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Predictive markers of abdominal aortic stiffness measured by echo-tracking in subjects with varying insulin sensitivity

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Abstract

Arterial stiffness is influenced by advancing age and vascular disease and is an independent risk factor for cardiovascular events and death. Using ultrasound measurements, arterial stiffness in a specific arterial segment can be assessed. The aim of this observational study was to explore the prospective and cross-sectional associations between arterial stiffness measured by ultrasound locally in the abdominal aorta and cardiovascular risk factors/markers including insulin resistance measured by the homeostatic model assessment - insulin resistance (HOMA-IR), lipids and abdominal obesity.

This study includes 335 subjects from Malmö, Sweden, examined in 1991-1994 and again at follow-up in 1998-2000 (mean age 64 years, 42% men). Ultrasound measurement of the abdominal aorta was performed at follow-up investigation.

In the female subgroup, there was a positive association between HOMA-IR at baseline and abdominal aortic stiffness at follow-up (beta=0.18, p=0.03) and a negative association between high density lipoprotein and aortic stiffness (beta=-0.23, p=0.005), independently of classical cardiovascular risk factors. These associations were not found among men. The results suggest a greater or different role of impaired glucose metabolism in the pathophysiology of arterial stiffness in women than in men.

Key Words: Abdominal aorta; Blood Pressure; Epidemiology; Insulin Resistance; Ultrasonography; Vascular Stiffness

Introduction

Stiffness of the large elastic arteries is a major risk factor for incident cardiovascular events and death [1]. With ageing, the walls of the elastic arteries stiffen and the important volume buffering function and pressure absorbing function is diminished [2]. This results in an increase in systolic and a decrease in diastolic blood pressure, thereby amplifying the pulse pressure (PP).

However, the arterial elastic properties are not anatomically homogenous and decrease when moving peripherally along the arterial tree [3]. The carotid-femoral Pulse Wave Velocity (c-f PWV) is the gold standard for measuring regional arterial stiffness estimating the average arterial stiffness of the carotid-femoral pathway [4]. Arterial stiffness can also be measured locally using echo-tracking ultrasonography which provides information of the arterial distensibility from a specific arterial area [3].

It is well known that diabetes is associated with arteriosclerosis and arterial stiffness [5]. Markers of an impaired glucose metabolism are linked to an increased arterial stiffness measured by c-f PWV [6] as well as brachial PP progression [7].

Aortic distensibility is also affected by atherosclerosis [8]. Therefore, the level of risk factors such as low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides, through their roles in atherogenesis, could also affect aortic stiffness.

The aim of this cohort study was to investigate the predictive value of baseline insulin sensitivity, lipid levels and other selected cardiovascular risk factors for local abdominal aortic stiffness as measured by echo-tracking ultrasonography in a population with varying degree of insulin resistance.

Materials and methods

The study population is part of the larger population-based Malmö Diet and Cancer (MDC) cohort consisting of middle-aged male and female subjects from the city of Malmö, Sweden [9, 10]. From this cohort, in all 6103 subjects took part in the Cardiovascular Arm of the MDC cohort (MDC-CV) investigated during 1991 to 1994, constituting the baseline examination in this study [11]. Follow-up took place in 1999-2000 when a total of 909 subjects from MDC-CV were reinvestigated for risk factors associated with insulin resistance. These subjects were selected according to degree of insulin sensitivity as estimated by the homeostatic model assessment (HOMA) levels [12], so that 15% were sampled from each of the lowest two quartiles of the HOMA distribution, 30% from the third quartile, and 40% were sampled from the subjects with baseline HOMA in the fourth quartile [13]. In total, 349 randomly selected subjects of the 909 subjects in this so called HOMA cohort also underwent an ultrasonographic investigation of the abdominal aorta. From the 349 individuals examined with ultrasonography, complete data were available on 335 subjects (mean age 64 years, 42 percent men). A written informed consent was collected from all participants and the study was approved by the ethical committee at the Lund University, Lund, Sweden.

Ultrasonographic measurements were performed using a phase-locked echo-tracking system (Diamove®, Teltec AB, Lund, Sweden) with a spatial resolution of less than 10 μm [14, 15]. The time resolution was 1.15 ms and the smallest detectable movement was 8 μm [14]. Briefly, the echo-tracking instrument consist of a 3.5 MHz linear array transducer interfaced with a real time ultrasound scanner (EUB 240; Hitachi, Tokyo, Japan)[15]. Two electronic markers automatically identify the posterior and anterior arterial wall respectively and follow its pulsatile movements. The real time interface indicates to the operator at which level the registration is performed. Through this procedure, maximum and minimum diameters of the abdominal aorta, 3-4 cm proximal to the aortic bifurcation, were assessed three times in each

subject. The brachial systolic and diastolic blood pressures (mmHg) were measured directly prior to the ultrasound investigation, with the subject in supine position, using a manual sphygmanometer. A mean of three readings was recorded.

Aortic stiffness index, β was calculated according to the formula:

$$Stiffness(\beta) = \ln\left(\frac{Ps}{Pd}\right) \cdot \frac{Dd}{Ds - Dd}$$

where ln is the natural logarithm, Ps is systolic and Pd is diastolic blood pressures in mmHg, Dd is diastolic aortic diameter and Ds is systolic aortic diameter [16]. Results are based on mean β stiffness index from three assessments.

At both baseline and follow-up, examinations included a physical examination and sampling of blood after an overnight fast. HOMA-insulin resistance (HOMA-IR) was calculated as described by Matthews *et al* [12], by use of the formula: (fasting insulin x fasting glucose)/22.5 where insulin is expressed as mIU/liter and glucose as mmol/liter. Smoking was defined as current smoking. Information about smoking and ongoing anti-hypertensive drug treatment was retrieved from a questionnaire.

Statistical analysis

Statistical calculations were carried out using IBM SPSS Statistics, version 21. Outliers were defined as >1.5 standard deviations (SD) from the median, extreme outliers as > 3 SD from the median. β stiffness index were compared between men and women using a two-sample t-test. Correlations between β stiffness index, waist circumference, HOMA-IR, triglycerides, LDL cholesterol and HDL cholesterol respectively were calculated in crude models using Spearman's univariate correlation analysis. β stiffness index, HOMA-IR, triglycerides and HDL cholesterol data were log-transformed before entered in multiple regression analysis, to achieve normal distributions. The determinants of β stiffness index reaching statistical

significance in univariate analysis was then analyzed in a multiple regression model adjusting for age, sex, smoking and ongoing anti-hypertensive drug treatment at ultrasound investigation. These analyses were also carried out for men and women separately. A p-value less than 0.05 was considered significant.

Results

The mean follow-up time was 6.7 years (±SD 0.7). The characteristics of the study population at baseline and follow-up are presented in Table 1. Of the 335 included subjects, five subjects were excluded from further analysis, including two subjects with β stiffness index > 60 (extreme outliers) and three subjects with PP at follow-up < 20 mmHg (outliers and suspected incorrect measurements).

The β stiffness index values showed a positively skewed distribution and was significantly higher in men than in women (mean 17.0 vs. 12.9, $p<0.001$).

Results from crude analysis using Spearman's univariate correlation are presented in Table 2. These analyses showed a statistically significant, positive association between aortic stiffness and both baseline and follow-up waist circumference (baseline: $r=0.35$, $p<0.001$, follow-up:0.32, $p<0.001$) as well as triglycerides (baseline: $r=0.15$, $p=0.005$, follow-up: $r=0.15$, $p=0.006$). HDL cholesterol at baseline and follow-up was inversely associated with aortic stiffness (baseline: $r=-0.28$, $p<0.001$, follow-up: $r=-0.26$, $p<0.001$). HOMA-IR was positively associated with aortic stiffness according to both baseline and follow-up measurements (baseline: $r=0.19$, $p=0.001$, follow-up: $r=0.16$, $p=0.004$), but for HOMA-IR none of these results were statistically significant in the male subgroup. Figure 1a and 1b show the mean β-coefficient in different quartiles of follow-up HOMA-IR for women and men respectively. There were no associations between LDL cholesterol and aortic stiffness in either sex.

Results from multiple regression analysis are presented in Table 3. In all subjects, there was a statistical significant association between age (baseline: beta=0.32, p<0.0001), sex (baseline: beta=-0.18, p=0.005) baseline and follow-up HDL cholesterol (baseline: beta=-0.16, p=0.01, follow-up: beta=-0.19, p=0.002) with abdominal aortic stiffness. In females, aortic stiffness was negatively associated with both baseline and follow-up HDL cholesterol (baseline: beta=-0.23, p=0.005, follow-up: beta=-0.22, p=0.004). Aortic stiffness in the female subgroup was positively associated with HOMA-IR at baseline (beta=0.18, p=0.03), but not at follow-up. In the male subgroup aortic stiffness was associated with waist circumference (baseline: beta=0.23, p=0.04, follow-up: beta=0.21, p=0.04) but not with HOMA-IR or HDL cholesterol.

Discussion

The results from this observational study indicate that age and male sex are predictors of local abdominal aortic stiffness. Our study shows a relatively strong (beta=0.18), positive relationship between (HOMA-IR) and follow-up aortic stiffness among women, but not among men. Only a few studies have previously investigated the abdominal aorta distensibility using echo-tracking techniques, reporting for example that the aorta is more prone to stiffening than the carotid arteries [17] and that aortic stiffness is positively associated with age and male gender [18]. However, none of previous publications using this method, to our knowledge, have stated objectives similar to our study. There exists strong evidence that diabetes is associated with increased arterial stiffness [5]. Associations have also been described between arterial stiffness and prediabetic states such as insulin resistance and impaired glucose tolerance [6, 19, 20]. In a study of 41 subjects a correlation between fasting glucose levels and local aortic distensibility was shown using a local invasive method [21]. In addition, one study using echo-tracking found an association between insulin resistance and femoral and carotid stiffness in patients with diabetes mellitus [22]. The proposed mechanisms of how

diabetes results in arterial stiffness are numerous and include for example formation of AGE-products, as well as the influence of oxidative stress, monocyte vascular adhesion and inflammation on the arterial wall [23]. Several studies using PWV have reported greater associations between diabetes and arterial stiffness in women than in men [24-26], results that are supported by our study. Such gender differences have also been shown in type 1 diabetics using echo-tracking system where diabetic women showed higher abdominal aortic stiffness compared to controls [27]. A greater impact of diabetes on arterial stiffness in women could, at least partially, explain the more marked increase in cardiovascular disease risk associated with diabetes in women than in men [5]. In univariate correlation analysis the association between β-coefficient and waist circumference was significant among women but it lost its significance in multiple regression analysis. Since insulin resistance is associated with abdominal obesity the lack of association between waist circumference and β-coefficient might be related to over-adjustment.

Since atherosclerosis also contributes to arterial stiffness (arteriosclerosis) and because of the central role of lipids in atherogenesis [28], a correlation between lipids and aortic stiffness was considered. In our study there were no associations between aortic stiffness and triglycerides or LDL cholesterol in multiple regression analysis. HDL cholesterol, however, was statistically significant inversely associated with aortic stiffness both prospectively and cross-sectionally. This association existed both in all subject analyzed together as well as in the female subgroup but not among men. In a systematic review from 2009 investigating cross-sectional determinants of PWV an association between LDL cholesterol and PWV was shown in only one of 21 studies but between HDL cholesterol and PWV in four of 37 studies [29]. None of the four studies reporting an association between HDL cholesterol and PWV indicated any evident sex differences. A study of predictive determinants for arterial stiffness from 2012 including 3769 subjects showed a negative association between HDL cholesterol

and PWV in both men and women [30]. The reason for the gender disparity in the current study, in our interpretation, is due to small differences in HDL cholesterol and β stiffness index distributions between men and women rather than a true difference in pathophysiology.

There are circumstances which have to be taken into account when comparing local abdominal aortic stiffness and regional assessments of arterial stiffness. The local distensibility measured by ultrasonography shows a correlation with c-f PWV but this is not particularly strong, depending both on the elastic heterogeneity along the arterial tree and on methodological difficulties [31]. The abdominal aorta is known to consist of less elastin and more collagen than the thoracic aorta and is therefore stiffer [32]. There are also pathophysiological differences where the aorta, as mentioned above, seems to be more to prone to stiffening than, for example, the carotid artery [17, 33] and the elasticity is also specific for a certain intra-arterial pressure [34].

Our study is one of few studies available investigating local abdominal aortic stiffness using echo-tracking technique. In a study investigating the abdominal aorta in 69 subjects, the β stiffness index, in contrast to the elastic modulus (Ep), was not affected significantly by an increase in blood pressure [18]. This is, of course, an important strength of the β stiffness index. However, the ultrasound technique is not uncomplicated, why some limitations should be considered. Pressure on the abdomen while performing the investigation can impair the normal expansion of the aorta during systole affecting the results. In the formula for calculating the β stiffness index we used brachial blood pressure instead of aortic blood pressure that we lacked. The usage of brachial blood pressure has previously been shown to underestimate pulse pressure by approximately 10 mmHg when compared to blood pressure obtained from intra-arterial measurements in the aorta but this underestimation was equal in all ages and for both sexes [35]. Subjects with an impaired glucose metabolism, as described in methods, are deliberately overrepresented in this study compared to the average population.

This makes it easier to reach significant results with regard to glucose metabolism but, on the other hand, impairs the external validity. On the other hand, a follow-up time of 6.7 years results in a healthy survivor bias effect which most likely attenuates the associations in our results. The adjustment for drug medication involved adjustment for ongoing anti-hypertensive drug treatment in multiple regression analysis while no consideration to treatment against dyslipidemia was taken. Furthermore no measurements of regional arterial stiffness such as c-f PWV were performed, which would have been interesting for comparison and to determine if any risk markers are more associated with arterial stiffness specifically in the abdominal aorta than other arterial segments. Ideally, hyperinsulinaemic, euglycaemic clamp methodology should be used instead of the more blunt marker HOMA-IR. Finally, there were no baseline measurement of aortic stiffness, and it would of course be of interest to study the changes in aortic stiffness during the follow-up period and its relation to changes in cardiovascular risk factors.

In conclusion, this study indicates a prospective positive independent association between insulin resistance, and a negative association between HDL cholesterol, and abdominal aortic stiffness among women while these relationships were not seen among men. In addition, age and male sex were also positively associated with aortic stiffness. These gender differences should be confirmed in larger studies from different populations.

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Conflicts of Interest

The authors declare no conflict of interest.

Summary table

What is known about the topic	What this study adds
Arterial stiffness predicts cardiovascular events and mortality.	Cross-sectional and retrospective data on the associations between cardiovascular risk factors and stiffness of the abdominal aorta specifically.
Diabetes, and in some studies also dyslipidemia, is associated with regional arterial stiffness.	
While regional stiffness has been extensively studied, for example with pulse wave velocity, local arterial stiffness of the abdominal aorta is not well characterized.	Gender difference in the impact of insulin resistance and dyslipidemia on abdominal aortic stiffness.

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Table 1: Characteristics of the study population (n=335) at baseline examination and at follow-up, with means, standard deviation (SD), number of subjects and proportions (%).

Characteristic	*Baseline*			*Follow-up*		
	All	**Men**	**Women**	**All**	**Men**	**Women**
	Mean ±SD			**Mean ±SD**		
Age (years)	58 ± 6	58 ± 6	58 ± 6	64 ± 6	64 ± 6	65 ± 6
Men, n (%)	139 (42)			139 (42)		
SBP (mmHg)	141 ± 18	143 ± 19	140 ± 18	139 ± 19	139 ± 20	138 ± 19
DBP (mmHg)	87 ± 9	88 ± 9	86 ± 9	82 ± 9	84 ± 8	81 ± 8
BMI (kg/m^2)	26.4 ± 3.8	26.1 ± 3.5	26.6 ± 4.0	27.0 ± 3.9	26.6 ± 3.5	27.2 ± 4.2
Waist circumference (cm)	86 ± 12	93 ± 10	80 ± 10	90 ± 11	96 ± 10	87 ± 11
β stiffness index				14.6 ± 9.1	17.0 ± 10.2	12.9 ± 7.8
LDL cholesterol (mmol/L)	4.22 ± 0.97	4.14 ± 0.86	4.29 ± 1.04	3.74 ± 0.84	3.71 ± 0.73	3.76 ± 0.91
HDL cholesterol (mmol/L)	1.37 ± 0.37	1.23 ± 0.32	1.48 ± 0.38	1.52 ± 0.40	1.38 ± 0.34	1.63 ± 0.40
Triglycerides (mmol/L)	1.39 ± 0.67	1.45 ± 0.68	1.35 ± 0.66	1.28 ± 0.65	1.29 ± 0.64	1.27 ± 0.66
Glucose (mmol/L)	5.06 ± 0.67	5.18 ± 0.77	4.98 ± 0.58	5.33 ± 0.87	5.49 ± 0.96	5.22 ± 0.78
Insulin (mIU/l)	9.26 ± 5.31	9.54 ± 5.97	9.06 ± 4.79	10.02 ± 6.01	10.36 ± 6.12	9.77 ± 5.94
HOMA-IR	2.15 ± 1.45	2.27 ± 1.71	2.06 ± 1.24	2.47 ± 1.81	2.63 ± 1.99	2.35 ± 1.67
Current smoking, n (%)	80 (24)	40 (29)	40 (20)	80 (24)	41 (30)	39 (20)
Hypertension treatment, n (%)	53 (16)	21 (15)	32 (16)	83 (25)	28 (20)	55 (28)

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance

Table 2: Spearman's univariate correlation analysis between aortic stiffness (β stiffness index) and HOMA-IR, LDL cholesterol, HDL cholesterol, triglycerides and waist circumference, at baseline and follow-up, for all subjects and stratified by gender.

		All		Men		Women	
		r	**p**	**r**	**p**	**r**	**p**
Follow-up	**HOMA-IR**	0.16	0.004	0.13	0.12	0.15	0.03
	Triglycerides	0.15	0.006	0.18	0.03	0.15	0.04
	LDL cholesterol	0.00	0.98	0.02	0.80	-0.01	0.92
	HDL cholesterol	-0.26	<0.001	-0.18	0.04	-0.18	0.01
	Waist circumference	0.32	<0.001	0.31	<0.001	0.20	0.005
Baseline	**HOMA-IR**	0.19	0.001	0.05	0.56	0.27	<0.001
	Triglycerides	0.15	0.005	0.10	0.23	0.16	0.02
	LDL cholesterol	0.09	0.09	0.08	0.33	0.14	0.06
	HDL cholesterol	-0.28	<0.001	-0.12	0.15	-0.24	0.001
	Waist circumference	0.35	<0.001	0.26	0.002	0.22	0.002

Abbreviations: r, Spearman's correlation coefficient; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 3: Multiple regression analysis of the independent determinants of aortic stiffness (β stiffness index) as the dependent variable, at baseline and follow-up calculated for all subjects, stratified by gender and adjusted for ongoing anti-hypertensive drug treatment at the ultrasound examination.

		All		Men		Women	
		Beta	**p**	**Beta**	**p**	**Beta**	**p**
Follow-up	**Age at follow-up**	0.34	<0.001	0.27	0.002	0.42	<0.001
	Female sex	-0.18	0.001				
	Waist circumference	0.14	0.03	0.21	0.04	0.09	0.24
	HOMA-IR	-0.03	0.68	-0.05	0.64	-0.01	0.88
	Triglycerides	-0.02	0.80	0.01	0.90	-0.05	0.52
	HDL cholesterol	-0.19	0.002	-0.12	0.23	-0.22	0.004
	Smoking	-0.01	0.84	0.03	0.74	-0.04	0.52
Baseline	**Age at baseline**	0.32	<0.001	0.23	0.01	0.42	<0.001
	Female sex	-0.18	0.005				
	Waist circumference	0.09	0.23	0.23	0.04	0.03	0.72
	HOMA-IR	0.06	0.33	-0.14	0.21	0.18	0.03
	Triglycerides	-0.06	0.36	0.04	0.67	-0.16	0.07
	HDL cholesterol	-0.16	0.01	-0.04	0.73	-0.23	0.005
	Smoking	-0.01	0.81	0.04	0.68	-0.05	0.47

Abbreviations: Beta, standardized correlation coefficient; HOMA-IR, homeostatic model assessment of insulin resistance; HDL, high density lipoprotein.

Legends to figures

Figure 1a: Mean and 95 % CI of β stiffness index for *women* in different quartiles of baseline HOMA-IR.

Figure 1b: Mean and 95 % CI of β stiffness index for *men* in different quartiles of baseline HOMA-IR.