European Journal of Radiology 111 (2019) 34–40

Contents lists available at ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

Research article

Relationship between carotid intima-media thickness and carotid artery stiffness assessed by ultrafast ultrasound imaging in patients with type 2 diabetes

Fu-shun Pan[a], Ming Xu[a], Liang Yu[b], Jia Luo[a], Man-ying Li[a], Jin-yu Liang[a], Yan-ling Zheng[a], Xiao-yan Xie[a,*]

[a] *Department of Medical Ultrasonics, Institute of Diagnostic and Interventional Ultrasound, the First Affiliated Hospital, Sun Yat-Sen University, 58 Zhongshan Road 2, Guangzhou, 510080, PR China*

[b] *Department of Vascular and Thyroid Surgery, The First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan Road 2, Guangzhou, 510080, PR China*

A R T I C L E I N F O

Keywords:
Carotid artery elasticity
Pulsed wave velocity
Ultrafast ultrasound imaging
Intima-media thickness
Diabetes

A B S T R A C T

Objectives: To evaluate the relationship between carotid stiffness and carotid intima-media thickness (CIMT) in patients with type 2 diabetes (T2DM).

Materials and methods: Carotid properties were evaluated in 317 consecutive subjects (98 volunteers for controls, 105 patients with normal CIMT for T2DM group 1, and 114 patients with thickened CIMT for T2DM group 2). The CIMT and carotid pulse wave velocity at the beginning (PWV-BS) and at the end of systole (PWV-ES) were measured.

Results: Apart from PWV-BS in T2DM group 1, CIMT and PWV-ES were significant higher in patients groups than those of in controls. In multiple regression analysis, diabetes was independently associated with PWV-ES and not with PWV-BS. Moreover, when adjusting for baseline covariates, only PWV-ES (odds ratio = 4.27, $P < 0.001$) distinguished carotid in T2DM group 1 from that of controls. Concerning the relationship between log(CIMT) and PWV-ES, when adjusting for baseline covariates, the association were still significant in controls and T2DM group 1, whereas it was no longer present in T2DM group 2 ($P = 0.091$). Additionally, the slope (β) after adjustment for the PWV-ES to log(CIMT) was significantly steeper in T2DM group 1 than that of in controls (β = 8.35 vs. 3.31, $P < 0.01$).

Conclusions: The PWV-ES seem to be a better biomarker candidate than PWV-BS to assess the carotid stiffness in diabetic patients. Compared with controls, diabetic patients showed more advanced functional changes than morphological changes despite normal CIMT, whereas the relationship trend was not present when thickened CIMT emerged.

1. Introduction

Type 2 diabetes (T2DM) is an important risk factor for atherosclerosis and cardiovascular disease (CVD) events [1]. Atherosclerosis is a complex process involving both thickening (morphological) and vessel stiffening (functional) changes of the arterial wall. Common carotid intima-media thickness (CIMT) is a well-established surrogate marker for evaluating morphological changes and predicting future CVD events [2]. However, recent consensus guideline stated that CIMT is not recommended for the risk assessment of the first CVD event [3]. With regard to T2DM, one study also demonstrated that CIMT progression can not be used as a surrogate end point in clinical trials [4]. These conflicting results might be attributable to different measurement protocol. After all, a small inter-assay variation in IMT measurements may mask actual morphological change.

Functionally, pulse wave velocity (PWV) is one of the classical indexes of arterial stiffness. From the Moens-Korteweg equation, the PWV is closely associated with artery elasticity, thickness, and radius [5]. Concerning the carotid stiffness assessment, there are various non-invasive techniques and the echo-tracking technique is a common used reference method [6]. However, the echo-tracking technique requires expensive equipment and a high level of technical expertise, which may hamper its clinical applicability. Recently, several studies by Konofagou et al [7,8] highlighted the potential value of ultrafast imaging for local

* Corresponding author.
E-mail addresses: panfushun123@163.com (F.-s. Pan), xu2004m@sina.com (M. Xu), yuliang6@mail.sysu.edu.cn (L. Yu), luojia7@mail.sysu.edu.cn (J. Luo), limany@mail2.sysu.edu.cn (M.-y. Li), awayfish@163.com (J.-y. Liang), zhyanl@mail.sysu.edu.cn (Y.-l. Zheng), xxy1992sys@163.com (X.-y. Xie).

https://doi.org/10.1016/j.ejrad.2018.12.016
Received 22 June 2018; Received in revised form 3 December 2018; Accepted 13 December 2018

measurement of arterial pulse waves. And then ultrafast ultrasound imaging has been developed to automatically measure the CIMT and carotid stiffness expressed as ultrafast pulse wave velocity (ultrafast PWV) [9–11]. This technique is based on sending a single plane wave in emission and focusing on reception only, which is characterized by extremely imaging frame rate (up to 10,000 frames per second) [9,12]. It can capture the local carotid PWV not only at the beginning of systole (PWV-BS) but also at the end of systole (PWV-ES). Other researchers and we have shown that ultrafast ultrasound imaging is an effective and user-friendly method with high reproducibility in evaluating carotid stiffness [13–16]. Our previous study showed that each ultrafast PWV acquisition was completed within 1 min. Zhu et al [16] demonstrated that ultrafast PWV was a reliable modality for the diagnosis and quantitative assessment of carotid stiffness in patients with hyperlipemia. Additionally, another study based on ultrafast ultrasound demonstrated that CIMT and PWV increased and were positively correlated with the hypertension stage in hypertensive patients [14].

To date, there are several studies addressed the relationship between CIMT and carotid stiffness based on ultrasound in patients with T2DM [17,18]. However, the conclusions were conflicted and it was difficult to compare results due to different technique criteria. One study based on a prototype US system showed that carotid artery elasticity correlated with the number of atherosclerosis risk factors independent of baseline covariates [17]. Whereas another study employed automated speckle-tracking method based on US demonstrated that carotid arterial mechanics changes preceded the wall thickening in hypertensive patients other than diabetic patients [18]. To our knowledge, there have been no reports on the evaluation of carotid stiffness using ultrafast PWV in patients with T2DM. In the present study, we aimed to evaluate the relationship between CIMT and carotid ultrafast PWV in patients with T2DM. In addition, the alteration trends of CIMT and ultrafast PWV for patients in different stages of T2DM were assessed as well when compared with controls.

2. Materials and methods

2.1. Study population

This prospective study was approved by the institutional review board, and written informed consent was obtained from all patients. Between January 2017 and April 2018, 810 patients with established T2DM from endocrine department in our medical center were recruited to perform carotid ultrasound and ultrafast PWV. T2DM was diagnosed according to American Diabetes Association criteria [19]. A total of 454 patients were excluded due to cardiovascular or cerebrovascular disease, chronic kidney disease or diabetic nephropathy, arrhythmias, common carotid artery plaques, failure of ultrafast PWV acquisition, and maintenance of ultrafast imaging apparatus (Fig. 1). Plaque was defined as either a focal region with maximal IMT ≥ 1.5 mm [20]. Out of the included 356 subjects, 105 patients with normal CIMT and absence of hypertension and/or hyperlipidemia were classified as T2DM group 1, and 114 patients with thicken IMT were classified as T2DM group 2. Upper limit values of the normal CIMT were defined as less than the upper 90th percentile of CIMT obtained in 24,871 healthy individuals according to age and gender groups [21]. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or taking antihypertensive drugs. Hyperlipidemia was defined as total cholesterol ≥5.7 mmol/L and/or triglyceride ≥1.7 mmol/L, or taking antihyperlipidemic drugs. Smoking was defined as consuming at least 1 cigarette daily.

The study also enrolled 98 healthy volunteers (control group) who visited our hospital for physical examination during the same interval. All the volunteers had normal CIMT and were free of plaque, diabetes, hypertension, hyperlipidemia, cardiovascular or cerebrovascular disease, chronic kidney disease and arrhythmias.

2.2. CIMT and carotid ultrafast PWV assessments

CIMT and carotid stiffness assessments were performed by one registered vascular radiologist (F.S.P.) with an Aixplorer ultrafast ultrasound imaging system (Supersonic Imagine, Aixen Provence, France) equipped with SL15-4 (frequency 4–15 MHz) probes. A routine ultrasound scan including common carotid artery, carotid bulb and portions of the internal and external carotid arteries on both sides were performed for each subject in supine position with the head tilted slightly to the contralateral side.

The CIMT and carotid ultrafast PWV measurements were consistent with our previous study [15]. The far CIMT was automatically measured by using an Aixplorer's tool when the intima-media layers were smooth (Fig. 2a); otherwise, IMT was measured manually at the thickest site in ZOOM pattern of B-mode US. Three consecutive CIMT measurements were obtained and averaged as the final CIMT value. After CIMT measurements, ultrafast PWV mode was activated and automatically deduces the PWV-BS, PWV-ES of the anterior artery wall, and their standard deviations (expressed as Δ ±) (Fig. 2b). The final ultrafast PWV was calculated as the average of three consecutive valid measurements. The valid ultrafast PWV acquisition was defined as simultaneously display of PWV-BS, PWV-ES, and the value of Δ ± less than 20% of the corresponding PWV values.

Previous studies have reported that both the CIMT and ultrafast PWV had no differences between sides [13,14,22] in healthy population or patients with atherosclerosis risk factors. With a reasonable assumption, these findings might apply to patients with T2DM. Thus, only unilateral CIMT and corresponding carotid ultrafast PWV data were used in the final analyses regardless of whether CIMT and carotid ultrafast PWV acquisitions were performed on one or both sides.

2.3. Baseline data acquisitions

The basic characteristics of study population were acquired via electronic medical record system. Blood pressure was measured with an electronic sphygmomanometer (Omron) after the subject had rested for at least 15 min. For laboratory test, serum glucose, total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured after an overnight fast. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation.

2.4. Statistical analysis

Statistical analysis was performed using MedCalc Software, version 11.2 (MedCalc program, Belgium). A P value less than 0.05 was accepted as indicating statistical significance. The quantitative variables were described as mean values and standard deviations. The qualitative variables were summarized as counts and percentages. Between-groups comparisons were performed by t-test or one way analysis of variance (ANOVA) with the Bonferroni post hoc test for continuous variables and chi-square for categorical variables. A multiple linear regression analysis with a stepwise manner was performed to determine independent parameters related to carotid wall properties. A multivariate logistic regression model controlling for baseline covariates was performed to identify independent vascular parameters distinguishing T2DM group 1 from control group. According to the Moens-Korteweg equation [5], the relationships between log(CIMT) and ultrafast PWV value after adjusting for baseline covariates in different groups were examined by linear regression analysis. Differences in association of CIMT with ultrafast PWV were compared by the slope (β) of the appropriate regression lines ($y = \alpha + \beta \times CIMT$).

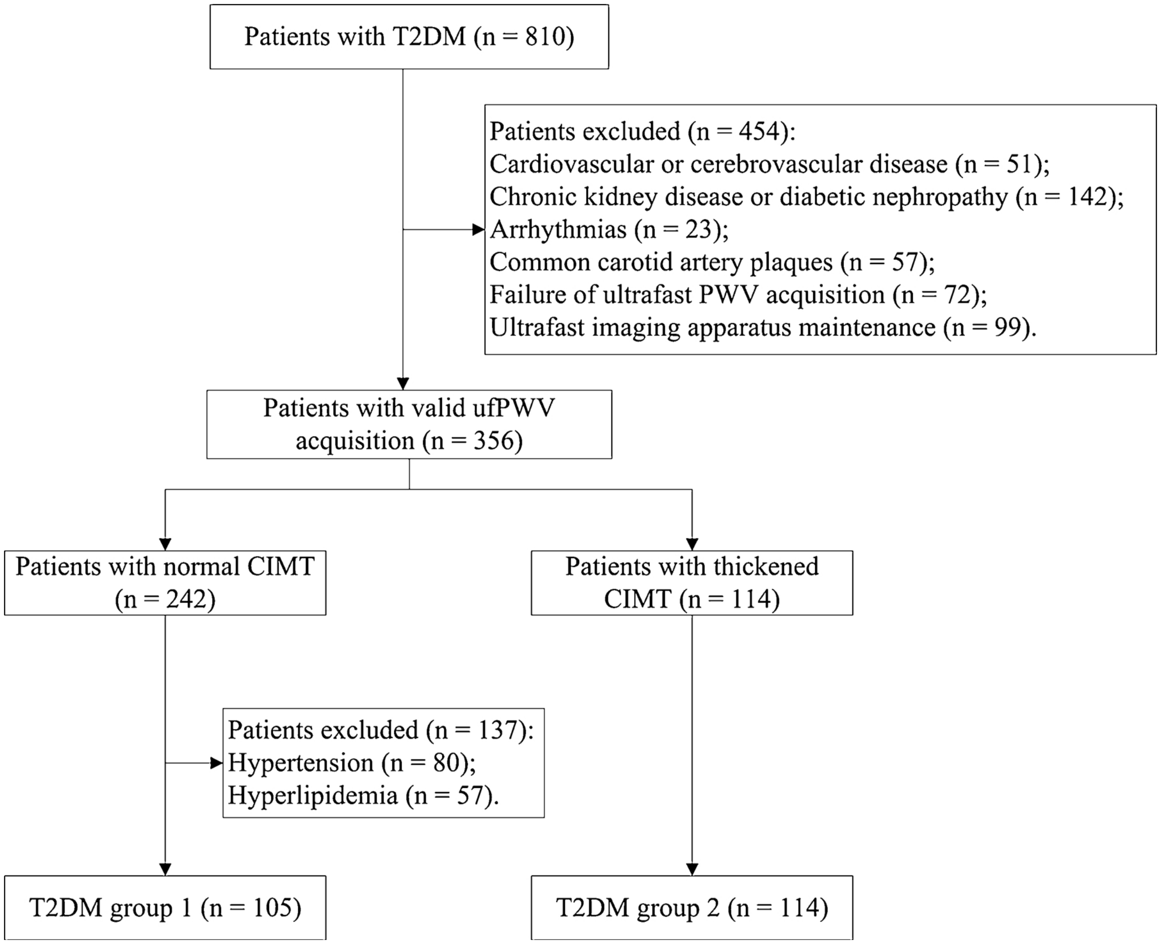

Fig. 1. Flow diagram of patients with T2DM.

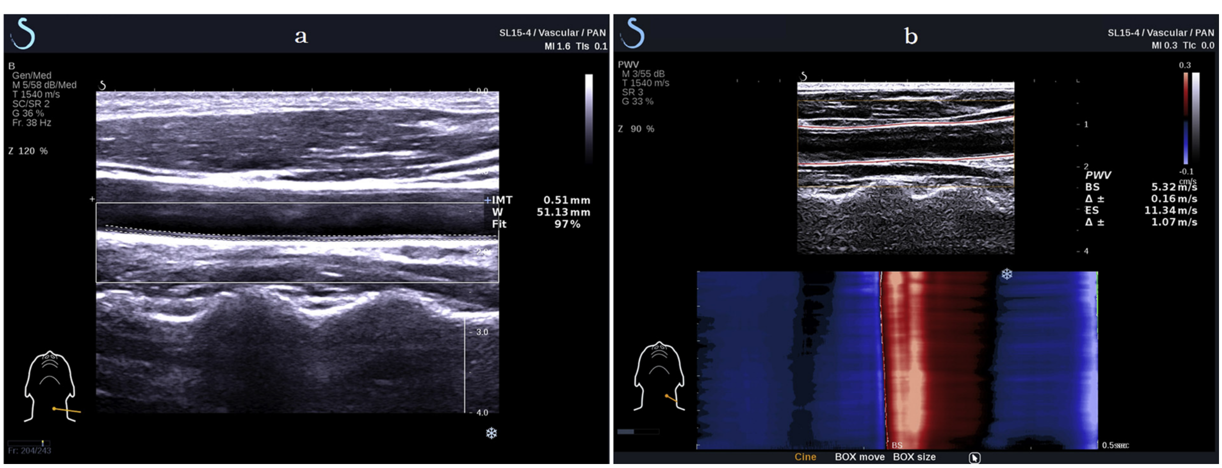

Fig. 2. Carotid properties in a 55-year-old man with type 2 patients. (a) B-mode US showed the CIMT was 0.51 mm, (b) Ultrafast PWV measurements at the beginning of systole (PWV-BS = 5.32 m/s, Δ ± = 0.16 m/s) and the end of systole (PWV-ES = 11.34 m/s, Δ ± = 1.07 m/s).

3. Results

3.1. Basic characteristics of the study population

The basic characteristics of the study population are summarized in Table 1. There was no significant difference between T2DM group 1 and control group in terms of age, whereas the patients in T2DM group 2 were significant older than those of T2DM group 1 and control group ($P < 0.01$). In T2DM group 2, hypertension was found in 82 (71.9%) patients and 64 (78.0) subjects were taking antihypertensive drugs. Additionally, there were 77 (66.7%) patients with hyperlipidemia and 18 (23.4%) subjects were taking antihyperlipidemic drugs.

Morphologically, the CIMT of T2DM group 2 was significant thicker than those of T2DM group1 and control group ($P < 0.01$). Although the CIMT of T2DM group 1 was within normal reference, it was thicker than that of control group (0.60 ± 0.11 vs. 0.53 ± 0.08, $P < 0.01$). Functionally, both PWV-BS and PWV-ES values of T2DM group2 were significant higher than those of T2DM group1 and control group ($P <$

Table 1
Basic characteristics of study population.

Parameters	Control group (n = 98)	T2DM group 1 (n = 105)	T2DM group 2 (n = 114)	*P* value
Age (years)	47.7 ± 11.4	50.3 ± 10.8	58.8 ± 9.2*†	< 0.001
Male (%)	45 (45.6)	63 (60.0)	58 (50.9)	0.122
BMI (kg/m^2)	20.8 ± 1.7	23.4 ± 3.3*	25.5 ± 3.8*†	< 0.001
Smoking (%)	20 (20.4)	29 (27.6)	32 (28.1)	0.372
Duration of T2DM (years)	NA	5.8 ± 5.8	9.6 ± 7.5†	NA
Fasting glucose (mmol/L)	4.8 ± 0.5	8.1 ± 4.1*	8.7 ± 3.7*	< 0.001
HbA1c (%)	NA	9.2 ± 2.4	8.9 ± 1.9	NA
SBP (mmHg)	111.5 ± 9.9	119.8 ± 11.1*	141.8 ± 19.4*†	< 0.001
DBP (mmHg)	72.5 ± 6.9	74.3 ± 7.3	86.7 ± 11.8*†	< 0.001
Total cholestrol (mmol/L)	4.1 ± 0.7	4.7 ± 0.7*	5.5 ± 1.4*†	< 0.001
Triglyceride (mmol/L)	1.17 ± 0.33	1.51 ± 0.37*	1.92 ± 1.61*†	< 0.001
HDL cholestrol (mmol/L)	1.6 ± 0.6	1.2 ± 0.3*	1.3 ± 0.5*	< 0.001
LDL cholestrol (mmol/L)	2.8 ± 0.9	2.9 ± 0.8	3.3 ± 0.9*†	< 0.001
Uric acid (μmol/L)	354.9 ± 86.7	334.8 ± 73.5	366.2 ± 80.2	0.438
Serum creatinine (μmol/L)	71.4 ± 15.4	67.0 ± 15.9	71.4 ± 15.4	0.131
Diabetic retinopathy (%)	NA	9 (8.6)	17(14.9)	NA
Diabetic neuropathy (%)	NA	21(20.0)	36 (31.6)†	NA
Diabetes therapy (%)	NA	9.2/62.2/28.6	1.8/34.2/64.0	NA
(diet/OHA /OHA + insulin)	0.53 ± 0.08	0.60 ± 0.11*	1.09 ± 0.17*†	< 0.001
CIMT (mm)	5.89 ± 1.03	5.97 ± 1.19	6.81 ± 1.36*†	< 0.001
PWV-BS (m/s)	6.95 ± 1.04	8.73 ± 1.92*	10.38 ± 1.84*†	< 0.001
PWV-ES (m/s)	6.28 ± 0.45	6.42 ± 0.41	6.31 ± 0.52	0.768
Carotid diameter (mm)				

Data are expressed as mean ± standard deviation or numbers of patients, with percentages in parentheses; * $P < 0.01$ vs. control group; † $P < 0.01$ vs. T2DM group 1; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood presure;
HDL, high-density lipoprotein; LDL, low-density lipoprotein; OHA, oral hypoglycemic agents; CIMT, carotid intima-media thickness; PWV-BS, pulse wave velocity at the beginning of systole; PWV-ES, pulse wave velocity at the end of systole; NA indicates not applicable.

0.01). Meanwhile, PWV-ES values of T2DM group1 were also significant higher than those of control group ($P < 0.01$), whereas there was no significant difference for PWV-BS between T2DM group 1 and control group ($P = 0.477$).

3.2. Independent variables affecting carotid properties

To elucidate the independent variables affecting CIMT and ultrafast PWV, multiple linear regression analysis was performed between baseline parameters and carotid properties in different model. As shown in Table 2, age was the only common independent risk factor associated with increased CIMT, PWV-BS, and PWV-ES in all the three model (β = 0.223 - 0.473, $P < 0.01$ for all). In model 2 and 3, diabetes was the common independent risk factor affecting CIMT and PWV-ES (β = 0.101 - 0.364, $P < 0.01$ for all). Hyperlipidemia and decreased HDL cholesterol were another independent risk factors associated with increased CIMT (β = - 0.083 - 0.520, $P < 0.01$ for both), whereas hypertension was another independent risk factor associated with increased PWV-BS and PWV-ES (β = 0.218 - 0.290, $P < 0.01$ for all). Importantly, apart from age and hypertension, neither diabetes nor hyperlipidemia was independent risk factor associated with increased PWV-BS in any model.

Table 2
Independent factors related to carotid arterial property in multiple regression analyses.

Parameters	CIMT		PWV-BS		PWV-ES	
	β	*P* value	β	*P* value	β	*P* value
Model 1	0.473	< 0.001	0.223	0.001	0.356	< 0.001
Age	0.194	< 0.001	0.295	< 0.001	0.418	< 0.001
Diabetes	0.238	< 0.001	0.218	0.003	0.114	0.018
SBP	0.165	< 0.001	0.275	< 0.001	0.333	< 0.001
Model 2	0.325	< 0.001	0.221	< 0.001	0.364	< 0.001
Age	−0.083	0.001			0.290	< 0.001
Diabetes	0.076	0.047			0.419	< 0.001
Hyperlipidemia	0.297	< 0.001			0.335	< 0.001
HDL cholestrol	0.101	0.001			0.256	< 0.001
Hypertension	0.520	< 0.001				
Model 3	0.056	0.026				
Age	−0.082	0.002				
Diabetes						
Hyperlipidemia						
Sex						
HDL cholestrol						
Hypertension						

β, standard regression coefficient; Model 1, control group + T2DM group 1; Model 2, Control group + T2DM group 2; Model 3, All subjects. Parameters evaluated in model 1 included age, sex, BMI, smoking, fasting plasma glucose, uric acid, total cholesterol, triglyceride, HDL and LDL cholesterol levels, systolic, diastolic blood pressure and the presence,duration and HbA1c level of diabetes. In model 2 and 3, the parameters based in model 1 plus hypertension and hyperlipidemia were included.

Table 3
Multivariate logistic regression analysis of carotid properties to distinguish T2DM group 1 from control group.

Parameters	Model 1 OR (95% CI)	P value	Model 2 OR (95% CI)	P value	Model 3 OR (95% CI)	P value
CIMT	0.53 (0.36 – 0.80)	0.008	0.55 (0.36 – 0.82)	0.011	0.58 (0.36 – 0.91)	0.116
PWV-BS	4.38 (1.02 – 7.86)	0.603	3.25 (0.68 – 5.85)	0.682	1.34 (0.12 – 2.63)	0.723
PWV-ES	3.95 (2.50 – 6.22)	< 0.001	3.75 (2.34 – 5.99)	< 0.001	4.27 (2.47 – 7.42)	< 0.001

OR, odds ratio; CI, confidence interval; Model 1, adjust for age, gender, and BMI; Model 2, adjust for model 1 plus SBP, DBP, and smoking; Model 3, adjust for model 2 plus total cholestrol, triglyceride, HDL cholestrol, and LDL cholestrol.

3.3. Carotid properties to distinguish T2DM group 1 from control group

The differences of vascular parameters adjusted for baseline covariables between T2DM group 1 and control group are demonstrated in Table 3. There was no significant difference for PWV-BS after adjustment (OR = 1.34–4.38, $P = 0.603 - 0.723$). The CIMT showed significant differences after adjusted for age, gender, BMI, systolic blood pressure, diastolic blood pressure, and smoking (OR = 0.55, $P = 0.011$). However, after adjusting serum lipid as well, the difference was no longer present (OR = 0.58, $P = 0.116$). With regard to PWV-ES, the difference was still present after adjustment (OR = 3.75–4.27, $P < 0.001$).

3.4. Relationships between morphological and functional features of carotid arteries

The relationships between morphological and functional features in different group are shown in Table 4, with univariate associations illustrated in Figs. 3 and 4. Apart from the unadjusted PWV-BS in control group, all the unadjusted and adjusted PWV-BS values were not associated with log(CIMT) in any group. Apart from in model 1 and model 3 for the T2DM group 2, all the PWV-ES values were positive associated with log(CIMT) in all the three group (β = 3.31–15.75, $P < 0.05$ for all). The slope (β) of the regression line for the PWV-ES to log(CIMT) was significantly steeper in the T2DM group 1 than that of in the control group (slope = 15.75 vs. 8.71, respectively; $P < 0.01$), whereas a reverse trend was observed when compared T2DM group 2 and control group (slope = 7.78 vs. 8.71, respectively; $P < 0.01$). After adjusting for baseline covariates including in a stepwise manner (Table 4), the associations between PWV-ES and log(CIMT) were still significant in control group and T2DM group 1, whereas it was no longer present in T2DM group 2 (slope = 4.97, $P = 0.091$). Additionally, the slope after adjustment for the PWV-ES to log(CIMT) was still significantly steeper in T2DM group 1 than that of in control group (slope = 8.35 vs. 3.31, respectively; $P < 0.01$).

4. Discussion

We demonstrated that T2DM was independent risk factor associated with increased PWV-ES other than PWV-BS. Additionally, the PWV-ES can distinguish T2DM group 1 from control group after adjusting for baseline covariates. Finally, we also showed that the slope of line for the PWV-ES to CIMT after adjusting for baseline covariates was still significantly steeper in T2DM group 1 than that of in control group, whereas PWV-ES was not associated with CIMT in the control group than that of in T2DM group 1. In other words, compared with controls, patients with T2DM showed more advanced functional changes than morphological changes despite normal CIMT, whereas the trend of relationship did not exist in T2DM patients with thickened CIMT, suggesting that carotid artery stiffening assessed by ultrafast ultrasound imaging might be not parallel with intima-media thickening in T2DM patients. To the best of our knowledge, this is the first study to evaluate the relationship between CIMT and carotid stiffness in patients with T2DM using ultrafast ultrasound imaging.

Histologically, intima-media thickening involves the enlargement and coalescence of separate pools of extracellular lipid particles in the muscular-elastic layer of the intima, then the smooth-muscle cells widely converge around the lipid core and lead to inflammation. The stiffness of the vessel wall is mainly dependent on 2 prominent scaffolding proteins: collagen and elastin. An overproduction of abnormal collagen, mainly by stimulation of an inflammatory context, diminished quantities of normal elastin and stiffened the vascular wall [23].Clinically, CIMT is the most widespread index for assessing atherosclerosis. However, it is only indicate morphological changes of the artery wall. Recently, great emphasis has been placed on the arterial stiffness changes in atherosclerosis and in the development of CVD. A recent meta-analysis has demonstrated that carotid stiffness was positively associated with stroke [24]. It was also observed that arterial stiffness changes are strongly associated with CIMT [25]. Meanwhile, it was recommended that assessment of both carotid stiffness and CIMT is optimal [6].

In our study, apart from PWV-BS between T2DM group 1 and control group, all the PWV-BS and PWV-ES values of T2DM group2 were significant higher than those of T2DM group1 and control group. According to the Moens-Korteweg equation [5], the carotid PWV is related to its elasticity, diameter and thickness. Thus, it is very well expected that PWV is positively associated with CIMT. Nevertheless, CIMT and PWV are well-established surrogate markers for evaluating atherosclerosis changes and predicting future CVD events [2]. As could be anticipated from the Moens-Korteweg equation, our results tend to

Table 4
Association between log(CIMT) and ultrafast PWV for study subjects.

Parameters	Unadjusted β (95% CI)	P value	Model 1 β (95% CI)	P value	Model 2 β (95% CI)	P value	Model 3 β (95% CI)	P value
PWV-BS								
Control group	3.16(2.06 - 4.27)	0.036	2.39 (- 2.07 -6.87)	0.290	2.17 (-2.40 - 6.74)	0.349	2.94 (-1.62 - 7.49)	0.203
T2DM group 1	1.67 (-1.25 - 4.59)	0.259	−1.87 (- 6.03 - 2.29)	0.374	−1.70 (-6.21 - 2.67)	0.431	−1.34 (-5.96 - 3.29)	0.567
T2DM group 2	3.17 (-1.31 - 7.65)	0.163	0.60 (- 3.88 - 5.07)	0.792	0.88 (-3.69 - 5.46)	0.703	2.32 (-2.73 - 7.37)	0.364
PWV-ES								
Control group	8.71 (6.25 - 11.18)	< 0.001	4.39 (0.87 - 7.91)	0.022	4.05 (0.57 - 7.53)	0.023	3.31 (0.55 - 6.08)	0.018
T2DM group 1	15.75 (12.10 - 19.39)	< 0.001	11.95 (6.74 - 17.16)	< 0.001	10.39 (5.05 - 15.73)	< 0.001	8.35 (4.12 - 12.60)	< 0.001
T2DM group 2	7.78 (1.84 - 13.72)	0.011	3.93 (- 1.66 - 9.53)	0.166	5.44 (0.09 - 10.79)	0.046	4.97 (-1.01 - 10.95)	0.091

β, slope of line; CI, confidence interval; Model 1, adjust for age, gender, BMI, and carotid diameter; Model 2, adjust for model 1 plus SBP, DBP, and smoking; Model 3, adjust for model 2 plus total cholestrol, triglyceride, HDL cholestrol, and LDL cholestrol.

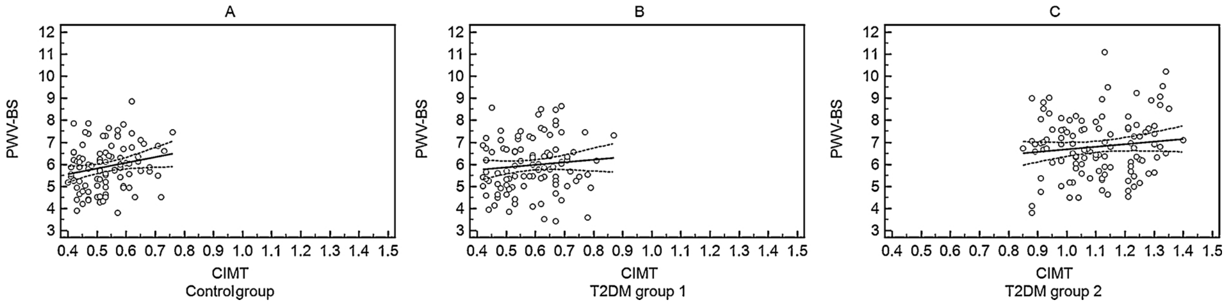

Fig. 3. Scatter diagrams and univariate associations between CIMT and PWV-BS in control group (A; r = 0.214, *P* = 0.034), T2DM group 1 (B; r = 0.106, *P* = 0.280), and T2DM group 2(C; r = 0.122, *P* = 0.196).

demonstrate the relationship between CIMT and carotid ultrafast PWV in patients with T2DM. In addition, the relationship trends of CIMT and ultrafast PWV for patients in different stages of T2DM were assessed when compared with controls. After all, the validity of the MK equation in vivo has not been fully demonstrated so far, especially due to the presence of pulse wave reflections [5,26,27].

In the present study, multiple linear regression analysis in different model showed that diabetes was the common independent risk factor affecting CIMT and PWV-ES. Importantly, apart from age and hypertension, neither diabetes nor hyperlipidemia was independent risk factor associated with increased PWV-BS. Previous study has demonstrated that systolic PWV might be more suited to quantify changes in arterial stiffness than diastolic PWV [28]. Correspondingly, both PWV-BS and PWV-ES represented the systolic PWV. However, another study suggests that aorta arterial stiffness in late systole is a good alternative due to less susceptible to disturbance from peripheral reflections [29]. Although carotid PWV is not equal to aortic PWV, we indeed found that T2DM was independently associated with increased PWV-ES other than PWV-BS. Furthermore, we also showed that the PWV-ES can distinguish T2DM group 1 from control group after adjusting for baseline covariates. Based on a recent review demonstrating that arterial stiffness can be increased in diabetic subject and even in pre-diabetic populations with impaired glucose tolerance [30], we speculate that PWV-ES other than PWV-BS is a more suited parameter to quantitatively assess the functional changes of carotid artery in T2DM patients. Our study showed that only age and hypertension were independent risk factors associated with increased PWV-BS. Thus, the validity of PWV-BS merits further study.

Current guidelines stated that CIMT > 0.9 mm can be regarded as an existing abnormalities [31], while another recent multicenter study showed that the upper 97.5th percentiles of CIMT was less than 0.8 mm in healthy individuals with age < 50 years old regardless of gender [21]. Thus, the one-size-fits-all criterion (CIMT > 0.9 mm) might omit existed morphological change, especially in young and middle-aged individuals with atherosclerosis risk factors. In our study, normal or thickened CIMT was judged on the basis of the recent study [21]. One study based on a murine atherosclerosis model showed that aorta stiffening might precede IMT thickening [32]. Another study of carotid properties using speckle-tracking method verified analogous findings in hypertension patients other than T2DM patients [18]. In our study, to elucidate carotid properties for distinguishing diabetic subjects with normal CIMT from controls, potential influence of other atherosclerosis risk factors should be excluded. Thus, patients with hypertension and/or hyperlipidemia were not included as T2DM group 1. Although the CIMT of T2DM group 1 was within normal reference, it was thicker than that of control group. Moreover, patients with T2DM showed more advanced functional changes than morphological changes compared with controls before CIMT thickening. However, after adjusting for baseline covariables, only the PWV-ES, instead of CIMT, showed significant difference between T2DM group 1 and control group, indicating that carotid artery stiffening assessed by ultrafast ultrasound imaging may precede CIMT thickening in patients with type 2 diabetes, which was contrary to the results of previous study [18]. We speculated that different technique and inclusion criteria might be explanations for the discordance. Nevertheless, apart from morphological changes, other mechanism may regulate arterial stiffness in diabetes.

Previous study has demonstrated that T2DM patients showed more advanced carotid morphological changes than aorta functional changes when compared with control subjects [33]. However, there was no study specifically designed for type 2 diabetes concerning on the variation tendency between functional changes and morphological changes in different stage of atherosclerosis. In our study, compared with controls after adjusting for baseline covariates, T2DM patients showed more advanced functional changes than morphological changes before intima-media thickening, whereas associations between PWV-ES and CIMT was no longer present after intima-media thickening. In T2DM patients, hyperlipidemia and abnormality of HDL lipoprotein and LDL lipoprotein (dyslipidemia) often coexist. Our study showed that hyperlipidemia and decreased HDL cholesterol were independent risk factors associated with increased CIMT, which was in line with previous study [33]. It remains unclear whether dyslipidemia and development of atherosclerotic lesions alone contribute to vessel stiffening. However, the dissociation between these wall changes in our study implies, to some extent, that carotid thickening and stiffening

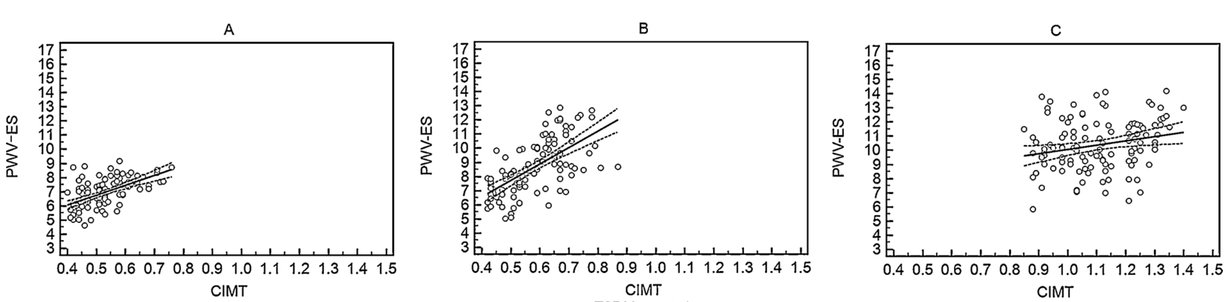

Fig. 4. Scatter diagrams and univariate associations between CIMT and PWV-ES in control group (A; r = 0.575, *P* < 0.001), T2DM group 1 (B; r = 0.634, *P* < 0.001), and T2DM group 2 (C; r = 0.229, *P* = 0.014).

may involve different atherosclerotic processes.

This study had some limitations. Firstly, we did not compare ultrafast ultrasound imaging to other imaging modalities measuring local carotid stiffness. Although echo-tracking technique is commonly used to evaluate carotid stiffness, it requires dedicated equipment and is time consuming, which limits its clinical practice. Secondly, part of control subjects has been accompanied by atherosclerosis risk factors including smoking, abnormality of HDL lipoprotein and LDL lipoprotein. Thirdly, considerable patients in T2DM group 2 were taking antihypertensive and/or antihyperlipidemic drugs. The possibility of the impact of the drugs on the carotid properties can not be excluded. Fourthly, thickening IMT or plaque in the femoral artery was detected in some subjects out of the T2DM subgroup with normal CIMT. In other words, the CIMT do not reflect the whole atherosclerotic burden of the artery tree.

In conclusion, our study demonstrated PWV-ES other than PWV-BS is a more suited parameter to quantitatively assess the functional changes of carotid artery in T2DM patients. Additionally, our results provide earlier detection of functional changes in T2DM patients despite a normal CIMT. Finally, we also showed that carotid artery stiffening might be not parallel with intima-media thickening in diabetic patients. These findings might be help to stratify patients with CVD risk factors.

Conflict of interest

None.

References

[1] N. Sarwar, P. Gao, S.R. Seshasai, R. Gobin, S. Kaptoge, E. Di Angelantonio, E. Ingelsson, D.A. Lawlor, E. Selvin, M. Stampfer, C.D. Stehouwer, S. Lewington, L. Pennells, A. Thompson, N. Sattar, I.R. White, K.K. Ray, J. Danesh, Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies, Lancet 375 (9733) (2010) 2215–2222.

[2] M.W. Lorenz, H.S. Markus, M.L. Bots, M. Rosvall, M. Sitzer, Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis, Circulation 115 (4) (2007) 459–467.

[3] D.C. Goff Jr., D.M. Lloyd-Jones, G. Bennett, S. Coady, R.B. D'Agostino Sr., R. Gibbons, P. Greenland, D.T. Lackland, D. Levy, C.J. O'Donnell, J.G. Robinson, J.S. Schwartz, S.T. Shero, S.C. Smith Jr., P. Sorlie, N.J. Stone, P.W. Wilson, 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, J. Am. Coll. Cardiol. 63 (25 Pt B) (2014) 2935–2959.

[4] M.W. Lorenz, J.F. Price, C. Robertson, M.L. Bots, J.F. Polak, H. Poppert, M. Kavousi, M. Dorr, E. Stensland, P. Ducimetiere, K. Ronkainen, S. Kiechl, M. Sitzer, T. Rundek, L. Lind, J. Liu, G. Bergstrom, L. Grigore, L. Bokemark, A. Friera, D. Yanez, H. Bickel, M.A. Ikram, H. Volzke, S.H. Johnsen, J.P. Empana, T.P. Tuomainen, P. Willeit, H. Steinmetz, M. Desvarieux, W. Xie, C. Schmidt, G.D. Norata, C. Suarez, D. Sander, A. Hofman, U. Schminke, E. Mathiesen, M. Plichart, J. Kauhanen, J. Willeit, R.L. Sacco, S. McLachlan, D. Zhao, B. Fagerberg, A.L. Catapano, R. Gabriel, O.H. Franco, A. Bulbul, F. Scheckenbach, A. Pflug, L. Gao, S.G. Thompson, Carotid intima-media thickness progression and risk of vascular events in people with diabetes: results from the PROG-IMT collaboration, Diabetes Care 38 (10) (2015) 1921–1929.

[5] T. Fukui, K.H. Parker, T. Yamaguchi, Pulse Wave Propagation in Large Blood Vessels Based on Fluid-solid Interactions Methods, (2012).

[6] S. Laurent, J. Cockcroft, L. Van Bortel, P. Boutouyrie, C. Giannattasio, D. Hayoz, B. Pannier, C. Vlachopoulos, I. Wilkinson, H. Struijker-Boudier, Expert consensus document on arterial stiffness: methodological issues and clinical applications, Eur. Heart J. 27 (21) (2006) 2588–2605.

[7] J. Luo, R.X. Li, E.E. Konofagou, Pulse wave imaging of the human carotid artery: an in vivo feasibility study, IEEE Trans. Ultrason. Ferroelectr. Freq. Control 59 (1) (2012) 174–181.

[8] J. Vappou, J. Luo, E.E. Konofagou, Pulse wave imaging for noninvasive and quantitative measurement of arterial stiffness in vivo, Am. J. Hypertens. 23 (4) (2010) 393–398.

[9] M. Tanter, M. Fink, Ultrafast imaging in biomedical ultrasound, IEEE Trans. Ultrason. Ferroelectr. Freq. Control 61 (1) (2014) 102–119.

[10] M. Couade, C. Flanagan, W.N. Lee, E. Messas, M. Fink, M. Pernot, M. Tanter, Evaluation of local arterial stiffness using ultrafast imaging: a comparative study using local arterial pulse wave velocity estimation and shear wave imaging, Ultrasonics Symposium, (2010), pp. 475–478.

[11] G. Montaldo, M. Tanter, J. Bercoff, N. Benech, M. Fink, Coherent plane-wave compounding for very high frame rate ultrasonography and transient elastography, IEEE Trans. Ultrason. Ferroelectr. Freq. Control 56 (3) (2009) 489–506.

[12] E. Messas, M. Pernot, M. Couade, Arterial wall elasticity: state of the art and future prospects, Diagn. Interv. Imaging 94 (5) (2013) 561–569.

[13] T. Mirault, M. Pernot, M. Frank, M. Couade, R. Niarra, M. Azizi, J. Emmerich, X. Jeunemaitre, M. Fink, M. Tanter, E. Messas, Carotid stiffness change over the cardiac cycle by ultrafast ultrasound imaging in healthy volunteers and vascular Ehlers-Danlos syndrome, J. Hypertens. 33 (9) (2015) 1890–1896 discussion 1896.

[14] X. Li, J. Jiang, H. Zhang, H. Wang, D. Han, Q. Zhou, Y. Gao, S. Yu, Y. Qi, Measurement of carotid pulse wave velocity using ultrafast ultrasound imaging in hypertensive patients, J. Med. Ultrason. 44 (2) (2001) 183–190 (2017).

[15] F.S. Pan, L. Yu, J. Luo, R.D. Wu, M. Xu, J.Y. Liang, Y.L. Zheng, X.Y. Xie, Carotid artery stiffness assessment by ultrafast ultrasound imaging: feasibility and potential influencing factors, J. Ultrasound Med. (2018).

[16] Z.Q. Zhu, L.S. Chen, H. Wang, F.M. Liu, Y. Luan, L.L. Wu, N. Liu, P. Wang, H. Huang, Carotid stiffness and atherosclerotic risk: non-invasive quantification with ultrafast ultrasound pulse wave velocity, Eur. Radiol. (2018).

[17] H. Okimoto, Y. Ishigaki, Y. Koiwa, Y. Hinokio, T. Ogihara, S. Suzuki, H. Katagiri, T. Ohkubo, H. Hasegawa, H. Kanai, Y. Oka, A novel method for evaluating human carotid artery elasticity: possible detection of early stage atherosclerosis in subjects with type 2 diabetes, Atherosclerosis 196 (1) (2008) 391–397.

[18] S.A. Kim, S.H. Park, S.H. Jo, K.H. Park, H.S. Kim, S.J. Han, W.J. Park, J.W. Ha, Alterations of carotid arterial mechanics preceding the wall thickening in patients with hypertension, Atherosclerosis 248 (2016) 84–90.

[19] Standards of medical care in diabetes–2008, Diabetes Care 31 (Suppl (1)) (2008) S12–54.

[20] J.H. Stein, C.E. Korcarz, R.T. Hurst, E. Lonn, C.B. Kendall, E.R. Mohler, S.S. Najjar, C.M. Rembold, W.S. Post, Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine, J. Am. Soc. Echocardiogr. 21 (2) (2008) 93–111 quiz 189-90.

[21] L. Engelen, I. Ferreira, C.D. Stehouwer, P. Boutouyrie, S. Laurent, Reference intervals for common carotid intima-media thickness measured with echotracking: relation with risk factors, Eur. Heart J. 34 (30) (2013) 2368–2380.

[22] G. Howard, A.R. Sharrett, G. Heiss, G.W. Evans, L.E. Chambless, W.A. Riley, G.L. Burke, Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. ARIC Investigators, Stroke 24 (9) (1993) 1297–1304.

[23] C.P. Johnson, R. Baugh, C.A. Wilson, J. Burns, Age related changes in the tunica media of the vertebral artery: implications for the assessment of vessels injured by trauma, J. Clin. Pathol. 54 (2) (2001) 139–145.

[24] T.T. van Sloten, S. Sedaghat, S. Laurent, G.M. London, B. Pannier, M.A. Ikram, M. Kavousi, F. Mattace-Raso, O.H. Franco, P. Boutouyrie, C.D.A. Stehouwer, Carotid stiffness is associated with incident stroke: a systematic review and individual participant data meta-analysis, J. Am. Coll. Cardiol. 66 (19) (2015) 2116–2125.

[25] R. Ravikumar, R. Deepa, C. Shanthirani, V. Mohan, Comparison of carotid intima-media thickness, arterial stiffness, and brachial artery flow mediated dilatation in diabetic and nondiabetic subjects (the Chennai Urban Population Study [CUPS-9]), Am. J. Cardiol. 90 (7) (2002) 702–707.

[26] E.G. Brennan, N.J. O'Hare, M.J. Walsh, Transventricular pressure-velocity wave propagation in diastole: adherence to the Moens-Korteweg equation, Physiol. Meas. 19 (1) (1998) 117–123.

[27] M. McGarry, P. Nauleau, I. Apostolakis, E. Konofagou, In vivo repeatability of the pulse wave inverse problem in human carotid arteries, J. Biomech. 64 (2017) 136–144.

[28] E. Hermeling, A.P. Hoeks, M.H. Winkens, J.L. Waltenberger, R.S. Reneman, A.A. Kroon, K.D. Reesink, Noninvasive assessment of arterial stiffness should discriminate between systolic and diastolic pressure ranges, Hypertension 55 (1) (2010) 124–130.

[29] E. Hermeling, K.D. Reesink, R.S. Reneman, A.P. Hoeks, Confluence of incident and reflected waves interferes with systolic foot detection of the carotid artery distension waveform, J. Hypertens. 26 (12) (2008) 2374–2380.

[30] S.B. Prenner, J.A. Chirinos, Arterial stiffness in diabetes mellitus, Atherosclerosis 238 (2) (2015) 370–379.

[31] G. Mancia, G. De Backer, A. Dominiczak, R. Cifkova, R. Fagard, G. Germano, G. Grassi, A.M. Heagerty, S.E. Kjeldsen, S. Laurent, K. Narkiewicz, L. Ruilope, A. Rynkiewicz, R.E. Schmieder, H.A. Struijker Boudier, A. Zanchetti, A. Vahanian, J. Camm, R. De Caterina, V. Dean, K. Dickstein, G. Filippatos, C. Funck-Brentano, I. Hellemans, S.D. Kristensen, K. McGregor, U. Sechtem, S. Silber, M. Tendera, P. Widimsky, J.L. Zamorano, S.E. Kjeldsen, S. Erdine, K. Narkiewicz, W. Kiowski, E. Agabiti-Rosei, E. Ambrosioni, R. Cifkova, A. Dominiczak, R. Fagard, A.M. Heagerty, S. Laurent, L.H. Lindholm, G. Mancia, A. Manolis, P.M. Nilsson, J. Redon, R.E. Schmieder, H.A. Struijker-Boudier, M. Viigimaa, G. Filippatos, S. Adamopoulos, E. Agabiti-Rosei, E. Ambrosioni, V. Bertomeu, D. Clement, S. Erdine, C. Farsang, D. Gaita, W. Kiowski, G. Lip, J.M. Mallion, A.J. Manolis, P.M. Nilsson, E. O'Brien, P. Ponikowski, J. Redon, F. Ruschitzka, J. Tamargo, P. van Zwieten, M. Viigimaa, B. Waeber, B. Williams, J.L. Zamorano, The task force for the management of arterial hypertension of the European Society of, C. The task force for the management of arterial hypertension of the European Society of, 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC), Eur. Heart J. 28 (12) (2007) 1462–1536.

[32] A. Gotschy, E. Bauer, C. Schrodt, G. Lykowsky, Y.X. Ye, E. Rommel, P.M. Jakob, W.R. Bauer, V. Herold, Local arterial stiffening assessed by MRI precedes atherosclerotic plaque formation, Circ. Cardiovasc. Imaging 6 (6) (2013) 916–923.

[33] H. Taniwaki, T. Kawagishi, M. Emoto, T. Shoji, H. Kanda, K. Maekawa, Y. Nishizawa, H. Morii, Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes. Vessel wall properties in type 2 diabetes, Diabetes Care 22 (11) (1999) 1851–1857.