Available online at
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Diabetes & Metabolism 38 (2012) 413–419

Original article

Relationship between tissue glycation measured by autofluorescence and pulse wave velocity in young and elderly non-diabetic populations

G. Watfa [a,b,c,d], G. Soulis [a], E. Tartagni [e], A. Kearney-Schwartz [a], C. Borghi [e], P. Salvi [a,e], A. Benetos [a,b,c,d,*]

[a] *Department of Geriatrics, University Hospital of Nancy, 54500 Vandœuvre-lès-Nancy, France*
[b] *Inserm, U961, Faculty of Medicine, Nancy, France*
[c] *University of Nancy, Nancy, France*
[d] *University Hospital of Nancy & Inserm Clinical Investigation Centre, CIC 9501, Nancy, France*
[e] *Internal Medicine, Aging and Kidney Disease Department, University of Bologna, Bologna, Italy*

Received 3 January 2012; received in revised form 6 April 2012; accepted 6 April 2012

Abstract

Objective. – Advanced glycation end-products (AGEs) and pulse wave velocity (PWV) are pivotal indices of the processes of arterial ageing and damage accumulation. The aim of the present study was to investigate the impact of AGEs, as measured by a non-invasive skin autofluorescence method, on arterial stiffness, estimated by PWV, in two different age groups of non-diabetic subjects.

Methods and patients. – A total of 116 non-diabetic subjects were classified into two groups, with 55 subjects in the group aged < 65 years and 61 in the group aged ≥ 65 years. AGEs were measured by skin autofluorescence while carotid–femoral PWV was assessed by tonometry.

Results. – A significant (positive) association was observed between PWV and AGE skin autofluorescence in the younger age group ($r = 0.51$; $P < 0.0001$). However, this association was no longer significant after further adjustments for age and other factors on multiple regression analyses. In contrast, this correlation was not found in the elderly group ($r = 0.098$; $P = 0.454$).

Conclusion. – Younger non-diabetic subjects exhibit a different correlation profile between AGEs accumulated in skin and cfPWV as an index of arterial stiffness compared with elderly subjects. AGEs were significantly associated with cfPWV in younger individuals, but not in the elderly. A further study with a larger number of subjects is proposed to confirm the contribution of AGEs, the formation of which is manageable, as a determinant of arterial stiffness in younger subjects.

Keywords: AGEs; PVW; Pulse wave velocity; Elderly; Non-diabetic

Résumé

Relation entre la glycation tissulaire mesurée par autofluorescence et la vitesse de l'onde de pouls dans des populations non diabétiques, jeunes et âgées.

Objectif. – Les produits de la glycation avancée (AGEs) et la vitesse de l'onde de pouls (VOP) sont des indices pivots du processus du vieillissement artériel et de l'accumulation de dommages tissulaires. Le but de la présente étude était d'étudier l'impact des AGEs, mesurés par une méthode non invasive de mesure de l'autofluorescence cutanée, sur la rigidité artérielle évaluée par la VOP dans deux groupes de sujets non diabétiques et d'âge différents.

Méthodes et patients. – Cent seize sujets non diabétiques ont été classés en deux groupes : 55 sujets dans le groupe sujets jeunes (< 65 ans) et 61 dans le groupe sujets âgés (≥ 65 ans). Les AGEs ont été mesurés par autofluorescence cutanée et la VOP carotido-fémorale a été évaluée par tonométrie.

Résultats. – Une relation positive significative a été observée entre la VOP et les AGEs mesurés par autofluorescence cutanée dans le groupe des sujets jeunes ($r = 0{,}51$, $P < 0{,}0001$). Cette relation n'était plus significative après ajustement sur l'âge et d'autres facteurs par l'analyse de régression multiple. En revanche, cette corrélation n'a pas été observée dans le groupe des sujets âgés ($r = 0{,}098$, $P = 0{,}454$).

* Corresponding author. Tel.: +33 3 83 15 33 22; fax: +33 3 83 15 76 68.
E-mail address: a.benetos@chu-nancy.fr (A. Benetos).

1262-3636/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.diabet.2012.04.004

Conclusion. – Les sujets non diabétiques jeunes présentent un profil de corrélation différent entre l'accumulation des AGEs et la VOP (comme indice de la rigidité artérielle) comparativement à ce qui a été observé chez les sujets âgés. Les AGEs sont significativement corrélés à la VOP chez les sujets jeunes, mais non chez les sujets âgés. Une étude complémentaire portant sur un plus grand nombre de sujets est nécessaire pour confirmer la contribution des AGEs, dont la formation est régulée comme un déterminant de la rigidité artérielle chez les sujets jeunes.

Mots clés : AGEs ; VOP ; Vitesse de l'onde de pouls ; Personnes âgées ; Non diabétiques

1. Abbreviations

AGEs	advanced glycation end-products
AS	arterial stiffness
a.u.	arbitrary units
BMI	Body Mass Index
bpm	beats per minute
cfPWV	carodito-femoral pulse wave velocity
DBP	diastolic blood pressure
ESRD	End-stage renal disease
HDL	high-density lipoproteins
HR	heart rate
LDL	low-density lipoproteins
MBP	mean blood pressure
PP	pulse pressure
SBP	systolic blood pressure
SD	standard deviation

2. Introduction

When Maillard [1] first described, in 1912, the respective reaction between glucose and amino acids forming a bond between the sugar and amino-acid groups of a protein, he could not imagine the biological significance of the reaction. Products of the Maillard reaction are commonly known as 'advanced glycation end-products' (AGEs), and are the final products of the non-enzymatic glycation reaction. The magnitude of accumulation of these non-enzymatic glycation products is dependent on glucose levels and duration of exposure [2].

The Maillard hypothesis for diabetes proposes that AGE formation impairs tissue protein function and structure [3], and is a hallmark of diabetic complications. Indeed, increased plasma levels of circulating AGEs are correlated with increased mortality and cardiovascular disease in type 1 diabetes patients [4]. In animal models, treatment with well-known AGE cross-link breakers has been shown to improve arterial and myocardial stiffness and cardiac function [5,6].

Recently, better characterization of the complex AGE structure and the evolution of specific analytical and biosynthetic methods have allowed the accurate quantification of various AGEs as markers of glycation stress in biological samples [7]. However, given that the blood and urine levels of these various AGEs do not necessarily reflect tissue levels, novel non-invasive techniques have been developed and validated that permit better analysis of tissue AGE accumulation. Skin autofluorescence measurement is one such technique, and has been demonstrated to be a rapid, inexpensive and accurate means of estimating AGE accumulation in various white subpopulations with and without disease [8].

Numerous chronic disorders such as diabetes and atherosclerosis are known to precipitate arterial wall ageing, a process marked by central arterial wall stiffness [9]. A convenient method for estimating arterial rigidity is carotid–femoral pulse wave velocity (cfPWV), a measure of intrinsic stiffness of the aortic wall, for which reference and normal values have recently been established [10]. It has been demonstrated that cfPWV values are predictive of cardiovascular disorders such as primary coronary events in patients with hypertension [11], and are considered a valuable prognostic tool [12]. Recently, cfPWV was also found to correlate with plasma levels of AGEs in non-diabetic subjects [13], and it was demonstrated that antihypertensive treatment lowered cfPWV [14]. Thus, these two newly emergent physiological parameters represent pivotal indices of the processes of ageing and damage accumulation in diabetic, hypertensive and other generally ill populations.

With ageing, there is an increase in both aortic stiffness and AGE production. It is therefore of interest to investigate whether the possible relationships between AGEs and PWV differ between younger and older non-diabetic subjects. Thus, the aim of the present study was to investigate the impact of skin AGEs, as measured by a non-invasive skin autofluorescence method, on aortic stiffness, as estimated by cfPWV, in two different age groups of non-diabetic subjects as potential markers of arterial ageing.

3. Methods

The older participants (61 subjects aged ≥ 65 years) were consecutively recruited from the geriatrics department at the University Hospital of Nancy, while the younger participants (55 subjects aged < 65 years) were derived from the cohort attending the final visit of the Brisighella Heart Study [15]. Briefly, participants were included if they were free of diabetes and had signed an informed consent form. Subjects were excluded if they had a poor health status.

Supine systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured on the left arm after a minimum 10-min rest period. Blood pressure was measured three times, from which the average of the three measurements was derived. Heart rate (HR) was assessed in beats per minute (bpm). A nurse measured height and weight, and calculated the body mass index (BMI) of all participating individuals. Also, total serum cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, creatinine and fasting blood glucose were all measured the same day after

an overnight fast, using a standardized venous blood-sampling technique.

AGE skin autofluorescence (AF) was assessed with the AGE Reader (DiagnOptics, Groningen, The Netherlands) as described previously [16–18], and expressed in arbitrary units (a.u.). This is a completely automated method requiring no intervention by the examiner. In addition, the values presented here are the means of three consecutive measurements. In the original AGE Reader publication [18], it had been demonstrated that repeated measurements over a single day had an overall Altman error percentage of 5.03%. The PulsePen device (DiaTecne srl, Milan, Italy) was used for measuring cfPWV [19,20], using the procedure as previously detailed elsewhere [20].

3.1. Statistical analysis

Descriptive values are expressed as either means ± standard deviation (SD) or number and percentages. Variables were compared using Student's *t* test or the Chi-square test as appropriate. A P value < 0.05 was regarded as statistically significant. Statistical analyses were performed using the NCSS 2000 statistical software package (Kaysville, UT, USA). Univariate correlations were performed using Pearson's parametric analysis.

Analyses of the factors associated with cfPWV and AGEs were performed using multiple regression analyses and an interactive backward-selection method. Validity of the model assumption was verified using analysis of model residuals and testing for heteroscedasticity.

4. Results

4.1. Study population characteristics

The clinical and biological characteristics of the present study population are shown in Table 1. Of the 116 subjects enrolled, women represented 53%. Also, in Table 1, the data are presented according to a cut-off at age 65 years (total mean age: 64 ± 17 years). Women represented 47% and 57% of the younger (< 65 years) and elderly (≥ 65 years) groups, respectively. The younger subjects were taller and heavier, but did not differ in terms of BMI compared with the older participants who, in turn, had higher SBP, pulse pressure (PP), cfPWV and AGE skin AF values. Smoking habits also did not differ, whereas a history of hypertension was more common in the elderly subpopulation. Except for total cholesterol, which was higher in the younger subgroup, the two subgroups did not differ significantly in terms of overall biological parameters.

A significant (positive) association was observed between cfPWV and AGE skin AF in the total population (r = 0.449; P = 0.000001) and in the younger age group (r = 0.51; P < 0.0001) (Fig. 1). In contrast, such a correlation was not found in the elderly subjects (r = 0.098; P = 0.45; Fig. 1). Separate analyses of the younger and older subjects not receiving antihypertensive or lipid-lowering treatment showed similar results: there was a relationship between AGEs and PWV observed only in the younger subgroup (data not shown).

Table 1
Clinical and biological characteristics of the study population.

	Total	< 65 years	≥ 65 years	P*
Number	116	55	61	
Age (years)	64.0 ± 17.0	49.1 ± 10.4	77.5 ± 8.4	
Gender (women, %)	53	47	57	0.276
Weight (kg)	71.8 ± 16.7	77.2 ± 17.9	67.0 ± 14.2	0.001
Height (m)	1.65 ± 0.09	1.69 ± 0.09	1.62 ± 0.09	0.0001
Body mass index (kg/m^2)	26.2 ± 5.0	27.2 ± 5.5	25.3 ± 4.5	0.058
Hypertension history (%)	49	29	67	< 0.0001
Treated hypertension (%)	44	25	61	0.0001
Past or current smoker (%)	52	61	43	0.058
Dyslipidaemia history (%)	47	53	43	0.276
Treated dyslipidaemia (%)	26	20	31	0.171
Cardiovascular history (n)	31	7	24	0.001
CHD history	6	1	5	0.126
SBP (mmHg)	136.4 ± 20.4	130.9 ± 16.3	141.3 ± 22.4	0.005
DBP (mmHg)	78.7 ± 10.9	80.1 ± 9.2	77.4 ± 12.2	0.187
MBP (mmHg)	97.0 ± 15.5	97.0 ± 10.7	97.0 ± 18.9	0.478
Pulse pressure (mmHg)	57.7 ± 15.7	50.8 ± 11.8	63.9 ± 16.3	< 0.0001
Heart rate (bpm)	66.3 ± 14.3	63.6 ± 11.2	68.7 ± 16.3	0.052
AGE skin AF (a.u.)	2.45 ± 0.62	2.11 ± 0.45	2.75 ± 0.60	< 0.0001
cfPWV (m/s)	9.76 ± 3.95	7.48 ± 1.92	11.83 ± 4.17	< 0.0001
Glucose (g/L)	0.97 ± 0.13	0.98 ± 0.11	0.96 ± 0.14	0.611
Cholesterol (g/L)	1.95 ± 0.48	2.07 ± 0.45	1.84 ± 0.49	0.028
LDL cholesterol (g/L)	1.33 ± 0.39	1.38 ± 0.41	1.26 ± 0.36	0.178
HDL cholesterol (g/L)	0.48 ± 0.14	0.45 ± 0.10	0.51 ± 0.17	0.060
Triglycerides (g/L)	1.12 ± 0.76	1.21 ± 0.95	1.04 ± 0.55	0.315
Creatinine (mg/L)	10.64 ± 2.46	10.14 ± 1.80	11.08 ± 2.86	0.070

Data are expressed as means ± SD and as percentages (%); *Student's *t* test (continuous variables) or Chi-square test (categorical variables); CHD: coronary heart disease; SBP/DBP/MBP: systolic/diastolic/mean blood pressure; bpm: beats per min; cfPWV: carotid–femoral pulse wave velocity; AGE: advanced glycation end-product; AF: autofluorescence; a.u.: arbitrary units; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

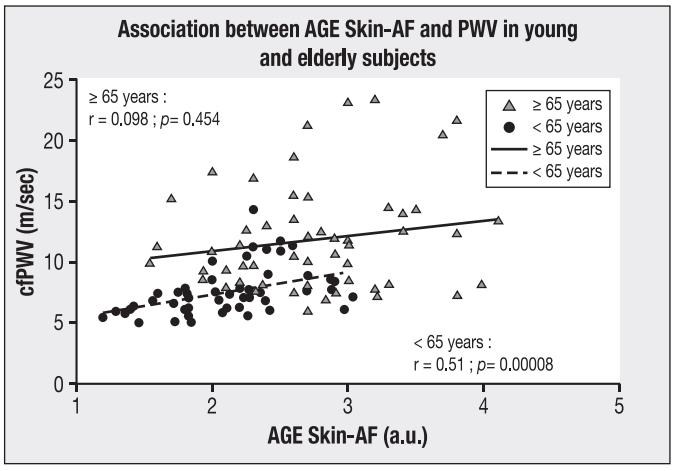

Fig. 1. Association between glycation and arterial stiffness in younger and elderly non-diabetic subjects. cfPWV: carotid–femoral pulse wave velocity; AGE: advanced glycation end-product; AF: autofluorescence; a.u.: arbitrary units.

In the younger subjects, the significant univariate correlates of cfPWV included BMI (r = 0.386; $P = 0.006$), dyslipidaemia (r = 0.38; $P = 0.004$), SBP (r = 0.556; $P = 0.0001$), DBP (r = 0.525; $P < 0.0001$), mean blood pressure (MBP; r = 0.569; $P < 0.00001$), PP (r = 0.406; $P = 0.002$) and HR (r = 0.443; $P = 0.0007$), with a trend towards an association with age (r = 0.262; $P = 0.053$). In the elderly group, the significant univariate correlates of cfPWV were age (r = 0.407; $P = 0.001$), smoking history (r = −0.303; $P = 0.019$), DBP (r = 0.279; $P = 0.029$) and MBP (r = 0.285; $P = 0.026$), with a trend towards associations with SBP (r = 0.246; $P = 0.055$) and HR (r = 0.22; $P = 0.088$).

According to the American Diabetes Association, prediabetic subjects are considered to be those who have fasting plasma glucose (FPG) levels > 1 g/L and < 1.25 g/L [21]. Our subjects were therefore divided, using the aforementioned values of measured FPG as cut-off points, into two further subgroups: prediabetic and normoglycaemic. No significant differences in skin AF measurements, using the AGE Reader, were observed between these two groups when compared by respective age category or when pooled together (Fig. 2).

To further investigate our primary findings regarding the determinants of cfPWV in younger and elderly non-diabetic subjects, additional multiple regression analyses were performed after adjusting for all variables associated with PWV in the univariate analyses described above. In younger subjects (Table 2), the correlation between cfPWV and AGEs disappeared after further adjustments for age and MBP, whereas the presence of dyslipidaemia was a significant determinant of cfPWV in this population. In the elderly group, age and HR were positively associated with cfPWV (Table 3).

Similar multiple analyses performed for AGE skin AF as a dependent variable resulted in a model correlating age and smoking history with skin glycation levels, as measured by AF, in both younger (r = 0.024, $P = 0.00001$ and r = 0.218, $P = 0.041$, respectively) and older (r = 0.332, $P = 0.03$ and r = 0.0372, $P = 0.01$, respectively) subjects.

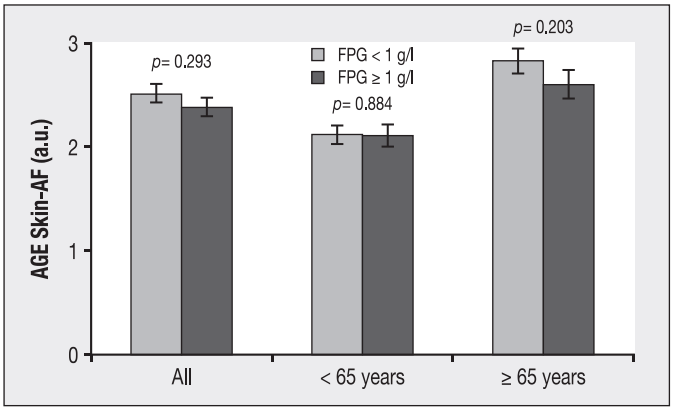

Fig. 2. Skin autofluorescence (AF) measurements comparing prediabetic and normoglycaemic subjects in different age subgroups. FPG: fasting plasma glucose; AGE: advanced glycation end-product; a.u.: arbitrary units.

Table 2
Multiple linear regression analyses using carotid–femoral pulse wave velocity (cfPWV) as a dependent variable in younger non-diabetic subjects aged < 65 years.

Source	Regression coefficient	Partial r^2	P
Intercept	−2.68 ± 1.95		0.176572
Age (years)	0.06 ± 0.02	0.157	0.003296
Dyslipidaemia history (%)	0.96 ± 0.42	0.093	0.026078
MBP (mmHg)	0.07 ± 0.02	0.192	0.001042
r^2	0.436		

MBP: mean blood pressure.

Table 3
Multiple linear regression analyses using carotid–femoral pulse wave velocity (cfPWV) as a dependent variable in elderly subjects aged ≥ 65 years.

Source	Regression coefficient	Partial r^2	P
Intercept	−4.20 ± 4.83		0.388
Age (years)	0.15 ± 0.06	0.999	0.014
HR (bpm)	0.06 ± 0.03	0.065	0.049
r^2	0.174		

HR: heart rate; bpm: beats per min.

5. Discussion

The present study aimed to non-invasively measure biomarkers of ageing in both younger and elderly non-diabetic subjects. Our present findings have added new details regarding the involvement of glycation product accumulation in arterial stiffness as evaluated by cfPWV. Indeed, the present results demonstrate that younger non-diabetic subjects exhibit a different correlation profile between AGEs accumulated in skin (as measured by AF) and cfPWV (as an index of arterial rigidity) compared with older non-diabetic subjects. In fact, AGEs were found to be significantly associated with cfPWV in the younger population, but not in the elderly. However, this association was not significant after multivariate adjustments for other covariates, particularly MBP.

In untreated younger hypertensive patients, McNulty et al. [22] demonstrated a positive correlation between plasma AGE

levels and PWV. A similar correlation was also found in relatively healthy subjects between the specific AGE serum carboxymethyl-lysine (CML) and PWV [13], whereas a positive association was demonstrated in type 1 diabetes patients between AGEs and PP, an indirect index of arterial stiffness [23].

On further analysis, a discrepancy was found between our younger and older subpopulations in terms of cfPWV determination that persisted even after multiple linear regression analysis for risk factors. In the younger subjects, age, MBP and the presence of dyslipidaemia were independent determinants of aortic stiffness while, in the elderly group, only age and HR were linked with cfPWV. Differences among distinct age groups with regard to parameters determining arterial stiffness have also been shown for another index, the augmentation index, which in younger subjects was correlated with somatometric and cardiovascular constituents different than those found in subjects aged > 60 years. In fact, the association of the augmentation index with classical risk factors for cardiovascular diseases (CVD) was attenuated in the elderly group [24].

The present results observed in our younger subjects suggest that the increase in arterial stiffness primarily stems from elevated BP, as MBP accounted for 19% of the variation in PWV in this age group (Table 2). Despite a significant contribution of AGEs in PWV determination with age, multivariate analyses showed that this contribution did not persist after taking MBP levels into account. However, several—but not all—studies have shown an involvement of dyslipidaemia in PWV determination [25], whereas HR was found to be independently associated with cfPWV in the elderly, but not in the younger population. Indeed, the presence of an increased HR is one of the major determinants of accelerating progression to aortic stiffness [26].

In a 2008 study by Ueno et al. [17], the association between AGEs, measured by the same technique as used here, and PWV was examined in 120 non-diabetic patients with end-stage renal disease (ESRD), and in 110 age- and gender-matched control subjects with neither renal disease nor diabetes. However, they had included an extremely fragile group (the ESRD patients) whereas our present study excluded any subjects with extreme co-morbidities. For this reason, the only comparison that can be made between that study and ours concerns their control group and our younger population group. They observed a positive correlation between AGEs and PWV (with an r coefficient of 0.25) in their controls that was also found in our younger subjects, but with a stronger correlation (r coefficient of 0.51). Similarly, they also observed that, after multiple regression analysis adjusted for other covariates, this correlation in their control group disappeared, as did our present observation in our younger subjects.

Corman et al. [27] investigated the effect of AGE blockade using aminoguanidine in old normotensive rats in the prevention of arterial stiffness progression. Their study revealed the complexity of the underlying mechanism, as the amelioration of arterial stiffening following aminoguanidine treatment was not due to changes in the collagen and elastin contents of the arterial wall. However, as our present study was not interventional, it is not possible to propose AGE blockade in arterial wall stiffening in humans; furthermore, 67% of our present group of elderly subjects were hypertensive. This and other differences in study features may therefore help to explain the discrepancies between the above-reported results and those of our present study.

Extracellular matrix proteins are more susceptible to significant glycation even under conditions of mild hyperglycaemia because of their slow turnover rate [28], thus leading to the formation of cross-links and, consequently, to undesirable effects such as decreased vessel elasticity [22], and increased arterial thickness and rigidity [29].

Reference values have recently been established to facilitate a broader use of the present technique [10]. The PulsePen is a novel device that has been demonstrated to reliably measure cfPWV [30]. The skin AF reader, on the other hand, is a recently introduced apparatus that enables the non-invasive measurement of AGE accumulation. Moreover, skin AF levels have been shown to be independently related to mortality and coronary heart disease, and are increased in patients with renal failure and diabetes [31].

Some limitations of our present study should be noted. Although the present technique for measuring skin AF has been validated to represent accumulation of AGEs, it is possible that other skin fluorophores not evaluated by the technique may be interfering with skin AF measurements. The technique is also limited by the fact that not all AGEs exhibit fluorescent properties. In addition, it is possible that the absence of an association between AGEs and PWV in elderly subjects might be related to the different representation of AGE molecules in such a population [32]. However, skin fluorescence as measured by the AGE Reader does correlate with skin levels of both fluorescent and non-fluorescent AGEs, thereby suggesting that skin AF may be a marker of total pooled skin AGE status [18]. Nevertheless, taking into account the relative weaknesses of our present study—namely, the modest sample size and missing data on blood biology—it may be that this is just one small step towards revealing the multifaceted profile of arterial senility. Another limitation of our study is related to the different nationality backgrounds of the two populations compared.

In any case, the combination of these readily available, non-invasive techniques could prove invaluable in clinical practice for the early detection and/or assessment of arterial ageing and damage accumulation.

6. Conclusion

To our knowledge, this is one of the first studies to concomitantly measure AGEs using the skin AF technique and cfPWV in the elderly, one of the original points of our study. In subjects without diabetes, the study investigated the relationship between skin accumulation of AGEs and arterial stiffness, pathophysiological changes that are linked to unfavourable clinical outcomes. It may be hypothesized that, early in the process of arterial ageing, there are numerous risk factors influencing arterial rigidity, most of which are potentially reversible, such as MAP, or at least manageable, such as AGE production. There may be clinical significance in terms of allowing early interventions in the pathway leading to stiffness. The lack of association

between MBP and PWV in the older subjects may be related to the more frequent use of antihypertensive treatment in this age group that could have altered MBP and, to a lesser degree, PWV in this subpopulation. There was also the emergence of AGE levels as a pivotal determinant, apparently early in the process of arterial senescence, that may be of value in proleptically identifying and managing its progression to prevent any possible clinically important implications. Further longitudinal and interventional studies are needed to establish firm conclusions and develop therapeutic means for fighting against accelerated arterial ageing. The present authors are currently organizing a larger study in diabetic patients.

Thus, younger non-diabetic subjects exhibited a correlation profile between AGEs accumulated in skin, a marker of tissue senescence, and cfPWV, an index of arterial stiffness, that was different from that in elderly subjects. In particular, AGEs were found to be significantly associated with cfPWV in younger individuals compared with the older age group, although this finding remains to be confirmed by further investigations.

Disclosure of interest

Professor Paolo Salvi is a consultant for DiaTecne s.r.l. (Milan, Italy).

Acknowledgments

We thank Mr Pierre Pothier for his helpful suggestions and critical reading of the manuscript.The sources of support: Fondation pour la Recherche Medical (FRM), Plan Pluriformation (PPF).

Authors' contributions: G.W: Investigator, study concept and design, analysis and interpretation of data, writing the manuscript; G.S: Subject recruitment, analysis and interpretation of data, writing the manuscript; E.T: Subject recruitment, study concept and design, contribution in manuscript preparation; A.K.S: Chief investigator and methodology; C.B: Chief investigator, study concept and design; P.S: Chief investigator, methodology and subject recruitment; AB: Project Coordinator, study concept and design, analysis and interpretation of data, writing the manuscript.

References

[1] Maillard LC. Action des acides aminés sur les sucres : formation des mélanoïdines par voie méthodique. C R Acad Sci 1912;154:66–8.
[2] Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 1988;318:1315–21.
[3] Monnier VM. Nonenzymatic glycosylation, the Maillard reaction and the aging process. J Gerontol 1990;45:B105–11.
[4] Nin JW, Jorsal A, Ferreira I, Schalkwijk CG, Prins MH, Parving HH, et al. Higher plasma levels of advanced glycation end products are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study. Diabetes Care 2011;34:442–7.
[5] Asif M, Egan J, Vasan S, Jyothirmayi GN, Masurekar MR, Lopez S, et al. An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness. Proc Natl Acad Sci U S A 2000;97:2809–13.
[6] Wolffenbuttel BH, Boulanger CM, Crijns FR, Huijberts MS, Poitevin P, Swennen GN, et al. Breakers of advanced glycation end products restore large artery properties in experimental diabetes. Proc Natl Acad Sci U S A 1998;95:4630–4.
[7] Beisswenger PJ. Glycation and biomarkers of vascular complications of diabetes. Amino Acids 2012;42:1171–83.
[8] Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands JL, Smit A. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. Cardiovasc Diabetol 2008;7:29.
[9] Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler Thromb Vasc Biol 2005;25:932–43.
[10] Mattace-Raso FU, Hofman A, Verwoert GC, Wittemana JC, Wilkinson I, Cockcroft J, et al. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. Eur Heart J 2010;31:2338–50.
[11] Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;39: 10–5.
[12] Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. Circulation 2003;107:2864–9.
[13] Semba RD, Najjar SS, Sun K, Lakatta EG, Ferrucci L. Serum carboxymethyl-lysine, an advanced glycation end product, is associated with increased aortic pulse wave velocity in adults. Am J Hypertens 2009;22:74–9.
[14] Ong KT, Delerme S, Pannier B, Safar ME, Benetos A, Laurent S, et al. Aortic stiffness is reduced beyond blood pressure lowering by short-term and long-term antihypertensive treatment: a meta-analysis of individual data in 294 patients. J Hypertens 2011;29:1034–42.
[15] Cicero AF, Magni P, Lentini P, Ruscica M, Dozio E, Strollo F, et al. Sex hormones and adipokines in healthy pre-menopausal, post-menopausal and elderly women, and in age-matched men: data from the Brisighella Heart Study. J Endocrinol Invest 2011;34:e158–62.
[16] Lutgers HL, Gerrits EG, Graaff R, Links TP, Sluiter WJ, Gans RO, et al. Skin autofluorescence provides additional information to the UK Prospective Diabetes Study (UKPDS) risk score for the estimation of cardiovascular prognosis in type 2 diabetes mellitus. Diabetologia 2009;52: 789–97.
[17] Ueno H, Koyama H, Tanaka S, Fukumoto S, Shinohara K, Shoji T, et al. Skin autofluorescence, a marker for advanced glycation end product accumulation, is associated with arterial stiffness in patients with end-stage renal disease. Metabolism 2008;57:1452–7.
[18] Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL, et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. Diabetologia 2004;47:1324–30.
[19] Joly L, Perret-Guillaume C, Kearney-Schwartz A, Salvi P, Mandry D, Marie PY, et al. Pulse wave velocity assessment by external noninvasive devices and phase-contrast magnetic resonance imaging in the obese. Hypertension 2009;54:421–6.
[20] Salvi P, Lio G, Labat C, Ricci E, Pannier B, Benetos A. Validation of a new non-invasive portable tonometer for determining arterial pressure wave and pulse wave velocity: the PulsePen device. J Hypertens 2004;22: 2285–93.
[21] American Diabetes Association. Standards of medical care in diabetes–2011. Diabetes Care 2011;34(Suppl. 1):S11–61.
[22] McNulty M, Mahmud A, Feely J. Advanced glycation end-products and arterial stiffness in hypertension. Am J Hypertens 2007;20:242–7.
[23] Schram MT, Schalkwijk CG, Bootsma AH, Fuller JH, Chaturvedi N, Stehouwer CD. Advanced glycation end products are associated with pulse pressure in type 1 diabetes: the EURODIAB Prospective Complications Study. Hypertension 2005;46:232–7.
[24] Janner JH, Godtfredsen NS, Ladelund S, Vestbo J, Prescott E. The association between aortic augmentation index and cardiovascular risk factors in a large unselected population. J Hum Hypertens 2011, http://dx.doi.org/10.1038/jhh.2011.59.

[25] Benetos A, Waeber B, Izzo J, Mitchell G, Resnick L, Asmar R, et al. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. Am J Hypertens 2002;15:1101–8.

[26] Benetos A, Adamopoulos C, Bureau JM, Temmar M, Labat C, Bean K, et al. Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. Circulation 2002;105:1202–7.

[27] Corman B, Duriez M, Poitevin P, Heudes D, Bruneval P, Tedgui A, et al. Aminoguanidine prevents age-related arterial stiffening and cardiac hypertrophy. Proc Natl Acad Sci U S A 1998;95:1301–6.

[28] Vlassara H, Fuh H, Makita Z, Krungkrai S, Cerami A, Bucala R. Exogenous advanced glycosylation end products induce complex vascular dysfunction in normal animals: a model for diabetic and aging complications. Proc Natl Acad Sci U S A 1992;89:12043–7.

[29] Yoshida N, Okumura K, Aso Y. High serum pentosidine concentrations are associated with increased arterial stiffness and thickness in patients with type 2 diabetes. Metabolism 2005;54:345–50.

[30] Salvi P, Magnani E, Valbusa F, Agnoletti D, Alecu C, Joly L, et al. Comparative study of methodologies for pulse wave velocity estimation. J Hum Hypertens 2008;22:669–77.

[31] Mulder DJ, Water TV, Lutgers HL, Graaff R, Gans RO, Zijlstra F, et al. Skin autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced glycation endproducts: an overview of current clinical studies, evidence, and limitations. Diabetes Technol Ther 2006;8: 523–35.

[32] Peppa M, Uribarri J, Vlassara H. Aging and glycoxidant stress. Hormones (Athens) 2008;7:123–32.