Original Article

Salivary Immunoglobulin A responses to 6-minute walk test in elderly women

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ABSTRACT

Objetive: The aim of the present study was evaluate the effect of the 6-minute walk test (6MWT) on the secretory immunoglobulin A (sIgA) kinetics in whole saliva of healthy elderly women. Methods: Nine healthy, elderly females (age 61 ± 6.7 years) performed the 6 MWT. Saliva samples and heart rate (HR) were measured in basal, immediately (IPE), and 30 minutes after the 6 MWT (30-Post Ex). Blood lactate [La] was measured at the same intervals. The ELISA assay was used to determine the sIgA concentrations [sIgA]. The Biuret assay was employed to determine the total proteins levels in saliva. Results: The exercise intensity reached by the participants was ≈ 84 $\pm$ 16% of maximum HR. Baseline [La] was 1.8 ± 0.9 mmol/L. [La] at IPE increased 3.4 mmol/L above baseline (p = 0.02). [sIgA] increased by 233.3 $\pm$ 109.3 % at IPE and remained 211.2 $\pm$ 100.1 % greater at 30-Post Ex compared with basal (p = 0.02). The 6 MWT did not modify total proteins levels (p > 0.05). Conclusion: The 6 MWT, performed to submaximal intensity, appears to be enough of a stimulus to increases sIgA levels in healthy elderly women independent of total proteins concentration in saliva. **Keywords:** 6 MINUTE WALK TEST, BLOOD LACTATE, ELDERLY WOMEN, SECRETORY IMMUNOGLOBULIN A.

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INTRODUCTION

The older population has become the main growth segment in the current society (Chang, Pan, Chen, Tsai, & Huang, 2012) , and is projected to accelerate in the coming decades (WHO, 2015). It has been estimated that by the 2030, the people in the world aged over 60 years will be 1.4 billon, and by the 2050, the older population will reach almost 2.1 billon (WHO, 2015). The aging is a time-dependent functional decline process that affects most living organisms (Carmona & Michan, 2016; López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013). In agreement with this, the elderly people show a high risk to suffer cognitive problems (Uemura et al., 2012), development of cardiovascular diseases (de Souza et al., 2015; Shanmugasundaram, Rough, & Alpert, 2010) and immunosenscense (Prcina, Novak, Cigankova, & Kontsekova, 2018). The last condition is caused by changes in the functions in both adaptive and innate immune system (van der Geest et al., 2017).

Secretory IgA (sIgA) is a predominant immunoglobulin on the mucosal surface of humans and other mammals (Corthésy, 2013). In saliva, the plasma cells adjacent to ducts and acini of salivary glands are the principal source of sIgA (Bishop, Walker, Scanlon, Richards, & Rogers, 2006). Previously, authors indicated that people with salivary sIgA deficiency are susceptible to recurrent infections, principally at the upper respiratory tract (Teixeira, Martins, Martins, & Cunha, 2008). On the other hand, studies performed in young adults, demonstrated that the acute (single bout) exercise increases sIgA concentration [sIgA] (Allgrove, Gomes, Hough, & Gleeson, 2008; Sari-Sarraf, Reilly, Doran, & Atkinson, 2007), reduces [sIgA] (Baralic et al., 2010; Usui et al., 2011) or does not change [sIgA] (Li & Rush, 2009). The controversies could stem from differences in fitness levels, exercise modes, intensities and fidelity of sample collection.

The acute sIgA responses to single episodes of physical exercise in older individuals are largely unexplored (Rikli & Jones, 1998). In detail the sIgA secretion was elevated after just twenty minutes of low-intensity calisthenics in older women (Sakamoto et al., 2005). On the other hand, Hwang et al (Hwang, Park, & Lim, 2016) reported that following 3-months of Pilates training, the sIgA secretion rate increased transiently, but the authors did not find any effect on [sIgA] with a high-intensity cycle ergometry test (i.e., Åstrand Cycle Ergometry Test) in older women. The Åstrand Cycle Ergometry Test is a protocol employed to determine the VO_2Max (Astrand & Ryhming, 1954). However, this test utilize expensive equipment. The exercise technicians or physicians, usually employ more accessible but validated test to determine the fitness level of the patients.

The 6-minute walk test (6 MWT) is an inexpensive and easy test commonly employed to determine the physical endurance in healthy people (Negreiros et al., 2017; Rikli & Jones, 1998; Someya, Mugii, & Oohata, 2015). Initially, Rikli & Jones indicated that during the 6MWT, subjects walked by 6 minutes as fast as they comfortably could, trying to cover the maximum distance possible (Rikli & Jones, 1998). However, others studies identified that if the tester, encourage verbally the participants, they get a better performance during the 6 MWT (Guimarães, Bellotti, Bacal, Mocelin, & Bocchi, 2002; Troosters et al., 2002). Concretely, with the encourage method, the participants walk almost to their maximum capacity (Guimarães et al., 2002; Guyatt et al., 1985). Consequently, the oxygen uptake is higher (Troosters et al., 2002). Regarding to these information, it is possible consider to the 6 MWT performed with the encouragement as a vigorous exercise. It is know that the high intensity exercise induces immunosuppression (Ihalainen, Schumann, Mero, & Ihalainen, 2016; Nieman, 1994). Particularly, the high intensity training reduces the [sIgA] in saliva (Ihalainen et al., 2016), increasing the risk to acquire recurrent upper respiratory tract infection (URTI) (Gleeson & Pyne, 2015; Teixeira et al., 2008). Thus, the 6 MWT performed with the encouragement method can result in a paradox situation. On one side, allows identify more accurately the fitness levels in the elderly population.

However, on the other side, could induce immunosuppression and make them prone to URTIs. Therefore, the aim of the present work was study the effect of the 6 MWT performed with the encouragement method on the salivary [sIgA] in healthy elderly women.

MATERIAL AND METHODS

The study was approved by the ethics committee of the Universidad Autónoma de Baja California, México. All experimental procedures were conducted in accordance with Helsinki Declaration.

Participants
Nine healthy elderly women (61 ± 6.7 years) of modest physical fitness living in Ensenada (Baja California, Mexico) were recruited. Participants were informed of the scope and procedure of the study and subsequently provided written consent. Participants completed the physical activity scale for elderly (PASE) to determine the physical activity in elderly people (Voorrips, Ravelli, Dongelmans, Deurenberg, & van Staveren, 1991). According to the PASE score, all participants could be classified as having moderate physical activity levels (Voorrips et al., 1991).

Study Design
The 6 MWT was performed after a 2-hr fasting period and between 9 and 11:00 AM. The blood lactate and heart rate (HR) were determined at baseline (just before exercise), immediately (IPE), and 30- minutes post-exercise (30 Post-Ex). Saliva and blood samples were collected with HR at each time point.

Anthropometry Measurements
Anthropometrics variables, such as body weight, height, and waist circumference (WC) were determined in all the subjects 24 hrs. after participants completed the PASE questionnaire. An electronic balance (RICE LAKE, Floor Level) was employed to determine the body weight. A stadiometer (Seca 213) was used to evaluate height. WC was determined following methodology previously reported (Howel, 2012). Body mass index (BMI) was calculated as body weight in kilograms divided by the square of body height in meters. All anthropometric measurements were performed between 10:00 AM and 12:00 PM, during which participants underwent the pre-exercise 2-hr fasting period.

6-minute Walk Test
The 6 MWT test, useful for estimating physical endurance in elderly people (Rikli & Jones, 1998), was performed following methodology previously reported (Guimarães et al., 2002; Rikli & Jones, 1998) with some modifications. In brief, one TANITA pedometer (PD-733F; Illinois: USA) was assigned to each participant. The pedometer was attached with a strap to the neck, and setting to display the steps. The pedometer was cleared of previous registers. The investigators made a mark on the ground, then, the participants performed 3 steps back from the mark. After this, the investigator requested participants to walk 10 steps from the mark previously made. One investigator evaluated the distance covered by 10 steps, with this, the stride length was determined. The pedometer was setting to zero. The participants were instructed to walk around a basketball field for 6 minutes, the aim was covered as much ground as possible. One tester walked alongside the participants encouraging to walk as fast as possible. This methodology encouraged the greatest effort in participants (Guimarães et al., 2002). Immediately after the 6 MWT, the tester registered the steps walked by the participant to determine the distance covered.

Cardiovascular and Metabolic Variables

Heart rate (HR) and blood pressure (BP) were measured after 5 minutes of seated rest and prior to completing the 6 MWT. BP was measured using the auscultatory method. HR was determined using the Polar FT1 heart rate monitor at the end of the 5-minute rest period and throughout exercise and recovery. The exercise intensity was determined with following formula: heart rate at 6 min/estimated maximal heart rate (220-age) (Someya et al., 2015). A hand-held lactate analyzer (Nova Biomedical) and test strips were employed to evaluate the lactate concentrations.

Saliva Collection

An unstimulated saliva sample was collected in each participant at the times indicated above. The saliva collection at baseline and 30 Post-Ex were performed following methodology previously reported (Akimoto et al., 2003; Baralic et al., 2010; Shimizu et al., 2007) with brief modifications. Participants sat quietly for 5 minutes, and then rinsed out their mouths with sterilized water three times. Participants remained in seated rest for another 5 minutes with slightly lowered head to allow the spontaneous saliva flow in the mouth. The participants expectorated three times for 2 minutes specifically at 0, 60 and 120 seconds into a falcon tube of 15 mL. During the saliva collection process, the participants were asked not to make any special effort to gather saliva in their mouths. But, they were asked to allow the saliva to flow naturally. Saliva collected IPE followed similar methodology, but without the prior rest period.

The saliva samples were immediately stored to -20ºC for future analysis. Saliva flow was determined following previously-published methodology (Sakamoto et al., 2005; Teixeira et al., 2008). In brief, the total sample volume (mL) was divide by the time taken to produce the saliva sample (minutes) and reported as mL/min.

Saliva Analysis

sIgA concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) kit (1-1602; Salimetrics, LLC, Carlsbad, CA) according to the manufacturer's instructions. To avoid inter-assay variability, all the samples were assayed on the same microtiter plate. The samples were analyzed in duplicate and the mean concentrations were calculated. The limit of sensitivity for sIgA was 2.5 µg/mL. Color was detected 10 min after the stop solution was added and the absorbance was read at 490 nm. (iMark microplate absorbance reader, BIO-RAD, Cat. 168-1130). The sIgA secretion rate (µg/min) was determined by multiplying the absolute sIgA concentration (µg/mL) with the saliva flow rate (SFR) (mL/min) (Baralic et al., 2010; Teixeira et al., 2008). Total proteins were determined by the Biuret protein assay (Spinreact). The bovine serum albumin was used as standard following the manufacturer's instructions.

Statistical Analysis

The data are presented as mean $\pm$ standard deviation (SD). Groups of data were compared in a one-way, repeated-measures analysis of variance (ANOVA) followed by Tukey's post hoc test; a p value of ≤ 0.05 indicated statistical significance. All statistical analyses and figures were performed using the statistical software package GraphPad Prism 6.0 (CA, USA).

RESULTS

Participant baseline characteristics are displayed in the Table 1. In general, the women could be described as overweight, but with normal resting hemodynamics and having moderate physical activity levels. The mean VO_2Max for the participants was 32.21 ± 2.87 mL/kg•min (Table 1).

Table 1. Baseline characteristics of the study subjects. (n = 9)

Variable	Mean	Standard Deviation
Age (yrs)	61	6.7
Body weight (kg)	67.7	7.1
Height (m)	1.56	0.05
Body Mass Index (kg/m^2)	27.5	2.2
Waist circumference (cm)	86.1	6.7
Resting HR (ppm)	73.6	8.7
Resting systolic BP (mmHg)	131.7	18.7
Resting diastolic BP(mmHg)	74.1	10.5
PASE Score (physical activity score)	12.2	4.7
VO$_2$Max estimated (mL/kg•min)	32.21	2.87

The mean distance covered during the 6 MWT was 590.8 ± 55.3 m – a rate of 1.64 m/sec. The mean exercise intensity was 84.1 ± 15.9% of HR max. Baseline HR (74 ± 9 bpm) increased to 134 ± 24 bpm IPE and then returned toward baseline by 30 Post-Ex (78 ± 11 bpm) ($p < 0.0001$) (Figure 1a). The Blood [La] increased 188% from baseline (1.8 ± 0.9 mmol/L) to 5.2 ± 3.1 mmol/L IPE ($p = 0.02$). Blood [La] remained 56% above baseline at 30 Post-Ex, but this difference was not significantly different (Figure 1b). There was a moderate but significant relationship (r=0.69, p=0.03) between the HR max and [La] at the end the 6 MWT (Figure 2).
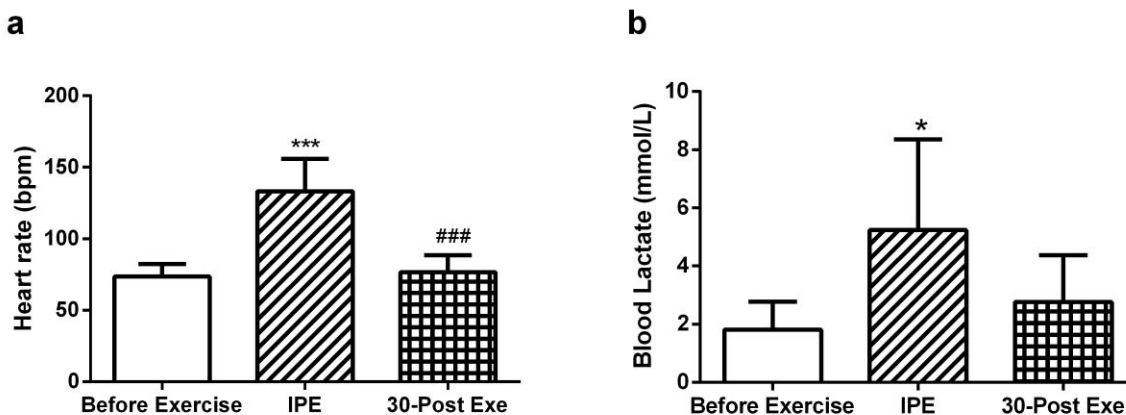
***p<0.00001 vs Before exercise. ###<0.0001 vs IPE *p=0.02 vs Before exercise. One way ANOVA. Tukey´s pos hoc test

Figure1. Changes in heart rate during the 6-minute walk test (6 MWT) in healthy elderly women. The heart rate was determined at basal state (Before exercise). Immediately finished the 6 MWT (IPE), and 30 minutes after finished the 6 MWT (30-Post Exe) (a). Effects of the 6-minute walk test (6 MWT) on the blood lactate levels in healthy elderly women. Blood lactate determined at baseline (Before exercise). Immediately finished the 6 MWT (IPE), and 30 minutes after finished the 6 MWT (30-Post Exe) (b)
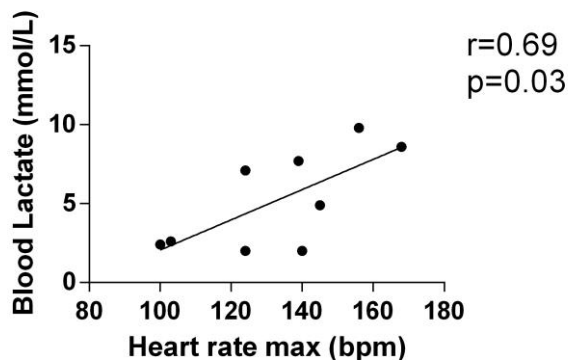
Figure 2. Correlation analysis of the blood lactate levels vs heart rate at finished the 6-minute walk test

The 6 MWT increased the salivary [sIgA] from 41.1 ± 20.9 μg/mL at baseline to 89.1 ± 39.6 μg/mL IPE (p = 0.028), at 30 Post-Ex, the mean [sIgA] was 78.9 ± 41.5 μg/mL (Figure3a). The Figure 3b shows that 6 MWT increased significantly the relative [sIgA] at both IPE and 30 Post-Ex with respect to basal state (F (2, 21) =5.57, p=0.01). Baseline SFR (0.35 ± 0.15 mL/min) remained unaltered IPE (0.22 ± 0.12 mL/min) or after exercise (0.28 ± 0.17 mL/min) (p = 0.20) (Figure 3c). In the same sense, the sIgA secretion rate did not change with the exercise (baseline = 13.7 ± 8.0 μg/min; IPE = 20.9 ± 14.8 μg/min; 30 Post-Exe= 19.9 ± 16.5 μg/min) (Figure 3d). Total [protein] in saliva did not change with the 6 MWT (baseline = 5.15 ± 1.64 mg/mL; IPE = 7.65 ± 2.86 mg/mL; 30 Post-Ex = 5.94 ± 1.85 mg/mL) (Figure 4).
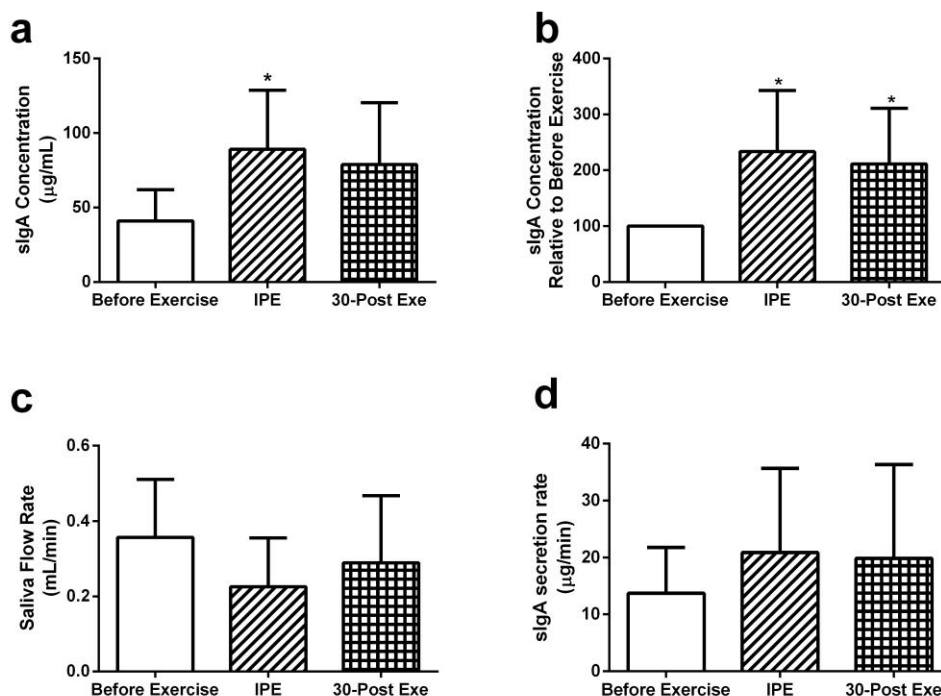
p<0.05 vs Before exercise. One-way ANOVA. Tukey´s pos hoc test

Figure 3. Effect of the 6-minute walk test (6 MWT) on the absolute sIgA concentration in saliva (a), relative sIgA concentration in saliva (b), saliva flow rate (c), and salivary sIgA secretion rate (d). Sample determined at basal state (Before exercise). Immediately finished the 6 MWT (IPE), and 30 minutes after finished the 6 MWT (30-Post Exe)
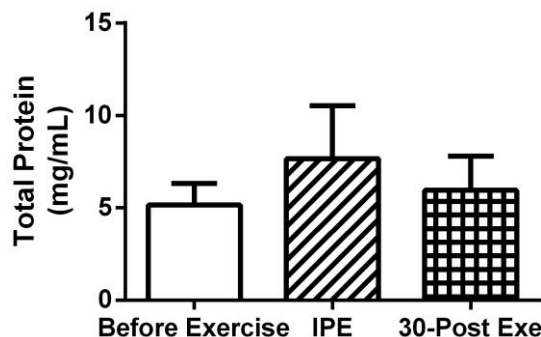
Figure4. Effect of the 6 MWT on the total saliva protein concentration. Sample determined at basal state (Before exercise). Immediately finished the 6 MWT (IPE), and 30 minutes after finished the 6 MWT (30-Post Exe)

DISCUSSION

The present study was designed to characterize the salivary sIgA response to the 6 MWT in healthy elderly women. Results of the current study, demonstrate that [sIgA] are elevated after a 6MWT, performed at greater than 80% of maximal HR, in moderately-active older women. The higher post-exercise [sIgA] was not accompanied by changes in SFR, sIgA secretion rate or total salivary proteins. Our results may be interpreted to indicate that a brief and moderate-vigorous field exercise testing in elderly women does not induce immunosuppression at the upper respiratory tracts (indicated by [sIgA]).

The mean 6 MWT distance covered by the present cohort is similar to that previously reported in older physically active women (Janaudis-Ferreira, Sundelin, & Wadell, 2010). Furthermore, the exercise intensity reached by the particians in the current study was ~ 84%. This data is in agrement with others studies in which the encouragement method was utilized during the 6MWT (Jenkins et al., 2009). The current study demonstrates an immune-simulative effect of a single episode (6 minutes) of physical exercise to submaximal effort in older individuals with moderate physical activity level. In concordance with our data, a recent study reported a higher absolute sIgA concentration in older women after a graded exercise test performed to >80 % HR (Hwang et al., 2016). However, in that study, the length of the exercise session was not reported. Besides, in the Hwang' work just reported the absolute [sIgA]. Others authors also reported a higher absolute sIgA concentration after a single exercise session to low intensity (Sakamoto et al., 2009). However, more than absolute concentrations, authors indicated that the relative [sIgA] are preferable data to determine the risk of URTI (Neville, Gleeson, & Folland, 2008). For this reason, in the present study, we determine the impact of the 6 MWT on the relative [sIgA]. Our data indicate that the 6MWT performed to submaximal effort, increases immediately the relative [sIgA], this effect is still present after 30 minutes of finished the 6MWT. To our best knowledge, this is a novel data. Currently, the molecular mechanism to explain the higher sIgA after the exercise is unknown. Therefore, further studies are necessary to identify the underlying molecular mechanism that explain the changes in salivary [sIgA] during the physical exercise in older population. The sIgA secretion rate was not modify by the 6MWT. Other reports indicated a higher sIgA secretion rate after a single session (Hwang et al., 2016; Sakamoto et al., 2005). In these studies, a higher SFR after the acute exercise also was reported. Contrary, in the current study, the field exercise testing did not change the SFR. Considering that SFR is a primary component for increasing the sIgA secretion rate during the exercise in elderly subjects (Sloan, Engels, Fahlman, Yarandi, & Davis, 2013), We suggest that the no effect of the 6 MWT on the SFR in the participants, could explain partially, the lack effect on the sIgA secretion rate.

In addition to the immunological results, the present work found a significant relationship between the blood lactate and the maximal heart rate during the 6 MWT. Together, the [La] and HR responses indicate that the 6 MWT was performed at sub-maximal effort. Previous authors reported that the blood lactate and the HR max during the 1-Mile walk test have a direct relationship in healthy elderly adults (Bazzano, Cunningham, Cama, & Falconio, 1998). These background and the data of the current study, suggest that the metabolic variable (lactate) and heart rate are good indicators to control the intensity during the field exercise testing at least in apparently healthy elderly people.

CONCLUSION

The results of the present data showed that, the 6 MWT performed to submaximal effort increased [sIgA] in elderly healthy women. Neither changes on the SFR, sIgA secretion rates, and the unaltered concentrations of salivary total protein measured through the exercise, indicate specific effect of the field exercise testing on the immunoglobulin. The mechanisms from moderate-to-high-intensity exercise that increase [sIgA] and potentially enhance immune function remain to be determined. Additionally, it is worth indicate that the encouragement method affect the performance during the 6MWT (Guyatt et al., 1985), this situation open the possibility to study the impact of the 6 MWT performed without the encouragement on immune response in healthy elderly women.

ACKNOWLEDGMENTS

COMPETING INTERESTS

The authors declare that they have no competing interests.

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