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Genetic and environmental factors on the relation of lung function and arterial stiffness

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Summary
Background: An association between reduced lung function and increased cardiovascular risk has been reported, but the underlying mechanisms are unknown. The aim of this study was to assess the heritability of lung function and to estimate its genetic association with arterial stiffness.
Methods: 150 monozygotic and 42 dizygotic healthy Hungarian and American Caucasian twin pairs (age 43 ± 17 years) underwent spirometry (forced vital capacity/FVC/, forced expiratory volume in 1 s/FEV_1/; MIR Minispir, USA); and their brachial and central augmentation indices (AIx), and aortic pulse wave velocity (PWV) were measured by oscillometric Arteriograph (TensioMed Ltd, Budapest, Hungary). Phenotypic correlations and bivariate Cholesky decomposition models were applied.
Results: Age-, sex-, country- and smoking-adjusted heritability of FEV_1, percent predicted FEV_1, FVC and percent predicted FVC were 73% (95% confidence interval /CI/: 45—85%), 28% (95% CI: 0—67%), 68% (95% CI: 20—81%) and 45% (95% CI: 0—66%), respectively. Measured and percent predicted FVC and FEV_1 values showed no significant phenotypic correlations with AIx or aortic PWV, except for phenotypic twin correlations between measured FEV_1, FVC with brachial or aortic augmentation indices which ranged between −0.12 and −0.17. No genetic covariance between lung function and arterial stiffness was found.
Conclusions: Lung function is heritable and the measured FVC and FEV are phenotypically, but not genetically, associated with augmentation index, a measure of wave reflection. This relationship may in turn reveal further associations leading to a better mechanistic understanding of vascular changes in various airway diseases.

Introduction

Heritability of lung function has been investigated in twin and family studies, with discordant results. In such studies, heritability of forced expiratory volume in 1 s (FEV_1) ranged between 10% and 77% and that of forced vital capacity (FVC) ranged between 26% and 91%. Environmental factors could explain a modest part of the variance in most of the studies.[1—5] Lung function also depends on gender.[6] Age differences also influence the variance of lung function.[3] A longitudinal study of twins found that the heritability of FEV_1 and FVC among never smoking female twins do not change remarkably (32% and 36% for FEV_1, 41% and 37% for FVC at baseline and at 3-year follow-up, respectively).[4] Genetic factors seem to modestly contribute to lung function variance as demonstrated in a longitudinal study.[7]

The relationship between impaired lung function and atherosclerosis or cardiovascular morbidity and mortality has been investigated by several authors.[8,9] Higher arterial stiffness is associated with poorer lung function when adjusted for age, sex, and ethnic group.[10] Elevated plasma levels of inflammatory markers may partially explain the increased cardiovascular risk among men with low FVC.[11] Obviously, smoking also contributes to the association between pulmonary function and coronary heart disease.[12] French epidemiological data showed that, even in healthy men, there is a relationship between pulmonary function and arterial stiffness.[13] To our knowledge, no study has ever investigated the genetic influence on the lung function-arterial stiffness relationship. In order to assess this association, twin study modeling is the most appropriate method. By comparing identical with non-identical twins, twin studies provide information on the relative contribution of genes and environment, and how the two interact.

The aim of our study was to assess the heritability of lung function, the phenotypic correlations between pulmonary function (FEV_1, FVC) and arterial stiffness measures, and furthermore, to determine whether there is a shared genetic relation between lung function and arterial stiffness.

Methods and materials

Subjects and study design

192 Hungarian and American healthy adult twin pairs (150 monozygotic [MZ] and 42 dizygotic [DZ]; age 43 ± 17 years (mean ± standard deviation) were recruited in this classic twin study. Exclusion criteria were chronic respiratory disease, pregnancy, ethnicity other than Caucasian, acute infection within three weeks of measurement or foreseeable lack of compliance with test procedures. Zygosity was assigned according to a seven-part self-reported response.[14] Studies were approved by local ethical committees (Institutional Review Board committee names and project approval numbers: Semmelweis University Regional and Institutional Committee of Science and Research Ethics, 29/2009; Twins Days Festival Ethical Board, 1/2009) and all study subjects gave informed consent prior to entering the study.

Anthropometric, respiratory and vascular measurements were obtained at two Hungarian twin festivals (Agfalva and Szigethalom), in two Hungarian hospitals and at the Twins Days Festival in the USA (Twinsburg, OH) in 2009 and 2010.

Anthropometric data

Body mass index (BMI) was measured by a clinically validated OMRON BF500 body consistency monitor (Omron Healthcare Ltd., Kyoto, Japan). Current height was verified simultaneously.

Pulmonary function measurement

Lung function was assessed by dynamic spirometry (Minispir Waukesha, WI, USA). The spirometer was calibrated daily using a 1-L syringe. The maneuvers were performed with the subject standing while wearing nose clips. The largest FEV_1 and the largest FVC from among all acceptable maneuvers were used in this analysis. FVC and FEV_1 measurements were performed in accordance with guidelines recommended by the American Thoracic Society.[15] Lung function variables were expressed in absolute (measured) values and as a percentage of predicted values (based on the subject's age, height, sex), using the reference values recommended by the ERS[16] and ECCS93 reference equation values.[17]

Arterial stiffness test

Aortic pulse wave velocity (PWV_{ao}) as a measure of arterial stiffness and brachial (AIx_{bra}) and aortic augmentation indices (AIx_{ao}) as measures of the arterial waveform were assessed by a clinically validated oscillometric device[18,19] (TensioMed Arteriograph, TensioMed Ltd., Hungary, software version 1.10.1.1.), calculating arterial stiffness parameters from oscillometrically recorded pressure waves of the brachial artery (pulse wave analysis), in supine position. PWV_{ao} is widely recognized as a direct marker of arterial stiffness[20,21] and inversely related to arterial distensibility; it is calculated by the formula: PWV_{ao} = distance (m)/transit time (sec). PWV_{ao} is correlated with cardiovascular risk factors[22] and it is a strong vascular risk factor for prediction of mortality in the elderly[23] and in the general population.[24] Dividing the augmentation pressure (difference between the second and first systolic peaks, P_2-P_1) by the pulse pressure gives the augmentation index, which is an indirect marker of endothelial dysfunction. The augmentation index is being used more and more frequently in studies as a parameter of peripheral vascular resistance and wave reflection.[25,26]

Study subjects were evaluated by the same researchers in order to decrease inter-observer variability and in accordance with guidelines recommended by the European Society of Cardiology.[27] The PWV is calculated from each pulse wave from the suprasystolic measurement, and the results are compared. The standard deviation of the PWV values is taken as a measure of good correlation, or similarity, between them. The software (TensioMed) accepts the measurements as good quality under SD = 1.0. Only the mean value of the best measurement (if automatic quality control showed less than 1.0 standard deviation for PWV_{ao}) was taken into consideration during the analysis. All subjects were restricted from smoking for 3 h, from eating for 1 h, and from drinking alcohol or coffee for 10 h prior to measurements. Adherence to these restrictions was ascertained by querying the subject.

Statistical analysis

Descriptive analysis (mean ± standard deviation for continuous variables, percentage for categorical variables) for BMI, FEV_1, FVC values, and vascular parameters was conducted using SPSS (SPSS 17.0 for Windows; SPSS, Chicago, IL). Differences between genders, zygosity and countries were calculated using independent-sample *t*-tests. *P* value <0.05 was considered significant. All analyzes were corrected for age, gender, country (significant effect of country, $p < 0.05$) and smoking (the phenotypic correlations were also calculated unadjusted to smoking). Smoking was adjusted according to two groups (never smokers and ex-smokers with a quitting period of at least one year and <5 pack year; and ex-smokers with >5 pack year and active smokers; 1 pack year is defined as smoking 1 pack of cigarettes daily for at least one year). Heritability estimates were determined based on the consideration that greater levels of MZ than DZ within pair similarity indicate a genetic influence on a phenotype, while similarity of co−twin correlations suggests that the variance is due to shared environmental factors. Three general sources of variance are calculated: (i) additive genetic factors or heritability (A) which represents the effects due to genes at multiple loci or multiple alleles at one locus; (ii) common environmental variance (C), which estimates the contribution of the common family environment of both twins (familiar socialization, diet, exposure to high levels of air pollution, parental smoking, shared womb, etc.); and (iii) nonshared environmental variance (E), which represents the effects that apply only to each individual twin and all sources of unique experiences and exposures that cause within-pair differences (e.g., discordance for smoking, differences in illnesses and occupational exposures). Structural equation modeling was performed using the

Mplus Version 6.1 (Muthén&Muthén) weighted least squares estimation due to the categorical variable of interest.[28] Empirical confidence intervals were calculated with a Bollen-Stine Bootstrap.[29] Univariate quantitative genetic modeling was performed to decompose the phenotypic variance of the considered parameters into heritability (A), shared (C), and unshared (E) environmental effects (ACE analysis).[30] Chi-square model fit *p*-values are presented where the desired results show insignificant model misfit. Instead of a covariance matrix, the estimation procedure used the raw data matrix. Given the small sample size, no component was fixed to 0 in the model.

A bivariate Cholesky decomposition was used to derive the magnitude of covariation between the investigated respiratory function and arterial stiffness phenotypes and to estimate what proportion of this correlation is attributable to common underlying genetic and environmental factors. In order to estimate the amount of overlap between genes or environment that influences the two parameters, genetic and environmental correlations between those phenotypes were calculated.

Results

Clinical characteristics and measures

Table 1 presents clinical characteristics of the sample by zygosity, sex and country. Opposite-sex twin pairs were excluded as their inclusion could bias the heritability estimates upward if gender-specific or X chromosome effects are present. A significant difference between males and females was observed concerning the arterial stiffness, BMI, measured FEV_1 and FVC ($p < 0.05$ for all). A significant difference in the rate of current and ex-smokers was detected across countries ($p < 0.001$ for both). Smoking years were higher in dizygotic twins than in monozygotic subjects ($p < 0.05$). Significant differences were observed in pulmonary function parameters concerning zygosity ($p < 0.05$) except for percent predicted FEV_1. Hungarian twins had significantly higher lung function values ($p < 0.001$ for FVC and $p < 0.05$ for FEV_1, respectively) compared to the Americans.

Heritability analysis of pulmonary function and arterial stiffness

Age-, sex-, country- and smoking-adjusted genetic and environmental variance, estimated with ACE analysis, and demonstrated 95% confidence intervals (CI), are shown in Table 2. Accordingly, genetic factors appear to contribute, at least in part, to the pulmonary function and arterial stiffness parameters, as the MZ twins had higher intrapair correlation compared to that of DZ twins. Models had a good fit.

Phenotypic twin correlations between pulmonary function and arterial stiffness

Table 3 presents phenotypic twin correlations, the correlations between phenotypes within the same individuals, for FEV_1, FVC and arterial stiffness considering age, family, sex and country as covariates with or without smoking adjustment. Phenotypic correlation ranged between −0.12 and −0.17 ($p < 0.05$) between measured pulmonary function values and both brachial and aortic augmentation indices, suggesting that better measured lung function corresponds to lower AIx values. Additionally, FVC and FEV_1 values showed no significant phenotypic correlations with AIx or aortic PWV. The estimated ACE model confirmed the role of genetic factors on lung function and arterial stiffness phenotypes; in addition, significant low phenotypic correlations were estimated between some pulmonary function and arterial stiffness measures, therefore, a possible genetic covariance of FEV_1, FVC and AIx can be estimated by the bivariate Cholesky decomposition model. Measured and percent predicted FVC and FEV_1 values showed no significant phenotypic correlation with aortic PWV; therefore, the influence of common genetic and environmental factors on those relationships were not investigated.

Genetic covariance of FEV_1, FVC, brachial and aortic augmentation indices

Standardized genetic, common and unique environmental components of the covariance were calculated in the investigated measures by the bivariate Cholesky decomposition model. Additive genetic components showed no significant influence for the covariance between lung function values and augmentation indices (therefore, data are not shown).

Discussion

To our knowledge, this is the first study that investigates the relative contribution of genetic and environmental factors to the relation between lung function and arterial stiffness in a relatively large twin sample. This study demonstrates that the heritability of lung function is high for observed absolute values and moderate for percent predicted values, and a significant negative low phenotypic correlation exists between measured FEV_1, FVC and augmentation indices. No significant phenotypic correlation was estimated between lung function parameters and aortic PWV. No genetic covariance was found between FEV_1, FVC and augmentation index.

Our findings concerning the heritability of lung function and vascular stiffness are in line with the literature.[1–3,30–37] In our population-based sample of twins, genetic effects accounted for 28–73% of the variability of lung function. Two twin studies showed that additive genetic effects on FEV_1 accounted for 61%, and 67% of the total variation; however, the mean age was higher with respect to our population.[1,3] Hubert and co-workers reported slightly higher heritability results than our data, but their study population contained only adult (42–56 years) male twins.[2] The genetic variance accounted for 50–58% for the brachial AIx, aortic AIx and PWV, similarly to other studies with reported heritabilities of 37–53%.[30–34]

Significant negative phenotypic correlation was found between FEV_1, FVC and AIx (but not with aortic PWV) which

Table 1 Clinical characteristics and measures according to zygosity, gender and country.

	Total	Zygosity		Gender		Country	
		Monozygotic	Dizygotic	Male	Female	Hungarian	American
Subjects, *n*	384	300	84	88	296	290	94
Monozygotic:dizygotic, *n*	300:84	N/A	N/A	70:18	230:66	212:78	88:6
Age, years	43 ± 17	42 ± 17[%]	48 ± 14[%]	43 ± 17	44 ± 17	43 ± 17	46 ± 17
FVC, % predicted	97.4 ± 16.0	96.1 ± 16.3[%]	100.6 ± 14.9[%]	99.3 ± 16.1	96.7 ± 16.0	100.0 ± 15.0[§]	89.1 ± 16.2[§]
FEV_1, % predicted	99.3 ± 15.5	98.8 ± 15.7	100.5 ± 15.0	99.1 ± 17.5	99.2 ± 14.8	100.3 ± 14.9[#]	95.9 ± 16.9[#]
FEV_1, l	3.0 ± 0.8	2.9 ± 0.8[%]	3.1 ± 0.9[%]	3.8 ± 0.8[$]	2.7 ± 0.6[$]	3.1 ± 0.8[§]	2.7 ± 0.8[§]
FVC, l	3.5 ± 1.0	3.4 ± 1.0[¥]	3.8 ± 1.1[¥]	4.6 ± 1.0[$]	3.2 ± 0.7[$]	3.7 ± 1.0[§]	3.0 ± 0.9[§]
Brachial AIx, %	−29.6 ± 32	−30.5 ± 33	−26.0 ± 29	−43.3 ± 26[†]	−25.6 ± 33[†]	−29.0 ± 32	−31.3 ± 30
Central AIx, %	22.5 ± 16	22.0 ± 16	24.2 ± 14	15.6 ± 13[†]	24.5 ± 16[†]	22.7 ± 16	21.8 ± 15
Aortic PWV, m/s	8.6 ± 2.5	8.4 ± 2.4	9.3 ± 2.4	8.1 ± 2.0[†]	8.8 ± 2.6[†]	8.7 ± 2.5	8.6 ± 2.4
BMI, kg/m^2	25.8 ± 5.5	25.6 ± 5.5	26.6 ± 5.4	27.0 ± 4.5[†]	25.6 ± 5.8[†]	25.4 ± 5.2[^]	27.4 ± 6.2[^]
Never smokers, %	70.3	71.4	66.3	67.4	71.1	70.5	69.5
Ex smokers, %	17.1	16.2	20.5	19.1	16.5	13.7[§]	27.4[§]
Current smokers, %	12.6	12.5	13.3	13.5	12.4	15.8[§]	3.2[§]
Smoking years	4.3 ± 9.7	3.5 ± 8.3[%]	7.3 ± 13.3[%]	5.1 ± 10.4	4.1 ± 9.4	4.3 ± 9.5	4.3 ± 10.2

Mean ± standard deviation. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; AIx, augmentation index; PWV, pulse wave velocity; BMI, body mass index.
[%]Monozygotic *vs* dizygotic $p < 0.05$.
[¥]Monozygotic *vs* dizygotic $p < 0.005$.
[†]Male *vs* female $p < 0.05$.
[$]Male *vs* female $p < 0.001$.
[^]Hungarian *vs* American $p = 0.052$.
[§] Hungarian *vs* American $p < 0.001$.
[#]Hungarian *vs* American $p < 0.05$.

Table 2 Age, gender, country and smoking adjusted genetic and environmental variance component parameter estimates and 95% confidence intervals of the best-fitting univariate ACE models with *p* value of model fit.

Measure	A	C	E	Model fit (*p* value)
FVC, % predicted	0.45 (0.00—0.66)	0.14 (0.00—0.56)	0.41 (0.31—0.52)	0.58
FEV_1, % predicted	0.28 (0.00—0.67)	0.31 (0.00—0.59)	0.41 (0.27—0.55)	0.49
FVC, l	0.68 (0.20—0.81)	0.08 (0.00—0.55)	0.24 (0.17—0.32)	0.49
FEV_1, l	0.73 (0.45—0.85)	0.00 (0.00—0.55)	0.26 (0.17—0.37)	0.26
Aortic AIx, %	0.58 (0.10—0.75)	0.10 (0.00—0.57)	0.32 (0.24—0.44)	0.12
Brachial AIx, %	0.55 (0.08—0.74)	0.13 (0.00—0.57)	0.33 (0.24—0.45)	0.17
Aortic PWV, m/s	0.50 (0.25—0.68)	0.00 (0.00—0.00)	0.50 (0.33—0.73)	0.0001

A, indicates heritability; C, shared environmental variance component; E, unique environmental variance component; Model fit, *p* value of Chi-square test of Model fit; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; AIx, augmentation index; PWV, pulse wave velocity.

indicates that impaired lung function is associated with increased peripheral vascular resistance and wave reflection, but not with aortic distensibility characterized by aortic PWV. The association of cardiovascular and respiratory system has been previously hypothesized because increased cardiovascular morbidity and mortality was observed in patients with impaired lung function.[8—10] However, novel hemodynamic measurements such as oscillometric arterial stiffness assessment, which is included in our study, had not been previously performed. Our study showed that there is a phenotypic but no genetic relationship between lung function and peripheral vascular resistance (wave reflection). AIx is accepted as measure of wave reflection[30] and it is closely correlated with the cardiovascular risk and an independent predictor of mortality in patients with end-stage renal disease.[26] Arterial stiffness was also correlated with the quality of life in older people.[35]

Although we report data collected from a healthy population, the relationship between arterial stiffness and respiratory diseases, such as chronic obstructive pulmonary disease (COPD) and bronchial asthma, might also be of clinical relevance and has in fact been investigated previously.[36—44] In particular, COPD and emphysema patients are at increased risk for cardiovascular morbidity and mortality, associated with endothelial dysfunction, arterial stiffness and atherogenesis, independently of tobacco smoke exposure.[36—40] The key elements causing an early subclinical cardiovascular involvement in COPD patients may include systemic and abnormal lung inflammation, hypoxia, oxidative stress, alterations in levels of matrix metalloproteinases and the functionality of endothelial nitric oxide synthase.[41,42] Emphysema severity is associated with arterial stiffness in COPD patients, which may be attributable to similar pathophysiological processes within the lung and arteries.[43] Significant correlations between arterial stiffness and FEV_1 were reported in asthmatic patients, suggesting the presence of a common systemic process, most likely an inflammatory pathway, involving both the cardiovascular and respiratory systems.[44]

Based on our results, the same non-genetic factors may play a role in lung function and endothelial function in healthy individuals.[11—13] Due to the possible non-genetic (although weak) link between impaired lung function and vascular resistance, as a possible clinical consequence of our study, early screening of augmentation index in patients with reduced lung function is warranted. Similarly, a recent prospective study reported that lung function assessment in mid-life may identify individuals at greater risk of future cardiovascular disease.[45] Augmentation index could be non-invasively assessed at an early age in healthy subjects with impaired lung function who are still free of respiratory illnesses, and conversely, pulmonary function could be assessed in subjects with increased augmentation

Table 3 Bivariate family, age, sex, population corrected phenotypic correlations and 95% confidence intervals with or without smoking adjustment from a bivariate structural equation saturated model of a genetic covariance decomposition model between lung function and arterial stiffness parameters.

		Brachial AIx, %	Central AIx, %	Aortic PWV, m/s
Non smoking adjusted	FVC, % predicted	0.038 (−0.079, 0.156)	0.037 (−0.081, 0.155)	−0.030 (−0.145, 0.085)
	FEV_1, % predicted	0.011 (−0.107, 0.129)	0.009 (−0.109, 0.128)	−0.055 (−0.172, 0.061)
	FVC	−0.147* (−0.267, −0.027)	−0.150* (−0.271, −0.030)	0.000 (−0.120, 0.119)
	FEV_1	−0.162* (−0.281, −0.043)	−0.166* (−0.285, −0.046)	−0.007 (−0.128, 0.115)
Smoking adjusted	FVC, % predicted	0.063 (−0.055, 0.181)	0.062 (−0.056, 0.180)	−0.017 (−0.134, 0.099)
	FEV_1, % predicted	0.040 (−0.078, 0.159)	0.039 (−0.080, 0.157)	−0.041 (−0.160, 0.077)
	FVC	−0.121 (−0.243, 0.001)	−0.123 (−0.246, −0.001)	0.012 (−0.108, 0.133)
	FEV_1	−0.133* (−0.254, −0.012)	−0.126* (−0.246, −0.006)	0.009 (−0.114, 0.131)

AIx, augmentation index; PWV, pulse wave velocity; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s.
*$p < 0.05$.

indices or atherosclerotic phenotypes if further studies confirm our results. To our knowledge, no previous study has investigated the relationship between PWV and lung function in healthy subjects; nonetheless, other authors reported higher PWV in the case of impaired lung function in chronic respiratory diseases.[37,39,43,44]

Our cohort consisted of healthy middle-aged individuals who may present endothelial dysfunction (increased AIx) but normal aortic PWV, which is mainly accelerated in advanced atherosclerosis in older age.[46] Of note, the mean aortic PWV value was not elevated in our sample. A correlation between impaired pulmonary function and elevated AIx but not with aortic PWV could be explained if our study population had subclinical atherosclerosis with increased Aix, which was not significant enough to result in increased aortic PWV. We suppose that this argument may serve as a possible interpretation for the discrepancy between the AIx and PWV findings. In addition, it must be noted that the statistical test (genetic covariance composition) traditionally calls for a large sampling in comparison to our sample size. This could be the reason why our results concerning the covariance analysis are not statistically significant.

The strength of our study is underpinned by the evaluation of all the lung function and arterial stiffness tests that were performed by the same trained researchers, with the same devices, on the same day. However, as in every human clinical study, there are unavoidable limitations. Data were pooled across countries to raise the power to identify genetic and environmental effects but no evidence of heterogeneity between states was detected.

In conclusion, our study was the first international twin study to investigate the possible genetic relationship between lung function and arterial stiffness. Our data suggest a phenotypic but not genetic link between lung function and peripheral vascular resistance (wave reflection). This relationship may explain the importance of inflammation, oxidative stress and hypoxia in the pathogenesis of atherogenesis. Improved understanding of the factors associated with increased cardiovascular risk in the setting of lung function impairment is needed. Our study could be a first step to find further associations of vascular changes in different airway diseases and help guide linkage studies toward better understanding of the cardiopulmonary system. Improved understanding of the factors associated with increased cardiovascular risk in the setting of lung function impairment is needed.

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Other contributions

The paper was presented at 20th Annual meeting of European Respiratory Society in Barcelona in September, 2010 and chosen to be in the 'top 4' media news of the congress. Commenting on the results, ERS president Nikolaos Siafakas, MD, said "this is an important study that shows a link between lung diseases and the whole body." (Twin Study Suggests Common Mechanism Driving Lung Function and Arterial Stiffness, http://www.medscape.com/viewarticle/729168).

Author contributions

Dr Tarnoki DL: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted.

Dr Tarnoki AD: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted.

Dr Lazar: the conception and design of the study, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted.

Mrs Medda: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted.

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Prof Horvath: the conception and design of the study, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted.

Conflict of interest

D. Tarnoki, A. Tarnoki and A. A. Molnar received travel reimbursements in order to perform the American measurements (Medexpert Ltd.). D. Tarnoki and A. Tarnoki received financial support for the equipment purchase by Balassi Institute - Hungarian Scholarship Board Office and Hungarian Scientific Research Fund [OTKA 68808]. Agnes Lannert is also an employee of Medexpert Ltd. Hungary, who provided the Arteriograph device (arterial stiffness and function measurement) for Drs. Tárnoki. Mrs. Lannert educated the investigators on the use of the device and facilitated meaurements as an application specialist. She also participated in the data analysis and manuscript preparation. Other authors declare no conflict of interest.

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