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Effects of Regular Moderate Exercise on Arterial Stiffness and PTX3 Protein and Some Cardiac Parameters

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ABSTRACT This study intends to show some of the effects of long-term and regular moderate exercise on arterial stiffness, Pentraxin 3 (PTX3) levels and some cardiac parameters in individuals of middle and advanced age. Twenty mountaineers and twenty sedentary individuals were subjects of this study. Each mountaineer and the individuals in the control group were measured for lipid profile, complete blood count, and underwent echocardiography, and exercise stress test. Central and peripheral pulse wave velocity was measured. The plasma Pentraxin 3 protein was assayed. Metabolic equivalent and PTX3 levels were found to be significantly higher in mountaineers, compared to the sedentary group. Femoral-ankle pulse wave velocity, carotid-femoral pulse wave velocity, left ventricular end-systolic diameter and the neutrophil to lymphocyte ratio were found to be significantly lower in mountaineers, compared to the sedentary group ($p<0.05$). Long-term and regular, moderate exercise significantly decreases indicators of systemic inflammation and arterial stiffness.

INTRODUCTION

Approximately eighty percent of cardiovascular diseases, the primary cause of mortality, are associated with arterial dysfunction and arterial disorders (Thom et al. 2006). Arterial stiffness is a sign of ageing characterized with reduced arterial elasticity resulting from diabetes, atherosclerosis and chronic renal diseases involving especially major arteries (Zieman et al. 2005). Arterial stiffness can be assessed both invasively and non-invasively through pulse wave velocity (PWV), wall thickness measurement, and arterial pressure wave analysis (Oliver and Webb 2003). The golden standard of non-invasive methods include measurements conducted on the carotid and femoral region due to

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their speed of wave travel and repeatability (Laurent et al. 2006). Pulse wave velocity measured arterial stiffness is an independent predictor of disease mortality and morbidity (Laurent et al. 2001; Benetos et al. 2002; Weber et al. 2004). Regular aerobic exercise is reported to reduce cardiovascular disease risk by reducing age-related central arterial stiffness in middle-aged and elderly women and men (Tanaka et al. 1998; Tanaka et al. 2000).

The role of the immune system and inflammatory pathway in the development of atherosclerotic diseases is well known and systematic markers are used to determine mid-aged individuals who are at risk of cardiovascular illness (Kritchevsky et al. 2005). One of these inflammatory markers is Pentraxin 3 (PTX3) from the C-Reactive Protein (CRP) family, which might play a role in the pathogenesis of atherosclerosis (Rolph et al. 2002). However, it is claimed that PTX3 has a cardioprotective effect in the atherosclerotic region (Miyaki et al. 2011). In men that underwent endurance training, PTX3 levels were found to be higher than in sedentary men (Miyaki et al. 2011). It was reported that moderate aerobic exercise elevated the PTX3 levels in middle-aged women due to the cardioprotective effect of exercise (Miyaki et al. 2012). Aerobic exercise reportedly increases PTX3 levels and decreases central arterial stiffness in mid-aged and advanced aged subjects (Zempo-Miyaki et al. 2015).

Moreover, in mice with PTX3 deficiency, the infarct area/space is larger and heart damage and inflammation improve with recombinant PTX3 treatment (Norata et al. 2010).

N/L ratio is an indicator of systemic inflammation in the body and tends to be elevated in progressive atherosclerosis (Kalay et al. 2012). It is a well-known fact that regular aerobic exercise increases maximal oxygen consumption, reduces left ventricular hypertrophy, total cholesterol, LDL and triglyceride levels, the systolic and diastolic blood pressure levels and plays a role in reducing the risk for cardiovascular disease. Nevertheless, there is the lack of adequate data showing that mountaineering or nature walks or trekking brings about the same effect.

The researchers aimed to show some of the effects of long-term and regular moderate exercise (trekking) on arterial stiffness, PTX3 levels and some cardiac parameters in middle-aged and elderly male subjects.

MATERIAL AND METHODS

Sample

Twenty mountaineers between the ages 35 to 68, who have been performing regular, moderate exercise (trekking) once a week for 5 to 20 years (mean 10.45 years), and 20 sedentary individuals of the same age group were included as the control group in this study. The control group consisted of volunteers who had not exercised in the last two years for more than 2 hours a week. Subjects with the presence of any of the following were excluded, that is, metabolic bone disease, coronary artery disease, hypertension, arthritis, diabetes, liver disease, kidney failure, corticosteroid use, antihypertensive drug use, smoking, use of alcohol and body mass index over 30 kg/m^2.

Tests and Analyses

All the mountaineers and the control group were measured for lipid profile and complete blood count and also underwent echocardiography. The protocol was adjusted to 'Bruce protocol' (Pollock et al. 1976). In the Bruce protocol participants started exercising at a treadmill speed of 2.74 km/h and a grade of ten percent. Every three minutes the speed and grade were automatically adjusted. The subjects continued to exercise under direct supervision until they could no longer perform, their heart rate exceeded the target heart rate zone, or peripheral capillary oxygen saturation (SpO_2) dropped to less than eighty percent. Either of these was marked as exhaustion, and time from the start of Bruce protocol to exhaustion was called "time to exhaustion". Maximum oxygen consumption (VO_2 max) was calculated accordingly using the formula, VO_2 max = (4.38×T-3.9), where 'T' is the recorded 'time to exhaustion' (Rahnama et al. 2010). All subjects were monitored for 15 minutes after the test. MET values were calculated according to the Bruce Protocol MET chart.

Arterial stiffness was measured using the PulseTrace PWV device (Micro medical PulseTrace, Rochester, 2009, UK). The velocity of blood between carotid-femoral arteries was used for central stiffness (cfPWV) and femoral-tibialis posterior arteries were used for peripheral stiffness (faPWV). An average of three measurements was taken. Serum levels of PTX3 were determined by BOST brand commercial kit (catalog no: EK0861) with sandwich type ELISA kit.

The study was approved by the local ethical committee. The study was performed according to the Helsinki Charter. All subjects gave their written informed consent to participate.

Statistical Analyses

Statistical analyses were completed via IBM SPSS v22. The mean and standard values were used for data that showed a normal distribution and median and 25-75 percentiles were used for data that did not show a normal distribution. Independent student t-test was used to compare groups that showed a normal distribution. Mann-Whitney U-test was used to compare PTX3 values that did not show a normal distribution. Correlation analysis was conducted by adjusting for age due to lack of fixed variables among PTX3, cfPWV, faPWV, MET and N/L values. The statistical significance level was $p < 0.05$.

RESULTS

There were no statistically significant differences in the demographic characteristics, haematologic and biochemical parameters (Table 1) between the two groups. MET (Table 1) and plasma PTX3 levels (Table 2) were found to be significantly higher in mountaineers, compared to the sedentary group ($p < 0.05$). Left ventricular end-systolic diameter (Table 1), femoral-ankle pulse wave velocity, carotid-femoral pulse wave velocity (Table 2) and the neutrophil to lymphocyte ratio (N/L) were found to be significantly lower in mountaineers, compared to the sedentary group ($p < 0.05$) (Table 2). A statistically significant and inverse relationship was found between PTX3 and cfPWV, faPWV ($p < 0.001$), N/L ratio ($p<0.05$) (Table 3). Moreover, a statistically significant correlation was found between cfPWV, faPWV and N/L ratio ($p<0.001$) (Table 3). The negative correlation was found between MET and cfPWV, faPWV, N/L ratio ($p<0.05$) (Table 3).

Table 2: cfPWV,faPWV, Pentraxin 3 Protein and N/L Ratio

	Mountaineers (n=20)	*Sedentary controls (n=20)*	*p*
cfPWV(m/s)	7.22± 1.59	8.61± 0.79	<0.05
faPWV (m/s)	8.27± 1.03	9.49± 0.70	<0.05
PTX3(ng/ml)	0.46± 0.16	0.2 ± 0.11	<0.05
N/L	1.80 ± 0.10	2.26± 0.30	<0.05

DISCUSSION

In this study, the central and peripheral stiffness values (cfPWV, faPWV respectively) of the mountaineering group were found to be significantly lower than in the sedentary group. This shows that long-term moderate exercise such as

Table 1: Haematological, biochemical characteristics, age, body mass index (BMI), exercise and cardiovascular characteristics

	Mountaineers (n=20)	*Sedentary controls (n=20)*	*p*
Age, years	52.75± 8.24	51.80± 5.99	0.681
BMI	27.85± 2.55	27.47± 1.31	0.556
Systolic blood pressure	122.25± 16.09	17± 11.66	0.246
Diastolic blood pressure	75.05± 9.19	78.1± 8.86	0.298
MET	12.95± 2.91	11.31± 1.46	0.032
Maximal heart rate	166.50± 9.58	163.95± 7.02	0.345
Hb (g/dl)	14.9± 1.64	15.3± 0.39	0.324
Triglycerides (mg/dL)	161.08± 16.39	152.42± 24.33	0.746
Total cholesterol (mg/dL)	201.60± 6.32	211.07± 11.29	0.423
LDL-C (mg/dL)	121.51± 6.12	145.11± 17.90	0.578
HDL-C (mg/dL)	48.50± 3.61	50.64± 2.95	0.614
Ejection fraction	64.00± 1.63	61.00± 1.63	0.218
Left ventricular end-systolic diameter (mm)	2.98± 0.11	3.32± 0.13	0.048
Left ventricular end-diastolic diameter (mm)	4.59± 0.16	4.60± 0.15	0.978

Body Mass Index (BMI), High Density Lipoprotein-Cholesterol (HDL-C), Low Density Lipoprotein-Cholesterol (LDL-C), Metabolic Equivalent Test (MET)

Table 3: Correlation between PTX3, MET, cfPWV, faPWV and N/L

			PTX3	*cfPWV*	*faPWV*	*MET*	*N/L*
Age	PTX3	Correlation	1.000	-.806	-.820	.180	-.442
		Significance (2-tailed)	.	P<0.00137	P<0.001	.274	P<0.0
		df	0		37	37	537
	cfPWV	Correlation	-.806	1.000	.940	-.421	.614
		Significance (2-tailed)	P<0.00	.	P<0.00	P<0.05	P<0.00
		df	137	0	137	37	137
	faPWV	Correlation	-.820	.940	1.000	-.455	.572
		Significance (2-tailed)	P<0.00	P<0.00	.	P<0.05	P<0.00
		df	137	137	0	37	137
	MET	Correlation	.180	-.421	-.455	1.000	-.464
		Significance (2-tailed)	.274	P<0.05	P<0.05	.	P<0.05
		df	37	37	37	0	37
	N/L	Correlation	-.442	.614	.572	-.464	1.000
		Significance (2-tailed)	P<0.05	P<0.001	P<0.001	P<0.05	.
		df	37	37	37	37	0

Carotid-femoral Pulse Wave Velocity (cfPWV), Femoral-Ankle Pulse Wave Velocity (faPWV), Metabolic Equivalent Test (MET), Neutrophil to Lymphocyte Ratio (N/L), Pentraxin 3 Protein (PTX3),

mountaineering and trekking, even if performed once a week, positively affects stiffness values. This effect might be due to increased arterial expansion-expandability. Different outcomes have been reported on the effect of various types of exercise on arterial stiffness. Resistance exercise is known to increase central arterial stiffness (Miyachi et al. 2004; Collier et al. 2008; Collier et al. 2010), while regular aerobic exercise is reported to reduce arterial stiffness, cardiovascular risk factors and mortality (Collier et al. 2008; Collier et al. 2010; Zempo-Miyaki et al. 2015). In a study aerobic exercise was found to lower central arterial stiffness but did not affect peripheral arterial stiffness (Hayashi et al. 2005), whereas in other studies central and peripheral arterial stiffness were reported to be reduced (Collier et al. 2008; Collier et al. 2010). Seals et al. (2009) claimed that long-term aerobic exercise improved age-related vascular endothelial dysfunction and arterial stiffness. Moreover, aerobic exercise is known to benefit stiffness improvement by stimulating prostaglandin, nitric oxide and c type natriuretic peptide genes at the aorta (Maeda et al. 2005). Schmidt-Trucksuss et al. (2000) found that femoral artery diameter and extensibility/ enlarge ability measured by ultrasound imaging and simultaneous aplanation tonometry was higher in men that performed endurance training compared to sedentary men. Dinneno et al. (2001) observed an increase in the femoral artery lumen diameter following a 3-month aerobic exercise program for middle-aged and elderly sedentary individuals that also entailed walking.

The MET values of the mountaineering group were higher than in the sedentary group showing that the walks and trekking increased aerobic capacity. There is a moderate negative correlation between MET values and faPWV and cfPWV. This shows that arterial stiffness is reduced by even a moderate increase in aerobic capacity. Low exercise intensity and frequency might be the reason why the potency of the correlation is reduced. Amoh-Tonto et al. (2009) found that reduced functional capacity increased the brachial-ankle PWV. Horta et al. (2015) and Endes et al. (2016) indicated that PWV values decrease with physical activity, and increase with a sedentary lifestyle. Short-term aerobic exercise also reduces arterial stiffness, but the effect is reversed in 4 weeks with the discontinuation of exercise (Kakiyama et al. 2005). Mora et al. (2007) reported that even moderate physical activity reduced the cardiovascular disease risk. This study also purports that the cardiovascular disease risk might be reduced by the lower arterial stiffness values of the mountaineers.

Enhanced systemic inflammation is known to play a role in the development mechanism of progression to cardiovascular disease. Old age that is associated with lack of physical activity/physical inactivity can stimulate systemic inflammation (Fischer et al. 2006). Long-term aerobic exercise is reported to protect from diabetes and cardiovascular diseases that are characterized by chronic low-grade infections (Mathur et al. 2008; Petersen et al. 2005). PTX3 that has a CRP-like structure increases in the course of cardiovascular disease. Nevertheless, it is claimed that increased PTX3 has anti-inflammatory and anti-atherogenic properties and prevents inflammation (Norata et al. 2010). In some studies, measurement of the inflammatory factor levels in the blood stream or circulation was a diagnostic tool for cardiovascular disease (Norata et al. 2010). It is reported that parallel to cardiac improvement of patients that exercised within the scope of a cardiac rehabilitation program PTX3 concentration levels in the plasma dropped (Fukuda et al. 2012).

Miyaki et al. showed in a study with post-menopausal women that regular aerobic exercise performed for 2 months 3-5 times per week for 30-45 minutes significantly increased PTX3 levels (Miyaki et al. 2012). Zempo-Miyaki et al. (2015) reported that aerobic exercises elevated PTX3 levels in mid-aged and advanced aged individuals. In another study, it was shown that young men performing endurance exercise had higher PTX levels in the plasma compared to sedentary controls of the same age (Miyaki et al. 2011). In a study by Nakajima et al. (2010) investigating the acute effect of high-intensity exercise on PTX3 and high-sensitive CRP levels in the blood stream were found to rise rapidly and significantly immediately after an exercise bout performed at an aerobic threshold of seventy percent. There is evidence showing that long-term exercise lowers serum CRP levels at rest and has anti-inflammatory effects (Kasapis and Thompson 2005).

In this study, the researchers found that PTX3 levels of mountaineers who performed mild and long walking exercises were higher than the control group. An inverse and statistically significant ($p<0.05$) relationship found between PTX3 and cfPWV, faPWV values in the correlation analysis shows that long trekking and mountaineering activities increase the PTX3 value. Low exercise intensity and frequency might explain the lack of significant correlation between PTX3 and MET values. Nevertheless, the researchers can say that long-term moderate mountaineering and nature walks and trekking might have cardioprotective effects by raising PTX3.

N/L ratio is a practical and cheap inflammatory marker used in the follow-up of cardiovascular disease. N/L ratio was found to be high in acute coronary syndrome and stable coronary artery disease (Bhat et al. 2013), which is associated with long-term mortality risk (Cakici et al. 2014). Tanindi et al. (2015) found a correlation between enhanced arterial stiffness and enhanced N/L ratio. Makras et al. (2005) reported a significant reduction of N/L ratio following military physical education compared to pre-exercise values. In this study, the researchers found that N/L ratio is lower in mountaineers compared to sedentary individuals ($p<0.05$). Although reduced N/L ratio is correlated with reduced arterial stiffness it shows a negative correlation with increased MET and PTX3. In the light of this result, the researchers can say that trekking exercises have beneficial effects on one of the inflammatory parameters, that is, N/L ratio in middle age and elderly individuals.

In this study, end systolic diameter was smaller in the mountaineers group compared to sedentary individuals, indicating that trekking or nature walks performed once a week positively affected heart muscle contractility. Nevertheless, the exercise frequency was not adequate enough to bring about significant differences in parameters such as hemoglobin, total cholesterol, HDL cholesterol, LDL cholesterol and tryglyceride levels. Trekking and mountain walk performed once a week for a long time reduce PWV and N/L ratio and increase PTX3 protein values independently from biochemical and hematologic parameters.

A possible limitation of the study is the relatively small number of participants in the study. The researchers do not have any information about their nutritional program, serum levels of interleukins, cytokines and adipokines and other inflammatory markers such as hsCRP, which could partially highlight the underlying mechanism.

CONCLUSION

In conclusion, long-term and moderate exercise performed at least once a week (trekking) increases the amount of the cardioprotective protein PTX3, lowers central and peripheric arterial stiffness, reduces the N/L ratio and the cardiovascular disease risk associated with advanced age.

RECOMMENDATIONS

This study has shown that trekking and mountaineering performed once a week had a positive effect on arterial stiffness in mid-aged and advanced-aged subjects. More evidence is needed from studies with a larger population and narrower age range to strengthen and reinforce the outcome of this study.

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