Large Artery Stiffness Is Not Related to Plasma Cholesterol in Older Subjects with Hypertension

Anthony M. Dart, Christoph D. Gatzka, James D. Cameron, Bronwyn A. Kingwell, Yu-Lu Liang, Karen L. Berry, Christopher M. Reid, Garry L. Jennings

Objective—Previous studies have demonstrated a prognostic role of large artery stiffness in hypertensive subjects and increased stiffness in subjects with coronary artery disease. Although plasma cholesterol is an established risk factor for cardiovascular disease, its relationship with large artery properties in a hypertensive population is unclear.

Methods and Results—Plasma cholesterol and large artery properties were measured at baseline in a subset of participants of a randomized controlled trial (ANBP2) evaluating hypertension treatment in older (65 to 84 years) subjects. Noninvasive measures of large artery behavior were central augmentation index (AI), systemic arterial compliance (SAC), and transverse expansion of the aortic arch (aortic distensibility). Arterial waveforms acceptable for analysis were obtained in ∼80% of cases yielding valid measurements of AI in 868, SAC in 846, and aortic distensibility in 680 subjects. Mean total and high-density lipoprotein (HDL) concentrations were 5.5±1.0 and 1.4±0.5 mmol L⁻¹. Total and HDL cholesterol and AI were greater in females than males, whereas SAC and aortic distensibility were greater in males. In multiple regression analyses there were no significant associations between stiffness parameters and total or HDL cholesterol. Significant independent associations in such analyses were found for mean arterial blood pressure, gender, age, height, and heart rate, in keeping with previous findings.

Conclusions—In the largest cohort of elderly hypertensive subjects studied to date, plasma cholesterol per se was not associated with large artery stiffness. Such independence from cholesterol increases the potential for artery stiffness measurements to additionally contribute to cardiovascular risk assessment in this population. (Arterioscler Thromb Vasc Biol. 2004;24:962-968.)

Key Words: risk factors ■ arterial compliance ■ arterial hypertension ■ hypercholesterolemia ■ population studies

The stiffness of the aorta and other large arteries increases with age.¹-³ Beyond ageing, a further increase in arterial stiffness is observed in the presence of coronary artery disease.⁴-⁸ This presumably reflects the widespread nature of the atherosclerotic process.⁹,¹⁰ Thus, conversely, an increase in arterial stiffness could be an early predictor of coronary risk useful in screening. An increase in pulse-wave velocity (a consequence of increased large artery stiffness) has been shown to relate to outcome in a hypertensive population.¹¹ A number of studies have also examined the relation between plasma total or low-density lipoprotein (LDL) cholesterol and large artery stiffness in normotensive, asymptomatic patients. These studies have reported no relation,¹²-¹⁴ reduced,⁵,¹⁵-¹⁷ and increased stiffness with elevated total or LDL cholesterol.¹⁵,¹⁸,¹⁹ The effects of cholesterol in a hypertensive population are of particular interest because of their high risk for cardiovascular events and thus potential for screening. The additional value of assessing large artery stiffness would be diminished if increased stiffness were simply associated with cholesterol, which could itself be easily measured. Studies of conduit artery stiffness in hypertensive subjects have, however, been limited to the carotid and radial artery.²¹

In the present study, we report on the association between total and high-density lipoprotein (HDL) cholesterol levels and a range of arterial properties in the largest cohort of older hypertensive subjects studied to date. Large artery properties were assessed locally at the level of the transverse aortic arch, globally as total systemic arterial compliance, and also in terms of the augmentation of central systolic pressure (augmentation index [AI]).

Methods

Subjects were recruited from the 6083 participants in the Australian Comparative Outcome Trial of Angiotensin-Converting Enzyme Inhibitor- and Diuretic-Based Treatment of Hypertension in the Elderly (ANBP2).²² This was a comparative outcome trial using a prospective, randomized, open-label design with blinding of endpoint assessments. Subjects were recruited through general practices. They were seen by a study nurse 3 times at least 1 week apart to assess eligibility for the trial. Subjects previously treated with blood pressure-lowering treatment were required to discontinue such therapy for at least 2 weeks before the eligibility assessments. Eligibility
required an age between 65 and 84 years, an untreated systolic blood pressure of $\geq 160$ mm Hg or diastolic pressure $\geq 90$ mm Hg if systolic pressure at least $\geq 140$ mm Hg, no stroke or myocardial infarction within the previous 6 months, serum creatinine $<2.5$ mg/dL, and no cardiac failure, dementia, or serious comorbidity. After ascertaining eligibility, but before randomization, participants recruited in the greater Melbourne area were asked to participate in a substudy on left ventricular and arterial properties, independent of their enrolment in the main trial, and informed consent was obtained. Of these, 1204 agreed to participate in the combined substudy. Arterial measurements were not available because of technical reasons (e.g., equipment failure, only single operator available, or time constraints) in 115 subjects. In a further 218 subjects, waveform measurements were found to be impossible to obtain or unsatisfactory either by the technical operator at the time of data acquisition or at subsequent waveform analysis (by K.L.B.). A further 3 subjects were excluded because of nonavailability of cholesterol measurements. Data exclusion occurred without knowledge of other patient data (cholesterol level, medical history, etc). Previous separate ethics approval for the substudy and use of associated trial data were obtained from the Ethics Committee of the Royal Australian College of General Practitioners.

Subject Characteristics
Information on past medical history (including diabetes and the presence of vascular disease), medication, and cigarette consumption was obtained from the ANBP2 data center for all participants in the substudy.

Biochemical Measurements
Plasma total and HDL cholesterol were determined on a random sample for all participants. At the request of the responsible general practitioners, blood samples were drawn and analyses performed by accredited local public or private pathology laboratories. All laboratories participated in an externally monitored quality-assurance program.

Biomechanical Measurements
All measurements in this study were made by 2 trained operators. Subjects were placed in the recumbent position in a quiet air-conditioned room. After at least 10 minutes of rest, a carotid pressure waveform was obtained via applanation tonometry of the proximal right carotid artery using a pencil-tip transducer (Micro-tip SPT-301; Millar Instruments, Houston, Tex). Blood flow was measured simultaneously with a continuous wave 4.0-MHz zero-crossing Doppler velocimeter in the suprasternal notch directed at the ascending aorta (Multi Dopplex MD1; Huntleigh Technology, Cardiff, United Kingdom). Brachial blood pressure was measured in triplicate with a Dinamap 1846 SXp (Critikon, Tampa, Fla), and the average of measurements was used for calculation of systemic arterial compliance and reported in the article.

Immediately afterward, 2-dimensional-guided M-mode echocardiography of the aortic root and transverse aortic arch (suprasternal, long axis) was performed as previously described using Hewlett-Packard Sonos 500 equipment and recorded on videotape.23-25

Calculation of Biomechanical Indices
From the waveform recordings, at least 3 and a maximum of 10 representative cardiac cycles were selected by a single operator (K.L.B.). Waveforms were linear detrended, assuming equality at the start and end of each cardiac cycle, scaled to brachial diastolic and mean pressure, truncated to the length of the shortest cycle, and an arithmetic average was used for subsequent analysis. Besides selection of representative waveforms, all analysis was fully automated.

On the carotid waveform as obtained by tonometry, we identified the augmentation point from the first zero-crossing from positive to negative of the fourth derivative occurring at least 50 ms after the foot of the waveform.26 Augmented pressure was determined as the difference between the pressure at the augmentation point and peak systolic pressure, with AI as the ratio between this and the pulse pressure in that particular cardiac cycle.

Systemic arterial compliance (SAC) was determined from the same averaged cardiac cycles using measurements of pressure and Doppler flow of the ascending aorta.23 From the videotape recordings, a single operator (Y.L.L.) measured internal dimensions of the transverse aortic arch in at least 3 and a maximum of 5 representative cardiac cycles. Aortic distensibility was calculated as relative aortic expansion (aortic systolic-diastolic divided by diastolic diameter) divided by the difference between average peak pressure on the scaled waveforms as obtained above and brachial diastolic pressure. The repeatability over 2 to 4 weeks of SAC, AI, and aortic distensibility have been reported previously, including Bland-Altman plots of the data.24,27,28

Statistical Analysis
All data are presented as mean±SD, except when indicated otherwise. Comparisons between means were evaluated by unpaired t test or ANOVA (with post hoc Scheffe test) for continuous variables and by $\chi^2$ for proportions. Linear regression was by the method of least squares with list-wise deletion of missing cases. Multiple regression statistics were calculated using SPSS for Windows version 11.5.1 (SPSS Inc, Chicago, Ill). Tertiles were defined for men and women separately based on available values for total, HDL, and total/HDL cholesterol ratios.

<table>
<thead>
<tr>
<th>Table 1. Differences Between Males and Females</th>
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<tr>
<td>Age (y)</td>
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<tr>
<td>Systolic blood pressure (mm Hg)</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Total cholesterol (mmol L⁻¹)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol L⁻¹)</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio (1/1)</td>
</tr>
<tr>
<td>Atheroma (%)</td>
</tr>
<tr>
<td>Lipid lowering therapy (%)</td>
</tr>
<tr>
<td>Cigarette smoking status (%)</td>
</tr>
<tr>
<td>Never/Ex/Current</td>
</tr>
<tr>
<td>Diabetes (%)</td>
</tr>
<tr>
<td>Previous antihypertensive therapy (%)</td>
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</table>

Results
Subject characteristics are given in Table 1 for all subjects combined, as well as for males and females separately. Total cholesterol values ranged from 2.7 to 10.3 mmol L⁻¹. Mean total and HDL cholesterol values were similar to those for the whole ANBP2 cohort of 6083 subjects: respective values in ANBP2 were 5.7±1.0 and 1.3±0.5 mmol L⁻¹ for all subjects, 5.4±1.0 and 1.2±0.4 mmol L⁻¹ for males, and 5.9±1.0 and 1.5±0.5 mmol L⁻¹ for females.

Female subjects were slightly older, had a lower diastolic blood pressure and higher total and HDL cholesterol levels, but a lower total/HDL ratio than male subjects. Females were less likely to have diabetes (Table 1) and more likely to have never smoked cigarettes. Atheroma (defined as the presence
Aortic Stiffness at the Level of the Aortic Arch

For the total population the average value of aortic distensibility was 0.52±0.36 · 10⁻³ mm Hg⁻¹. Values ranged from 0 to 3.33 with 25th and 75th percentiles of 0.29 and 0.67 and a median of 0.44 · 10⁻³ mm Hg⁻¹.

Although aortic distensibility was not related to the level of total cholesterol in either males or females (Figure 1, bottom), it was significantly higher at lower levels of HDL cholesterol in males (Figure 2, bottom, P=0.02). No such effect was observed in women, and distensibility in women with HDL <1.1 mmol L⁻¹ (N=33, distensibility index 0.48±0.42 · 10⁻³ mm Hg⁻¹) was not significantly different from women with HDL above that value. Consistently, distensibility was greatest in males with a higher total/HDL cholesterol ratio (Figure 3, bottom, P=0.005). Aortic distensibility was less in women than in men.

Augmentation Index

For the total population, the average value of AI was 34%±13%. Values ranged from −3 to 71, with 25th and 75th percentiles of 23 and 43 and a median of 34%.

AI was not related to the level of total, HDL, or total/HDL cholesterol ratio (Figures 1 through 3, middle) in either males or females. AI was greater in women than in men. In multiple regression analysis, AI was greater at increased mean arterial blood pressure and less with increase in heart rate, height, and age (Table 2) with AI (%)=100.6−0.403 · heart rate (min⁻¹) + 6.56 · gender (female=1, male=0) + 0.198 · mean arterial pressure (mm Hg) − 0.272 · height (cm) − 0.273 · age.
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with increase in height (Table 2) with SAC (mL increase in mean arterial blood pressure and age and greater females. In multiple regression analysis, SAC was less with either males or females. SAC was greater in males than in the total/HDL cholesterol ratio (Figures 1 through 3, top) in the range.

In separate analyses of males and females (Table 1), associations of AI were similar to the overall population, except that heart rate was significant in males.

Discussion

In this study of older hypertensive subjects with a low prevalence of known symptomatic atheromatous disease, large artery stiffness was not related to the level of total cholesterol. There were univariate relationships with both HDL cholesterol and total/HDL cholesterol ratio for aortic distensibility in men, but these effects were no longer significant in multiple regression analyses, because of confounding by age, mean arterial pressure, and heart rate. The large size of the cohort together with the use of several measures of arterial stiffness makes it unlikely that an independent effect of plasma cholesterol would have been missed if one had in fact existed. The study had a power of >90% to detect an r2 of 1% or more for such an association.

Major mechanisms by which cholesterol might affect the arterial indices reported in this study are through alteration in endothelial function and the development of atheroma. Effects of circulating lipoproteins on endothelial function are well recognized. A subsequent effect on indices of large artery function seems more likely for those particularly influenced by wave reflection from smaller vessels, because a direct effect from the endothelium on relatively thick vessels seems less likely. A study of the effect of altering endothelial function pharmacologically concluded that the observed changes in large artery properties were most likely passive and caused by concomitant changes in arterial blood pressure. A second plausible mechanism linking lipoproteins and artery stiffness could be through the presence of atheroma. Increased large artery stiffness has been found in subjects with coronary artery disease in a number of studies, although an association with a composite vascular endpoint (peripheral, cerebral, and coronary) was not apparent in the present cohort.

Other associations of large artery stiffness generally were in agreement with previous findings. Thus large artery stiffness was greater at higher mean arterial blood pressure and greater in women. Similarly, the association between AI and height and heart rate, which will influence the relation between the time of reflected pressure waves and left ventricular ejection, is also well known. Although increasing age is generally found to be associated with increased stiffness, the restricted age range of the elderly cohort limited its significance.

The absence of an association between artery stiffness and cholesterol in this cohort is perhaps not surprising, given that 2 of the major determinants of artery stiffness, namely older age and hypertension, were present in all subjects, thereby reducing the potential role for other factors. In addition, it is possible that in this older population, subjects with elevated total cholesterol (or low HDL cholesterol) and increased
artery stiffness are underrepresented because of such subjects having died or having become seriously ill before reaching age 64 years. Such a phenomenon may also have contributed to the previous finding of a less steep increase in aortic stiffness with age among asymptomatic subjects with hypercholesterolemia than among controls. The lack of association with cholesterol in older hypertensives might, however, increase the additive predictive power of artery stiffness measurements because they would not be interchangeable with the (far more readily determined) measures of plasma cholesterol, as would be the case if there were a close relationship between these parameters.

There have been several other previous studies examining the relationship between plasma lipids and various measures of large artery stiffness. Studies in normotensive, asymptomatic populations have given conflicting results, perhaps in part because of limited group sizes, with reports of no relationship,12,13 because of limited group sizes, with reports of no relationship12,13 or greater stiffness at high HDL levels.17 Equivalent studies in a hypertensive population are limited to measurement of AI and showed no additional effect of elevated total cholesterol.20 There have also been studies of the local mechanical properties of the carotid20 and radial21 arteries, which also failed to show an additional effect of cholesterol in hypertensive populations.

Table 2: Results of Multiple Regression Analysis for the Dependent Variables

<table>
<thead>
<tr>
<th>Gender</th>
<th>All</th>
<th>0 Male</th>
<th>1 Female</th>
<th>Height</th>
<th>MAP</th>
<th>HR</th>
<th>Total C</th>
<th>HDL C</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>Age</td>
<td></td>
<td></td>
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<tr>
<td>Subjects</td>
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<tr>
<td>AI</td>
<td>0.54</td>
<td>0.10</td>
<td>0.26</td>
<td>0.24</td>
<td>0.35</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SAC</td>
<td>0.54</td>
<td>0.15</td>
<td>0.15</td>
<td>0.41</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Aort Dist</td>
<td>0.40</td>
<td>0.15</td>
<td>0.18</td>
<td>0.12</td>
<td>0.36</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

| Males |        |        |         |        |     |    |        |       |
| AI     | 0.51  | NS    | NA     | 0.35  | 0.39 | NS | NS |
| SAC    | 0.52  | 0.19  | NA     | 0.45  | 0.11 | NS | NS |
| Aort Dist | 0.42  | 0.18  | NA     | 0.37  | 0.11 | NS | NS |

| Females |        |        |         |        |     |    |        |       |
| AI     | 0.43  | 0.17  | NA     | 0.19  | 0.34 | NS | NS |
| SAC    | 0.49  | 0.14  | NA     | 0.43  | 0.11 | NS | NS |
| Aort Dist | 0.38  | 0.11  | NA     | 0.35  | NS | NS | NS |

AI indicates augmentation index; SAC, systemic arterial compliance; Aort Dist, aortic distensibility; NS, not significant; NA, not applicable.

Independent variables entered were age, gender, height, mean arterial blood pressure (MAP), heart rate (HR), total plasma cholesterol (Total C), HDL cholesterol (HDL C), presence of atheroma, use of lipid-lowering therapy, previous use of blood pressure-lowering therapy, presence of diabetes, and cigarette smoking status (never/ex/current).

The composite r values and standardized beta coefficients are shown for each of the analyses.

Intervention studies with cholesterol-lowering medication have been performed in younger subjects. A comparison with the current study is not only complicated by the marked difference in patient characteristics between those studies and the study reported here but also by the possibility of vascular effects of statins, which are unrelated to cholesterol reduction.

In the current study, a variety of measures were used to assess large artery stiffness. However, all fundamentally relate to the capacity of the artery wall to become displaced by an increase in intraluminal pressure. This is directly assessed at the level of the aortic arch by aortic distensibility. Aortic expansion is determined by transthoracic echocardiography with the use of central arterial pressure determined by applanation tonometry. AI is partly determined by the timing of reflected pressure waves and hence depends on the velocity of pulse-wave transmission. An increase in arterial wall stiffness produces an increase in pulse-wave velocity, an earlier return of reflected pressure waves, and consequently an increase in AI measured centrally. Systemic arterial compliance is a functional measure of the capacitance of the arterial system derived from the 2-element Windkessel model. Acceptable waveforms were obtained in 80% of those in whom measurements were attempted. Given the similarity in total and HDL cholesterol between this population and the main ANBP2 cohort of >6000 subjects, it seems unlikely that this introduced any bias.

Lipid estimates were limited to total and HDL cholesterol. There was no stipulation that such estimates should be performed on fasting samples; hence, triglycerides (and calculated LDL) were not obtained. However, it is likely that, in keeping with usual practice, a substantial proportion would
have been obtained on fasting samples. In addition, total cholesterol levels are little-influenced by factors such as time of day, posture, and meal consumption.\textsuperscript{45,46} Furthermore, total cholesterol determined on fasting and nonfasting samples has been shown to significantly predict future congenital heart disease events in a large population of hypertensive subjects.\textsuperscript{47} The predictive value of a low HDL cholesterol is well recognized, and risk estimates based on total and HDL cholesterol have also been substantiated.\textsuperscript{48,49} Previous studies reported very similar associations between aortic stiffness and both LDL cholesterol and total/HDL cholesterol ratio.\textsuperscript{17} In the latter study, total/HDL cholesterol ratio could be substituted for LDL cholesterol with no change in the results of multiple regression analysis. This would suggest that our results would be similar even if LDL cholesterol had been available.

An underlying association between cholesterol levels and measures of large artery stiffness could have been obscured by the use of lipid lowering-therapy, especially if this had been only recently introduced, such that a life-long relationship between cholesterol and large artery stiffness were disturbed. However, such therapy was only used in $\approx 13\%$ of the population and use of lipid-lowering therapy was not a significant term in the multiple regression analyses. In addition, omission of those using lipid-lowering therapy from the analysis did not alter the findings (data not shown).

In summary, in a large cohort of older hypertensive subjects, there was no independent association of plasma total or HDL cholesterol with a variety of measures of large artery stiffness. Such independence from cholesterol increases the potential for artery stiffness measurements to additionally contribute to cardiovascular risk assessment in this population.

Acknowledgments

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References

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