

## **The effects of resistance elastic bands exercises on salivary IgA and salivary cortisol levels in elderly women.**

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### **Abstract**

**This study investigated the effect of a resistance exercise program using elastic bands for the enhancement of immune function and prevention of metabolic disorders, as measured by levels of salivary cortisol (sCor) and salivary immunoglobulin A (sIgA). Twenty-two elderly women were divided into an exercise group ( $77.91 \pm 1.41$  y) and a control group ( $78.73 \pm 1.51$  y). The exercise group performed resistance exercises with an elastic band at a frequency of 3 times/week and 60 min/day for 4 months. Physical fitness and levels of sCor and sIgA compared between before and after the exercise program. A significant increase in the number of lifts made in a lift dumbbell test was observed after the exercise program for both the left ( $p<0.05$ ) and right ( $p<0.01$ ) arms, indicating an improvement in upper limb muscular endurance. The number of steps on a 2 min walking in place test was also significantly increased following the exercise program ( $p<0.01$ ), indicating improved cardiorespiratory function. Levels of sCor and sIgA reduced following the exercise program, but not by a statistical significance. A negative, but not significant, correlation was found between sIgA and sCor levels. In conclusion, the resistance exercise program using elastic bands conducted in our study improved the physical fitness. However, the exercise durations were too short to achieve a meaningful reduction of stress factors and improvements in the immune function. In future research, duration and intensity of exercise is modified to achieve greater improvements in the immune function of elderly people.**

**Keywords:** Salivary IgA, Salivary cortisol, Resistance exercise, Elderly women, Immune system, Stress.

*Accepted on November 27, 2017*

### **Introduction**

The reduced immune function of an elderly person can cause rapid progression from influenza to pneumonia due to reduced resistance to infections [1]; furthermore, reduced immune function is associated with mortality from chronic inflammatory metabolic disorders and cancer [2]. Stress is one of the major factors in the reduction of immune function, and the increase of cortisol release means the inhibition of immune function in elderly people [2-4]. Levels of hormones released from the hypothalamic-pituitary-adrenal [HPA] axis are used to a marker of physiological stress [5]. Cortisol is a hormone released from the adrenal cortex [6], and its hypersecretion negatively affects metabolism by inducing inflammation, muscle mass reduction, and bone loss [7].

While blood examination is commonly suggested as a health indicator test for elderly people, recently saliva has been gaining increasing attention as a diagnostic tool. Saliva contains hormones, peptides, electrolytes, mucous, antibacterial compounds, and various enzymes [8]. Components of saliva are sensitive to the method of collection and the strength of the stimulus. The secretion rate and concentration of saliva can vary depending on the timing of salivary collection, sex [9], and age [10]. In addition, the

amount and components of saliva may change due to the effects of disease or drugs, and proteolytic enzymes in saliva can affect the stability of specific compounds in a test. Certain molecules may be broken down while diffusing into saliva. Despite these drawbacks, non-invasive saliva testing has become popular since saliva could be collected without affecting hormone and immune marker test results, and without the use of special equipment. Additionally, there are very few side effects, and it is cost-effective and efficient for large-scale testing.

For saliva is used to as a diagnostic tool in place of plasma, the association between saliva and plasma levels must be identified. Cycling exercise [11], resistance exercise [12], and resistance exercise for patients with osteoporosis [13] have been significantly associated with serum and salivary cortisol levels. Salivary cortisol levels reflect free cortisol and bioactive steroid derivative levels in blood, and measured stress reactions more accurately than free cortisol levels in blood [5].

Salivary immunoglobulin A (sIgA) is an antigen-specific antibody that mediates the primary immune response [14], and has protective functions. Secretory immunoglobulin A (sIgA; >85%), which is one of various antimicrobial components, is

directly produced by B lymphocyte around the salivary glands. Immunoglobulin A (IgA) is known to inhibit the entry of foreign substances, including bacteria and viruses, into the body, and defend against intraoral or upper airway infections [15-17]. The sIgA is associated with the exercise intensity. Exercise of low to moderate intensity is reported to improve immunoglobulin function [18], while high intensity overtraining is reported to reduce sIgA levels and the saliva flow rate to weaken immune function [3,4,15,19]. Stress also affects the rate of sIgA secretion, which increased by acute psychological stress, and reduced in chronic psychological stress. Continuous exercise training may reduce chronic stress and increase the rate of sIgA secretion in elderly people [16].

However, research on the improvement of immune function due to exercise training and the association between salivary cortisol (sCor) and sIgA is still in the early stages, and further investigation of the treatment and prevention of aging-related diseases is demanded. The purpose of present study investigates the effects of a resistance exercise program using elastic bands on sCor and sIgA levels in elderly women.

## Materials and Methods

### Subjects

Twenty-two elderly women were divided into a resistance elastic band exercise group (Ex: 77.91 ± 1.41 y) and a control group (Con: 78.73 ± 1.51 y) (Table 1). Physical fitness, sCor levels, and sIgA levels compared before and after the exercise program. The study protocol followed with the Declaration of Helsinki 1975.

**Table 1.** Characteristic of subjects (n=22).

	Exercise group (n=11)	Control group (n=11)
Age (y)	77.91 ± 1.41	78.73 ± 1.51
Height (cm)	145.60 ± 1.76	150.18 ± 1.44
Body weight (kg)	52.99 ± 3.03	54.16 ± 2.50
BMI (kg/m <sup>2</sup> )	24.85 ± 1.13	23.88 ± 0.94
Fat%	26.67 ± 2.01	24.86 ± 1.93
WHR	0.93 ± 0.03	0.90 ± 0.02

Values are Means ± Standard Errors; BMI: Body Mass Index; WHR: Waist Hip Ratio.

### Elastic band resistance exercise program

Since it is recommended for elderly people to exercise 3 times/week for at least 30 min/session to strengthen their health and increase muscular strength [20], subjects performed resistance exercises with an elastic band at a frequency of 3 times/week and 60 min/day for 4 months (warm-up: 10-15 min; main exercise: 35-40 min; cool down: 10 min).

Exercise intensity determined with the elongation percent of the elastic band. Yellow bands were used to the beginning of

the training, which replaced with red bands 4 weeks later depending on the individual's muscular strength. Muscle-strengthening exercises consisted of lower limb exercises (long-sitting ankle plantar flexion, leg press, and calf raise), and upper limb exercises (arm flexion, and range of motion of the shoulder joint).

Exercise intensity was also determined by heart rate (HR) using the Karvonen formula, which calculates the target HR as follows:

$$\text{Target HR} = ((\text{HR}_{\text{max}} - \text{HR}_{\text{rest}}) \times \text{exercise intensity (60\%)} + \text{HR}_{\text{rest}})$$

Maximum HR determined by a formula based on age. Exercise intensity maintained at a moderate level with a rating of perceived exertion (RPE) of 10-12, 60% HR<sub>max</sub>.

### Measurement of body composition

Body weight was measured using InBody 3.0 (Biospace, Seoul, Korea), and body mass index (BMI) was calculated as follows: BMI=body weight/height<sup>2</sup> (kg/m<sup>2</sup>). Subcutaneous fat thickness was measured with a skinfold caliper (Skyndex: Caldwell, Justice and Co., AR, USA), and percentage of fat (%fat) was calculated [21]. Waist-to-hip ratio (WHR) was found by measuring the waist circumference from the bottom of the upper part of the iliac crest, and the hip circumference from the middle part of the iliac crest [22].

### Physical fitness test

The upper limb endurance test consisted of a 30-s 2 kg dumbbell lifting test using the right arm and then the left arm [23]. The number of repetitions achieved by each arm recorded as the score. The lower limb endurance test consisted of a 30-s chair leg squat test using a 46 cm-tall chair without an armrest. The number of repetitions recorded as the score. The static balance test involved a one-leg stance test, in which a subject spread her arms out while looking to the front, stood on the one foot she felt the most comfortable with, and the duration of the stance measured in seconds [24]. For the dynamic balance test, the Timed Up & Go Test (TUG) was performed in which the time from when a subject stands up from a 46 cm-tall chair without an armrest to when she sits back on the chair after walking for 3 m was measured [24]. For the cardiorespiratory endurance test, a 2-min walking in place test performed, and the number of repetitions recorded as the score [25].

### Saliva collection

Saliva collected during the same period for all subjects in a fasting state [26]. The temperature of saliva was maintained at 22. The subjects were refrained from brushing their teeth and eating on the morning of saliva collection, and from drinking beverages 30 min before collection. The purpose of saliva collection explained once subjects were at rest. The subjects rinsed their mouth with 100 ml of distilled water for three times, and rested for 5 min until saliva accumulated from the salivary glands. After swallowing the saliva for the last time, the subjects chewed on a sterile cotton swab from the salivette

tube for 1 min when commanded. They spat out the cotton swab, which had absorbed the saliva, into the salivette tube. The duration of saliva collection recorded. After centrifuging the salivette tube at 4, and 3000 rpm for 5 min, the saliva at the bottom of the tube was placed in an e-tube, and the saliva volume was measured. The e-tube containing the collected saliva was stored in a freezer at temperatures below 80°C until analysis. The cotton swab container was stored in a refrigerator.

**Analysis of salivary IgA and salivary cortisol levels**

Salivary cortisol levels measured by performing an enzyme immunoassay with a cortisol EIA Kit [19]. Salivary immunoglobulin A levels were measured via an enzyme-linked immunosorbent assay (ELISA) [27]. SIgA levels measured in duplicate by sandwich ELISA, by using commercial kits (#ab137989Abcam, UK). SIgA levels measured by using standard samples with known levels of IgA provided by the manufacturer and expressed as ng/ml. Optical density was read on a standard automated plate reader at 450 nm (BioTek, VT, USA). The concentration of salivary IgA calculated upon the linear curve fit of the related four parameters.

**Statistical analysis**

Means and standard errors calculated for all data on different periods using SPSS 17.0. An independent t-test compared the pre-test values between the two groups, and a paired t-test compared by period. A pearson’s correlation analysis performed to analyze the association between sCor and sIgA levels. The level of statistical significance was set at p<0.05.

**Table 3.** Comparisons of physical fitness between pre and post exercise in exercise group (n=11).

	Items	Pre-Ex	Post-Ex
Upper limb muscle endurance	Left lifting dumbbell (reps)	25.50 ± 0.82	26.80 ± 1.07*
	Right lifting dumbbell (reps)	25.18 ± 0.94	27.09 ± 1.20**
Lower limb muscle endurance	Chair leg squat (reps)	16.90 ± 1.72	20.90 ± 1.61
Static balance	One leg stance (sec)	13.20 ± 2.60	9.84 ± 1.99
Dynamic balance	Time Up & Go Test(TUG) (reps)	9.50 ± 0.70	13.60 ± 3.79
Cardiopulmonary endurance	2 min walking in place (steps)	76.00 ± 6.95	95.78 ± 7.03**

Values are means ± standard errors; \*p<0.05; \*\*p<0.01: between time

**Salivary cortisol and salivary IgA levels**

The saliva sample volumes collected after the 4-month resistance elastic bands exercise increased after exercise, but not significantly. The sCor and sIgA levels reduced after exercise, but not significantly (Table 4). A negative correlation was find between sCor and sIgA levels, but did not reach statistical significance (Table 5).

**Results**

**Body composition and physical fitness**

In the exercise group, no significant differences in BMI and WHR found after 4 months of elastic band resistance exercise training (Table 2). The number of lifts in the dumbbell lift test was significantly increased for both the right (p<0.05) and left (p<0.01) arms after exercise, indicating an improvement in muscular endurance.

**Table 2.** Comparisons of body compositions between pre and post exercise in exercise group (n=11).

Items	Pre-Ex	Post-Ex
Body weight (kg)	52.99 ± 3.04	53.15 ± 3.05
BMI (kg/m <sup>2</sup> )	24.85 ± 1.13	24.95 ± 3.87
WHR	0.93 ± 0.03	0.93 ± 0.01

Values are means ± standard errors

The chair leg squat test for lower limb muscle endurance and the TUG results of the dynamic balance test increased from pre-exercise to post-exercise, but the difference was not significant. The number of steps in the 2-min walking in place exercise significantly increased (p<0.01), and thus, cardiorespiratory endurance was improved (Table 3). During the 4-month exercise program using elastic bands, the elderly subjects maintained their sedentary lifestyle due to difficulty moving, which may explain why the upper limb muscular strength relatively more improved than lower limb muscular strength.

**Discussion**

Immunoreactive hormones, such as immunoglobulins or adrenal androgens involved in the primary immune response that protects the body function from stress, undergo changes in response to stress [5]. The production and secretion of cortisol in the adrenal cortex is control by the autonomic nervous system, and the HPA axis [28]. Increased anger, depression due to aging, and exercise intensity are directly proportionate to the amount of cortisol secretion [5,29,30]. A study that

investigated a correlation between saliva and plasma found a significant correlation between salivary and plasma cortisol levels in the resting state ( $r=0.50$ ,  $p<0.05$ ), and after exercise ( $r=0.62$ ,  $p<0.01$ ) in a resistance exercise group [12]. In our previous study, we found a significant positive correlation between levels of cortisol in serum and saliva in patients with osteoporosis ( $r=0.265$ ,  $p<0.05$ ) [13]. Therefore, non-invasive saliva testing may be useful method in research concerning aging- and immune-associated with diseases such as metabolic disorders, cancer, and cerebrovascular diseases.

**Table 4.** Comparisons of salivary factors between pre and post exercise in exercise group ( $n=11$ ).

Items	pre Ex	post Ex
Salivary weight (g)	1.387 ± 0.220	1.458 ± 0.322
Salivary Cortisol (ng/ml)	2.6 ± 0.25	2.04 ± 0.39
Salivary IgA (ng/ml)	45549.45 ± 4781.99	32507.85 ± 3632.61

Values are means ± standard errors

**Table 5.** Correlation between salivary IgA and salivary cortisol in exercise group ( $n=11$ ).

Items	Salivary cortisol (ng/ml)		
	Pre	Post	Total
Salivary IgA (ng/ml)	-0.588 ( $p=0.035$ )	-0.150 ( $p=0.624$ )	-0.198 ( $p=0.333$ )

Values are r correlation coefficient (p values)

Cortisol is a catabolic hormone, and an anti-inflammatory agent that regulates stress within the body [11]. Thus, it plays a significant role in the function of the immune system. Cortisol levels increase during high-intensity exercise with 60-70% of  $VO_2$ max and above [31], and levels of IgA, which provide the first line of defence, increase during moderate-intensity exercise, and decrease during stressful and intense exercise [32]. Therefore, cortisol and IgA secreted at different rates according to exercise intensity, duration, and amount [33].

The IgA secretion rate reported a significant increase in elderly subjects after 12 months of moderate exercise training [27]. This demonstrates that habitual and consistent exercise training can improve immune function that compromised due to aging. In previous study, a 24-month resistance and endurance exercise program continuously increased IgA levels and secretion rates [34], suggesting the need for continuous exercise for strengthening immune function. In the present study, sCor and sIgA levels decreased after the 4-month resistance exercise program using elastic bands, but did not reach statistical significance. Similarly, no significant reduction in sCor levels showed after a 12-week exercise program in patients with osteoporosis in a previous study [13]. The lack of reduction in cortisol levels and the lack of IgA proliferation shown in previous research [35] on elderly people who completed short-term exercise programs (<24 weeks), which altogether indicates a lack of improvement in immune

function, has been attributed to less than careful selection of exercise durations [36].

An increase in muscle mass and muscular strength in an elderly person affects the expression of anti-inflammatory cytokines, and enhances immune function. A guideline advising the inclusion of resistance exercise in training for healthy aging and increased protein synthesis has been reported [20]. Resistance exercise effectively increases bone mass and muscle function in elderly people, increases the size of type II muscular fibers, and prevents a decrease in the number of satellite cells due to aging [37]. Previously, elderly subjects aged 61-77 years showed a 2-kg increase in bone mass, and a 2.7 kg decrease in body fat following 26 weeks of resistance exercise training [38].

In a previous study, resistance exercise using elastic bands for improving muscular strength in elderly subjects increased muscular strength and gait ability in elderly women [39]. In the present study, upper limb muscular strength increased to a greater degree than lower limb muscular strength by the end of the 4-month exercise program using elastic bands. This attributed to the sedentary lifestyle of the elderly subjects, who have difficulty walking, outside of exercise time. However, based on a report that resistance exercise increases aerobic capacity in elderly people and patients with coronary artery diseases (CAD) [40], and the increase in cardiorespiratory function as results of upper limb resistance exercises observed in this study, upper limb resistance exercise using elastic bands may be used to prevent cardiovascular diseases. Following observation of a simultaneous increase in plasma cortisol levels and decrease in muscle mass, hyper-cortisolism considered as a cause of sarcopenia [41]. The exercise durations in this study were too short compared to previous study to achieve a meaningful increase in muscle mass and improvements in immune function in our elderly subjects.

The rate of saliva secretion tends showed the lower among elderly people than young adult subjects [10], and the overall saliva secretion rate is lower among women than men due to the smaller salivary gland size [9]. Therefore, no significant difference in saliva secretion rate observed in this study because the subjects were all women with low salivary secretion rates, and only a small number of subjects were included in this study. In addition, the effects of disease, drugs, and proteolytic enzymes on the amount and components of saliva, and the stability of the tests could not disregard.

Most studies have reported cortisol, and levels of immune substances and androgen in the mucous to be associated. However, sCor and sIgA levels have been found to be negative correlation [42], and more significantly associated with long-term exercise than short-term exercise [43]. Research results have varied depending on exercise intensity, duration, and amount [33]. Due to this inconsistency in research results, the association between cortisol and adrenal androgen during exercise training according to aging is still unclear, and further research is necessary. In this study, a negative correlation was found between sCor and sIgA levels, but it was not significant. Therefore, setting an appropriate exercise intensity for a

muscle mass increase and an improvement in immune function is important for elderly people for whom accurate measurement of muscle mass and muscle strength is difficult. Appropriate exercise intensity settings should be applied during an exercise program for elderly people to achieve significant improvements in levels of stress and immune response.

The results of this study in which hyper-secretion of cortisol negatively affected muscle metabolism and strength [44], and compromised immune function, demonstrate the possibility of implementing exercise programs aimed at preventing cortisol hyper-secretion to reduce muscle loss in elderly people. However, considering that the neuroendocrine system and complex physiological mechanisms work simultaneously to improve immune function, future research must consider using long-term exercise programs to achieve improvements of immune function in elderly people. With the increasing use of saliva for diagnostic purposes, it is necessary that diseases in elderly people, sensitivity due to drugs, specificity, the association between saliva and established diagnostic criteria, and reproducibility be identified in future research in order to verify the effectiveness of saliva as a diagnostic tool.

This study had several limitations. Firstly, we only included women in this study. Secondly, the number of patients experimented was relatively small study. Thirdly, the exercise duration may have been too short to achieve significant reductions in stress factors and improvements of immune function.

Elderly women ( $77.91 \pm 1.41$  y) showed a significant improvement in the dumbbell lift test for both the left ( $p < 0.05$ ) and right ( $p < 0.01$ ) arms after completing our 4-month resistance exercise program using elastic bands (3 times/week, 60 min/day). The subjects also showed a significant improvement in the 2-min walking in place test in the cardiorespiratory endurance test after performance the program ( $p < 0.01$ ). sCor and sIgA levels decreased after exercise compared to before exercise, but not reach statistical significance. In addition, a negative correlation found between levels of sCor and those of sIgA, but did not reach statistical significance. While the resistance exercise program using elastic bands in this study improved physical fitness in elderly women, the exercise durations were too short to achieve significant reductions in stress factors and improvements in immune function. Therefore, in future research, exercise duration and intensity should be modifying to achieve a more significant improvement on immune function in elderly people.

## Acknowledgements

This research was supported by the Bisa Research Grant of Keimyung University in 2015.

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