Original Research

The Impact of Physical Activity on Endothelial Function in Middle-Aged and Elderly Subjects: The Ikaria Study

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Introduction: Exercise training and physical activity (PA) have substantial vascular and cardiac health benefits. Ikaria Island has been recognised as having one of the highest longevity rates worldwide and a high percentage of healthy ageing. We examined the relationship between endothelial function and levels of habitual PA to evaluate the factors related to healthy ageing in this population.

Methods: The study was conducted on a subgroup population of the IKARIA study consisting of 185 middle-aged (40-65 years) and 142 elderly subjects (66-91 years). Endothelial function was evaluated by ultrasound measurement of flow-mediated dilatation (FMD). PA was evaluated using the shortened version of the self-reported International Physical Activity Questionnaire (IPAQ). Subjects in the low PA group (<500 MET/min/week) were considered as physically inactive and the rest as active.

Results: In the overall study population FMD was inversely associated with age (r=-0.24, p<0.001) and middle-aged subjects had higher FMD compared with the elderly (6.26 ± 3.31% vs. 5.21 ± 2.95%, p=0.003). Multiple linear regression analysis revealed that among middle-aged subjects the physically active had higher FMD compared with the physically inactive. Physically active subjects in the middle-aged group showed higher FMD compared with the physically active elderly (p=0.008). However, there was no difference in FMD values between middle-aged inactive subjects and the elderly physically active (p=NS).

Conclusion: The present study revealed that increased PA was associated with improved endothelial function in middle-aged subjects and that PA in elderly subjects can ameliorate the devastating effects of ageing on arterial wall properties.

Epidemiological and clinical evidence indicate that exercise training and physical activity (PA) have substantial vascular and cardiac health benefits and can reduce cardiac risk.[1-3] The benefits from PA have been mainly attributed to better control of blood pressure and glucose levels, as well as body weight.[2,4]

Endothelium is known to play a key role in vascular function.[5] In particular, endothelial function, which is usually quantified by flow-mediated dilatation (FMD) in the brachial artery, reflects the "health" of the vessel wall and has been associated with various clinical factors (e.g. high blood pressure) and with the risk of cardiovascular disease, while it is affected by lifestyle habits (e.g. smoking) and ageing.[6-9] Recently, it has been suggested that PA is associated with an improvement in endothelial function, arterial wall properties and healthy status, with a parallel reduction in systolic blood pressure, inflammatory process, and oxidative stress.[10-12]

The purpose of the present study was to evaluate the relationship between endothelial function and levels of habitual PA in the context of the Ikaria Study, after considering various demographic, lifestyle and clinical characteristics. The reason for selecting Ikaria Island was because this island's inhabitants have recently been recognised as having one of the highest longevity rates worldwide, as well as a high percentage of healthy ageing.[13,14] Specifically, although in Europe only the 0.1% of the population lives to over 90 years old, in Ikaria Island the percentage of these people is roughly 1%. As Ikaria's elderly population comprises an isolated rural group with established lifestyle conditions, it provides an ideal context for the study of the factors related to cardiovascular risk.[15]

Methods

Study sample

The IKARIA epidemiological study[14,16-20] was carried out in the province of Ikaria Island, in Greece. In brief, during 2009, a volunteer-based, multistage sampling method was used to enroll 631 men (65 ± 13 years) and 698 women (64 ± 13 years), from all areas of Ikaria Island. Individuals residing in assisted-living centers were not included in the survey. The participation rate was 95%. Of the aforementioned sample we randomly selected every fourth participant entering the study, in order to evaluate their endothelial function; thus, 155 men (64 ± 12 yrs) and 172 women (62 ± 13 yrs) were studied in this project. Participants between 40 and 65 years old (n=185, 53 ± 7 years) were considered as middle-aged, while those over 65 years old (n=142, 75 ± 6 years) were considered as elderly. All participants were interviewed by trained personnel (cardiologists, general practitioners and nurses) who used a standard questionnaire developed for the purposes of the study.

An *a posteriori* statistical power analysis revealed that a sample size of 65 subjects per group was adequate to evaluate differences in FMD values between physically active and inactive participants equal to 1% ± 2% at 0.05 type I error, achieving a statistical power >80%.

Bioethics

The study was approved by the Medical Research Ethics Committee of our institution and was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. All subjects were informed about the aims of the study, agreed to participate, and signed an informed consent.

Assessment of PA

PA was evaluated using the short version of the self-reported International Physical Activity Questionnaire (IPAQ), which has been validated for the Greek population.[21,22] Frequency (times per week), duration (minutes per time) and intensity of PA during sports, occupation and/or free-time activities were assessed. Participants who did not report any PA were defined as sedentary. In accordance with the standard IPAQ scoring procedures, physically active participants were classified into one of the following groups: upper tertile, "vigorous" PA (>2500 metabolic equivalent of task, MET/min/week), middle tertile, "moderate" PA (500-2500 MET/min/week), or lower tertile, "low" PA (<500 MET/min/week).

Anthropometric, clinical and biochemical characteristics

Weight and height were measured using standard procedures, and body mass index (BMI) scores were calculated as weight divided by height squared (kg/m^2). Resting arterial blood pressure was measured three times in the right arm, at the end of the physical examination with the subject in sitting position. Patients whose average blood pressure levels were ≥140/90 mmHg or were under anti-hypertensive medication were classified as hypertensive subjects. Fasting blood samples were collected from 08.00 to 10:00 hours. Blood lipid examinations (serum total cholesterol, high density lipoprotein cholesterol and triglycerides) were measured using a chromatographic enzymic method in an automatic analyzer (RA-1000). Low-density lipoprotein cholesterol was calculated using the Friedewald formula: total cholesterol - HDL cholesterol - 1/5 triglycerides. The intra- and inter-assay coefficients of variation did not exceed 3% for cholesterol levels, 4% for triglycerides, and 4% for HDL-cholesterol. Hypercholesterolaemia was defined as a total serum cholesterol level >200 mg/dl, or the use of lipid lowering agents. All biochemical evaluations were carried out in the same laboratory, which followed the criteria of the World Health Organization Reference Laboratories. Diabetes mellitus type 2 was determined by fasting plasma glucose tests and was analysed in accordance with the diagnostic criteria of the American Diabetes Association: fasting blood glucose levels >125 mg/dl (7 mmol/L), or the use of special medication, indicating the presence of diabetes.

Evaluation of vascular function

Endothelial function was evaluated by estimating the FMD in the brachial artery, using established procedures.[23-25] Briefly, all subjects were instructed to refrain from smoking at least six hours before the examination time, and after 10 minutes' rest the right brachial artery was scanned in longitudinal section, 5 cm above the antecubital fossa, using a Vivid e PRO ultrasound imager (General Electric, Milwaukee, Wisconsin, USA) equipped with a linear array (4-13 MHz) U/S transducer. A pneumatic cuff placed distal to the ultrasound probe was then inflated to supra-systolic pressure on the forearm for 5 minutes to induce reactive hyperaemia. After the release of the ischaemia cuff, brachial artery diameter was measured every 15 seconds for 2 minutes, and FMD was defined as the percentage change of vessel diameter from rest to 60 s after cuff release. The repeatability of the technique for determining FMD has been determined using the repeatability coefficient (British Standards Institution), which is given by the formula: $2 \times (\Sigma d_i^2 / n)$ (where n is the sample size and d_i is the difference between the consecutive measurements, the lower the better). According to this formula the coefficient was found to be equal to 5.0%, which suggests good repeatability.

Socio-demographic and lifestyle variables

The age and sex of the participants were recorded. As regards smoking habits, current smokers were defined as those who smoked at least one cigarette per day or had stopped smoking for less than a year, while non-current smokers were defined as those who had stopped smoking for at least one year or had never smoked.

Statistical analysis

All variables were tested for normal distribution using P-P plots. Normally distributed variables were expressed as mean ± SD. Student's t-test or analysis of variance (ANOVA) were used to compare mean values of normally distributed variables (i.e. age, BMI, FMD) between different categories of qualitative variables (i.e. gender, PA status). *Post hoc* analysis after Bonferroni correction was applied to test for pairwise differences between study subgroups. The chi-square test was used to evaluate dependency between categorical variables (i.e. diabetes mellitus, smoking status, and PA status). Correlations between normally distributed quantitative variables were tested with Pearson's r coefficient. Multiple linear regression analysis was used to test for the association between FMD and PA levels, after adjustment for several covariates known to affect endothelial function. P-values <0.05 were considered to indicate statistical significance, while p-values <0.1 were considered to indicate a statistical trend. Plots of standardised residuals against predicted values were used to evaluate models' assumption of homoscedacity, while the Durbin–Watson statistic was used to test for serial dependency. Collinearity between independent variables was tested using the VIF criterion. Cook's distances were used to check for influential points in the regression analyses. All statistical analyses were performed using SPSS software (version 18.0; SPSS Inc, Chicago, IL).

Results

Basic characteristics of the participants

The basic characteristics of the participants are shown in Table 1. In the middle-aged group, 24% of the participants were classified as physically inactive (i.e. low PA) and the remaining 76% were classified as physically active (i.e. moderate or vigorous PA); in the elderly group, 22% were classified as inactive and the remaining 78% were classified as active. No differences were observed in the distribution of PA levels between middle-aged and elderly subjects (p=0.31).

Vascular function and PA

In the overall study population FMD was inversely associated with age (r=-0.24, p<0.001). The mean FMD was 5.79 ± 3.19%, while middle-aged subjects had higher FMD compared with the elderly (6.26 ± 3.31% vs. 5.21 ± 2.95%, p=0.003). Moreover, in the whole study group, FMD was higher in physically active compared to physically inactive subjects (6.06 ± 3.23% vs. 4.95 ± 3.02%, p=0.008).

Age-specific analysis revealed that middle-aged physically active subjects had higher FMD compared to the elderly physically active participants (6.64 ±

Table 1. Characteristics of the study sample according to physical activity status.

	Low physical activity (<500 MET/min/week)	Moderate physical activity (500-2500 MET/min/week)	Vigorous physical activity (>2500 MET/min/week)	p
Subjects, (%)				
Middle-aged, n=185	24	59	17	
Elderly, n=142	22	66	12	
Age (years)				
Middle-aged, n=185	55 ± 6	53 ± 7	53 ± 6	0.12
Elderly, n=142	75 ± 6	75 ± 6	73 ± 5	0.35
Female (%)				
Middle-aged, n=185	64	56	36	0.02
Elderly, n=142	61	50	19	0.04
Body mass index (kg/m^2)				
Middle-aged, n=185	30.8 ± 5.2	28.6 ± 4.8	29.8 ± 4.7	0.04
Elderly, n=142	31.5 ± 5.2	29.1 ± 3.9*	27.7 ± 3.2*	0.006
Hypertension (%)				
Middle-aged, n=185	39	31	48	0.68
Elderly, n=142	86	80	71	0.54
Antihypertensive medication (%)				
Middle-aged, n=185	14	16	23	0.57
Elderly, n=142	65	47	38	0.14
Hypercholesterolaemia (%)				
Middle-aged, n=185	75	62	65	0.31
Elderly, n=142	65	78	62	0.18
Diabetes mellitus (%)				
Middle-aged, n=185	21	9	21	0.08
Elderly, n=142	21	25	19	0.82
Current smokers (%)				
Middle-aged, n=185	41	40	42	0.97
Elderly, n=142	10	17	25	0.38
Cardiovascular disease (%)				
Middle-aged, n=185	2	5	3	0.78
Elderly, n=142	35	19	19	0.19
FMD (%)				
Middle-aged, n=185	5.04 ± 3.32	6.83 ± 3.32*	5.99 ± 2.96	0.009
Elderly, n=142	4.81 ± 2.58	5.25 ± 2.87	5.86 ± 4.11	0.52

Data are presented as mean ± standard deviation or as percentage of subjects (%). *p<0.05 "moderate", "vigorous" vs. "low" physical activity group. FMD – flow-mediated dilatation.

3.24% vs. 5.34 ± 3.07%, p=0.008). There was no difference in FMD values between middle-aged physically inactive and elderly physically active subjects (5.04 ± 3.32% vs. 5.34 ± 3.07%, p=0.99). Furthermore, subjects classified as having low PA had a greater BMI in both study groups. No significant difference was observed between the three PA groups with respect to smoking habits, prevalence of hypertension, hypercholesterolaemia, diabetes mellitus or cardiovascular diseases, in both middle-aged and elderly subjects (Table 1).

However, residual confounding may exist; thus, multiple linear regression analysis, after adjusting for sex, BMI, smoking habits, systolic arterial pressure, antihypertensive treatment, the presence of diabetes mellitus, hypercholesterolaemia, and cardiovascular disease revealed that among middle-aged subjects those engaging in vigorous or moderate PA had higher FMD compared with subjects with a low level of PA (Table 2). However, this association was not significant among elderly participants (Table 2, Figure 1).

In addition to the previous analysis, further linear regression analysis (Table 3) showed that there was no difference in FMD values between middle-aged physically inactive and elderly active participants, whereas there was a significant difference in FMD values between middle-aged and elderly physically active subjects (Table 3).

As regards the other characteristics, hypertensive subjects had significantly lower FMD compared to normotensives (5.37 ± 3.05% vs. 6.37 ± 3.46, p=0.009), while normotensive patients who report-

Table 2. Results from multiple linear regression analysis that evaluated the association between flow-mediated dilation (dependent outcome), physical activity level (main effect), and various other covariates.

	Middle-aged (n=185)			Elderly (n=142)		
	b coefficient	95% confidence interval	p	b coefficient	95% confidence interval	p
Physical activity status						
Low (ref)	-	-			-	-
Moderate	2.12	0.75 to 3.48	0.002	-0.33	-1.88 to 1.22	0.67
High	1.76	0.039 to 3.49	0.045	1.246	-1.033 to 3.527	0.28
Sex	-1.11	-2.20 to -0.028	0.044	-0.408	-1.633 to 0.817	0.51
Age (per 1 year)	-0.12	-0.204 to -0.039	0.004	-0.049	-0.152 to 0.052	0.33
Body mass index (per 1 kg/m^2)	0.10	-0.009 to 0.217	0.07	-0.052	-0.200 to 0.095	0.48
Cardiovascular disease (Y/N)	-1.31	-4.51 to 1.88	0.41	-0.86	-2.274 to 0.553	0.23
Systolic blood pressure (per 1 mmHg)	0.008	-0.023 to 0.039	0.61	-0.017	-0.050 to 0.016	0.31
Antihypertensive treatment (Y/N)	0.095	-1.61 to 1.803	0.91	0.42	-0.737 to 1.744	0.42
Diabetes mellitus (Y/N)	-0.87	-2.53 to 0.79	0.30	0.68	-0.737 to 2.102	0.34
Hypercholesterolaemia (Y/N)	0.383	-0.732 to 1.499	0.49	0.037	-1.326 to 1.400	0.95
Smoking (Y/N)	0.114	-0.967 to 1.196	0.83	-0.091	-1.758 to 1.576	0.91

Y/N – yes/no. "No" was set as reference group in Y/N variables, and "female" was as the reference category for sex.

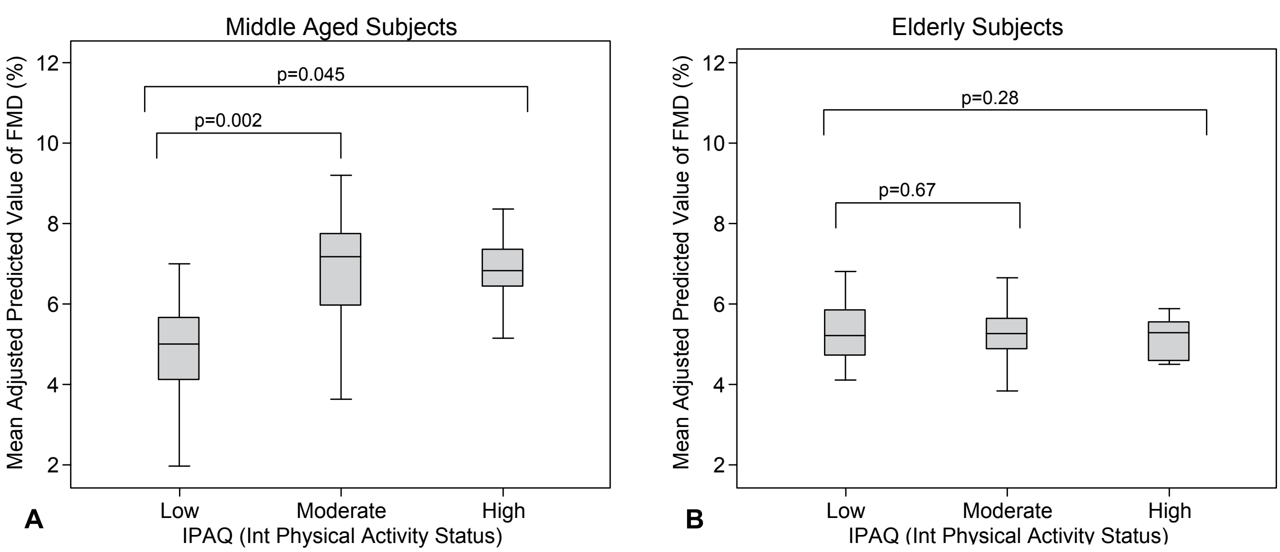

Figure 1. Box-plot of mean predicted values of flow-mediate dilation (FMD) according to the IPAQ physical activity status, after adjustment for age, sex, body mass index, smoking habits, systolic arterial pressure, antihypertensive treatment, and the presence of diabetes mellitus, hypercholesterolaemia and cardiovascular disease. A: middle-aged subjects; B: elderly subjects.

ed low PA levels had significantly lower FMD compared with subjects reporting moderate or vigorous PA (4.56 ± 2.88% vs. 6.99 ± 3.51% vs. 6.73 ± 3.38%, p=0.005). In addition, patients suffering from diabetes mellitus had marginally lower FMD compared with subjects with normal blood glucose levels (5.18 ± 2.86% vs. 5.96 ± 3.26%, p=0.09). Similarly, in subjects with normal blood glucose levels, those with low PA had significantly lower FMD compared with subjects engaging in moderate or vigorous PA (4.94 ± 3.11% vs. 6.25 ± 3.23% vs. 6.25 ± 3.49%, p=0.03).

Discussion

The present study revealed that habitual PA was associated with improved vascular function in middle-aged participants. These findings indicate the beneficial effects of PA on vascular endothelium, mainly in middle-aged subjects. More importantly, although FMD was inversely correlated with age, PA among the elderly seems to moderate the adverse effect of ageing on vascular function, since FMD values were similar in middle-aged physically inactive and elder-

Table 3. Results from multiple linear regression analysis of the association between flow-mediated dilation (dependent outcome), physical activity level in different agegroups (main effect), and various other covariates.

	b coefficient	Middle-aged 95% confidence interval	p
Middle-aged "inactive" vs. elderly "active" subjects	-0.87	-2.28 to 0.53	0.22
Middle-aged "active" vs. elderly "active" subjects	1.17	0.19 to 2.16	0.02
Elderly "inactive" vs. elderly "active" subjects	-0.09	-1.64 to 1.46	0.91
Sex (male vs. female)	-0.69	-1.49 to 0.10	0.08
Body mass index (per 1 kg/m^2)	0.03	-0.05 to 0.12	0.43
Cardiovascular disease (Y/N)	-0.99	-2.32 to 0.33	0.14
Systolic blood pressure (per 1 mmHg)	-0.006	-0.02 to 0.02	0.58
Antihypertensive treatment (Y/N)	0.058	-0.95 to 1.07	0.91
Diabetes mellitus (Y/N)	-0.24	1.31 to 0.82	0.64
Hypercholesterolaemia (Y/N)	0.05	-0.80 to 0.90	0.90
Smoking (Y/N)	0.45	-0.43 to 1.34	0.31

Y/N – yes/no. "No" was set as reference group in Y/N variables.

ly active subjects. The aforementioned findings are of importance for public health, since they support the hypothesis that PA may ameliorate the devastating effects of ageing, which compromise the restraining effect of endothelial function on the atherosclerotic process.

Impairment of arterial endothelial function is an important early step in the atherosclerotic process[26] and is associated with resistant hypertension.[27] PA has a beneficial effect in individuals with endothelial dysfunction, such as asymptomatic individuals with one or more risk factors for cardiovascular disease, and patients with coronary artery disease or heart failure.[28-30] Moreover, long-term endurance training counteracted the loss of endothelium-dependent vasodilatation associated with ageing.[31] Habitual PA enhances endothelial function in conduit arteries, skeletal muscle arterioles, and coronary arterioles in adults.[28] It has been suggested that the mechanism mediating the beneficial effects of PA on arterial endothelial function is an exercise-induced increase in blood flow, leading to augmented shear stress and cyclic stretch, further causing enhanced nitric oxide production.[32] Shear stress represents the frictional force that the flow of blood exerts at the endothelial surface of the vessel wall; it plays a central role in cell function and structure via managing several processes and contributes to the progress of atherosclerosis.[33] It is well established that exposure of endothelial cells to shear stress stimulates the production of nitric oxide (NO) from L-arginine by the action of nitric oxide synthase and regulates numerous actions of endothelium related to atherogenesis.[34-36] Shear stress induces a sharp yet transient elevation in intracellular calcium, enhancing calmodulin binding to endothelial nitric oxide synthase (eNOS), hence increasing eNOS activity.

The beneficial effect of PA was independent of changes in body weight or BMI and was attenuated substantially after training cessation.[37] Three months of moderate intensity cycling increased FMD in patients with metabolic syndrome who had no symptoms of cardiovascular disease.[38] Similarly, increased levels of PA during daily life are associated with improved endothelial function in patients with peripheral arterial disease.[39]

Data concerning the beneficial effect of PA on endothelial health are most consistent in subjects with impaired endothelial function.[40] The discrepancy in the results of studies of healthy adults may relate to differences in the intensity and amount of PA.[41] Furthermore, continuous training is needed to maintain the vascular benefits of PA.[37]

In this study elderly subjects showed no significant benefit from increased PA in terms of endothelial function. Elderly subjects also showed a great prevalence of other comorbidities known to impair endothelial function, such as hypertension, diabetes mellitus, hypercholesterolemia, and known cardiovascular disease. The additive unfavourable effect of increased age and of the aforementioned risk factors may have cancelled out any beneficial effect of habitual PA on endothelial function in the elderly group in our study. In favor of this hypothesis is also the fact that, although in our study PA was associated with better endothelial function in normotensive subjects and in subjects with normal blood glucose levels, there was no association between PA levels and endothelial function in subjects suffering from hypertension and diabetes mellitus.

The main finding of this study, that habitual PA was positively associated with FMD levels, is in accordance with previous studies that showed the beneficial effect of exercise on endothelial function in healthy subjects,[42] in subjects with metabolic syndrome,[43] and in patients with diabetes mellitus.[29] The present study revealed that endothelial function in elderly active subjects was similar to that in inactive middle-aged subjects, introducing the hypothesis that habitual exercise and training can modulate endothelial dysfunction caused by the impact of cardiovascular risk factors and ageing.

Further studies are needed to determine the exact mechanism by which specific types of PA, with varying quality and intensity, affect endothelial function. In addition, more research is required to identify the patients with cardiovascular risk factors who will gain the greatest benefit from specific types of PA, as well as the exact effect of PA in healthy subjects with normal endothelial function.

Conclusion

The present study revealed that in middle-aged subjects endothelial function is associated with the level of habitual PA, independently of the presence of cardiovascular disease risk factors known to affect arterial wall properties. These findings indicate the beneficial effects of PA and exercise training on vascular endothelium and suggest another cardioprotective effect of habitual exercise on vascular ageing and on atherosclerosis progression.

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