Does the Augmentation Index of Pulse Waves Truly Increase With Progression of Atherosclerosis? An Experimental Study With Hypercholesterolemic Rabbits

Shin-ichiro Katsuda,1 Masao Miyake,1 Daisuke Kobayashi,1 Akihiro Hazama,1 Masahiko Kusanagi,2 and Kenji Takazawa3

BACKGROUND
Recently, the central augmentation index (AIx) has been reported to show a nonlinear correlation with age. We investigated whether the AIx of the central artery changes with the progression of atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits.

METHODS
We simultaneously recorded pressure and flow waves in the ascending aorta in normal and KHC rabbits aged 10–12, 22–24, and 34–36 months, under pentobarbital anesthesia.

RESULTS
The systolic, diastolic, and mean arterial pressures and total peripheral vascular resistance were significantly higher in KHC rabbits than in their age-matched controls. The systolic pressure of the KHC rabbits increased with age. Additionally, the AIx of the KHC rabbits was significantly higher than that of their age-matched controls, although the AIx did not show a significant age-dependent increase in either of the two rabbit groups. However, the development of atherosclerotic lesions progressed markedly in KHC rabbits, and the early and late (pulse pressure [PP]) systolic waves increased progressively in amplitude with age in the KHC rabbits. On the other hand, no significant differences were seen in the normal and KHC rabbits’ cardiac output (CO), stroke volume (SV), or heart rate (HR) at any age, nor did the two strains show significant age-related changes in these variables. Aortic compliance (SV/PP) was significantly lower in the 22–24- and 34–36-month-old KHC rabbits than in their age-matched controls, and decreased with age in the KHC rabbits.

CONCLUSIONS
Although the progression of atherosclerosis stiffened the aortic wall, it did not affect the AIx. This was partly the result of the decreased distensibility of the wall, in which the pressure waves used to determine AIx were measured.

Keywords: atherosclerosis, augmentation index, early systolic pressure, late systolic pressure, KHC rabbit, distensibility of the aortic wall
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Pressure waves produced by cardiac contraction reflect the impedance-mismatch at the peripheral arterial site at which they reach the arterial wall, travel anterogradely toward the heart, and are superimposed on the waves moving forward toward the arterial wall. The percentage of reflected waves in whole pressure waves is defined as the augmentation index (AIx)1 which has recently been used as an indirect measure of arterial stiffness.2 The AIx of the central artery includes useful information about cardiac function and peripheral circulation, as well as arterial stiffness,3 although the central AIx is usually estimated from peripheral pressure waves measured noninvasively through use of the generalized transfer function (GTF).4–6 The AIx has been reported to increase in patients with hypertension7–9 and atherosclerosis,10,11 and in aging healthy subjects.12–14 Increased peripheral vascular resistance can amplify wave reflection, and increased arterial stiffness accelerates pulse-wave velocity (PWV), which contributes in part to the early arrival of reflected waves at the aortic root. The central AIx is also useful for evaluating the hypotensive effect of vasodilating drugs.15

Recently, McEniery et al.2 reported data from the Anglo-Cardiff Collaborative Trial (ACCT) demonstrating that the AIx increased substantially in younger patients and changed less with age in older subjects, whereas PWV increased progressively after the age of 50 years. Shimizu and Kario9 also noted a nonlinear relationship between age and AIx. These findings suggest that arterial stiffness tends to proceed to a lesser degree with aging in younger subjects but progresses dramatically in patients over the age of 50 years. It is therefore dubious whether the AIx is truly a surrogate index of arterial rigidity in elderly, hypertensive, or atherosclerotic patients. Consequently, it is necessary to clarify experimentally the
reason for the nonlinear change in AIx observed with the progression of atherosclerosis. In the present study we investigated the age-associated changes in the AIx of the central artery in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits, a heritable animal model for hypercholesterolemia and atherosclerosis, in relation to the age-related changes in cardiac function and aortic distensibility that have been observed.

METHODS

Animals

Male normal and KHC rabbits aged 10–12, 22–24, and 34–36 months were included in the study (n = 8 for each age group in each strain). The rabbits were bred in an individual wire cage at room temperature (21–25 °C), at a relative humidity of 50–60% and with 12-hour light/dark cycle. Cholesterol-free commercial rabbit chow (RC-4; Oriental Yeast, Tokyo, Japan) was given at 100 g/day for each animal. The animals were allowed free access to water throughout the study. All experiments were approved by the Animal Ethics Committee of Fukushima Medical University and performed according to the Guidelines of Animal Care and Handling of the U.S. National Institutes of Health (NIH).

Surgical procedure

The rabbits were anesthetized through the intravenous administration of pentobarbital sodium (Nembutal, Abbott Laboratories, Chicago, IL) at a dose of 30 mg/kg, and were fixed in a supine position. Procaine chloride was applied to the incised portion of each rabbit as needed to reduce pain. An additional dose of pentobarbital sodium (2 or 3 mg/kg) was injected every 15 or 20 minutes if necessary. The surgical procedure used in the study was nearly the same as that reported in a previous study.17 A catheter with a micromanometer at the tip (3Fr, SPS-330; Millar Instruments, Dallas, TX) was introduced into the aortic root via the left common carotid artery. The chest was carefully opened through a median sternotomy with the animal breathing voluntarily, and an ultrasonic flow probe (6 or 8 mm inner diameter (ID) in diameter; Transonic Systems, Ithaca, NY) was placed at the level of the ascending aorta. Pressure and flow waves at the ascending aorta were recorded simultaneously with a personal computer (PowerBook M9691J/A; Apple, Cupertino, CA) through an analogue-to-digital converter (PowerLab system 16s; AD Instruments, Sydney, Australia) at intervals of 0.1 msec for approximately 30 seconds after a stable recording had been established.

Calculation of the total percent lesioned area

The aorta was incised from the aortic root to the bifurcation of the common iliac arteries, and was opened longitudinally after the length of the aorta had been measured. The methods used for calculating the ratio of the total lesioned area to total surface area of the aorta were similar to those described previously.18 The intimal surface of the aorta was photocopied and the outline of the aorta and atheromatous plaques were carefully traced, with the traced outline then fed into a computer through an image scanner. The percent lesioned area (PLA) was determined with NIH Image software (Scion Corporation, Houston, TX).

Data processing

Twenty successive pressure and flow waves were analyzed. The fourth derivative of the pressure waves was determined from the 20 original, successive, computer-stored pressure waves. The point at which the fourth derivative crossed the baseline (from above to below) for the second time corresponded to the inflection point of the pressure waves, and was assigned to the first systolic shoulder of the pressure waves according to the definition given by Kelly et al.1 All of the pressure waves in the ascending aorta in the present study were classified as having a type A pattern, as reported by Murgo et al.19 In the type A pattern, the height of late systolic waves is equivalent to the amplitude of pressure waves, or the pulse pressure (PP). The AIx of the pressure waves was determined from the ratio of P2/P1, where P1 and P2 represent the amplitudes of the early and late (PP) systolic waves, respectively.20–22 Values of mean arterial pressure (MAP) and cardiac output (CO) were derived from the pressure and flow waves recorded in the study by passage through a low-pass filter with time constants of 2.5 and 2.0 seconds, respectively. Heart rate (HR) was determined from the foot-to-foot intervals of the pressure waves. Total peripheral vascular resistance (TPR) was calculated as MAP/CO. Stroke volume (SV) was estimated as CO/HR for a mean of 1 minute. Aortic compliance was estimated as SV/PP.

Statistical analysis

All data with the exception of those for PLA were analyzed through two-level nested analysis of variance (ANOVA) using commercial statistical software (StatFlex version 6.0; Arttech, Osaka, Japan). If this two-level nested ANOVA revealed a significant difference in the KHC and normal control rabbits in any of the age groups (10–12 months vs. 22–24 months, 10–12 months vs. 34–36 months, and 22–24 months vs. 34–36 months) examined in the study, the relevant data were compared through the use of Tukey’s multicomparison test, using commercial statistics software (StatFlex version 6.0). The data for PLA in two different age groups of KHC rabbits were also compared through Tukey’s multicomparison test, followed by one-way ANOVA. Differences were considered significant at P < 0.05.

RESULTS

Body weight

Body weight tended to be higher in the normal rabbit group than in the KHC rabbit group at each of the ages examined in the study, although this difference was not significant (Table 1). An age-related change in body weight was not observed in either the KHC or normal rabbits, nor was a significant difference in the length of the aorta found at any age or in different age groups of the normal and KHC rabbits (Table 1).
Pressure waves and blood pressure

Figure 1 provides examples of pressure and flow waves and of the fourth derivative of the original pressure wave at the ascending aorta in normal and KHC rabbits aged 12, 24, and 36 months. The amplitude of the wave increased gradually with age in the KHC rabbit group, whereas it changed only slightly in the normal rabbit group. The older KHC rabbits demonstrated "peaking," in the form of a marked increase in wave amplitude. The point at which the fourth derivative crossed the baseline for the second time (from above to below) corresponded primarily to the first systolic shoulder of the pressure wave (Figure 1, arrow) and peak of the flow wave. The first systolic shoulder (peak of the early systolic wave) also increased gradually with age in the KHC rabbits. The data for the cardiovascular parameters measured in the study are shown in Table 1.

The systolic blood pressure (SBP; \(P < 0.01\) for each age group), diastolic blood pressure (DBP; \(P < 0.01\) for each age group), and MAP (\(P < 0.01\) for each age group) were significantly greater in the KHC groups than in their age-matched control groups. The 34–36-month-old KHC group showed a significant increase in SBP and a slight but significant decrease in DBP as compared with the corresponding blood pressures in the 10–12-month-old KHC group. The TPR of the KHC groups was significantly higher than that of the age-matched control groups (\(P < 0.01\) for each age group), although it did not increase significantly with age in either the KHC or normal control rabbits.

There was no significant difference in CO, HR, or SV in the KHC and age-matched control rabbit groups at different ages or in any two age groups of the normal and KHC rabbits. The SV/PP value was significantly lower in the 22–24- and 34–36-month-old KHC rabbit groups than in their age-matched controls, and was significantly lower in the 34–36-month-old KHC rabbits than in the 10–12-month-old KHC rabbits.

Augmentation index

The amplitude of the early systolic waves in the 34–36-month-old KHC rabbit group was significantly greater than in the normal rabbits.
than that in their age-matched control group ($P < 0.01$) or in the 10–12-month-old KHC rabbit group ($P < 0.01$) (Figure 2A). The late systolic waves (PP) were significantly greater in amplitude in the 22–24- and 34–36-month-old KHC rabbits than in their age-matched control groups ($P < 0.01$), and were also significantly greater in the 34–36-month-old KHC group than in the 10–12-month-old ($P < 0.01$) and 22–24-month-old ($P < 0.01$) KHC groups (Figure 2B). There was no significant age-related change in the early or late systolic waves in the normal rabbit group (Figure 2A, 2B). Figure 3 illustrates the AIX of the normal and KHC rabbit groups according to age group. The AIX was significantly greater in the KHC rabbit groups than in the age-matched control groups ($P < 0.01$ for 10–12 months group and $P < 0.05$ for 22–24 and 34–36 months age groups). The AIX did not show age-related changes in either the KHC or normal rabbits, although the atherosclerotic lesions in the KHC group progressed markedly.

**Pathology**

The pathological findings in the study were similar to those reported previously.23 The development of atherosclerotic lesions progressed markedly toward the peripheral aortic region with age, although atheromatous plaques were also located in the ascending aorta and around the bifurcations of branch arteries such as the mesenteric and renal arteries at the age of 12 months. Marked intimal thickening with abundant foam cells was observed at 12 months of age. The foam cells were gradually replaced by proliferative fibers as the age of the rabbits increased.

**DISCUSSION**

**Age-related changes in the pressure waveform**

Murgo et al. classified the pressure waveform in the ascending aorta in young and elderly subjects into the three major categories of types A, B, and C.19 Type A shows an early systolic shoulder followed by an augmented late systolic peak composed mainly of reflected waves, which is usually observed in subjects beyond middle age and patients with hypertensive or atherosclerotic disease. In waves of type C, an inflection point occurs in diastole after a dominant initial pressure peak. This type of wave is usually observed in young normal subjects. Waves of type B exhibit a pattern intermediate between that of types A and C waves, characterized by an initial peak with an amplitude that is almost equal to the late systolic peak. The type of pressure waveform in a particular subject is affected by the type of agent used to produce anesthesia, the depth of anesthesia, the subject’s vasomotor tone, and vasoactive drugs. In the present study, all pressure waves measured in the normal and KHC rabbits at different ages were of type A. We considered this to have
Change in AI with Progression of Atherosclerosis

been partly the result of pentobarbital anesthesia increasing the blood pressure by enhancing vasomotor tone, and partly the result of an earlier return in systole of reflected waves from the upper body near the ascending aorta. The age-related changes in the pressure waveform seemed to be negligible until 36 months of age in anesthetized normal rabbits. In contrast, the KHC rabbits showed a progressive increase in the early and late systolic waves with increased age. This may have resulted from the earlier return of the reflected waves and from an increase in the early and late systolic waves resulting from the decreased distensibility of the aortic wall with the progression of atherosclerosis.

**Figure 2.** Amplitude of early (A) and late (B) systolic waves in the ascending aorta of normal and Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits of different ages. The data are presented as mean ± SD. *P < 0.05, **P < 0.01.

**Figure 3.** Augmentation index of pressure waves in the ascending aorta of normal and Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits of different ages. The data are presented as mean ± SD. *P < 0.05, **P < 0.01.

Age-related changes in the augmentation index of the normal rabbit group

Aging causes a variety of morphological alterations in cardiac and vascular properties, including hypertrophy, intimal thickening, and luminal dilatation, which lead to alterations in cardiovascular function. The effect of aging on arterial stiffness has been evaluated through increases in PWV or AIX as direct or surrogate measures, respectively, of arterial stiffness. Pulse-wave velocity may be mainly influenced by changes in elastic modulus, thickness, and diameter of the arterial wall, whereas AIX may be affected by cardiac function, peripheral vascular state, and arterial stiffness. Most researchers have reported that the AIX exhibits a positive linear correlation with age in healthy subjects. Recently, McEniery et al. showed that in healthy volunteers, AIX increased progressively from 20–50 years of age, whereas PWV increased at a slow rate until about the age of 50 years and increased at a greater rate in individuals over 50 years of age. The pattern of age-related changes in AIX and PWV was found to differ. Fantin et al. observed that AIX increased with age until 55 years of age, after which it reached a plateau. Mitchell et al. found that AIX reached a maximum value in men and decreased in women over 60 years of age. Shimizu and Kario also reported a nonlinear relationship between age and AIX.

Rabbits can live for 8–12 years, although their lifespan varies widely depending on genetic factors and the environment in which they are bred. Rabbits of 34–36 months of age are thought to be approximately 30 years old in terms of human age, although it is difficult to precisely extrapolate...
age from rabbits to humans. In the present study, the AIx of the central artery did not show a significant age-related change in the normal rabbit group; the early and late systolic pressures were approximately the same in the 10−12-, 22−24-, and 34−36-month-old control groups.

Sex,27 height,28 and HR29 can also affect AIx. We used mature male adult rabbits (over 10−12 months old) of similar body weight and aortic length, which would contribute in part to minimizing variations in AIx among the three age groups of rabbits examined in our study. The major factors affecting early systolic pressure, such as CO, HR, and SV, did not change according to age.2 However, late systolic waves could be influenced by the TPR and PWV. The TPR in the normal rabbit group in our study changed slightly with age, but the change was not significant. We previously reported that the aortic PWV in older normal animals was nearly the same as that of young normal rabbits.30 There were no significant age-related alterations in the rheological properties of the central artery, such as incremental elastic modulus or wall thickness, nor any significant change in histological findings in the ascending, proximal thoracic, or proximal abdominal aorta among 10−12-, 22−24-, and 34−36-month-old normal rabbit groups.31 This finding suggests that normal rabbits do not show marked morphological and functional alterations in their cardiovascular system, which is responsible for preserving the AIx at a relatively low value until these animals reach an age of 34−36 months. This pattern of age-related changes in AIx in normal rabbits was different from that in most human studies, in which AIx was found to increase after the age of 20 years. In normal rabbits, AIx may increase with age after 36 months.3,25

Effect of progression of atherosclerosis on the augmentation index in the Kurosawa and Kusanagi-hypercholesterolemic rabbit group

The AIx has been shown to increase in patients with hypertension3−9 and atherosclerosis,10,11 whereas Nürnberger et al.12 found no correlation between AIx and age in patients with atherosclerotic disease. Hypertensive or atherosclerotic disease could induce rigidity of the aorta, which is thought to contribute in part to an increased AIx. In the present study, the AIx of the central artery did not show significant changes during aging in KHC rabbits, but remained significantly higher than in normal rabbits of each corresponding age group with the progressive development of sclerotic lesions. This contradicts the notion that AIx may become further elevated with the progression of atherosclerosis.

The AIx could be affected by changes in amplitude of the early and late systolic waves. In the present study, AIx was not affected by age, whereas the amplitudes of the early and late systolic waves increased significantly with increasing age. This is because AIx is expressed as the ratio of the amplitudes of the late to early systolic waves. Late systolic waves may be affected by the two major factors of rigidity of the arterial wall and the magnitude of reflected waves. We estimated the former from PWV and the latter from TPR. We previously observed a significant increase in aortic PWV in older as compared with young KHC rabbits.30 Early systolic waves are influenced by the ejection of blood from the heart and distensibility in the ascending aorta. The SV did not show significant changes with aging in either the normal or KHC rabbit groups. However, SV/PP decreased with increased age as the development of atherosclerotic lesions progressed. We also previously investigated the rheological properties of the aorta in normal and KHC rabbits aged 10−12, 22−24, and 34−36 months.23 The circumferential incremental elastic modulus of the ascending aortic wall in KHC rabbits in these three age groups was 91.7±8.0, 257.2±46.3, and 583.2±206.8 Kpa (mean ± SD), respectively, suggesting a drastic increase in wall stiffness from the progression of atherosclerosis.31

We conclude that AIx is not affected either by the progression of atherosclerotic lesioning or by alterations in the distensibility of the aortic wall during aging.

Limitations of the present study

It is difficult to directly extrapolate to humans the data obtained from the KHC rabbits in our study. In most human studies and clinical practices, the AIx of the central artery has been estimated from pressure waves measured noninvasively in the radial artery with application tonometry using the GTF.4−6 In humans, atherosclerotic lesions are relatively scarce in the ascending aorta as compared with the abdominal aorta.32 Furthermore, the localization and development of atherosclerosis differ in rabbits and humans.

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DISCLOSURE

The authors have no conflicts of interest in this work.

REFERENCES