Abstract—The relation between arterial function indices, such as pulse wave velocity and augmentation index with parameters derived from input impedance analysis, is still incompletely understood. Carotid pressure, central flow waveforms, and pulse wave velocity were noninvasively acquired in 2026 apparently healthy, middle-aged subjects (1052 women and 974 men) 35 to 55 years old at inclusion. Input and characteristic impedance, reflection coefficient, the ratio of backward-to-forward pressure amplitude (reflection magnitude), and augmentation index were derived. Pulse wave velocity increased by 15% (from 6.1 to 7.0 m/s) both in men and women. In qualitative terms, input impedance evolved from a pattern indicative of wave transmission and reflection to a pattern more compatible with a windkessel-like system. In women, a decrease in total arterial compliance led to an increased input impedance in the low frequency range, whereas few changes were observed in men. Characteristic impedance did not change with age in women and even decreased in men (P<0.001) and could not be identified as the primary determinant of central pulse pressure. Augmentation index increased with age, as was expected, and was systematically higher in women (P<0.001). Reflection coefficient and reflection magnitude increased with age (P<0.001) without gender differences. We conclude that, in healthy middle-aged subjects, the age-related increase in arterial stiffness (pulse wave velocity) is not fully paralleled by an increase in arterial impedance, suggesting a role for age-dependent modulation of aortic cross-sectional area. Wave reflection increases with age and is not higher in women than in men. (Hypertension. 2007;49:1248-1255.)

Key Words: cardiovascular physiology ■ blood pressure ■ vascular capacitance ■ arteries ■ biomechanics

The analysis of arterial stiffness and function and of pressure wave reflection received increasing attention for the past 3 decades. Several methods to describe arterial stiffness in a clinical setting emerged,1 the most investigated being pulse wave velocity (PWV) and augmentation index (AIx). Some aspects related to AIx still need further investigation, such as the systematically higher AIx in women even after adjustment for body size and heart rate, and the observation that AIx tends to levels off to a plateau value above the age of 60 years.2 Although PWV and AIx are related, they seem to reflect different aspects of arterial function.2,3

A more global view on the arterial system can be obtained from impedance analysis,4-6 requiring measurement of central pressure and flow waveforms. In addition, pressure waveforms (Pwfr) can be separated into their forward and backward components.7 In a recent study in hypertensive patients, Mitchell et al8 applied impedance analysis and found an increased aortic characteristic impedance (Zc) to play an important role in the elevated pulse pressure in patients with systolic hypertension. There are no integrated large-scale studies where both impedance analysis and newer indices like PWV and AIx were simultaneously acquired.

We have set up a broad prospective, longitudinal study where we aim to assess the development and progression of cardiovascular disease in the general population, referred to as the Asklepios Study.9 The first inclusion round, on which we report here, was finished in October 2004, and the baseline data were measured in middle-aged individuals (age range: 35 to 55 years; >2500 participants) free from overt cardiovascular disease. In addition to PWV and AIx, we estimated arterial Za and direct estimates of wave reflection.

The aims of the present investigation in apparently healthy middle-aged men and women were as follows: (1) to provide reference data of arterial input impedance (Zin) and wave reflection parameters; (2) to investigate the relation between aortic PWV and Zc; (3) to assess the contribution of aortic Zc to central pulse pressure; and (4) to investigate the relation between AIx and wave reflection.
Methods

We refer to the online supplement for an extended version of the Methods section (available at http://hyper.ahajournals.org). Only a brief summary will be given here. A total number of 2026 subjects (1052 women) met the requirements to be included into this study. The ethical committee of the Ghent University Hospital approved the study protocol, and all of the subjects gave written informed consent.

Noninvasive Assessment of Aortic Flow Waveform and Carotid Pressure

The aortic flow waveform, stroke volume (SV), and cardiac output were assessed using ultrasound (Vivid7, GE Vingmed Ultrasound) from the cross-sectional area of and blood velocities in the left ventricular outflow tract (LVOT). Carotid Pao was obtained using applanation tonometry at the left common carotid artery following an earlier described calibration scheme based on brachial artery tonometry. The maximum of Pao is carotid systolic pressure (SBPcap), the systolic-diastolic pressure difference is carotid pulse pressure (PPcap). In 417 subjects, brachial artery Pwf could not be recorded with exclusion of values

Averaged for men and women, (Figure 3A and 3B). Averaged for men and women, (Figure 3A and 3B). Averaged for men and women, (Figure 3A and 3B). Averaged for men and women, (Figure 3A and 3B). Averaged for men and women, (Figure 3A and 3B). Averaged for men and women, (Figure 3A and 3B). Averaged for men and women, (Figure 3A and 3B).

Derived and Measured Parameters

Zw was derived from time-aligned pressure and flow as the ratio of the corresponding pressure and flow harmonics. Zw was assessed in the frequency domain (Zin/H1), average of harmonics 3 to 10 with exclusion of values >3 times the median value of Zw over that range of harmonics). Because this method may introduce a bias to lower values of Zw, we also calculated Zw in the time domain (Zw/TD) following an approach proposed by Mitchell et al.

The modulus of Zw at 0 Hz equals systemic vascular resistance (SVR). Total arterial compliance was estimated using the pulse pressure method (CPPM) as well as the area method (Carea).

We calculated the reflection coefficient (r) of the vascular bed and used the amplitude of r at the heart frequency (r,fh) to represent the reflection coefficient. We also separated Pao into its forward (Pf) and backward (Pb) traveling component, with their ratio (Pf/Pb) giving the reflection magnitude. We further calculated the Afx and measured carotid-femoral PWV.

Determinants of Central Pulse Pressure

To assess the major determinants of central pulse pressure, we constructed forward linear regression models with CPPM as the dependent variable. In a first model, the included independent variables were age, sex, cardiac performance (SV, heart rate, and maximal aortic flow), and the parameters related to the arterial system: SVR, aortic Zw (Z-TD and Z-FD), total arterial compliance (CPPM and Carea), parameters following from wave reflection analysis (Γ1 and P0/Pf), PWV, and Afx. In a second model, we excluded the total arterial compliance CPPM and Carea to assess the contribution of Zw and wave reflection to pulse pressure. To retain the predominant parameters in both models, only parameters entering the model improving the model R² by >2% were retained.

Statistical Analysis

In the text and tables, data are given as mean values (SD). In the figure, SEMs are displayed. Subjects were subdivided into 4 half-decades of age (Q1 to Q4), defined as Q1: 35 to 40 years; Q2: 41 to 45 years; Q3: 46 to 50 years; and Q4: 51 to 56 years. Effects of age and sex were assessed with ANOVA techniques. For parameters depending on body size, height and weight were included as covariates to adjust for these confounding factors (ANCOVA) and eventually for mean arterial pressure if appropriate. Differences between men and women within an age stratum were assessed by checking for overlap of the 95% CIs of the estimated marginal mean. Nonoverlapping intervals indicated differences with P<0.05. All of the analyses were done in SPSS 12.0 (SPSS Inc).

Results

Population and general hemodynamic data are given in Tables 1 and 2. There was a relation of body length with age (P<0.001), with the subjects in Q4 being on average 3 cm taller than in Q1. Body mass index increased with age, both in men and in women. As expected, heart rate was higher in women than in men, and SV and cardiac output were lower in women, but none of these hemodynamic variables varied with age over the studied range.

Input Impedance, Zw and Total Arterial Compliance

The modulus and phase angle of Zw are displayed in Figure 1. Because body size is a major determinant of Zw, the data were adjusted by including height and weight as covariants in the statistical analysis. In men, there was a significant increase in the modulus of Zw in the low-frequency range (harmonics 1 and 2, P<0.05 and P<0.001, respectively), but the statistical power was modest (F values 3.4 and 8.1, respectively). There were pronounced oscillations in Zw modulus at the higher harmonics in the youngest subjects (most pronounced at harmonic 7), which leveled off with age. In women, the impedance modulus at harmonics 1 and 2 increased from Q1 to Q4 (all P<0.001; F value >26), whereas the oscillations at the higher harmonics (at harmonic 6) also cancelled out with age. It can be noticed that between harmonics 2 and 4, the phase angle became more negative with increasing age both in men and women (P<0.001 for harmonics 2 and 3 with F value >17; P<0.01 for harmonic 3 with F value >4.7).

Parameters describing the impedance patterns are summarized in Figure 2, with SVR being the value at 0 Hz, CPPM reflecting the impedance in the low-frequency range (harmonics 1 and 2), and Zw representing the high-frequency values of Zw. SVR increased with age and was not different between men and women. CPPM and Carea were overall higher in men (P<0.001), whereas Z-TD and Z-FD were overall higher in women (P<0.001). All of the parameters were adjusted for weight and height; CPPM, Z-TD, and Z-FD were additionally adjusted for mean arterial blood pressure. For these 3 parameters, the age–sex interaction term was significant or borderline significant (P<0.05 for Zw; P<0.07 for CPPM and Z-FD), and data were further analyzed separately. In men, CPPM (P=0.182) did not change with age, whereas it decreased in women (P<0.001). Zw increased with age in men (P<0.001) and tended to remain at the same level in women (P=0.101). Similarly, Z-TD decreased with age in men (P<0.001) and did not change in women (P=0.657). None of the 3 parameters was different between men and women over the age range 35 to 40 years (Figure 2). Comparing the 2 methods to assess Zw, we found that Zw was slightly higher than Z-TD (P<0.001, paired t test), but their values correlated well (correlation coefficient: 0.82; P<0.0001).

Reference Indices of Wave Reflection: Γ1 and P0/Pf

Both Γ1 and P0/Pf increased with age in men and women (P<0.001), but there was no gender difference for these parameters (Figure 3A and 3B). Averaged for men and women, Γ1 increased from 0.414 (0.080) in Q1 to 0.463 (0.106) in Q4, whereas P0/Pf increased from 0.449 (0.080) to...
The most obvious changes in $Z_{\text{in}}$ patterns between the age of 35 and 55 years (Figure 1) were a (modest) progressive age-related increase in SVR ($Z_{\text{in}}$ at 0 Hz) and a progressive “smoothening” of the oscillations in the modulus of $Z_{\text{in}}$ in the higher harmonic range (>5 Hz). In the phase angle, progressively more negative values for harmonics 3 to 5 with age were found, consistent with the invasive data from Murgo et al.14 Nichols and O’Rourke4 explained this shift in pattern on the basis of an asymmetrical T-tube model, where, in the young, 2 distinct reflection sites (from the upper and lower body) appear to determine the impedance spectrum. With aging and with a more rapid increase in PWV to the lower body, the timing of wave reflections from the upper and lower body becomes more similar, with the system presenting itself as a single tube with a discrete reflection site.4

Approaching the $Z_{\text{in}}$ pattern from the perspective of a windkessel model, a more negative phase angle, as we observed with increasing age (Figure 1B and 1D), is characteristic for an increase in the total arterial compliance of the model. This is a counterintuitive finding, because aging is generally associated with a loss in arterial compliance. It is possible that this finding is because of the fact that we calculated impedance combining carotid pressure with central aortic flow and, therefore, an artifact. We speculate, however, that the explanation could also be found in the basic assumptions underlying the windkessel model concept. In these lumped parameter models, the changes in pressure and flow take place simultaneously throughout the arterial tree.15 This is an assumption not entirely fulfilled, because there is wave travel at finite wave speed in the arterial tree. The faster waves travel (the higher the PWV), the better this assumption
is fulfilled, and the more the arterial tree will resemble a windkessel model. As such, it is possible that the more negative phase angles with increasing age are also a reflection of the fact that the arterial tree is better mimicked by a windkessel model in the older age range, whereas in younger subjects with slow wave travel, a model accounting for wave travel is probably more appropriate. We speculate that this “transitional behavior” of the arterial tree also explains why the AIx (an index intrinsically determined by pressure wave travel and reflection) is probably a more useful concept in young than in older subjects, where its value tends to reach a plateau.2

Although standard textbooks display age-related changes in impedance patterns,4,5,14 few data are available in larger populations for comparison with our data. Mazzaro et al16 recently measured $Z_{\text{in}}$ in 71 healthy men and women aged between 20 and 69 years and reported an increase in the minimum of the frequency spectrum ($f_{\text{min}}$) with age. In this study, we did not calculate $f_{\text{min}}$, because it is often not easy to identify the minimum in the impedance spectrum, which has a resolution limited to the fundamental frequency ($\approx 1$ Hz in humans). Nevertheless, when we calculated a theoretical $f_{\text{min}}$ based on the theory of wave reflections and the so-called quarter-wavelength formula,17 we found an increase in $f_{\text{min}}$ from $\approx 3.4$ Hz in $Q_1$ to 4.5 Hz in $Q_4$ (values averaged for men and women). These values are higher than the numbers reported by Mazzaro et al (who locate $f_{\text{min}}$ 2.6 Hz for subjects of $\approx 65$ years old)16 but are in the range reported in standard textbooks using invasive high-fidelity technology.4,5

Although the overall patterns appear qualitatively similar in men and women, there are distinct quantitative differences in $Z_{\text{in}}$ between both sexes. The modulus of the first 2 harmonics of $Z_{\text{in}}$ systematically increases with age in men and women, but the evolution is most clear in women (Figure 1). This is consistent with the data on total arterial compliance (Figure 2): CPPM systematically decreases with age in women, whereas it remains relatively constant in men. Interestingly, $Z_c$ also demonstrated a different age-related evolution in men and women: whereas it remained constant in women, $Z_c$ actually decreased in men over the studied age range. Because $Z_c$ is a parameter that can be estimated in different ways18 and that generally exhibits considerable variance, we estimated this parameter using both a time ($Z_{c-TD}$) and frequency domain ($Z_{c-FD}$) approach to exclude the possibility

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$Q_1$</th>
<th>$Q_2$</th>
<th>$Q_3$</th>
<th>$Q_4$</th>
<th>Variable</th>
<th>Age</th>
<th>Gender</th>
<th>Age×Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP$_{ba}$, mm Hg</td>
<td>Women 124.0 (13.2)</td>
<td>127.1 (14.8)</td>
<td>130.2 (14.8)</td>
<td>135.6 (17.7)</td>
<td>$P$ &lt;0.001</td>
<td>&lt;0.001</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>Men 131.1 (10.7)*</td>
<td>132.8 (12.4)*</td>
<td>134.1 (13.3)*</td>
<td>137.1 (16.5)</td>
<td>F</td>
<td>32.9</td>
<td>50.5</td>
<td>3.43</td>
<td></td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>Women 73.8 (10.8)</td>
<td>75.5 (10.6)</td>
<td>76.3 (10.1)</td>
<td>77.7 (11.2)</td>
<td>$P$ &lt;0.001</td>
<td>&lt;0.001</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Men 74.5 (9.5)</td>
<td>77.8 (10.6)</td>
<td>79.1 (9.7)*</td>
<td>79.7 (11.7)</td>
<td>F</td>
<td>17.7</td>
<td>17.2</td>
<td>1.068</td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>Women 95.9 (11.6)</td>
<td>98.1 (11.7)</td>
<td>100.1 (11.6)</td>
<td>102.9 (13.0)</td>
<td>$P$ &lt;0.001</td>
<td>0.003</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Men 97.2 (9.5)</td>
<td>100.5 (11.2)*</td>
<td>102.0 (10.6)</td>
<td>103.5 (13.2)</td>
<td>F</td>
<td>29.5</td>
<td>8.79</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>SBP$_{ca}$, mm Hg</td>
<td>Women 123.2 (14.6)</td>
<td>127.0 (16.3)</td>
<td>131.3 (16.7)</td>
<td>136.9 (19.7)</td>
<td>$P$ &lt;0.001</td>
<td>0.19</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>Men 126.6 (11.7)</td>
<td>129.4 (13.8)</td>
<td>131.1 (13.7)</td>
<td>134.9 (17.4)</td>
<td>F</td>
<td>43.9</td>
<td>1.69</td>
<td>2.91</td>
<td></td>
</tr>
<tr>
<td>PP$_{ca}$, mm Hg</td>
<td>Women 49.3 (8.9)</td>
<td>51.4 (10.9)</td>
<td>54.9 (12.7)</td>
<td>59.1 (15.2)</td>
<td>$P$ &lt;0.001</td>
<td>0.031</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Men 52.0 (9.8)*</td>
<td>51.5 (8.6)</td>
<td>51.9 (9.4)*</td>
<td>55.2 (11.6)*</td>
<td>F</td>
<td>32.7</td>
<td>4.64</td>
<td>9.59</td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>Women 65.3 (9.0)</td>
<td>65.8 (8.8)</td>
<td>65.2 (8.5)</td>
<td>65.4 (8.1)</td>
<td>$P$ 0.43</td>
<td>&lt;0.001</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Men 61.1 (9.1)*</td>
<td>61.2 (9.2)*</td>
<td>63.1 (10.3)*</td>
<td>61.8 (9.9)*</td>
<td>F</td>
<td>0.93</td>
<td>78.3</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>SV, mL</td>
<td>Women 70.8 (13.5)</td>
<td>70.7 (14.2)</td>
<td>70.8 (13.5)</td>
<td>69.5 (15.2)</td>
<td>$P$ 0.87</td>
<td>&lt;0.001</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Men 86.8 (18.7)*</td>
<td>87.1 (19.0)*</td>
<td>86.1 (18.6)*</td>
<td>86.6 (18.1)*</td>
<td>F</td>
<td>0.24</td>
<td>488</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>CO, L/min</td>
<td>Women 4.59 (0.94)</td>
<td>4.63 (1.05)</td>
<td>4.59 (0.95)</td>
<td>4.51 (0.98)</td>
<td>$P$ 0.69</td>
<td>&lt;0.001</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Men 5.23 (1.03)*</td>
<td>5.28 (1.20)*</td>
<td>5.37 (1.19)*</td>
<td>5.31 (1.16)*</td>
<td>F</td>
<td>0.49</td>
<td>224</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>LVOT, cm$^2$</td>
<td>Women 2.87 (0.46)</td>
<td>2.84 (0.49)</td>
<td>2.83 (0.43)</td>
<td>2.82 (0.43)</td>
<td>$P$ 0.11</td>
<td>&lt;0.001</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Men 3.79 (0.59)*</td>
<td>3.72 (0.60)*</td>
<td>3.72 (0.61)*</td>
<td>3.68 (0.58)*</td>
<td>F</td>
<td>2.02</td>
<td>1400</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). SBP$_{ba}$ and DBP indicate systolic and diastolic brachial blood pressure; MAP, mean arterial blood pressure; SBP$_{ca}$ and PP$_{ca}$, carotid systolic and pulse pressure; HR, heart rate; CO, cardiac output. In the 3 last columns, the $P$ and F value of the factors age and gender and their interaction term are given.

Per age category, it was verified whether the difference between men and women was statistically significant (*$P$<0.05).
that our findings were dependent on the method used to estimate $Z_c$. Both values correlated well, and both demonstrated the different trend between men and women in the evolution of $Z_c$ with age, although the effect was somewhat more pronounced for $Z_{c-TD}$.

These findings stand, at first glance, in contrast with the systematic age-related increase in PWV (+15% from Q1 to Q4), which is considered as a good marker of true arterial stiffness. Assuming that the aorta can be approximated as a reflectionless tube with an “effective cross sectional area”...
(A_{eff}) and that carotid–femoral PWV represents the PWV of this tube, one can relate PWV to changes in stiffness (distensibility coefficient), total compliance, and Zc. PWV is proportional to \(1/\sqrt{DC}\), and a 15% increase in PWV as observed both in men and women thus reflects a decrease in \(\sqrt{DC}\) of 25%. Zc varies proportional to PWV and inversely proportional to A_{eff}. In women, Zc was found to vary little over the studied age range, which suggests that the increase in PWV is paralleled by equal changes in A_{eff} (\(+15\%\)). Assuming that total compliance is proportional to A_{eff}distensibility coefficient, one therefore expects a net drop of 14% in C_{\text{PRM}} from Q1 to Q4, which is indeed the order of magnitude that we found in this study. In men, Zc actually decreased by \(\approx 15\%\), suggesting that the increase in A_{eff} (+30%) outweighed the increase in PWV. This increase in A_{eff}, together with the 25% decrease in distensibility coefficient, roughly matches with the unchanged C_{\text{PRM}} that we found in men. Mitchell et al\(^8\) already stressed the role of a reduced A_{eff} as an important mechanism contributing to elevated pulse pressure in hypertension, and they suggested a role for vessel tone in the modulation of aortic dimensions and Zc. Using MRI techniques, we found previously that an (age-related) increase in PWV can accompany aortic enlargement in patients with Marfan disease but also in control subjects. The sample size in that study (26 patients and 26 control subjects) was, however, too small to differentiate between men and women.\(^{19}\) Nevertheless, the interpretation of A_{eff} is not straightforward in the context of the aorta with its complex topology, and it is difficult to pinpoint A_{eff} to a specific anatomic location. We could not relate the cross-sectional area of the LV outflow tract (see Table 1 for the data) to the anticipated changes in A_{eff} (\(+15\%\)). Assuming that total compliance is proportional to A_{eff}distensibility coefficient, one therefore expects a net drop of 14% in C_{\text{PRM}} from Q1 to Q4, which is indeed the order of magnitude that we found in this study. In men, Zc actually decreased by \(\approx 15\%\), suggesting that the increase in A_{eff} (+30%) outweighed the increase in PWV. This increase in A_{eff}, together with the 25% decrease in distensibility coefficient, roughly matches with the unchanged C_{\text{PRM}} that we found in men. Mitchell et al\(^8\) already stressed the role of a reduced A_{eff} as an important mechanism contributing to elevated pulse pressure in hypertension, and they suggested a role for vessel tone in the modulation of aortic dimensions and Zc. Using MRI techniques, we found previously that an (age-related) increase in PWV can accompany aortic enlargement in patients with Marfan disease but also in control subjects. The sample size in that study (26 patients and 26 control subjects) was, however, too small to differentiate between men and women.\(^{19}\) Nevertheless, the interpretation of A_{eff} is not straightforward in the context of the aorta with its complex topology, and it is difficult to pinpoint A_{eff} to a specific anatomic location. We could not relate the cross-sectional area of the LV outflow tract (see Table 1 for the data) to the anticipated changes in A_{eff} (as did Mitchell et al in their response to a comment of O’Rourke et al). The question is, however, how well LVOT dimensions reflect (changes in) aortic dimensions. Although the latter have been shown to increase with age,\(^{19–21}\) there was no relation between LVOT cross-sectional area and age in our (middle-aged) population. In summary, although it is acknowledged that Zc is an estimated value (it is derived from a carotid tonometry curve combined with blood flow measured in the LVOT, and its value depends on appropriate realignment in time of this pressure and flow curve) and depends on body size and inherent assumptions on aortic physiology, the different evolution with time in men and women over this specific age range is at least remarkable and deserves our further attention.

One of the goals of the study was to assess the relation between wave reflection and AIx. This relation is relatively modest, with correlation coefficients <0.6. Also, unlike for AIx, there was no gender difference in \(\Gamma_1\) or P/Pf. As mentioned before, AIx is a composite measure, and although

### TABLE 3. Determinants of Carotid Pulse Pressure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cumulative (R^2)</th>
<th>(\beta)</th>
<th>SE</th>
<th>Normalized (\beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(constant)</td>
<td>2.65</td>
<td>1.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C_{\text{PRM}})</td>
<td>0.314</td>
<td>-34.65</td>
<td>0.52</td>
<td>-0.99</td>
</tr>
<tr>
<td>SV</td>
<td>0.783</td>
<td>0.690</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>SVR</td>
<td>0.833</td>
<td>5.69</td>
<td>0.53</td>
<td>0.15</td>
</tr>
<tr>
<td>(Z_{10})</td>
<td>0.853</td>
<td>113.3</td>
<td>4.7</td>
<td>0.36</td>
</tr>
<tr>
<td>(\Gamma_1)</td>
<td>0.873</td>
<td>23.41</td>
<td>1.35</td>
<td>0.19</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(constant)</td>
<td>-38.02</td>
<td>1.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Z_{10})</td>
<td>0.244</td>
<td>335.5</td>
<td>5.1</td>
<td>1.08</td>
</tr>
<tr>
<td>(Q_{\text{max}})</td>
<td>0.443</td>
<td>0.120</td>
<td>0.002</td>
<td>0.87</td>
</tr>
<tr>
<td>AIx</td>
<td>0.707</td>
<td>0.433</td>
<td>0.010</td>
<td>0.57</td>
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</tbody>
</table>

Results of the multiple linear regression analysis. Model 1 includes age and gender, SV, heart rate, and all parameters obtained from wave reflection and impedance analysis as independent variables. It leads to a model that adheres to the “windkessel” view of the arterial tree, with pulse pressure determined by SV, CPPM, and SVR. Model 2, excluding total arterial compliance, shows that \(\Gamma_1\) and \(Q_{\text{max}}\) (which largely determine the magnitude of the forward pressure wave) account for 44% of total variance, and that AIx explains an additional 26%. \(R^2\) is the cumulative value after adding the parameter to the model, \(\beta\) the parameter value, and SE the SE on \(\beta\).
it is related to the magnitude of wave reflection, it is obvious that other factors codetermine its value. Given the fact that PWV was not different between men and women and that we statistically corrected for differences in ejection duration and subject height, the origin of the gender difference in AIx is likely to be sought in gender-related differences in the relative distance to the reflection sites, differences in LV ejection patterns or other factors that are overlooked here. Possibly an approach as recently published by Westerhof et al. to assess reflection coefficients from $P_e$, alone (they assume a triangular approximation for the flow waveform and apply a wave decomposition similar to what we presented here) may be applicable in clinical practice.

There has been some recent discussion on the importance of wave reflection and its contribution to elevated pulse pressure. The prevailing view is that high pulse pressure (isolated systolic hypertension) is caused by a progressive degeneration and dilatation of the aorta and increased stiffening, causing an early return of pressure waves and boosting systolic pressure. Our data (Table 3, model 2) show that in a representative middle aged population, peak aortic flow and $Z_{TD}$ (which largely account for the amplitude of the forward pressure wave as $P_e - Z_{TD}$) maximal aortic flow) account for $\approx 44\%$ of the variance in central pulse pressure and that wave reflection (AIx) explains an additional $26\%$. This supports the findings of Mitchell et al., who stressed the importance of the amplitude of the forward pressure wave as the major factor determining pulse pressure. However, in a model that also included total arterial compliance (model 1), the parameters determining PPCA were total arterial compliance ($C_{PPM}$), SV, and SVR, explaining $83\%$ of the variance in PPCA. One might argue that this finding is mainly driven by the inherent high dependency of $C_{PPM}$ on $PPCA$. Excluding $C_{PPM}$ yielded a slightly different model with $C_{sec}$, SV, and $Z_{TD}$ as determinants, with the first 2 parameters accounting for $65\%$ and $Z_{TD}$ for an additional $10\%$ of the variance of PPCA. These results thus match with the “windkessel” notion that pulse pressure is predominantly determined by the low frequency properties of the arterial system (total arterial compliance). 9

Finally, we also wish to stress some limitations of our work. Although the noninvasive character of the methodology is a prerequisite for large-scale studies such as ours, it also implies some methodologic limitations. We used handheld carotid applanation tonometry recordings as surrogates for central pressure curves and combined these with flow waveforms measured at the LVOT. Although both curves were carefully realigned in time, the contours of central and carotid pressure are not entirely equal, which may have an effect on our impedance data and calculated reflection coefficients. We could not complete the tonometer calibration protocol (which includes brachial tonometry) in all of the subjects. Although we accounted for the brachial-to-radial amplification using a regression equation, this approach has introduced some additional variability in the data. On the other hand, these data sets were distributed randomly in the population, and the impact of this alternative calibration procedure in the subgroup should be limited. Also, the age range in our study is rather narrow. This has the advantage of focused analysis over this specific age range but precludes analysis of effects of aging. We did not perform a subgroup analysis in women to assess the potential effect of menopause or hormonal therapy.

**Perspectives**

Over the age of 35 to 55 years, the $Z_o$ of the systemic arterial system evolves from a pattern indicative of wave transmission and reflection to a pattern more compatible with a windkessel-like system. Indices targeting the quantification of wave reflection may therefore have more importance in young and middle-aged subjects than in the elderly. In healthy, middle-aged subjects, the magnitude of wave reflection increases with age both in men and women without any gender difference. There is also a progressive stiffening of the arterial tree as evidenced by an increasing PWV, but this evolution is not paralleled by increases in aortic $Z_e$. This is suggestive for adaptive mechanisms modulating the (“effective”) aortic cross-sectional area. Total aortic compliance is found to be the primary determinant of carotid pulse pressure in our population, and it outweighs the contributions of aortic $Z_e$ or wave reflection. As such, measurement of central pressure and flow for the assessment of global arterial parameters and arterial impedance remains most relevant and provides important mechanistic information that is, at least, complementary to the more frequently measured PWV and the AIx.

**Acknowledgments**

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**Disclosures**

None.

**References**


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on behalf of the Asklepios investigators

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Non-invasive (Input) Impedance, Pulse Wave Velocity and Wave Reflection
in Healthy Middle-Aged Men and Women

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Running title: Arterial function in healthy men and women

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Of the 2524 subjects (1301 women, 1223 men) participating at the Asklepios study, 2026 (1052 women, 974 men) met the requirements to be included into this study. Three hundred and sixty five (365) subjects were excluded because of antihypertensive and/or lipid lowering drug treatment and an additional 133 data sets were incomplete due to missing flow data (n=7) or left ventricular outflow tract dimensions (n=8), inability to accurately assess carotid tonometry (n=32) and/or technical failure in the non-invasive pressure measurement set-up (n=109). A complete description of the Asklepios study and its methodological details can be found elsewhere\textsuperscript{1}. All recordings were performed by one single investigator trained in Doppler echocardiography and applanation tonometry (ERR) and analysed by a second investigator (PS) who was blinded to the basic clinical data (with exception of the blood pressure values that were required for the analysis). The study was approved by the ethical committee of the Ghent University Hospital and all subjects gave informed consent to participate at the study.

*Basic clinical data*

After measurement of height, weight, waist and hip circumference, distances from sternal notch to carotid, radial and femoral arteries, subjects were allowed 10-15 minutes of rest in a temperature-controlled environment before examinations. Body mass index was calculated as weight(kg)/height(m\(^2\)) while body surface area (BSA) was calculated using the formula of Dubois and Dubois: BSA (m\(^2\)) = 0.20247 x Height(m\(^{0.725}\) x Weight(kg\(^{0.425}\). Blood pressure was recorded at various time points throughout the protocol; the value reported here is the blood pressure measured in between the echographic and tonometer measurements using a validated oscillometric Omron HEM-907 device (Omron Matsusaka Co. Ltd., Japan). Subjects were subdivided into 4 half-decades of age (Q\(_1\) to Q\(_4\)), defined as Q\(_1\): 35 - 40; Q\(_2\): 41 - 45; Q\(_3\): 46 - 50; Q\(_4\): 51-56.
Central flow waveform ($Q_{wf}$): echocardiography

The subjects underwent a resting echocardiographic examination using a commercially available ultrasonographic system (VIVID 7, GE Vingmed Ultrasound, Horten, Norway) equipped with a cardiac (M3S) probe. Subjects were examined in the left lateral recumbent position. All measurements were ECG-gated and consisted of cineloops or recordings of at least 5 (up to 30) cardiac cycles during normal breathing. The internal diameter of the left ventricular outflow tract (LVOT) was measured in the parasternal long-axis view at the valve annulus. LVOT area was calculated assuming circularity. Flow velocities were acquired in the LVOT using pulsed wave Doppler in the apical 5-chamber view. Images were exported in raw DICOM format and processed off-line within a dedicated software interface written in Matlab (The Mathworks, Natick, MA). For each cardiac cycle, the onset and end of systolic ejection were visually delineated with two cursors, after which the contours in the systolic phase were automatically traced using the transition in pixel intensity above a user-defined threshold value. Two to three cycles were averaged, and the average cycle sub-sampled to 500 sample points and smoothed using a Savitsky-Golay filter (order 3, frame width 31). Maximal velocities were multiplied with the LVOT cross-sectional area to obtain the aorta flow waveform ($Q_{wf}$). Stroke volume (SV) was obtained by integration of $Q_{wf}$, and cardiac output (CO) as the product of SV and heart rate (HR).

Central pressure waveform ($P_{wp}$): applanation tonometry

Applanation tonometry was performed with a Millar pen-type tonometer (SPT 301, Millar Instruments, Houston, Texas, USA) using a dedicated hard- and software platform. The measurement set-up, processing and calibration procedure were previously described\textsuperscript{2,3}. Briefly, tonometry was first performed at the level of the left brachial artery, and the
tonometric recording was calibrated by designating oscillometric systolic (SBP_{BA}) and diastolic (DBP) blood pressure to the peak and trough of the curve (see also Figure IA). Numerical averaging of the scaled brachial pressure waveform yielded mean arterial pressure, MAP. Next, tonometry was performed at the left common carotid artery, and this waveform was calibrated using DBP and MAP, and considered as a surrogate for central pressure waveform (P_{wf}). The maximum of P_{wf} is carotid systolic pressure (SBP_{CA}); the systolic-diastolic pressure difference PP_{CA}. In 417 subjects, brachial artery pressure waveforms could not be recorded with satisfactory quality within a reasonable time frame. In these subjects, MAP was calculated from a scaled radial artery waveform. For the scaling, we used DBP and an estimated value for radial systolic blood pressure (SBP_{RA}) obtained as: SBP_{RA} = 4.974 + 1.11SBP_{BA} - 0.178 DBP, r^2 = 0.914, an expression derived from linear regression analysis using a subset consisting of 1863 subjects where brachial and radial artery applanation tonometry were available. With this approach, we accounted for the brachial-to-radial amplification of systolic blood pressure^2.

\textit{Input impedance, total arterial compliance and indices of wave reflection: } |\Gamma|, P/P_f

P_{wf} and Q_{wf} were visually time-aligned, using (a) the rapid systolic upstroke of pressure and flow and (b) the dicrotic notch in the pressure signal and cessation of flow as reference points for the alignment. To assess input impedance (Z_{in}), pressure, P_{wf}, and flow, Q_{wf}, were decomposed into a series of sinusoidal harmonics using a Fourier transform, each represented by its amplitude (modulus) and phase angle. Z_{in} was calculated as the ratio of the pressure and flow harmonics. Characteristic impedance, Z_{c}, was assessed in the frequency domain (Z_{c-FD}) by averaging the modulus of the 3\textsuperscript{rd} to 10\textsuperscript{th} harmonic of Z_{in}, excluding, however, those frequencies where the modulus was higher than 3 times the median value of the modulus of Z_{in} over the 3\textsuperscript{rd} to 10\textsuperscript{th} harmonic. As this method may introduce a bias to lower values of Z_{c},
we also calculated $Z_c$ in the time domain ($Z_{c-TD}$) as the slope of the linear flow-pressure relation in the early systolic phase following an approach proposed by Mitchell et al.\textsuperscript{4}: $Z_{c-TD} = \Delta P/(0.95*Q_{max})$, with $\Delta P$ the difference between the pressure at the moment where the flow reaches 95% of max flow ($Q_{max}$) and diastolic blood pressure. The modulus of input impedance at 0 Hz equals total systemic vascular resistance (SVR). Total arterial compliance was estimated using the pulse pressure method ($C_{PPM}$)\textsuperscript{5}, an iterative method based on a 2-element windkessel model, as well as the area method ($C_{area}$) which is based on the area under the diastolic portion of the pressure wave\textsuperscript{6}.

The reflection coefficient ($\Gamma$) of the vascular bed is given by:

$$\Gamma = \frac{Z_{in} - Z_{c-FD}}{Z_{in} + Z_{c-FD}}$$

Similar to $Z_{in}$, $\Gamma$ is also a complex number (represented by amplitude and a phase angle) and function of frequency. We used the amplitude of $\Gamma$ at the heart frequency, $|\Gamma|$, to represent the reflection coefficient.

As demonstrated by Westerhof et al.\textsuperscript{7}, the pressure wave can be separated into its forward ($P_f$) and backward ($P_b$) traveling component (i.e., the reflected wave) with knowledge of pressure ($P_{wf}$), flow ($Q_{wf}$) and local characteristic impedance ($Z_{c-FD}$):

$$P_f = (P_{wf}+Z_{c-FD}Q_{wf})/2$$

$$P_b = (P_{wf}-Z_{c-FD}Q_{wf})/2$$

The ratio of the amplitudes of $P_b$ and $P_f$ ($P_b/P_f$) yields the reflection magnitude.

*Pulse wave contour analysis: the augmentation index*
The augmentation index (AIx) formally quantifies the pressure waveform classification scheme of Murgo et al.\textsuperscript{8}, and was calculated as

\[
\text{AIx} = 100 \frac{P_2 - P_1}{\text{SBP}_{CA} - \text{DBP}}
\]

P\textsubscript{1} and P\textsubscript{2} are either SBP\textsubscript{CA}, either the pressure associated with the “characteristic point” identified on P\textsubscript{wf}, depending on which occurs first (=P\textsubscript{1}). We automatically detected the timing of this characteristic point (T\textsubscript{cp}) using an algorithm described by Takazawa et al\textsuperscript{9}, based on the 4\textsuperscript{th} derivative of the pressure signal. In young subjects with a late return of the reflected wave, AIx is negative. It is positive in older subjects with an early return of the reflected wave. The augmentation pressure (AP) is defined as P\textsubscript{2} – P\textsubscript{1}. The left ventricular ejection duration (T\textsubscript{sys}) was taken as the time interval between the foot of the carotid pressure wave and the dicrotic notch.

\emph{Pulse Wave Velocity}

Pulse wave velocity (PWV) was estimated as \((\Delta L_{S,F} - \Delta L_{S,C})/(\Delta T_{Q,F} - \Delta T_{Q,C})\) with \(\Delta L_{S,F}\) and \(\Delta L_{S,C}\) the distance measured from sternum to femoral and carotid measuring sites, respectively. \(\Delta T_{Q,F}\) and \(\Delta T_{Q,C}\) are the time delay between the start of the QRS complex and the upstroke of flow measured with Doppler echography in the femoral and carotid artery, respectively.

\emph{Determinants of central pulse pressure}

To assess the major determinants of central pulse pressure, we constructed forward linear regression models with PPC\textsubscript{CA} as dependent variable. In a first model, included independent variables were age, gender, cardiac performance (SV, HR and maximal aortic flow ,Q\textsubscript{max}) and the parameters related to the arterial system: SVR, aortic characteristic impedance (Z\textsubscript{c-TD}, Z\textsubscript{c-FD}), total arterial compliance (C\textsubscript{PPM}, C\textsubscript{area}), parameters following from wave reflection analysis
(|Γ1|, P_b/P_f), PWV and AIx. In a second model, we excluded the total arterial compliance 
C_{PPM} and C_{area} to assess the contribution of Z_c and wave reflection to pulse pressure. To retain 
the predominant parameters in both models, only parameters entering the model improving 
the model $R^2$ by more than 2% and with an arbitrarily chosen F-value > 200 were retained.

Statistical analysis

In the text and tables, data are given as mean values (standard deviation). In the figures, 
standard errors on the mean are displayed. Effects of age and gender were assessed with 
analysis of variance techniques (ANOVA), using gender and age stratum as fixed factors. For 
parameters depending on body size, height and weight were included as covariants to adjust 
for these confounding factors (ANCOVA) and eventually for MAP if appropriate. Values for 
AIx were adjusted for systolic duration and body length. P<0.05 is considered statistically 
significant. F-values are given as well when relevant as an indication of the statistical power. 
All analysis was done in SPSS 12.0 (SPSS Inc, Chicago, Illinois).
References


Figure legend

Figure SI. The calibrated carotid artery pressure waveform ($P_{wf}$, panel C) is obtained from brachial (panel A) and carotid (panel B) artery applanation tonometry. Pulsed Doppler measurement of blood flow velocity in the left ventricular outflow tract (LVOT, panel D), together with LVOT cross sectional area, yields central blood flow waveform ($Q_{wf}$, panel E). $P_{wf}$ and $Q_{wf}$ yield input impedance modulus (panel F) and phase angle (panel G). Linear wave separation theory allows to separate the pressure wave into a forward ($P_f$) and backward ($P_b$) component (panel H).
Table SI. Parameters indicating the timing and magnitude of reflected waves.

<table>
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<tr>
<th>parameter</th>
<th>gender</th>
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<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>statistical power</th>
<th>age</th>
<th>gender</th>
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<td></td>
<td></td>
<td>age</td>
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<td>$T_{sys}$ (ms)</td>
<td>F</td>
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<td>356.1 (31.9)</td>
<td>360.0 (31.2)</td>
<td>364.8 (31.0)</td>
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<td>350.1 (33.5)</td>
<td>354.2 (33.2)$^*$</td>
<td>$F$ 4.99</td>
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<td>$T_{cp}$ (ms)</td>
<td>F</td>
<td>122.8 (44.7)</td>
<td>113.5 (35.1)</td>
<td>112.9 (37.6)</td>
<td>106.7 (24.5)</td>
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<td>152.9 (56.4)$^*$</td>
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<td>$F$ 44.3</td>
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<td>AP (mmHg)</td>
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</tbody>
</table>

Data are expressed as mean (standard deviation). F: female gender; M male gender. $T_{sys}$: systolic ejection duration; $T_{cp}$: time of occurrence of the characteristic point identified using 4th order derivatives; $P_{*cp}$: pressure associated with $T_{cp}$; AP: pressure augmentation; $\Delta T_{f,b}$: time delay.
between forward and backward wave; PWV: carotid-femoral pulse wave velocity adjusted for MAP. In the 3 last columns, the $P$- and $F$-value of the factors "age", "gender" and their interaction term are given. Per age category, it was verified whether the difference between men and women was statistically significant (*; $P<0.05$).
Figure SI