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The plasma concentration of advanced oxidation protein products and arterial stiffness in apparently healthy adults

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Abstract

Background: Oxidative stress plays an important role in the pathogenesis of atherosclerosis. Advanced oxidation protein products (AOPP) are markers of oxidative stress and mediators of inflammation. Increased arterial stiffness is associated with increased risk of cardiovascular mortality and morbidity. The aim of this study was to evaluate the relationship between an indirect marker of arterial stiffness and the AOPP level in apparently healthy individuals.

Methods and results: Arterial stiffness was estimated with the use of the stiffness index (SI_{DVP}) which significantly correlated with age, mean blood pressure, body fat content and AOPP. The SI_{DVP} was associated with AOPP concentration in both single ($R = 0.22$, $p = 0.03$) and multiple regression models adjusted for age, sex, mean blood pressure and body fat content ($R^2 = 42\%$, $p < 0.0001$).

Conclusions: The AOPP concentration is elevated in healthy people with increased values of stiffness index. This finding supports the concept that oxidative stress may contribute to arterial stiffening in humans.

Keywords: *Advanced oxidation protein products, oxidative stress, plasma concentration, arterial stiffness*

Introduction

Vascular stiffening is a hallmark of aging [1]. Increased arterial stiffness may also be a consequence of various pathological processes associated with diabetes, hypertension, metabolic syndrome or chronic renal disease [2–5]. It was recently suggested that an inflammatory process may be involved in large artery stiffening [6]. Arterial stiffness is also an independent marker for increased risk of cardiovascular complications in a number of diseases as well as in apparently healthy subjects [7,8]. The stiffness index (SI_{DVP}) is an indirect and non-invasive measure of arterial stiffness obtained with digital volume pulse (DVP) waveform recorded by photoplethysmography [9–12].

Oxidative stress plays an important role in the pathogenesis of cardiovascular complications associated with aging and in diabetes or hypertension [13–15]. Various markers are thought to be associated with inflammation and/or oxidative stress in patients suffering from cardiovascular complications as well as in apparently healthy subjects [16,17]. C-reactive protein is an independent predictor of outcome in survivors of myocardial infarction as well as healthy individuals [18,19]. Moreover, Yasmin et al. [6] recently demonstrated that C-reactive protein is independently associated with arterial stiffness in healthy subjects. However, so far only circumstantial evidences indicate that oxidative stress contributes to the process of arterial stiffening. Advanced oxidation

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protein products (AOPP) are markers of oxidative stress—mediated protein damage and possess some proinflammatory properties [20,21]. The AOPP plasma concentration is increased in patients with renal failure, diabetes, coronary artery disease and in subjects with chronic rheumatic valvular disease [20,22–24]. We hypothesized that AOPP levels could be correlated with SI_{DVP} in apparently healthy individuals.

Material and methods

Subjects

A total of 94 healthy volunteers was studied. The subjects who declared themselves as being healthy were recruited through a local advertisement and from the staff of our department. Careful history taking and physical examination revealed no abnormalities, their resting ECG was completely normal. None of the study subjects was taking any medication. We did not exclude patients with blood pressure $>$ 140/90 on a single measurement from the study. Total cholesterol (Synchron, CX, USA) and blood glucose (Ebiobasic Eppendorf, Germany) were determined in the subset of 58 subjects. The University Ethics Committee, approved the study protocol, and informed consent was obtained from all the participants.

Study protocol

Anthropometric measurements were made according to standard guidelines. Brachial blood pressure was obtained in the supine position, after 10-min rest. Brachial blood pressure was obtained by an oscillometric method (M-5, Omron Healthcare Co., Ltd, Kyoto, Japan).

Determination of the stiffness index by digital volume pulse analysis (SI_{DVP})

The DVP waveforms were recorded in the morning, after fasting for $\geq$8 h, at rest with the use of a photoplethysmograph (Pulse Trace 2000, MicroMedical, UK). Ten consecutive cardiac cycles were analyzed and then automatically averaged. The stiffness index of the DVP (SI_{DVP}) was obtained from the subject's body height (h) divided by the time between the systolic and diastolic peaks of the DVP (Figure 1). The SI_{DVP} is an estimate of pulse wave velocity (PWV) in large arteries and is regarded as a measure of large artery stiffness [9–12]. The within-subject coefficient of variation for estimation of SI_{DVP} was $<$5%.

AOPP assay. Determination of AOPP was by spectrophotometric detection according to

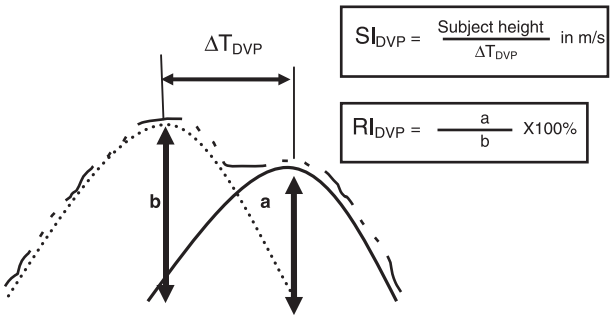

Figure 1. The SI_{DVP} is obtained from subject height divided by the time between the systolic and diastolic peaks of the DVP. The reflection index (RI_{DVP}) is determined as the height of the peak (a) component of the DVP expressed as a percentage of the systolic peak (b).

Witko-Sarsat et al. [20] as modified by Kalousova et al. [25]. Briefly, 200 µl of blood serum diluted 1:5 with 0.1 mol/l, pH 7.4, phosphate-buffered saline (PBS) was placed in a microtiter well, and 20 µl of acetic acid (14 mol/l) and 10 µl of 1.16 mol/l potassium iodide were added. Control wells contained PBS. Chloramine-T solution (0–100 µmol/l) was used for calibration. Absorbance was measured at 340 nm (spectrophotometer Multiscan LS, Labsystems) and the AOPP concentration is expressed in chloramines-T units (µmol/l).

Body fat amount

We used a bio-impedance analyser (Bodystat 1500, Bodystat Ltd, UK) to assess the fat content as a proportion of total body mass. Bio-impedance analysis was performed with a single frequency (50 kHz) device.

Statistical analysis

The results of continuous variables are expressed as mean values ± SD. Pearson correlation coefficients between the SI_{DVP} and the other continuous variables were calculated. The association of SI_{DVP} with other clinical variables was examined with the use of multivariable regression. R^2 was calculated in order to find how well each fitted model is useful in predicting the dependent variables. Statistical analyses were performed using StatSoft, Inc. (2005) STATISTICA (data analysis software system), version 7.1. www.statsoft.com, significance was set at $p < 0.05$.

Results

Table I presents the characteristics of the participants (65 women, 29 men). We have also performed a random evaluation of glucose level in 40 subjects (88 ± 14 mg/dl) and total cholesterol level in 58 subjects (195 ± 32 mg/dl). Neither glucose nor

Table I. Clinical characteristics of the study participants.

Characteristic	All subjects		Men		Women		
	Mean	SD	Mean	SD	Mean	SD	*p*
Age (years)	47	15	51	16	46	15	NS
M/F	29/65						
BMI (kg/m^2)	25	4	26	4	24	3	<0.05
Fat (%)	30	9	34	9	28	8	<0.05
Systolic blood pressure (brachial), (mm Hg)	121	15	123	15	120	16	NS
Diastolic blood pressure (brachial), (mm Hg)	72	9	73	9	72	9	NS
Mean blood pressure (mm Hg)	89	10	90	10	88	10	NS
Stiffness index (SI_{DVP}), (m/s)	8.5	2.0	8.4	2	8.5	2	NS
AOPP (μmol/l)	48	14	47	12	48	14	NS

M–male; F–female; BMI–body mass index; AOPP–advanced oxidation protein products; *p*–significance of differences between men and women; NS–non-significant difference.

cholesterol level correlated with SI_{DVP} (data not shown).

Relationships of SI_{DVB} with age, mean blood pressure, relative body fat content and AOPP

In the univariate analysis SI_{DVP} correlated significantly with age ($r = 0.60$, $p < 0.0001$ Figure 2A), mean blood pressure ($r = 0.29$, $p = 0.004$, Figure 2B), relative body fat content ($r = 0.44$, $p < 0.0001$, Figure 2C) and AOPP ($r = 0.22$, $p = 0.03$, Figure 2D).

Multiple regression analysis demonstrated that SI_{DVP} is independently correlated with age and AOPP (Table II). This model explaines the 42% variability in SI_{DVP}.

Since menopause may contribute to increased arterial stiffness we additionally evaluated a second model of multiple regression. The evaluated population consisted only of women and was subdivided into those below age of 55 years ($n = 45$, mean age 38 ± 11 years) and above 55 years of age ($n = 20$, mean age 63 ± 7 years). Multiple regression analysis demonstrated that SI_{DVP} is independently correlated

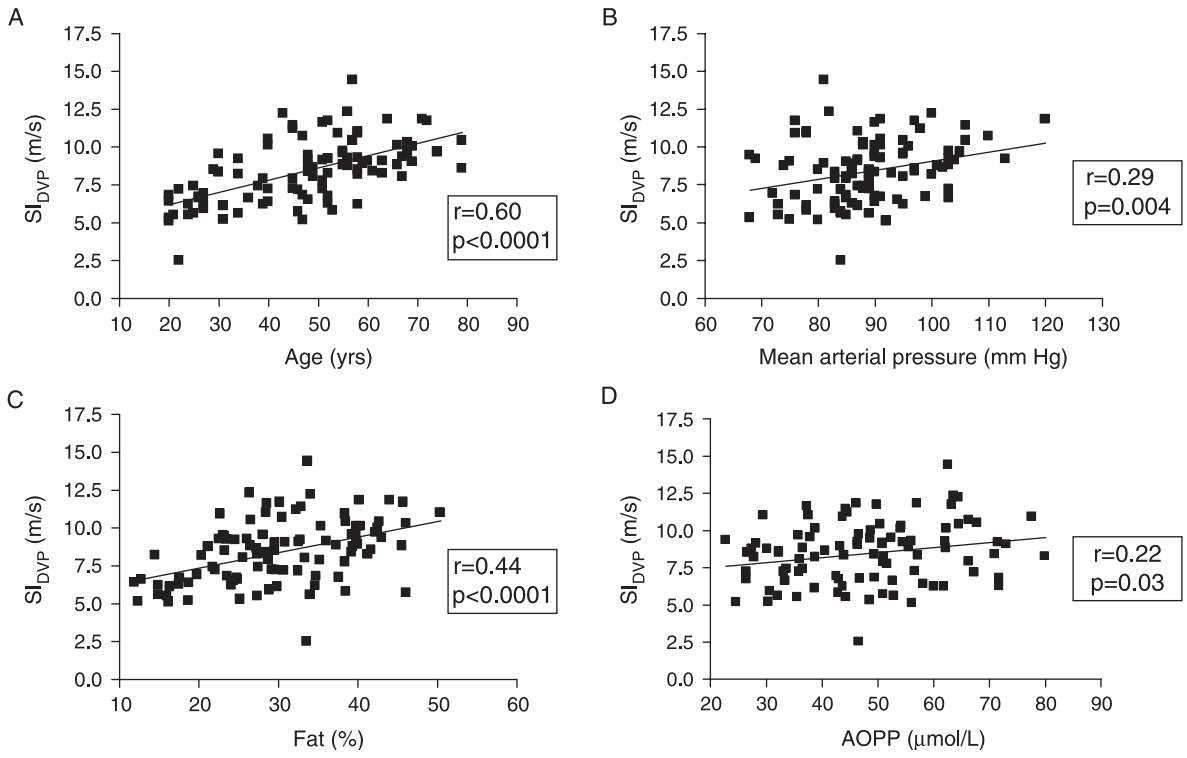

Figure 2. (A) Correlation between age and stiffness index. (B) Correlation between mean blood pressure and stiffness index. (C) Correlation between relative body fat amount and stiffness index. (D) Correlation between advanced oxidation protein products and stiffness index.

Table II. Standardized coefficients for a multiple linear regression of SI_{DVP} on age, sex, relative fat content, mean arterial pressure and advanced oxidation protein products.

Variable	Standardized coefficient	Significance level (p-value)
Constant		0.43
Age	0.46	$p < 0.0001$
Sex	−0.15	0.09
Fat (%)	0.17	0.12
Mean pressure (mm Hg)	0.11	0.18
AOPP(μmol/l)	0.16	0.04

$R^2 = 42\%$, $p < 0.0001$, SI_{DVP}–stiffness index; AOPP–advanced oxidation protein products.

with age and AOPP irrespective of menopause status. This model explaines the 44% variability in SI_{DVP} (Table III).

Discussion

The main finding of this study is that the AOPP, an index of oxidative stress and inflammation, is significantly associated with the SI_{DVP}, i.e. a marker of general arterial stiffness, in healthy people. The relationship between AOPP and SI is independent of age, mean blood pressure and body fat content. These results further support the hypothesis that inflammation and oxidative stress play an important role in arterial stiffening.

Aging is associated with stiffening of arterial vessels and premature arterial stiffness is regarded as an important risk factor for cardiovascular diseases [8]. Arterial stiffening is a complex process that involves both cellular and structural components of the arterial wall. The changes in arterial wall composition and function are age-dependent but are often accelerated by pathological processes associated with diabetes, hypertension, renal failure or obesity [2,5,26,27]. As the cardiovascular complications are associated with inflammation it is likely that some inflammatory mediators may play a role in arterial stiffening, at least in the mentioned above conditions. Moreover, it was recently demonstrated that even in apparently healthy subjects C-reactive protein, a marker and mediator of inflammation, is independently associated with PWV, a measure of arterial stiffness [6]. This association was significant, even after controlling for age and mean arterial pressure, which are the major determinants of PWV. Further support for the concept of inflammation being involved in the process of arterial stiffening comes from the study of Roman et al. [28] which demonstrated that arterial stiffness is increased in chronic inflammatory disorders independent of the presence of atherosclerosis and is related to, among other factors, C-reactive protein and IL-6 concentration. AOPP are elective markers of oxidative stress [20]. A study by Witko-Sarsat et al. [21], showed that AOPP concentration in plasma was closely related to neopterin, a selective monocyte activation marker. Moreover AOPP levels are closely related to the basal production of reactive oxygen species by circulating neutrophils (PMN). AOPP are also capable, *in vitro*, of stimulations an oxidative burst of monocyte and PMN [29]. We have recently shown that the superoxide anion production by activated PMN correlated with the augmentation index, a surrogate measure of arterial stiffness [30]. In our present study AOPP were significantly correlated with SI_{DVP}, independently of age, sex, menopause and mean blood pressure, major confounding factors of a measure of arterial stiffness. SI_{DVP} is closely related to PWV [10] and only recently was demonstrated to be independently associated with target organ damage in untreated hypertension [31]. Therefore the association of AOPP with SI_{DVP} seems to suggest that oxidative stress, inflammation and arterial stiffness are, to some extent, related. Moreover, Drueke et al. [32], recently showed that AOPP in patients with end-stage renal disease are significantly associated with common carotid artery intima-media thickening and an increased carotid wall to lumen ratio. Previous findings showing that AOPP concentration may depend, at least partly, on phagocyte activation suggest that a sustained cellular-associated, inflammatory process may lead to oxidative stress-mediated vascular remodeling [33].

Table III. Standardized coefficients for a multiple linear regression of SI_{DVP} on age, relative fat content, mean arterial pressure, advanced oxidation protein products and menopause status.

Variable	Standardized coefficient	Significance level (p-value)
Constant		0.49
Age	0.38	0.04
Menopause	0.04	0.81
Fat (%)	0.19	0.11
Mean pressure (mm Hg)	0.09	0.39
AOPP(μmol/l)	0.29	0.005

$R^2 = 44\%$, $p < 0.0001$, SI_{DVP}–stiffness index; AOPP–advanced oxidation protein products.

In summary, the AOPP concentration is independently correlated with arterial stiffness in healthy subjects. It is tempting, therefore, to speculate that oxidative stress may be involved in the process of arterial stiffening. It is also impossible to exclude the opposite option that arterial stiffening leading to enhanced shear stress of arterial walls may enhanced oxidative stress.

Acknowledgements

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