Innate Immunity Response to Treadmill Walking Exercise in Elderly

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ABSTRACT

Background and purpose: *The number of elderly people in increasing markedly, and their needs are growing rapidly. Several measures of immunity are altered with aging and this is believed to be a contributing factor in the increased incidence of diseases and high mortality rates so the aim of the present study was to examine the effect of moderate intensity treadmill walking on natural killer cells in young elderly.* ***Subjects and methods:*** *Forty five sedentary elderly men and women participated in this study. They were randomly assigned into two groups; group (A), 30 subjects who received moderate treadmill walking with intensity 40-70% of heart rate reserve and group (B) who participated in mild stretching and flexibility exercises. Natural killer cells percentage and time taken to complete 400 meters walking distance were measured before and after two months in both groups.* ***Results:*** *Natural killer cells percentage was increased in both men and women of the training group associated with reduction in the time taken to complete the walked distance, while control group showed a non-significant reduction in neutral killer cell percentage.* ***Conclusion:*** *Moderate intensity treadmill walking is effective to improve innate immunity, represented as natural killer cell percentage, and aerobic fitness in young elderly men and women.*

Key words: *Aerobic training; Natural killer cells; Cardiopulmonary fitness; Elderly.*

INTRODUCTION

Immune function in human begins undergoes some deterioration and loss of functions due to ageing. The increased incidence of malignancy, infectious disease, and autoimmune disorders with age is thought to be related to the decline of immunocompetence[2,26]. The immune system functions as the leading defense mechanism to protect organisms from pathogenic microorganisms. It is well know that functions of physical strength such as muscle strength, endurance power and balance decline with age, so does the function of the immune system in protective physical strength[11]. The immune system can be divided into two broad functions: innate (natural or non-specific and acquired (adaptative or specific) immunity, which work synergistically. Any attempts of an infectious agent to enter the body immediately activate the innate system. This so called "first line of defense" comprises three general mechanisms with the common goal of restricting micro organisms entry into the body: (1) Physical structural barriers (skin, epithelial linings and mucosal secretions); (2) chemical barriers (pH of bodily fluids and soluble factors such as lysozymes, and complement proteins); (3) Phagocytic cells (neutrophils and macrophages) and cytotoxic cells (natural killer cells). Exercise induced immune dysfunction seems to be mostly due to the immunosuppressive actions of stress hormones such as adrenaline and cortisol. Since many of the immunological changes to acute exercise appear to arise in response to stress hormones, factors such as exercise intensity, duration and subject fitness, which influence stress hormone secretion, will affect the immune response[10].

Accordingly, several randomized controlled trials have investigated the effect of moderate intensity aerobic exercise training on natural killer (NK) cell cytotoxicity and the most convincing data come from trials with at least 10 individuals per intervention and control group and < 15% dropout. Studies that meet these criteria indicate essentially no effect of exercise intervention on immune function and percentage of neutral killer cells. On the other hand, most of these studies included elderly men[3,20]. So the aim of the present study was to determine the effect of treadmill walking on innate immunity, measured as natural killer cell percentage (CD 16%) as a primary outcome and cardiopulmonary endurance measured as time taken for 400 meters walking test, in elderly men and women.

SUBJECTS AND METHODOLOGY

Subjects

A total of 45 healthy elderly (men and women), ranging in age from 60 years to 70 years. They were recruited from Faculty of Physical Therapy outpatient clinic. Potential subjects completed a preliminary medical history and a complete history was obtained prior to participation. Subjects were also screened for dementia using the mini-mental status scale and excluded from participation if dementia was present. Eligibility criteria also included; non smoker, sedentary [less than 60 minutes/week of moderate and vigorous recreational activity], women not taking postmenopausal hormones in the past six months. Exclusion criteria included; cancer, diabetes, obese, cardiovascular disease, asthma, regular use of aspirin or other non steroidal anti-inflammatory medications, corticosteroids or other medications known to affect immune function. The study procedures were explained and informed consent was obtained from eligible participant. Subjects were randomly assigned into two groups; group (A); 30 elderly who received treadmill walking training and grouped (B); 15 elderly as a control group who performed simple stretching exercises at home.

Instrumentation

Assessment instrument

Flow cytometry

A laboratory investigation was performed before and after the study period to measure the percentage of the main surface marker of natural killer cells (CD_{16}) in the study and the control groups also used during the training sessions.

Stop watch: it was used to measure the time taken to complete the 400-meter walking test.

Training Instrumentation

Computerized electronic treadmill. It was used to conduct the walking program, 3 times/week for two months.

Pulseometer: it was used to monitor the heart rate during training sessions according to the target or training heart rate.

Assessment procedure

Flow cytometry

Whole blood was collected in evacuated tubes containing EDTA or heparin as anticoagulant. It's a quantitive determination of CD_{16} (cluster of differentiation 16) in human blood using monoclonal antibody to CD_{16}, fluorescein isothiocyanate (FITC) conjugated (CD_{16}FITC). Samples should be analyzed immediately using flow cytometer or store samples at 2-8^{o}C in the dark and analyzed within 24 hours.

400-meter walking distance test

Participants were instructed to walk 400 meters (10 laps of 20 meters per segment (40 meters per lap) as quickly as possible at a pace they could maintain. Standard encouragement was given each lap. Heart rate was monitored before the test, at rest and at recovery 2 minutes later, as well as during the test. The test was stopped if the heart rate exceeded 135 beats /minute or if the participant reported chest pain or dyspnea during the test[13]. The time taken for the elderly to complete the 400 meters distance was calculated. A time of more than 7 minutes to walk 400 meters equates to a cardiopulmonary fitness level of less than 12 ml O_2/kg/min[23]. The fitness level for each participant was calculated according to the time taken to complete the distance, very fit: walk time less than 5 minutes, somewhat fit: walk time from 5 to less than 5.45 minutes, moderately fit: walk time from 5.45 to less than 7 minutes, and least fit: walk time equals to or more than 7 minutes[13].

Training procedure

Patients in group (A) participated in treadmill walking exercises for three times per week for two months. Each session composed of 15 minutes warming up exercises, using simple foot-work, such as marching in place and dynamic stretching such as side leg rises from side lying position, hamstring stretching with extended leg from crock lying position, quadriceps stretch from standing position, then the participant walked on the treadmill. The speed and the inclination of the treadmill was adjusted according to the target or training hear rate which was adjusted individually according to the following formula; target or training heart rate = ($HR_{max} - HR_{rest}$) x percent intensity + HR_{rest}[21].

The maximal heart rate was calculated based on the following equation; HR_{max} = 208 – age x 0.7[24]. The training percent intensity was adjusted at 40% that increased till

reaching 70% (mild to moderate exercise intensity). Cool down was performed for 5-10 minutes on treadmill with low speed to gradually decrease the heart rate.

The heart rate was monitored during the training sessions using pulse meter. The control group, group (B) performed the normal daily living activities, in addition to performing simple stretching exercises at home. The training session duration was 20 minutes which was increased by 5 minutes each session till reaching 45 minutes at the end of the training program.

Statistical analyses

All data were expressed as mean + SD. Statistical significance was evaluated using paired t-test to compare the mean difference before and after training inside each group and unpaired test to compare between both groups. Also t-test was used to compare between men and women inside the training group as regards to the percentage of neutral killer cells. Mc-Nemar test was used to evaluate the change in fitness grade before and after the study period inside each group. Chi square (x^2) was used to compare the change in fitness grade before and after the study period between both groups.

RESULTS

The study was conducted on 45 healthy elderly men and women. The demographic profile of the participants was shown in Table 1, at base line there were no statistically significant differences in age, height, weight.

Table (1): Mean and standard deviation of age, height and weight of groups.

	Group (A)		Group (B)		T-value	P-value	S
	Mean	+ SD	Mean	± SD			
Age (yrs)	64.125	±3.065	64.7	±3.283	0.809	0.421	NS
Height (cm)	162.93	±2.97	161.85	±2.49	1.757	0.08	NS
Weight (Kg)	68.57	±2.47	169.65	±1.19	1.68	0.06	NS

NS: non significant

As regards to sex distribution inside each group, group (A) included 15 men and 15 women and group (B) included seven men and seven women.

Natural killer cells concentration ($CD_{16\%}$) results.

At pretreatment, no significant difference was found between groups for $CD_{16\%}$; see Table 2, 3 and Fig. 1. In post training measurement, there was a statistical significant difference between groups (P = 0.0001). Group (A) showed an increase about 22.69%, while group (B) showed a non statistical significant decrease about 0.25%.

Table (2): Comparison between pre and post training as regards to $CD_{16\%}$ in group A and group B.

Item $CD_{16\%}$	Pre		Post		Relative change	T-test	
	Mean	+ SD	Mean	± SD		t-value	p-value
Group (A)	20.363%	±1.515	24.978%	±2.119	↑ 22.7%	14.026	0.0001 (S)
Group (B)	19.924	±1.891	19.874	±1.882	↓ 0.25%	0.595	0.56 (NS)

S: significant
NS: Non significant

Table (3): Comparison for mean ± SD of CD16% between both groups before and after study period and their relative changes.

	$CD_{16\%}$			
	Group A		Group B	
	Pre	Post	Pre	Post
Mean ± SD	20.36% ± 1.515	24.98% ± 2.119	19.92% ± 1.891	19.874% ± 1.882
Mean difference		4.62	- 0.05	
Relative change %		↑22.69%	↓0.25%	
P-value between both groups	0.0001 (S)			
P-value between both groups regarding relative change	0.0001 (S)			

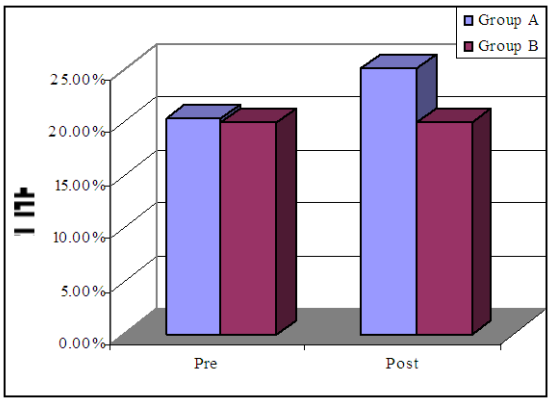

Fig. (1): Comparison between pre and post training values of CD16% for both groups.

Statistical comparison for mean ± SD of CD16% between men and women of the training group (group A) before and after training and their relative changes %.

As regards to $CD_{16\%}$ for men of the exercise training group, the mean was 20.355% ± 1.647 before training and 25.029% ± 2.082 after the training period. On the other hand, the mean of $CD_{16\%}$ for women inside the training group was 20.3721% ± 1.415 before training and 24.927% ± 2.209 after training. There was no statistical significant difference between men and women of the training group, P value 0.577, see Table 4 and Fig. 2.

Table (4): Comparison between mean and women inside the training group as regards to CD16% before and after training.

	$CD_{16\%}$			
	Group A (men)		Group B (women)	
	Pre	Post	Pre	Post
Mean + SD	20.372% ± 1.415	24.927% ± 2.209	20.355% ± 1.647	25.029% ± 2.082
Mean difference	4.56		4.674	
Relative change %	22.36%		23%	
P-value inside each group	0.0001 (S)		0.0001 (S)	
P-value between men and women	0.577			

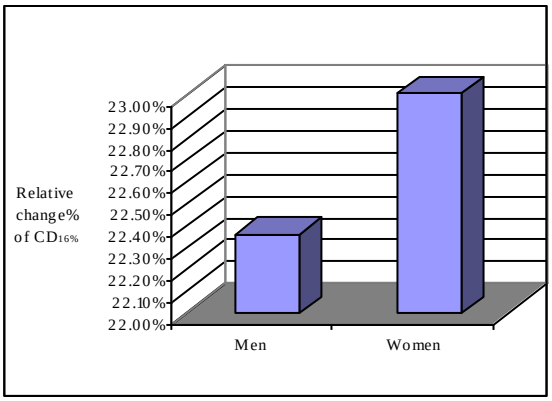

Fig. (2): Comparison between pre and post training values of CD16% for men and women as regards to relative change%.

Results of 400 meters walking distance time.

There was no statistical significant difference between groups in the grade of fitness before training, while there was high statistical significant difference between groups in the grade of fitness after training, see Table 5, 6.

Table (5): Comparison between group A and group B before training.

Before intervention	Group A		Group B	
	No	%	no	%
Least fit	19	63.3	9	60
Moderately fit	11	36.7	6	40
P	X2= 0.05, P=0.8278			

Table (6): Comparison between group A and group B after training.

After intervention	Group A		Group B		X2	P
	no	%	no	%		
Least fit	0	0	7	46.7	16.58	0.0001(S)
Moderately fit	0	0	8	53.3	16.46	0.0001(S)
Some what fit	12	40	0	0	8.18	0.0042(S)
Very fit	18	60	0	0	15	0.0001(S)

There was high statistical significant difference in grade of fitness after training in group A, while there was no statistical significant difference in grade of fitness after training in group B, see Table 7,8.

Table (7): Difference in grade of fitness in group A before and after training.

After intervention	Group A		Group B		X2	P
	no	%	no	%		
Least fit	19	63.3	0	0	27.8	0.0001(S)
Moderately fit	0	0	12	40	15	0.0001(S)
Some what fit	11	36.7	0	0	13.47	0.0002(S)
Very fit	0	0	18	60	25.71	0.0001(S)

Table (8): Difference in grade of fitness in group B before and after training.

Grade of fitness	Before intervention		After intervention		McNemar's X2	P
	no	%	no	%		
Least fit	9	60	7	46.7	0.54	0.4642(NS)
Moderately fit	6	40	8	53.3		

DISCUSSION

Consistent reports of the positive relationship between regular physical activity and immunosenescence have generated much excitement in the field of exercise immunology. It is generally accepted that natural killer (NK) cell activity per NK cell decreases with age and this had been associated with infection and death in the aged[5]. The effects of exercise and training on natural killer cells, components of innate immune system, have been studied extensively in young people. However, the published research on the elderly population is limited. On the other hand, these studies are often difficult to compare due to differences in their methods and presentation of the results. The present study was designed to investigate the impact of treadmill walking training with adjusted intensity on neutral killer cell percentage and aerobic fitness in young elderly. The outcome measurements are considered to improve innate immunity and cardiopulmonary fitness and to be measured objectively through flow cytometry to measure $CD_{16\%}$ and time taken to complete 400 meters walking distance. The study results showed that innate immunity ($CD_{16\%}$) increased by 22.69% in group (A), while group (B) showed a decrease by 0.25%, but it was non-significant. As regards to time taken to complete 400 meters distance, two categories of fitness were observed in the present study before training; moderately fit: patients with walking time 5.45 to less than 7 minutes and least fit: patients with walking time more than and equal to seven minutes the previous classification for the fitness level based on the time taken to complete the distance was cited after[13].

Cell surface receptor proteins, known as cluster of differentiation (CD), are recognizable through the use of monoclonal antibodies allowing NK cells and their subsets to be defined by their CD expression pattern Miller et al., and Moffett et al., [15,16] proved that the percentage of $CD_{16\%}$ NK cells in peripheral blood in normal healthy middle aged individuals reach to 20%. Based on the previous findings, participant in the present study showed a decrease in NK cells concentration (20.363% and 19.924% in group A and B, respectively). The results of the present study agreed with the study of DeLa-Rosa et al.,[4] who showed that there was a decrease in NK cell numbers in old individuals. They concluded that elderly people with low number of NK cells were reported to have three times mortality risk in the first 2 years of follow-up than those with high NK cell number. In another study, it was concluded that the independent increase in cell number was a compensation for decreased NK cell activity, as seen in healthy centenarians who tend to have a higher number of NK cells compared to the unhealthy group[22].

Several studies have examined the effect of endurance training and the corresponding effects on immune function in the elderly, but these studies most commonly selected neutral killer cytotoxic activity (NKCA) not NK cells number as an outcome measure. In addition, a correlation between basal NKCA and percentage or number of NK cells was found in the young group but not for elderly group[5]. So, studies showing contradicting results as regards to NKCA (cytotoxicity), with some reports an increase after endurance training and others show no change. A study done by Fehlman et al.,[7] reported an increase in NKCA after 10 weeks of endurance training and their result may be attributed to choose active elderly participant in their study.

The results of the present study contradicted with the study of Woods et al.,[25] who demonstrated that 6 months of moderate exercise in sedentary elderly (65.3 ± 0.8 yrs) did not change the percentage of neural killer cells. This contradict may be attributed to the increased NK cell cytotoxic activity in the study of woods et al., who selected a smaller age group than the age selected in the present study. It could be concluded from woods and colleagues study that an increase in cytotoxic activity of neutral killer cells may compensate for the decreased percentage of NK cells. This response to exercise training may be a possible mechanism for immune modulation young elderly.

The results of the present study was confirmed by Yan and Colleagues,[27] who tested the immune function of men ages 20-73 years who had participated in moderate exercise at least twice a week for more than 3 years. The study reported an increased concentration of neutral killer cells ($CD+_{56}$) in the 28 elderly participant compared to their age-matched control and to the young group. In spite of this increase in cell concentration, there were no significant differences in NK cytotoxic activity between groups.

The results of the present study go a head with Buyukyazi,[2] who examined the association between active versus in active lifestyle on NK percentage. The sample consisted of eleven elderly male athletes performing regular aerobic exercise for about 38 years and eleven male individuals of similar ages leading a sedentary life as a control group. He concluded that NK cell percentage of athletes was significantly greater than that of the control group and this increase is an indirect marker of enhanced NK cell function.

The results of the present study also contradicted by other studies, such as the study done by Campbell et al.,[3] who conducted a training program consisted of treadmill walking and stationary bicycling at intensity adjusted according to 40% increased to 60-75% of the maximal heart achieved during maximal graded treadmill test, in addition to resistance training, 5 days/week for 12 months. The sample consisted of postmenopausal women aged 50-75 yr. the study results revealed no significant change in NK cell counts which may be attributed high exercise intensity causing decrease in the innate immunity. Also Kimura et al.,[11] showed a decrease in NK (cells/ micro litters) count after walking exercise training for 3 months with intensity adjusted to make heart rate reach 80% of ventilatory threshold. Also Fahlman et al.,[7] reported no difference between exercise and control groups as regards to NK cells count (cells/ml) after 10 weeks of endurance training in the form of walking with intensity about 70% of heart rate reserve in a group of active elderly women. They attributed their results to higher exercise intensity as it is prescribed as a percentage of heart rate reserve rather than using the expected percentage of maximal oxygen consumption. On the other hand, they conducted their study during winter season. The results of the present study revealed an improve (increase) in aerobic fitness represented by a decrease in the time taken to complete 400 meters distance walk. Before training, the percentage of least fit elderly was about 63.3% and 60% in group (A) and group (B); respectively. The percentage of moderately fit was about 36.7% and 40% in group (A) and group (B); respectively. After training, group (A) showed a significant change in fitness grade, 0%, 0%, 40% and 60% for least fit, moderately fit, somewhat fit and very fit; respectively . On the other hand, group (B) showed a non statistical significant change in fitness grade after the study period.

Many factors may be involved in the reduced fitness for elderly. Muscular factors like decrease in motor units impaired muscular activity and substitution of type II by type I fibers, or neurological factors like decreased nerve conduction velocity and reaction time, decreased grey matter volume with white matter lesions have all been linked with diminished gait speed[1]. On the other hand, accelerated muscle proteolysis and sacropenia associated with aging has been associated with increased cytokine activity and dysfunction in the immune system[9,19]. Based on the previous findings, improvement seen in innate immunity may be attributed to improvement in aerobic fitness which may indirectly reflect increase in muscle mass and so decline in cytokines (inflammatory marker) associated with an increase in the anti inflammatory cytokines.

This assumption may be supported by Drela et al.,[6] who reported that increased expression of interleukin-2 (anti-inflammatory cytokine) may increase the activity of NK cells after long term endurance training in elderly male subjects. Adaptation behind the repeated exercise bout effect are thought to include a shift towards greater recruitment of slow-twitch motor units and the generation of new sacromeres in series, thereby reducing the extent of micro trauma[7,12]. Moderate exercise increases the recirculation of neutrophils and natural killer cells, two cells that play a critical role in innate immune defense[17,18]. Other explanations for the improved immune response seen in the present study may be exercise-induced cortisol concentration due to enhanced elimination and suppressed secretion through suppression of hypothalamic- adrenal pituitary axis[10]. Another explanation may be through release of neuroendocrine factors such as β-endorphin which may cause an increase in NK cell percentage[14].

Conclusion

Aerobic training in the form of treadmill walking exercise with prescribed intensity about 40-70% of heart rate reserve is effective to increase neutral killer cell percentage and aerobic fitness in young elderly men and women.

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الملخص العربي

استجابة المناعة الفطرية لتمرينات المشي على جهاز السير المتحرك في كبار السن

أصبح تزايد أعداد و احتياجات كبار السن الأصحاء ملحوظاً وهناك الكثير من مقاييس المناعة الت ي تتغير مع كبر السن ويعد هذا عاملاً مساعداً في حدوث الكثير من الأمراض مع تزايد معدلات الوفاة ولهذا فإن هذه الدراسة تهدف إلى تحديد تأثير برنامج التدريبات بواسطة المشي على السير الكهربائي المتحرك على تحسن المناعة الفطرية في صورة تحديد نسبة الخلايا الطبيعية القاتلة وكذلك على كفاءة الجهاز الدوري التنفسي في كبار السن. تم اختيار خمسة وأربعون من كبار السن الأصحاء (رجال وسيدات) وتم توزيعهم عشوائياً على مجموعتين، المجموعة الأولى (مجموعة أ) وهى مجموعة الدراسة وقد تلقت تمرينات المشي على جهاز السير الكهربائ ي بسرعة معادلة إلى 40 – 75% من احتياطي ضربات القلب وقد اشتملت هذه المجموعة على ثلاثين مريضا ومجموعة (ب) وهى المجموعة الضابطة وقد تلقت بعض تمرينات الاستطالة. تم قياس نسبة الخلايا الطبيعية القاتلة وكذلك الوقت اللازم لقطع مسافة مقدارها أربعمائة متر قبل وبعد شهرين. أظهرت النتائج وجود زيادة ذات دلالة إحصائية فـي نسبة الخلايا الطبيعية القاتلة وكذلك نقص ف ي الوقت اللازم لقطع مسافة 400 متر بعد إنهاء برنامج المشي كما أظهرت النتائج وجود نقص ليس له دلالة إحصائية ف ي المجموعة الضابطة ومن هذه النتائج يمكن استنتاج أن برنامج المشي على السير الكهربائي بشدة متوسطة مؤثراً في زيادة المناعة الفطرية في صغار كبار السن .

الكلمات الدالة : التمرينات الهوائية – الخلايا الطبيعية القاتلة- كفاءة الجهاز القلبي الرئوي – كبار السن الأصحاء .