

ORIGINAL ARTICLE

Reference values of one-point carotid stiffness parameters determined by carotid echo-tracking and brachial pulse pressure in a large population of healthy subjects

Olga Vriz¹, Victor Aboyans², Rosalba Minisini³, Julien Magne², Nicole Bertin¹, Mario Pirisi³ and Eduardo Bossone⁴

Arterial stiffness can predict cardiovascular events, and the aim of this study was to produce age- and sex-specific reference values for echo-tracking carotid stiffness in healthy subjects. A total of 900 subjects (500 males, mean age 45.8 ± 19 years) were enrolled. Common carotid artery stiffness and compliance, using a high-definition echo-tracking ultrasound system, were evaluated. To compare stiffness parameters across the different age groups, individual scores were transformed into *T*-scores, indicating how many standard deviation (s.d.) units an individual's score was above or below the mean that was observed in the group including same-sex individuals aged 36 to 44 years. Carotid stiffness was similar among genders, except compliance, which was lower in women ($P < 0.0001$). These characteristics were also maintained when the studied population was divided into seven age groups. Stiffness parameters increased significantly with age, but the opposite occurred for compliance. The *T*-score was found to increase significantly across all age groups, with a steeper increase in stiffness around the age of 60 years in women. For each *T*-score s.d., the corresponding carotid absolute values for arterial stiffness and compliance were obtained. In a multivariate model, carotid stiffness parameters were constantly and independently associated with age, mean arterial pressure, pulse pressure, heart rate and body mass index. Our study provides a normogram of carotid arterial stiffness and compliance indices obtained with the echo-tracking method in a large population of healthy subjects stratified by gender and age that can be used in clinical practice.

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INTRODUCTION

Arterial stiffness has emerged as a major independent predictor of cardiovascular risk and has been recognized as a marker of target organ damage.¹ It can be measured via systemic or local arterial compliance or regionally via carotid–femoral pulse wave velocity (PWV). PWV is considered to be the 'gold standard' method for determining regional arterial stiffness and has been proven to be an independent predictor of cardiovascular events.¹ However, this methodology has certain limitations, such as inaccuracy of distance measurements for the carotid–femoral distance measurement, and it is affected by properties of mixed elastic and muscular parts of the arterial tree. The less used echo-tracking system allows the assessment of local arterial stiffness by deriving the pressure–diameter curve of the artery (the measurements of changes in diameter over the cardiac cycle and the local pulse pressure (PP) should be determined

simultaneously) and calculating local arterial stiffness from the time delay between two adjacent distension waveforms. Local arterial stiffness can be determined at different arterial sites, but the carotid artery is of special interest because it is more easily assessed and has been shown to be more stable, enabling evaluation of the stiffness of elastic arteries.^{2,3} Recently, Engelen *et al.*⁴ reported the reference values of local carotid stiffness in a large group of healthy subjects. However, the measurements were derived from different systems and different anatomical locations of the carotid artery. Van Sloten *et al.*⁵ demonstrated the predictive value of carotid stiffness for cardiovascular events. In addition, an ultrasound-derived carotid stiffness estimation can be assessed simultaneously with the presence of plaques and precise measurements of intima–media thickness (IMT).

The aim of the present study was to produce reference values of local carotid arterial stiffness parameters using a single echo-tracking

¹Cardiology and Emergency Department, San Antonio Hospital, Udine, Italy; ²Department of Cardiology, Dupuytren University Hospital, Limoges, France; ³Department of Translational Medicine, University of Eastern Piedmont 'A Avogadro', Novara, Italy and ⁴Department of Cardiology 'Cava de' Tirreni and Amalfi Coast' Hospital, University of Salerno, Fisciano, Italy

Correspondence: Dr O Vriz, Cardiology and Emergency Department, San Antonio Hospital, Via Trento-Trieste, San Daniele del Friuli, Udine 33038, Italy.
E-mail: olgavriz@yahoo.com

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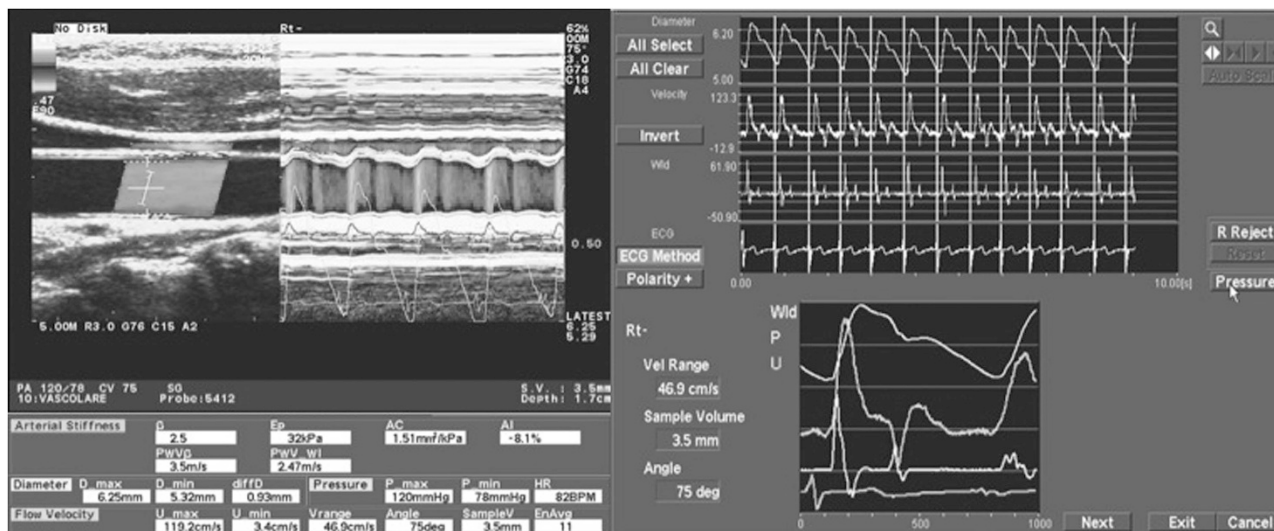


Figure 1 Measurement of arterial stiffness parameters with echo-tracking. Left panel: Echo-tracking gate at the level of the right common carotid artery and the change in diameter during the cardiac cycle. Right panel: Arterial stiffness parameters. AC, arterial compliance; β , beta-index; Ep, pressure-strain elastic modulus; PWV- β , one-point pulse wave velocity. A full color version of this figure is available at the *Hypertension Research* journal online.

system and a standardized anatomical location in a large population of healthy subjects and to assess the relative impact of age, gender, anthropometric and hemodynamic indices, as well as lifestyle habits.

METHODS

A total of 968 subjects were prospectively enrolled and recruited from a single center (San Antonio Hospital, San Daniele del Friuli, Udine, Italy), and a final number of 900 were considered healthy. Participants were selected from subjects who were investigated for work eligibility, subjects who were healthy blood donors, subjects who underwent electrocardiogram for obtaining access to spa facilities and subjects who were university members of the third age. All subjects underwent physical examination and anthropometry; they also completed questionnaires regarding their medical history, physical activity, coffee intake, alcohol use and smoking.⁶ None of the postmenopausal women were on estrogen replacement therapy. Weight (in kilograms) and height (in meters) were measured using standard techniques, and body mass index (BMI) was calculated as body weight divided by height squared. Body surface area was calculated using the DuBois formula ($0.20247 \times \text{height}^{0.725} \times \text{weight}^{0.425}$).

To generate a healthy sample, subjects were excluded if they had diabetes mellitus, hypertension (systolic blood pressure (SBP) ≤ 140 mm Hg and diastolic blood pressure (DBP) ≤ 90 mm Hg), nephropathy, cardiovascular disease including valve diseases or any other condition requiring chronic medication.⁷ All subjects underwent blood pressure and heart rate (HR) measurements in the supine position with an oscillometric semiautomatic sphygmomanometer. These were measured two times in the right arm after 10 min of rest in a quiet room and then before the carotid study; the two readings were taken 30 min apart. Phase V Korotkoff sounds were considered to be DBP, except for subjects with sounds tending towards zero, in whom phase IV was taken. PP (SBP-DBP) and mean arterial pressure (MAP) ($((2 \times \text{DBP}) + \text{SBP})/3$) were calculated.

Carotid artery stiffness parameters

Local arterial stiffness was evaluated at the level of the left common carotid artery 1 to 2 cm before its bifurcation using a high-definition echo-tracking ultrasound system (Alpha 10; Hitachi Aloka, Tokyo, Japan) as described previously.^{7,8} (The measurements carried out at the right and left common carotid were similar.) A wide-band multifrequency 5–13 MHz linear probe was used. Echo-tracking uses the raw radiofrequency signals that are based on the video signals with an accuracy of 0.01 mm. The optimal angle between the ultrasound beam and the vessel wall for diameter change measurements by echo-tracking is 90°. However, blood flow velocity perpendicular to the beam

cannot be detected. A different ultrasound beam was used to overcome this problem that is independently steerable. Figure 1 (top left panel) shows a long-axis view of the common carotid artery and the ultrasound beam configuration with the independent beam steering function. The solid line shows the ultrasound beam direction for velocity measurements; the dotted line shows the beam direction for diameter change measurements. Both beams were steered to intersect at the center of the range gate. The ultrasound beam steering angle can be changed from -30° to $+30^\circ$, with 5° angular increments. The echo-tracking gates were manually set at the boundaries between the intima and media of the anterior and posterior walls. The rate gate for velocity measurements was automatically positioned at the center of the diameter using echo-tracking gates. Flow velocity was corrected for the angle between the ultrasound beam and the blood flow velocity vector. Carotid arterial pressure and diameter change waveforms were similar during systole, whereas the carotid arterial pressure-diameter relationship showed slight nonlinearity and hysteresis during diastole (Figure 1, right panel).⁹ Brachial cuff pressure was measured just before starting ultrasound imaging and was entered into the system for calculation of carotid stiffness indices. At least five consecutive beats were averaged to obtain a representative waveform.

The following indices were calculated for the common carotid artery (Figure 1, left panel):

1. β -stiffness (stiffness parameter), index of arterial stiffness: $\beta = \ln(P_s/P_d)/[(D_s - D_d)/D_d]$
2. Ep (pressure-strain elastic modulus), index of vessel stiffness:

$$E_p = (P_s - P_d)/[(D_s - D_d)/D_d]$$

3. PWV (one-point PWV): $PWV\beta = \sqrt{(\beta P_d/2\rho)}$
4. AC (arterial compliance), index of blood vessel compliance:

$$AC = \pi(D_s \times D_s - D_d \times D_d)/[4 \times (P_s - P_d)]$$

where P_s and P_d are systolic and diastolic brachial pressure, respectively (used as a surrogate for carotid SBP and DBP), D_s and D_d are carotid arterial systolic and diastolic diameter, respectively, and ρ is blood density (1050 kg m^{-3}).

Reproducibility was assessed with determination of interobserver and intraobserver variability in 50 randomly selected healthy subjects. Intraobserver intersession variability was assessed by one observer by performing two sessions on different days in the same subject. The time interval between sessions ranged

from 2 days to 1 week. Interobserver intrasession variability was evaluated by two observers who measured echo-tracking in each subject consecutively.

One-point carotid PWV obtained by the echo-tracking system implemented in the ultrasound machine was compared with the gold standard carotid-femoral PWV assessed with the SphygmoCor system (Model SCOR-Px, Software version 7.01; AtCor Medical, Sydney, NSW, Australia).¹⁰

Statistical analysis

Summary statistics are presented as the median, mean and s.d. and 5–95% confidence interval. The study population was divided into seven age groups (group 1: 16–19 years, *n* = 58; group 2: 20–29 years, *n* = 172; group 3: 30–39 years, *n* = 147; group 4: 40–49 years, *n* = 159; group 5: 50–59 years, *n* = 133; group 6: 60–69 years, *n* = 107; and group 7: ≥ 70 years, *n* = 124) and then stratified by gender. An unpaired Student's *t*-test was used to compare gender differences, and analysis of variance (ANOVA) was performed to evaluate differences among age groups. Pearson's partial correlation was used to assess clinically relevant variables in men and women separately, which were then entered into the multivariate model. To easily compare stiffness and compliance

parameters across the different age groups, individual scores were transformed into *T*-scores, indicating how many s.d. units an individual's score was above or below the mean that was observed in the referred group, including same-sex individuals who were aged 36 to 44 years. The formula to calculate the *T*-scores was as follows:

$$T\text{-score} = (\text{individual stiffness parameter value} - \text{stiffness parameter mean value in age group 36–44 years}) \div \text{stiffness parameter s.d. value in age group 36–44 years.}$$

Inter- and intraobserver variability was examined using correlation coefficients (Pitman's test of differences in variation) and mean differences, and limits of agreement are reported. Statistical significance was set at *P* < 0.05.

All statistical analyses were performed using SYSTAT v.12.0 (Systat Software, Chicago, IL, USA).

RESULTS

General characteristics

A total of 900 healthy subjects (500 males; mean age 45.8 ± 19 years; range 16–94 years) were included in the study. Demographic and clinical characteristics, blood chemistry and one-point carotid stiffness compliance of the population are reported in Table 1. Females were slightly older with lower blood pressure and higher HR; carotid stiffness parameters were significantly higher in females, but after adjustment for age and HR, only arterial compliance was still significantly lower in females. IMT was lower in females, and the difference reached statistical significance when the data were adjusted for age and HR. Blood chemistry was characterized by higher levels of lipid profiles in males as well as higher hemoglobin and serum creatinine.

After stratification by gender and age, height progressively decreased as age increased, and weight increased up to 60 years in men and then decreased, whereas in women, it started to decrease at 50 years. BMI increased up to 50 years in both genders and then stabilized in men and continued to increase in women (Table 2). SBP was lower in women until the age of 50 years when it became similar to that of men. DBP was comparable between genders in the two youngest groups; then, it was lower in women and became similar again in the oldest group (Table 3). MAP and PP were lower in women than in men across all age groups (Table 3). One-point carotid stiffness parameters increased significantly with age in both genders, and AC was constantly and significantly lower in women in the two youngest groups (Table 4). IMT increased with age (group 1: 0.44 ± 0.07 mm and 0.43 ± 0.05 mm in males and females, respectively, NS; group 2: 0.47 ± 0.1 mm and 0.48 ± 0.08 mm, NS; group 3: 0.58 ± 0.1 mm and 0.49 ± 0.09 mm, *P* < 0.0001; group 4: 0.59 ± 0.1 mm and 0.59 ± 0.1 mm, NS; group 5: 0.69 ± 0.17 mm and 0.63 ± 0.14 mm, NS; group 6: 0.71 ± 0.16 mm and 0.69 ± 0.16 mm, NS; and group 7: 0.82 ± 0.17 mm and 0.82 ± 0.16 mm, NS) (ANOVA *P* < 0.0001 for both genders).

The *T*-score was found to increase significantly across all age groups in both genders, with women being slightly stiffer. In particular, there was a sharp increase in stiffness ~60 years of age in women and 65 years of age in men, with carotid stiffness in elderly women exceeding values for men. The opposite was observed for AC. Stiffness parameters did not change between the 16 and 25 years age group in women, whereas a progressive increase with age was observed in men from the age of 16 years (Figure 2).

Factors affecting carotid stiffness

In a simple correlation, one-point carotid stiffness parameters correlated positively with age, blood pressure parameters, HR, weight and BMI and negatively with height in men (Table 5) and with age, blood pressure parameters, weight and BMI in women, whereas HR

Table 1 Anthropometric, clinical and hemodynamic characteristics of the study population

| Variable | P-value | | P-value | |
|--|----------------------------|------------------------------|---------|--------------------------------|
| | Males (<i>n</i> = 500) | Females (<i>n</i> = 400) | P-value | Age, HR adjusted for age |
| Age (years) | 44.7 ± 18.4 | 47.2 ± 19.5 | 0.05 | — |
| Weight (kg) | 77.7 ± 11.7 | 61.9 ± 9.9 | 0.0001 | 0.0001 |
| Height (cm) | 175.8 ± 7.0 | 162.2 ± 7.3 | 0.0001 | 0.0001 |
| BSA (m ²) | 1.94 ± 0.16 | 1.65 ± 0.13 | 0.0001 | 0.0001 |
| BMI (kg m ⁻²) | 25.1 ± 3.28 | 23.72 ± 4.06 | 0.0001 | 0.0001 |
| SBP (mm Hg) | 125.8 ± 11 | 121.5 ± 13.6 | 0.0001 | 0.0001 |
| DBP (mm Hg) | 76.1 ± 9.2 | 73.0 ± 9.2 | 0.0001 | 0.0001 |
| PP (mm Hg) | 49.9 ± 10 | 48.5 ± 11.8 | NS | 0.002 |
| MAP (mm Hg) | 91.2 ± 14.3 | 88.9 ± 11.1 | 0.0001 | 0.0001 |
| HR (b.p.m.) | 63.7 ± 11 | 67.5 ± 10.1 | 0.0001 | 0.0001 |
| <i>Carotid stiffness</i> | | | | |
| β-stiffness | 6.5 ± 3.19 | 7.2 ± 3.5 | 0.02 | NS |
| Ep (kPa) | 86.7 ± 44.17 | 93.31 ± 42.59 | 0.037 | NS |
| PWV (m s ⁻¹) | 5.48 ± 1.28 | 5.68 ± 1.41 | 0.026 | NS |
| AC (mm ² kPa ⁻¹) | 0.97 ± 0.38 | 0.82 ± 0.37 | 0.0001 | 0.0001 |
| IMT (mm) | 0.66 ± 0.18 | 0.60 ± 0.18 | 0.13 | 0.037 |
| <i>Blood chemistry</i> | | | | |
| Hemoglobin (g dl ⁻¹) | 15.2 ± 2.02 | 13.4 ± 1.22 | 0.000 | |
| Glucose (mg dl ⁻¹) | 92.9 ± 9.6 | 89.2 ± 9.3 | 0.000 | |
| Total cholesterol (mg dl ⁻¹) | 193 ± 27.8 | 189 ± 25.4 | NS | |
| HDL-cholesterol (mg dl ⁻¹) | 55.3 ± 13.3 | 68.8 ± 16.6 | 0.0000 | |
| LDL-cholesterol (mg dl ⁻¹) | 112.2 ± 39.7 | 103.6 ± 38.7 | NS | |
| Tryglicerides (mg dl ⁻¹) | 96.7 ± 38.2 | 88.1 ± 32 | 0.003 | |
| Serum creatinine (mg dl ⁻¹) | 0.97 ± 0.16 | 0.78 ± 0.13 | 0.000 | |

Abbreviations: AC, arterial compliance; BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; Ep, pressure-strain elastic modulus; HDL, high-density lipoprotein; HR, heart rate; IMT, intima-media thickness; LDL, low-density lipoprotein; MAP, mean arterial pressure; PP, pulse pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

Data are presented as mean ± s.d.

Table 3 Hemodynamic characteristics of the study population

| | | SBP (mm Hg) | | DBP (mm Hg) | | PP (mm Hg) | | MAP (mm Hg) | | HR (b.p.m.) | | |
|---|--------|-------------|-----------|-------------|--------|------------|--------|-------------|--------|-------------|-----------|-----------|
| <i>Group 1: 16–19 years, n = 58 (M = 42, F = 16)</i> | | | | | | | | | | | | |
| | M | F | NS | M | F | NS | M | F | M | F | P = 0.003 | |
| Mean | 118.5 | 112.4 | | 66.9 | 64.8 | | 51.6 | 44.5 | 84.1 | 76.6 | 61.2 | 71.3 |
| s.d. | 11.3 | 17.1 | | 8.1 | 11.1 | | 11.2 | 10.5 | 7.7 | 11.3 | 10.2 | 14.9 |
| <i>Group 2: 20–29 years, n = 172 (M = 90, F = 82)</i> | | | | | | | | | | | | |
| | M | F | P < 0.001 | M | F | NS | M | F | M | F | P < 0.001 | P = 0.001 |
| Mean | 121.50 | 112.01 | | 72.34 | 69.80 | | 49.12 | 42.20 | 88.72 | 83.87 | 63.20 | 68.43 |
| s.d. | 11.76 | 12.69 | | 9.76 | 8.30 | | 10.5 | 10.63 | 8.24 | 8.64 | 10.34 | 9.03 |
| <i>Group 3: 30–39 years, n = 147 (M = 76, F = 71)</i> | | | | | | | | | | | | |
| | M | F | P = 0.009 | M | F | P = 0.009 | M | F | M | F | P = 0.02 | NS |
| Mean | 126.16 | 117.28 | | 77.18 | 73.26 | | 49.05 | 44.39 | 92.25 | 88.06 | 64.02 | 67.17 |
| s.d. | 10.67 | 9.02 | | 8.90 | 9.02 | | 9.0 | 8.77 | 13.24 | 8.5 | 12.7 | 10.8 |
| <i>Group 4: 40–49 years, n = 159 (M = 98, F = 61)</i> | | | | | | | | | | | | |
| | M | F | P = 0.018 | M | F | P = 0.01 | M | F | M | F | NS | P < 0.001 |
| Mean | 124.24 | 119.5 | | 77.9 | 74.5 | | 46.48 | 44.09 | 92.48 | 89.5 | 61.44 | 69.74 |
| s.d. | 12.43 | 11.9 | | 7.9 | 8.6 | | 10.05 | 8.23 | 12.32 | 9.08 | 11.23 | 10.59 |
| <i>Group 5: 50–59 years, n = 133 (M = 83, F = 50)</i> | | | | | | | | | | | | |
| | M | F | NS | M | F | P = 0.01 | M | F | M | F | P < 0.001 | NS |
| Mean | 127.2 | 128.8 | | 80.49 | 76.42 | | 49.57 | 49.6 | 97.02 | 91.21 | 64.16 | 65.28 |
| s.d. | 10.9 | 11.99 | | 8.12 | 9.47 | | 8.77 | 8.9 | 8.3 | 15.4 | 12.12 | 9.25 |
| <i>Group 6: 60–69 years, n = 107 (M = 54, F = 53)</i> | | | | | | | | | | | | |
| | M | F | NS | M | F | P = 0.02 | M | F | M | F | NS | NS |
| Mean | 127.49 | 129.28 | | 77.76 | 73.67 | | 50.94 | 54.88 | 87.74 | 90.35 | 65.1 | 65.1 |
| s.d. | 11.06 | 12.11 | | 7.88 | 9.83 | | 8.85 | 10.9 | 24.08 | 14.44 | 13.32 | 10.1 |
| <i>Group 7: ≥ 70 years, n = 124 (M = 57, F = 67)</i> | | | | | | | | | | | | |
| | M | F | NS | M | F | NS | M | F | M | F | NS | NS |
| Mean | 131.8 | 130.6 | | 76.8 | 75.07 | | 55.65 | 55.5 | 93.0 | 93.45 | 65.16 | 68.9 |
| s.d. | 10.8 | 9.3 | | 8.6 | 7.3 | | 11.8 | 10.3 | 15.69 | 6.5 | 12.2 | 9.8 |
| ANOVA | 0.0001 | 0.0001 | | 0.0001 | 0.0001 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | NS | NS |

Abbreviations: ANOVA, analysis of variance; DBP, diastolic blood pressure; F, female; HR, heart rate; M, male; MAP, mean arterial pressure; NS, not significant; PP, pulse pressure; SBP, systolic blood pressure; s.d., standard deviation.

Table 4 One-point carotid stiffness

| | | β | | E_p (kPa) | | AC ($\text{mm}^2 \text{kPa}^{-1}$) | | PWV (m s^{-1}) | | |
|--|--|---------|--------|-------------|--------|--------------------------------------|--------|---------------------------|--------|--|
| | | M | F | M | F | M | F | M | F | |
| <i>Group 1: 16–19 years, n = 58 (M = 42, F = 16)</i> | | | | | | | | | | |
| Mean | | 3.88 | 4.42 | 46.13 | 49.67 | 1.37 | 1.27 | 4.0 | 4.21 | |
| s.d. | | 0.99 | 1.04 | 11.09 | 12.58 | 0.39 | 0.33 | 0.48 | 0.45 | |
| | | | | | | | | | | |
| <i>Group 2: 20–29 years, n = 172 (M = 90, F = 82)</i> | | | | | | | | | | |
| Mean | | 4.42 | 4.52 | 55.82 | 53.82 | 1.26 | 1.21 | 4.46 | 4.42 | |
| s.d. | | 1.22 | 1.27 | 16.29 | 15.59 | 0.44 | 0.42 | 0.63 | 0.58 | |
| | | | | | | | | | | |
| <i>Group 3: 30–39 years, n = 147 (M = 76, F = 71)</i> | | | | | | | | | | |
| Mean | | 5.19 | 5.75 | 68.89 | 71.92 | 1.34 | 0.84 | 5.0 | 5.13 | |
| s.d. | | 1.42 | 1.69 | 21.57 | 21.51 | 0.29 | 0.29 | 0.7 | 0.76 | |
| | | | | | | | | | | |
| <i>Group 4: 40–49 years, n = 159 (M = 98, F = 61)</i> | | | | | | | | | | |
| Mean | | 6.12 | 6.65 | 81.50 | 84.28 | 0.98 | 0.73 | 5.45 | 5.5 | |
| s.d. | | 1.89 | 2.27 | 27.28 | 28.34 | 0.3 | 0.20 | 0.85 | 0.82 | |
| | | | | | | | | | | |
| <i>Group 5: 50–59 years, n = 133 (M = 83, F = 50)</i> | | | | | | | | | | |
| Mean | | 7.25 | 7.28 | 101.82 | 96.92 | 0.79 | 0.63 | 6.07 | 5.95 | |
| s.d. | | 2.26 | 2.3 | 30.75 | 34.97 | 0.25 | 0.19 | 0.91 | 0.95 | |
| | | | | | | | | | | |
| <i>Group 6: 60–69 years, n = 107 (M = 54, F = 53)</i> | | | | | | | | | | |
| Mean | | 8.67 | 9.56 | 114.62 | 125.32 | 0.73 | 0.63 | 6.36 | 6.55 | |
| s.d. | | 3.13 | 3.74 | 41.18 | 48.01 | 0.23 | 0.27 | 1.04 | 1.14 | |
| | | | | | | | | | | |
| <i>Group 7: ≥ 70 years, n = 124 (M = 57, F = 67)</i> | | | | | | | | | | |
| Mean | | 10.9 | 11.35 | 147.7 | 153.7 | 0.71 | 0.61 | 7.1 | 7.4 | |
| s.d. | | 4.7 | 4.0 | 63.47 | 57.35 | 0.28 | 0.18 | 1.42 | 1.55 | |
| ANOVA | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | |

Abbreviations: AC, arterial compliance; ANOVA, analysis of variance; E_p , pressure-strain elastic modulus; F, female; M, male; NS, not significant; PWV, pulse wave velocity; s.d., standard deviation.

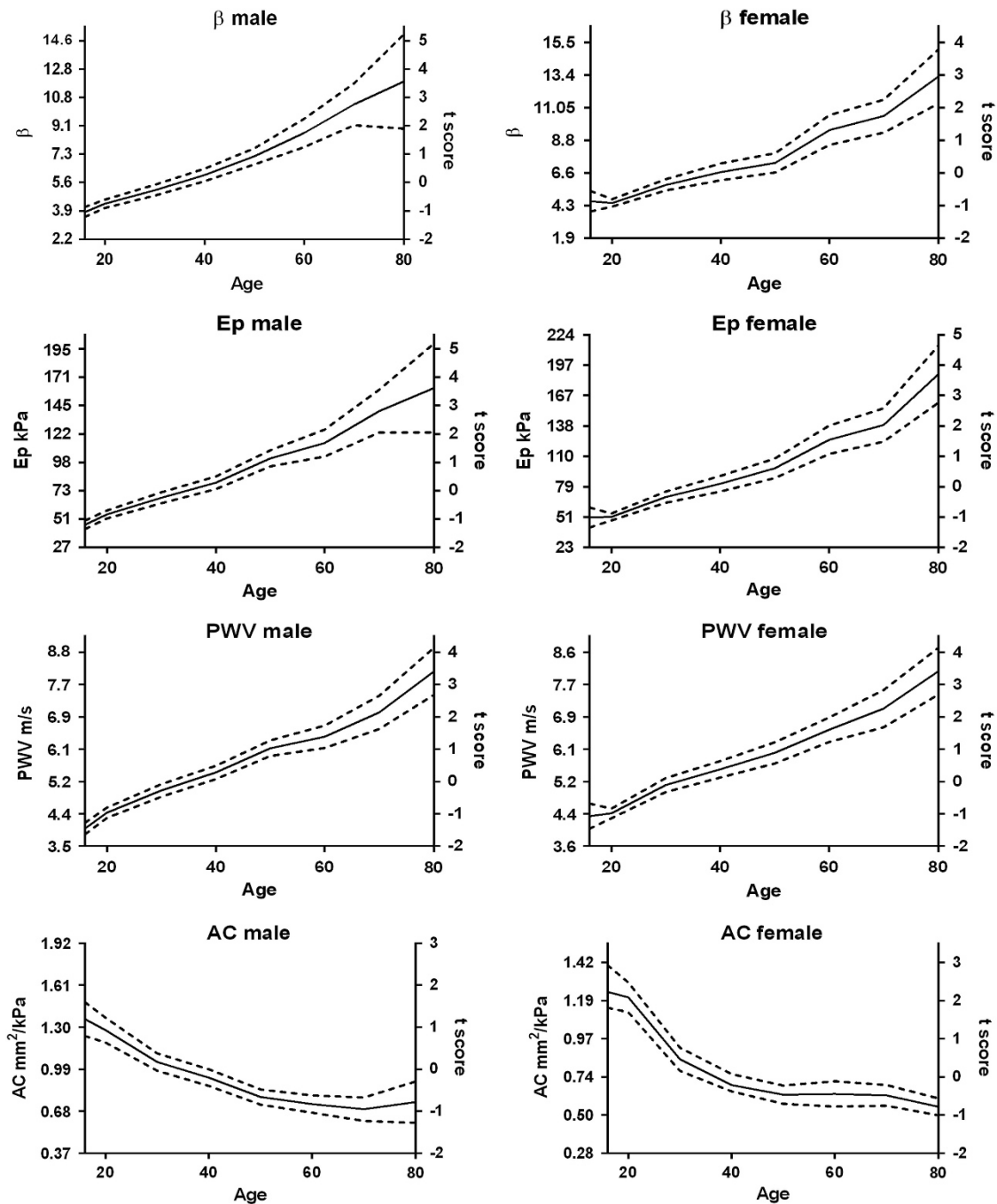


Figure 2 T-score of carotid stiffness parameters: s.d. (right axis) and corresponding absolute values (left axis).

correlated positively with PWV and negatively with height (Table 5). Gender correlated positively with β -index ($r=0.106$, $P=0.014$), Ep ($r=0.07$, NS) and PWV ($r=0.076$, NS) and negatively with AC ($r=-0.199$, $P<0.0001$). Physical activity correlated positively with AC ($r=0.306$) and negatively with β -stiffness ($r=-0.328$), Ep ($r=-0.335$) and PWV ($r=-0.374$) ($P<0.0001$ for all). Only 10% ($n=92$) of subjects were smokers, whereas 96% were coffee drinkers and light alcohol consumers; these parameters did not correlate with carotid arterial stiffness (NS). A multivariate model was constructed using all variables that were statistically significant in simple correlation analyses. Age, BMI, PP, MAP, HR and weight were independently correlated with one-point carotid stiffness. AC was

correlated with age, PP, MAP, HR, physical activity and smoking. IMT was associated with age, BMI, PP and HR (Table 6).

Reproducibility

Interobserver correlation. The results of Pitman's test of difference in variants for local arterial stiffness/compliance were as follows: β -stiffness $r=-0.153$ (NS), Ep $r=0.173$ (NS), PWV $r=0.018$ (NS) and AC $r=0.146$ (NS). On Bland-Altman analysis, the limits of agreement (reference range for difference) were -0.2027 to 1.830 , with a mean difference of -0.098 (confidence interval (CI): -0.354 to 0.157) for β -stiffness; limits of agreement (reference range for difference) of -29.718 to 31.456 , with a mean difference of 0.914

Table 5 Simple correlation between arterial stiffness and anthropometric data, MAP and IMT

| | β -stiffness | Ep | AC | PWV |
|---------------------------|-------------------------|-------------------------|--------------------------|-------------------------|
| Age (years) | $r=0.67$ $P<0.0001$ | $r=0.70$ $P<0.0001$ | $r=-0.547$ $P<0.0001$ | $r=0.733$ $P<0.0001$ |
| PP (mm Hg) | $r=0.41$ $P<0.0001$ | $r=0.44$ $P<0.0001$ | $r=-0.41$ $P<0.0001$ | $r=0.377$ $P<0.0001$ |
| MAP (mm Hg) | $r=0.167$ $P<0.0001$ | $r=0.347$ $P<0.0001$ | $r=-0.331$ $P<0.0001$ | $r=0.425$ $P<0.0001$ |
| HR (b.p.m.) | $r=0.162$ $P<0.0001$ | $r=0.178$ $P<0.0001$ | $r=-0.138$ $P<0.0001$ | $r=0.199$ $P<0.0001$ |
| Height (cm) | $r=-0.32$ $P<0.0001$ | $r=-0.29$ $P<0.0001$ | $r=0.297$ $P<0.0001$ | $r=-0.28$ $P<0.0001$ |
| Weight (kg) | $r=0.03$ NS | $r=0.09$ NS | $r=-0.012$ NS | $r=0.122$ $P=0.002$ |
| BMI (kg m ⁻²) | $r=0.31$ $P<0.0001$ | $r=0.357$ $P<0.0001$ | $r=-0.224$ $P<0.0001$ | $r=0.383$ $P<0.0001$ |
| BSA (m ²) | $r=-0.90$ NS | $r=0.04$ NS | $r=-0.12$ $P=0.003$ | $r=-0.017$ NS |
| IMT (mm) | $r=0.49$ $P<0.0001$ | $r=0.516$ $P<0.0001$ | $r=-0.306$ $P<0.0001$ | $r=0.513$ $P<0.0001$ |

Abbreviations: AC, arterial compliance; BMI, body mass index; BSA, body surface area; Ep, pressure-strain elastic modulus; HR, heart rate; IMT, intima-media thickness; MAP, mean arterial pressure; NS, not significant; PP, pulse pressure; PWV, pulse wave velocity.

(CI: -3.150 to 4.978) for Ep; limits of agreement (reference range for difference) of -1.050 to 0.984, with a mean difference of -0.033 (CI -0.168 to 0.102) for PWV; and limits of agreement (reference range for difference) of -0.185 to 0.215, with a mean difference of 0.015 (CI -0.012 to 0.041) for AC (Supplementary Figure 1, top 4 graphs).

Intraobserver correlation. The results of Pitman's test of difference in variants for local arterial stiffness/compliance were as follows: β -stiffness $r=-0.387$ ($P=0.003$), Ep $r=-0.104$ (NS), PWV $r=-0.181$ (NS) and AC $r=0.013$ (NS). In the Bland-Altman analysis, the limits of agreement (reference range for difference) were -4.048 to 3.460, with a mean difference of -0.294 (CI -0.792 to 0.204) for β -stiffness; limits of agreement (reference range for difference) of -69.263 to 72.238, with a mean difference of 1.487 (CI -7.899 to 10.874) for Ep; limits of agreement (reference range for difference) of -1.424 to 1.265, with a mean difference of -0.079 (CI -0.258 to 0.099) for PWV; limits of agreement (reference range for difference) of -0.305 to 0.308, with a mean difference of 0.002 (CI -0.039 to 0.043) for AC (Supplementary Figure 1, bottom 4 graphs).

DISCUSSION

At present, there are no reference values in the literature for local carotid stiffness and compliance parameters derived from echo-tracking ultrasound methods. In this study, we determined the age- and sex-specific reference values for local carotid stiffness and compliance parameters in a group of healthy subjects aged 16 to 100 years using the one-point ultrasound wall-tracking method. As expected, a progressive increase in carotid stiffness with age and the opposite for compliance was recorded in both genders. The studied indices included β -stiffness (a measure of arterial wall stiffness independent of blood pressure),¹¹ Ep (a measure of the mechanical properties of the arterial wall),¹² AC (describing the absolute change in diameter or area for a given pressure change)¹³ and PWV (derived from β -stiffness and providing information on arterial stiffness at a specific region of interest).² In a previous study from our group,

one-point carotid PWV was found to correlate with carotid-femoral PWV measured with the SphygmoCor device in a heterogeneous population, identifying the cutoff of 6.65 m s⁻¹ as the best predictor of carotid-femoral PWV >12 m s⁻¹.⁹ SphygmoCor is considered the 'gold standard' for arterial stiffness measurements, and its predictive value for cardiovascular events and mortality is well established.¹⁴ The guidelines for the management of arterial hypertension¹⁵ as well as the guidelines for the diagnosis and treatment of aortic diseases¹⁶ suggest measuring both vascular stiffness and target organ damage.

Local carotid arterial stiffness provides similar information to carotid-femoral PWV on the impact of aging on arterial stiffness in normal subjects, although some argue that this is not the case for hypertensive and/or diabetic patients.¹⁷ However, recent evidence demonstrated an association between carotid stiffness (also measured by echo-tracking),¹⁸ target organ involvement^{7,8,19,20} and cardiovascular events in different pathophysiological scenarios also including healthy subjects.^{5,21-24}

In the present study, carotid stiffness, β -stiffness, Ep and PWV were similar in men and women for all of the age groups, whereas AC was consistently lower in women. These are not unexpected data, and the lower AC is related to a smaller body size in women and smaller arterial diameter. However, because arterial stiffness is similar between genders, carotid distensibility that better reflects the arterial wall characteristics is not different.²⁵ Boutouyrie *et al.*²⁶ described regional stiffness, carotid-femoral PWV, in a large cohort of subjects and reported higher arterial stiffness in men than in women but the difference almost disappeared after adjusting for age. More recently, Vermeersch *et al.*²⁷ showed that carotid-femoral PWV increased at the same rate in men and women and did not differ between genders in any of the age groups. They also found that local stiffness (carotid artery) increased more rapidly than carotid-femoral aortic stiffness in women than in men over the studied age range when considering all of the variables (compliance, distensibility and PWV), suggesting a different response of the two arteries to increasing age in the two genders.

Normal reference values for one-point carotid stiffness parameters are of clinical utility when evaluating an individual subject as compared with what is expected in a reference population. To compare stiffness parameters across different age groups, *T*-scores were considered instead of mean stiffness values because they make it easier to determine how much individual stiffness values vary or deviate from those expected in healthy adults. In addition, for each *T*-score s.d., the corresponding carotid stiffness and compliance absolute values were obtained. All parameters showed a non-linear positive relationship between age and carotid stiffness and a negative relationship with compliance in both genders, with a steeper increase after 60 years of age in women. An earlier and steeper increase in carotid stiffness in women was also reported by Engelen *et al.*⁴ Mean age at natural menopause is around 51 years,²⁸ and both aortic and carotid stiffness were found to be higher as the time since menopause increased, either in patients or normotensive postmenopausal women.²⁹ In our study, local carotid stiffness was somewhat lower in women than in men until the age of 60 years but increased thereafter and exceeded that of men, suggesting that menopause is a major contributor to the age-dependent increase in arterial stiffness. It is well known that estrogen is a potent vasodilator with antiatherosclerotic effects, and estrogen deficiency in postmenopausal women leads to rapid arterial stiffening.³⁰ In our study, BMI was also shown to have a role; until the age of 59 years, women were constantly lighter than men, but thereafter, no differences in weight were observed between genders. Therefore, it is likely that menopause, along with an

Table 6 Backward multiple regression analysis

| | r ² | β | P-value |
|-------------------|----------------|----------|---------|
| β-stiffness | 0.46 | | |
| Age | | 0.56 | 0.0001 |
| BMI | | 0.108 | 0.0001 |
| PP | | 0.159 | 0.0001 |
| MAP | | -0.09 | 0.001 |
| HR | | 0.124 | 0.0001 |
| Height | | -0.108 | 0.0001 |
| Ep | 0.511 | | |
| Age | | 0.544 | 0.0001 |
| BMI | | 0.125 | 0.0001 |
| PP | | 0.174 | 0.0001 |
| HR | | 0.139 | 0.0001 |
| Height | | -0.0.071 | 0.009 |
| PWV | 0.56 | | |
| Age | | 0.60 | 0.0001 |
| BMI | | 0.146 | 0.0001 |
| MAP | | 0.126 | 0.0001 |
| HR | | 0.153 | 0.0001 |
| AC | 0.201 | | |
| HR | | -0.055 | 0.021 |
| Gender | | -0.211 | 0.0001 |
| Physical activity | | 0.10 | 0.004 |
| IMT | 0.529 | | |
| Age | | 0.666 | 0.0001 |
| PP | | 0.125 | 0.0001 |
| HR | | -0.083 | 0.005 |

Abbreviations: AC, arterial compliance; BMI, body mass index; Ep, pressure-strain elastic modulus; HR, heart rate; IMT, intima-media thickness; MAP, mean arterial pressure; PP, pulse pressure; PWV, pulse wave velocity.

increase in BMI, may have contributed to the sharp increase in local arterial stiffness after 60 years of age. In an analysis of data derived from the Multi-Ethnic Study of Atherosclerosis, Stern *et al.*³¹ found that women between 45 and 54 years of age had lower arterial stiffness than men, but beyond the age of 55 years, arterial distensibility was similar between sexes. These data also suggest that postmenopausal women bear a greater cardiac afterload than men and may be more predisposed to heart failure.³²

Several studies demonstrated that elevated resting HR is a predictor of adverse cardiovascular events, even after adjustment for physical activity.³³ Similarly, in our population of healthy subjects, HR correlated positively with arterial stiffness and negatively with arterial compliance. The association between HR and arterial stiffness and arterial compliance is based on decreased dynamic compliance, shorter time available for arterial wall recoil, a greater number of pulsatile strain cycles and elastic fiber fracture, all of which contribute to age-related arterial stiffening and promote atherosclerotic lesion formation.³⁴⁻³⁶

PP showed a J-curve pattern from adolescence to old age, as a result of greater pressure amplification from the aorta to peripheral arteries in younger subjects and increased aortic stiffening in older adults.³⁷ However, MAP increased progressively from the youngest group to the elderly. Blood pressure parameters, such as MAP or steady components, and PP or pulsatile components were independently associated with carotid stiffness/compliance.

In our study, age, BMI, blood pressure parameters and HR were the key determinants of local arterial stiffness and compliance, with the exception of BMI and weight in relation to AC where gender, physical activity and smoking were included in the multiple regression model. Recently, Brunner *et al.*³⁸ reported the same correlation between adiposity and arterial stiffness in both genders, demonstrating that the BMI to carotid-femoral PWV relationship accounted for 12% of the increment in cardiovascular risk resulting from higher BMI. In the univariate correlation analysis, female gender and physical activity were positively and negatively correlated with carotid stiffness, respectively; the opposite was observed for AC. On multivariate analysis, female gender and physical activity were independently associated only with AC in addition to age, PP, MAP, HR and smoking. A favorable and independent association has been reported between regular physical activity and central arterial compliance, counterbalancing the effect of aging.^{39,40} The mechanism by which aerobic exercise improves arterial compliance resides in the intrinsic characteristics of the artery, including the composition of the arterial wall (elastin and collagen fibers and structural determinants) and functional properties (e.g., vasoconstrictor effect of smooth muscle cells). One-point carotid compliance is also affected by smoking. The two most toxic compounds of cigarettes are nicotine and carbon monoxide, where nicotine causes endothelial cell proliferation and intimal hyperplasia and carbon monoxide increases circulating endothelial cells.^{41,42}

The IMT data of our study are consistent with those reported in the literature, in particular with regard to a positive correlation with age. In a multicenter experience comparing mean values between genders, IMT was significantly higher in men than in women, but when the difference between genders was tested within age groups, IMT was similar.⁴³ Independent determinants were found to be SBP, BMI, PP and HR in a negative way. The negative relation between HR and IMT could be an interaction between different factors such as SBP, BMI and age. In fact, age is positively related to BP and weight and negatively related to HR with a decrease in HR of 1 pulse per minute per year. It could also be partially mediated by SBP; in fact, a lower HR with a prolonged diastolic time will shift the reflecting wave into a systolic phase, increasing the central arterial stiffness that in turn contributes to an increase in IMT.⁴⁴ In another setting, the association between IMT and metabolic syndrome (hypertension, dyslipidemia, hyperglycemia and central obesity) was demonstrated, and arterial hypertension was demonstrated to be the most important determinant of IMT.⁴⁵ The correlation between IMT and stiffness suggests a close relationship between structural and morphological modifications.⁴⁶⁻⁴⁸

Reproducibility in terms of inter- and intraobserver variability was good and similar to that reported by Magda *et al.*,⁴⁹ although it was slightly lower in this study.

Limitations

Some limitations should be acknowledged. First, peripheral blood pressure measurements are known to overestimate central blood pressure, especially in young populations, and brachial pressure was used to calculate one-point carotid stiffness parameters. Second, all calculations were based on the assumption reported by Sugawara *et al.*⁹ who demonstrated a good linear relationship between carotid arterial pressure and diameter. Third, brachial pressure measurements were taken immediately before starting the carotid study, assuming that hemodynamic parameters did not change significantly during the exam. Fourth, the present results are derived from a single-center experience.

CONCLUSION

Our study provides a sonographic normogram of carotid arterial stiffness/compliance indices obtained with the echo-tracking method in a large population of healthy subjects stratified by gender and age, including anthropometry, hemodynamic parameters and lifestyle habits. These results may improve ultrasonic determination and interpretation of local arterial stiffness measures.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Hypertension Research website (<http://www.nature.com/hr>)