1 was 26.9 ± 68.4 cells/μl and 10.8 ± 12.1 cells/μl, Th/Tc cell rate was 2.3 ± 1.7 and 1.8 ± 1.0 before and after training, respectively. None had any significant change between them. NK, T, Th, Tc, mTh cell population, and Th/Tc cells rate were within the range of standard values. Th1 cells did not set a standard value.

4. Discussion

The result of this study showed that three-month continuous walking increased salivary SIgA levels of the elderly and that some lymphocytes did change but not enough to exceed standard values. Accordingly, it is possible that walking enhances mucosal immune function inside the buccal cavity of the elderly.

4.1. Walking Effect on Organism

Various walking-associated effects on organisms include weight loss by energy consumption, carbohydrate metabolism, muscle tissue, and fat tissue. There are hardly any studies reporting improvement of insulin resistance by walking. Izumi reported the effect of twelve-month walking on insulin resistance by a glucose tolerance test with diabetic subjects and subjects almost developing insulin resistance (Izumi, T., 2003). The result suggested that walking improved insulin values after loading glucose. In addition, three-month walking by a 59 year-old male subject improved an index of insulin resistance, Homeostasis Model Assessment Index (HOMA index), normalized from 2.6 to 0.9. In the present study, HOMA index accounted for 1.42 ± 0.64 on average before and 1.23 ± 0.68 after training. It did not have any significant change but the number of subjects having an index of more than 1.73, which indicated that they had insulin resistance, decreased from eight subjects to five after training (data not shown). Accordingly, a three-month session of walking, which is set in the present study, is thought to be enough load to affect the organism.

4.2. SIgA Change by Walking

The effect of walking on salivary SIgA has not been clarified. We examined the relationship of physical activity rate per day and SIgA in the early elderly. As a result, the velocity of SIgA secretion in the group of physical activity rate of 115 kcal to 250 kcal showed significant higher values than the groups of less than 115 kcal or more than 250 kcal. Since physical activity rate of 250 kcal in this measurement was equivalent to 10,000 steps, a daily physical activity rate of 115 kcal to 250 kcal may be appropriate for immune function (data not shown). This physical activity rate, however, is not expected in regular walking but in everyday life. The result of the present study has clarified that the three-month walking activity increases salivary SIgA levels of the middle elderly. A study on SIgA change by dividing

Table 3 WBC and Lymphocyte subpopulations at Pre and 3 months.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>3 months</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (cells/μl)</td>
<td>5130.0 ± 1320.7</td>
<td>4873.3 ± 1126.5</td>
<td>0.236</td>
</tr>
<tr>
<td>Lymphocyte (cells/μl)</td>
<td>1999.2 ± 524.0</td>
<td>1751.4 ± 521.8</td>
<td>0.017</td>
</tr>
<tr>
<td>NK (cells/μl)</td>
<td>362.2 ± 267.3</td>
<td>264.9 ± 161.6</td>
<td>0.035</td>
</tr>
<tr>
<td>T (cells/μl)</td>
<td>1083.3 ± 481.9</td>
<td>1139.0 ± 417.6</td>
<td>0.646</td>
</tr>
<tr>
<td>mTh (cells/μl)</td>
<td>392.3 ± 300.4</td>
<td>235.6 ± 127.4</td>
<td>0.009</td>
</tr>
<tr>
<td>Th (cells/μl)</td>
<td>651.5 ± 375.0</td>
<td>639.4 ± 321.4</td>
<td>0.908</td>
</tr>
<tr>
<td>Tc (cells/μl)</td>
<td>365.0 ± 257.8</td>
<td>401.0 ± 136.9</td>
<td>0.490</td>
</tr>
<tr>
<td>Th1 (cells/μl)</td>
<td>26.9 ± 68.4</td>
<td>10.8 ± 12.1</td>
<td>0.242</td>
</tr>
<tr>
<td>Th/Tc</td>
<td>2.3 ± 1.7</td>
<td>1.8 ± 1.0</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Values are means ± SD.
the subjects according to age and sex acknowledged significant increase in the elderly, under 64yrs and female. The male subjects did not statistically show a significant difference. However, the tendency of increase ($p = 0.104$) observed in them demonstrates that walking increases SIgA of the middle elderly.

Previously, we conducted resistance training using machine and endurance training using a bicycle ergometer twice a week for twelve months to examine salivary SIgA change of the elderly (Akimoto, T, et al, 2003). As a result, SIgA significantly increased four months and further increased twelve months after training compared with before. The present study sets walking exercise five times a week for three months. We obtained the same result as the previous studies. Accordingly, it may suggest that different training events increase salivary SIgA levels with continuous exercise. Future study on the relationship between intensity and frequency of exercise or types of events and SIgA may be effective in planning exercise programs which aim to improve immune function of the elderly.

4.3. Change of Lymphocyte Subsets by Walking

It has been reported that the lymphocyte subsets such as T, Th and Tc cells decrease with age (Utsuyama, M, et al., 1992). It may explain the reason that immune function declines with age. Fietta, et al. reported that in the ages of 25 to 45yrs, 46 to 65yrs, and 66 to 100yrs lymphocyte subset cell population does not have any sex-related differences (Fietta, A, et al., 1994). Therefore, we analyzed them in this study irrelevant to sex. Previously, we examined how 12 months of exercise training affect lymphocyte subsets of the elderly (Koizumi, K, et al., 2003). The content of training conducted was resistance training using a machine and endurance training using a bicycle ergometer twice a week for twelve months. As a result, T, Th, mTh and NKT cells observed significant increase twelve months after training compared with before. The increase of T and Th cells, which take a commanding role on the immune system, may suggest improvement of immune function by training. In the present study, NK and mTh cells significantly decreased after training while T and Th cells did not significantly change. One factor may be a decrease of the total population of lymphocytes. Although the cause of lymphocyte decrease is not clear, the lymphocyte population was within the range of standard value both before and after training. Other lymphocyte subsets, as well, were within the range of standard values. Although decrease in the lymphocyte population may be attributed to increase of granulation such as mature granulocyte, the mature granulocyte population did not show significant change between before (2553.8 ± 930.5 cells/μl) and after training (2690.6 ± 913.6 cells/μl). One more factor may slightly affect the influence of walking on lymphocytes. Nieman, et al. reported that elderly female subjects performing 30 to 40 minutes of walking five days a week for twelve weeks did not change in NK cell or T cell activation (Nieman, D. C, et al., 1993). Nehlsen-Cannarella, et al. examined obese female subjects in performance of 45 minutes of walking five days a week for fifteen weeks and the total populations of lymphocytes and T cells significantly decreased (Nehlsen-Cannarella, S. L, et al., 1991). Koizumi, et al., in their study of resistance training in addition to endurance training, found that T and Th cells increased and concluded that muscle damage and inflammation by resistance training induced cytokine production such as interleukin-1 by macrophage activation, which resulted in increase of T and Th cells (Koizumi, K, et al., 2003). It may suggest that a walking activity for three months is not effective to increase the lymphocyte subsets of the elderly. It is also possible that three months of training is not enough for increase of T cells and Th cells. For further study, exercise intensity and frequency as well as training period should be examined in order to increase the lymphocyte subsets of the elderly.

5. Conclusion

The present study examined how a three-month continuous walking activity affected salivary SIgA and lymphocyte subsets of the elderly. As a result, salivary SIgA level significantly increased. Especially, elderly, 64-year-olds and under, and female subjects significantly increased salivary SIgA levels. Although lymphocyte, NK cells, and mTh cells significantly decreased, they did not change greatly exceeding standard values. It is suggested that three months of walking training may enhance mucosal immune function inside the buccal cavity of the elderly.
References


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