The Effect of Lifelong Exercise Frequency on Arterial Stiffness

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Running Title: Lifelong Exercise and Arterial Stiffness

Key Words: Arterial stiffness, Exercise training, Aging

Key points summary:

- This study examined the effect of different "doses" of lifelong (>25 yr) exercise on arterial stiffening (a hallmark of vascular aging) in older adults.
- There are clear dose-dependent effects of lifelong exercise training on human arterial stiffness that varies according to the site and size of the arteries.
- Similar to what we have observed previously with ventricular stiffening, 4-5 days/week of committed exercise over a life-time are necessary to preserve "youthful" vascular compliance, especially of the large, central arteries.
- Causal exercise training of 2-3 times per week exercise training may be sufficient for middle-sized arteries like the carotid in order to minimize arterial stiffening with aging.
- However, there is little effect of exercise training on the small-sized peripheral arteries at any dose.

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I originally had great interest in the field of exercise physiology and aging when I graduated from School of Medicine, Niigata University (1998). After completing an initial clinical practice of anesthesiology in Japan, I worked on a PhD in Anesthesiology and Environmental Physiology focusing on cardiovascular physiology, exercise training and anesthesia in Nihon University (2002-2006). In order to extend my skills and knowledge, I worked in the Institute for Exercise and Environmental Medicine, where this work was performed (2005-2011). Currently, I am working in School of Medicine, Kyorin University to further explore my original research interest (2011-).

Abstract

Central arterial stiffness increases with sedentary aging. While near-daily, vigorous lifelong (>25 yr) endurance exercise training prevents arterial stiffening with aging, this rigorous routine of exercise training over a lifetime is impractical for most individuals. The aim was to examine whether a less frequent 'dose' of lifelong exercise training (4-5 sessions/wk for >30 min) that is consistent with current physical activity recommendations elicits similar benefits on central arterial stiffening with aging. A cross-sectional examination of 102 seniors (>60 yrs old), who had a consistent lifelong exercise history was performed. Subjects were stratified into 4 groups based on exercise frequency as an index of exercise 'dose': sedentary: <2 sessions/wk; casual exercisers: 2-3 sessions/wk; committed exercisers: 4-5 sessions/wk; Masters athletes: 6-7 sessions/wk plus regular competitions. Detailed measures of arterial stiffness and left ventricular afterload were collected. Biological aortic age and central PWV were younger in committed exercisers and Masters athletes compared to sedentary seniors. TACi (total arterial compliance) was lower, while Carotid β-stiffness index and Eai

(effective arterial elastance) were higher in sedentary seniors compared to the other groups. There appeared to be a dose-response threshold for carotid β-stiffness index and TACi. Peripheral arterial stiffness was not significantly different among the groups. These data suggest that 4-5 weekly exercise sessions over a lifetime is associated with reduced central arterial stiffness in the elderly. A less frequent dose of lifelong exercise (2-3 sessions/wk) is associated with decreased ventricular afterload and peripheral resistance, while peripheral arterial stiffness is unaffected by any dose of exercise.

Introduction

A notable consequence of sedentary aging is large-vessel arterial stiffening. This pathophysiological process is characterized by the development of fibrosis and collagen cross-linked products in the arterial wall. Central arterial stiffening increases the risk of cardiovascular-related morbidity and mortality in older adults (Vlachopoulos *et al.*, 2010); thus, the development of strategies to forestall age-associated cardiovascular (CV) diseases has important clinical implications.

Sustained, regular endurance exercise training is one such favourable strategy. We and others have previously shown that Masters athletes who have performed near-daily (6-7 sessions/wk), vigorous endurance exercise training plus competition for the majority of their adult lives have more compliant central arteries compared to their sedentary peers (Vaitkevicius *et al.*, 1993; Gates *et al.*, 2003; Shibata & Levine, 2011, 2012). While these findings support others (Arbab-Zadeh *et al.*, 2004; Bhella *et al.*, 2014) from our laboratory that underscore the critical role of sustained, lifelong physical activity in mitigating ventricular and arterial stiffening with aging, the rigorous training and competition routine of Masters athletes is not feasible or practical for most individuals.

Previously, we showed in these same subjects that a less frequent 'dose' of lifelong exercise training (4-5 sessions/wk for >30 min) that is consistent with current physical activity recommendations (≈150 min/wk), prevents cardiac atrophy and stiffening associated with sedentary aging (Bhella *et al.*, 2014). Moreover, this volume of exercise training was associated with higher maximal oxygen uptake (VO_2max), stroke index and effective arterial elastance (Eai) during exercise (Carrick-Ranson *et al.*, 2014). Since resting Ea increases with sedentary aging as a result of ventricular and central arterial stiffening (Redfield *et al.*, 2005), 4-5 sessions/wk of dynamic exercise over a lifetime may also be an effective and practical exercise frequency to prevent both ventricular

and central arterial stiffening with aging. However, the effects of different dose of life-long exercise training on arterial compliance with aging is still unclear.

Accordingly, to test the hypothesis that a threshold dose of 4-5 weekly exercise sessions over a lifetime would be associated with a reduction in central arterial stiffness, we performed a cross-sectional examination of detailed measures of central arterial stiffness in seniors (>60 yrs) who had performed a consistent frequency of exercise training for >25 yrs, focusing on weekly exercise frequency as an index of exercise dose.

Methods

Ethical approval

All subjects signed an informed consent approved by the institutional review boards of the University of Texas Southwestern and Texas Health Resources Presbyterian Hospital of Dallas and performed in accordance with the Declaration of Helsinki.

Subject Recruitment

Details of the subject recruitment process and study design were reported previously (Bhella *et al.*, 2014; Carrick-Ranson *et al.*, 2014). One hundred and two (n = 102) healthy seniors were recruited and stratified into four groups based on lifelong frequency of exercise training. Sedentary subjects (n = 27) exercised no more than once per week during the previous 25 yrs, 'casual' exercisers (n = 25) engaged in 2-3 sessions per week, 'committed' exercisers (n = 25) performed 4-5 sessions per week and competitive Masters athletes (n = 25) trained 6-7 times per week and participated in regular competitions. Exercise sessions were defined as periods of aerobic exercise of at least 30 minutes.

Subjects were recruited primarily from the Cooper Center Longitudinal Study (CCLS)(Wei *et al.*, 1999), a cohort of more than 80,000 individuals in whom physical activity and CV risk factors have been quantified and followed for >40 yrs. Using the CCLS database, investigators identified healthy subjects who had consistently reported the same level of regular exercise on clinic questionnaires over multiple visits spanning at least 20 years. Interested subjects underwent a comprehensive exercise history examination conducted by an experienced exercise physiologist and assisted by family members when possible. If exercise histories could be corroborated, subjects were invited to

participate in the next phase of screening. The sedentary population was enriched with subjects recruited from local senior groups such as bingo, gardening, volunteer groups, and health fairs (most subjects in this group came from non-Cooper Clinic sources). The Masters athlete population was enriched by direct recruitment from the top performers (10-15%) at regional and national endurance events (Arbab-Zadeh *et al.*, 2004) with most selected from race results. Regardless of the source of referral, however, all subjects were equally well vetted and rigorously screened in terms of medical history, physical examination, and detailed exercise training history.

All recruited subjects underwent the following screening protocol. First, a medical history and physical exam were recorded by a study physician and/or nurse. Obesity (BMI >30 kg/m^2), regular tobacco use within the past 10 years, hypertension (24 hr ambulatory blood pressure >140/90 mmHg), diabetes, chronic obstructive pulmonary disease, atrial fibrillation, obstructive coronary artery disease or significant valvular disease were exclusion criteria. Second, an exercise stress test was performed on all subjects, with ECG or echocardiography changes suggestive of ischemia or abnormal wall motion criteria for exclusion.

Assessment of arterial stiffness

Biological aortic age

Biological aortic age was determined from the central aortic arterial pressure waveform using the Modelflow algorithm as previously described (Shibata & Levine, 2011). First, using input ages from 20 to 90 yrs, Modelflow stroke volume (SV) was generated from a central blood pressure waveform reconstructed from a finger blood pressure waveform (Beatscope 1.1a; FMS)(Wesseling *et al.*, 1993). Biological aortic age was then determined by an inverse function of the linear regression between input age and generated Modelflow SV equation by using SV from the acetylene rebreathing method (Jarvis *et al.*, 2007) as an input signal. This index has been previously validated in our laboratory, demonstrating a high age specificity in sedentary adults, and high reproducibility in response to changes in hemodynamic loading conditions (Shibata & Levine, 2012).

Pulse wave velocity (PWV)

Central, and upper and lower limb peripheral PWV was measured with Doppler ultrasound (iE 33, Phillips) and calculated as the distance between measurement sites divided by the time delay between the two waveforms (Laurent *et al.*, 2006). Pulse transit time was calculated by subtracting

the time between the peak of the R-wave and the foot of the carotid flow profile from the time between the peak of the R-wave and the foot of the femoral flow for central PWV, and carotid from radial and femoral from dorsal flow profiles for upper limb and lower limb peripheral PWV, respectively. Peak of R-wave and the foot of the flow profile were visually determined by the same researcher. The distance between arterial measurement sites was calculated by subtracting the distance between the carotid site and the sternal notch from the distance between the sternal notch and the femoral site, and between the sternal notch and the radial site for central and upper limb peripheral PWV, and between femoral and dorsal for lower limb peripheral PWV, respectively (Supplemental Figure). Central PWV (carotid-femoral) and upper limb peripheral PWV (carotid-radial) were also measured with SphygmoCor Mx device. Blood pressure waveforms were measured just before Doppler flow measurements and these pressure waveforms were used for analyzing augmentation indices and carotid artery stiffness indices. Intraclass correlation coefficient (ICC) of central PWV between Doppler method and SphygmoCor was relatively high (IC: 0.742, 95% Confidence Interval: 0.576-0.843), while that of upper limb peripheral PWV was low (IC: 0.168, 95% Confidence Interval: -0.350-0.488).

Local arterial stiffness

β-stiffness index and distensibility coefficient of the common carotid artery were calculated from systolic and diastolic carotid dimensions and pressures (Hirai *et al.*, 1989; Laurent *et al.*, 2006). Sequential measurement of right common carotid and brachial pressure waveforms with applanation tonometry (SphygmoCor, Mx) was immediately followed by brachial arm cuff blood pressure measurement (Korotkoff sounds detected using electrosphygmomanometry; Suntech Medical Systems). Systemic diastolic and mean blood pressures were estimated from the brachial blood pressure waveform calibrated with arm-cuff systolic and diastolic blood pressures. These mean and diastolic blood pressures were used to calibrate a right common carotid blood pressure waveform to obtain carotid systolic (Ps) and diastolic blood pressures (Pd)(Kelly & Fitchett, 1992; Laurent *et al.*, 2006). The cross-sectional area of the right common carotid artery was measured from the images acquired with a high-resolution (Sono-CT) linear-array ultrasound (iE33, Phillips) transducer (~9 MHz). The measurements were made 1–2 cm proximal to the carotid bulb, with the transducer was placed at a 90° angle to the vessel so that near and far wall interfaces were clearly discernible. Acoustic quantification was applied for the edge detection of the internal arterial wall

(Q-Lab, Phillips), and maximum and minimum areas were considered systolic (As) and diastolic (Ad) areas.

Ascending and descending aortic β-stiffness index were calculated from simultaneous cardiac MRI aortic cross-sectional area and brachial cuff blood pressure measurements. Moreover, ascending and descending aortic strain were also calculated only from the aortic cross-sectional area since central blood pressures were not available during the MRI measurement. MRI of the aortic arch was obtained in the transverse plane at the level of the right pulmonary artery using 1.5-T clinical magnetic resonance scanner (NT model; Philips Corp., Amsterdam, the Netherlands) to assess aortic pulsative dimension (Redheuil *et al.*, 2011). MRI data was analyzed using commercially available software (Q-flow, NEXA Group Pty Ltd) and maximum and minimum areas were considered systolic (As) and diastolic (Ad) areas. β-stiffness index of the carotid artery, and the ascending and descending aortas, distensibility coefficient of the carotid artery and strain of the ascending and descending aortas were calculated by the following equations, respectively: ln(Ps/Pd)/(As-Ad)/Ad, (As-Ad)/(Ps-Pd)/Ad, and (As-Ad)/Ad.

Augmentation index

Carotid augmentation blood pressure was quantified as the difference between the first and second systolic peaks (Pauca *et al.*, 2001; Laurent *et al.*, 2006). Carotid blood pressure augmentation index was calculated as augmentation pressure expressed as a percentage of the pulse pressure determined with SphygmoCor Mx (Pauca *et al.*, 2001; Laurent *et al.*, 2006). Central augmentation pressure and index were calculated with a central pressure waveform reconstructed from radial blood pressure waveform by inverse transfer function method with SphygmoCor Mx (Chen *et al.*, 1997; Pauca *et al.*, 2001). To reduce the confounding effect of heart rate, augmentation indices were additionally normalized to a heart rate of 75 bpm.

Total arterial compliance (TAC), effective arterial elastance (Ea), total peripheral resistance (TPR)

TAC was calculated from stroke volume (SV) from acetylene rebreathing method (Jarvis *et al.*, 2007) divided by central arterial pulse pressure reconstructed from radial blood pressure waveform (SV/central pulse pressure). Ea and TPR were calculated by central systolic pressure/SV and 80*cardiac output/central mean pressure, respectively (Sunagawa *et al.*, 1983; Kelly *et al.*, 1992; Chemla *et al.*, 2003). To reduce the confounding effect of body size, stroke index (SVi) was used for the calculation of TACi, Eai and TPRi.

Statistics

A one-way analysis of variance (ANOVA) was used to determine differences in variables among the four groups. Post-hoc analysis (Student-Newman-Keuls method) was performed when a significant main effect was found. The partialη^2 of the one-way ANOVA was used to estimate effect size for primary outcome variables. A chi-square test was used to determine gender differences between groups. Commercially available software was used to perform all analyses (SigmaStat 3.5, SPSS 22.0). $P<0.05$ was considered statistically significant for ANOVA and post-hoc analysis. Data are presented as mean ± standard deviation in tables and figures.

Results

Subjects characteristics

Age and male/female ratio were not significantly different among groups (Table 1). The subjects were predominantly white (one black for casual and one Asian for committed). Body mass index, total body mass and body fat were significantly lower in Masters athletes, while maximal oxygen uptake (VO_2max) increased in a dose-dependent manner as reported previously (Bhella *et al.*, 2014; Carrick-Ranson *et al.*, 2014) (Table 1).

Blood pressure, stroke volume and related indices

Resting SV and SVi increased with a greater frequency of lifelong exercise training (Table 2). Arm cuff measures of systolic blood pressure tended to be higher, and central systolic and mean blood pressures were significantly higher in sedentary seniors ($P\leq0.016$ versus casual and committed exercisers). (Table 2). TACi was lower, while Eai was higher in sedentary seniors compared to the other groups (Table 2). Augmentation indices were not different among the groups (Table 3).

Arterial stiffness

Biological aortic age was younger, while central PWV was lower in committed and Masters athletes compared to a lower frequency of lifelong exercise training ($P\leq0.066$ versus casual exercisers and

sedentary seniors) (Figs. 1 & 2). In contrast, carotid artery β-stiffness index was significantly higher in sedentary seniors compared to the other groups (P≤0.047), but not between the 3 exercise-trained groups (Fig. 3). Similarly, carotid distensibility coefficient was significantly lower in sedentary seniors compared to the other groups. Upper and lower limb peripheral PWVs were not significantly different among groups (Table 3).

Discussion

The major findings from the present study were as follows: 1) 4-5 weekly sessions of exercise over a lifetime was associated with a reduction in central arterial stiffness in seniors, similar to what we have previously observed regarding myocardial stiffness; 2) a lifelong casual exercise frequency (2-3 sessions/wk) was associated with lower carotid artery stiffness, left ventricular afterload (Eai), and central blood pressures in the seniors, while this dose of exercise training did not affect the central arterial stiffness; and 3) Peripheral arterial stiffness was unaffected by lifelong exercise training, irrespective of "dose". These current findings extend previous observations from our laboratory (Bhella *et al.*, 2014; Carrick-Ranson *et al.*, 2014) and others (Vaitkevicius *et al.*, 1993; Gates *et al.*, 2003) underscoring the favourable effects of aerobic exercise for >30 min, 4-5 times per week throughout a lifetime on the adverse consequences of aging on CV stiffening.

Central artery stiffness

In the present study, central arterial stiffness was comprehensively examined using established approaches such as central PWV and aortic β-stiffness index, as well as a newer index estimating the biological (as opposed to the chronological) age of the aorta. Previous work from our laboratory demonstrates that this latter approach, which conceptually reflects structural changes of the aortic wall, accurately identifies sedentary aging and lifelong exercise training-related changes in aortic stiffness (Shibata & Levine, 2011). It is especially compelling that all indices studied in the current study, which used completely different sensors and analytic approaches to quantify large vessel stiffening, found similar patterns in large vessel arterial stiffening in response to graded lifelong exercise frequency; specifically, engaging in >3 sessions per week over a lifetime resulted in more "youthful" measures compared to sedentary seniors, which could be estimated to be approximately 10 and 25 years younger in committed exercisers and competitive Masters athletes, respectively. These findings are important as large epidemiological studies have shown that central arterial

stiffness assessed by PWV is a strong independent predictor of CV-related and all-cause mortality in older adults (Vlachopoulos *et al.*, 2010). Moreover, although the clinical impact of biological aortic age for predicting future CV events has not been confirmed by large epidemiological studies, this index provides strong physiological confirmation that changes in central arterial stiffness with exercise training measured in this and other studies (Vaitkevicius *et al.*, 1993; Tanaka *et al.*, 1998; Tanaka *et al.*, 2000; Gates *et al.*, 2003; Pierce *et al.*, 2013) are not secondary to central blood pressure changes.

In contrast, central aortic β-stiffness index was not significantly improved with lifelong exercise training, irrespective of weekly exercise frequency. The apparent absence of a training effect in our findings may be related to study methodology including the use of cuff pressure rather than central blood pressure, due to the technical difficulties in using the tonometry instrumentation in the MRI due to the strong magnetic field. The simultaneous collection of aortic dimensions and central blood pressure would provide the most precise assessment of aortic β-stiffness. Thus, given that we found divergent effects on cuff and central blood pressures in relation to lifelong exercise frequency, some caution should be exercised regarding the interpretation of the current aortic β-stiffness findings.

Carotid artery stiffness

We found that carotid β-stiffness index was higher in sedentary seniors, but similar among the exercise-trained groups, suggesting that only small beneficial effects are obtained above a low frequency of lifelong exercise training (2-3 sessions/wk). Thus, a large beneficial effect was obtained with relatively low levels of exercise, which is consistent to what has been previously reported in several epidemiological studies of exercise and CV risk. However, Tanaka et al. (Tanaka *et al.*, 2000) found that carotid β-stiffness index was significantly improved only in highly endurance trained (≥5 sessions/wk) but not in recreationally active (3-4 sessions/wk) senior men compared to their sedentary peers. We speculate that the discrepancy between study findings is related to methodological differences in the exercise histories of the exercise trained subjects, as Tanaka et al. examined senior men with a consistent exercise history of >2 yrs, while we examined senior men and women with a consistent exercise training history >25 yrs. This point is important, as the age when exercise training is begun may determine the effectiveness of exercise training to improve CV structure and function, particularly in properties like ventricular or large blood vessel compliance (Fujimoto *et al.*, 2010; Shibata & Levine, 2012). Therefore, the initiation of exercise training earlier in

life coupled with a longer exercise training history may explain why we observed significant improvements in carotid stiffening with a less frequent dose of lifelong exercise training.

Similar to carotid β-stiffness, systolic blood pressures were increased in sedentary seniors. Since the carotid sinus is the site of the carotid baroreflex, it is possible that improved baroreflex function due to a compliant carotid artery lowers systolic blood pressure. In addition, central systolic blood pressure is influenced by alterations in the interaction between forward traveling and reflected waves, mostly by reductions in timing and amplitude of reflected waves. Although augmentation indices did not show significant differences, changes in these factors may be another underlying mechanism of lower central blood pressure. These findings suggest that even a modest frequency of lifelong exercise training provokes favorable effects in central blood pressure regulatory mechanisms.

Peripheral artery stiffness

In contrast to central arterial stiffness, we were unable to show any clear effect on peripheral blood vessels. Previous studies have reported conflicting findings regarding the effect of aerobic exercise training on peripheral arterial stiffness, with significant improvements reported in young, healthy middle-aged (Rakobowchuk *et al.*, 2008; Currie *et al.*, 2009) or pre-hypertensive individuals (Collier *et al.*, 2008), while either no effect or a very small favorable effect has been observed in healthy adults of varying ages and fitness levels (Tanaka *et al.*, 1998; Hayashi *et al.*, 2005; Cook *et al.*, 2006).

Differences among previous findings appear to be influenced by study design, as the majority of longitudinal training studies have shown positive findings (Collier *et al.*, 2008; Rakobowchuk *et al.*, 2008; Currie *et al.*, 2009), while cross-sectional examinations including the current study report no effect (Tanaka *et al.*, 1998; Cook *et al.*, 2006). A likely explanation for these findings is that the inter-individual difference in peripheral arterial compliance is larger than that of intra-individual difference resulting from exercise training. The smaller effect size of peripheral PWV (partialη^2=0.021 for upper limb and 0.018 for lower limb) compared to central PWV (partial η^2=0.191) observed in the present study supports this contention.

In addition, we visually detect the foot of flow waveform to calculate time differences. More sophisticated approaches such as the intersecting tangents or 2nd derivative method may have revealed positive findings by reducing variability.

Potential underlying mechanisms

Arteriosclerosis with sedentary aging is characterized by profound structural remodelling of the arterial wall including the accumulation of connective tissue and extracellular collagen cross-linked products (advanced glycation endproducts), and the degeneration of elastin (Lakatta, 2003; Lakatta & Levy, 2003). Previously, we have reported that biologic aortic age is younger, indicative of more compliant central blood vessels in Masters athletes compared to age-matched sedentary controls (Shibata & Levine, 2011). This finding provides evidence that sustained, vigorous lifelong endurance exercise training may inhibit the adverse vascular remodelling associated with human aging. Conversely, 1 yr of exercise training, encompassing over 200 minutes per week of moderate and high-intensity exercise, failed to substantially improve biological aortic age in previously sedentary seniors (Shibata & Levine, 2012). This latter finding suggests that the age-related changes in large elastic blood vessel structure are not reversible by exercise training alone when initiated later in life.

Vascular functional adaptations, which are influenced by smooth muscle tone and endothelial function, also characterise the improved arterial compliance with exercise training. For example, endothelial function is improved with lifelong exercise training and with several months of exercise training in previously sedentary seniors (Luk *et al.*, 2012; Shibata & Levine, 2012; Cornelissen *et al.*, 2014; Kim *et al.*, 2014). It is likely that the peripheral vasculature, particularly the arterioles where the majority of vascular resistance is produced, is strongly influenced by these training-related effects. In the present study, total arterial compliance, blood pressures and cardiac afterload, which are all influenced by smooth muscle tone, were improved with even a modest (2-3 sessions/week) amount of lifelong exercise. These current findings are similar to that reported with 1 yr of endurance training in previously sedentary seniors (Shibata & Levine, 2012) suggesting that the functional components of arterial compliance appears to be more readily influenced by exercise training compared to structural components, particularly if exercise is initiated later in life.

Perspective

Given the importance of vascular stiffening to health and clinical outcomes with human aging, it is important to develop strategies to forestall age-related CV diseases. Exercise training is one approach; however, as noted in a recent review (Seals, 2014), the minimal and/or optimal dose of exercise training to preserve or improve vascular structure and function with human aging has yet to be clearly established. The present findings constitute an important step in this process by

demonstrating the minimal frequency of lifelong exercise required to preserve compliant central arteries in older age. Importantly, this minimum exercise frequency is consistent with and strengthens current recommendations for weekly physical activity (≈150 min/wk).

Moreover, several indices from the present study showed significant differences between sedentary and causal exercisers (carotid β-stiffness index, Central SP, Central MP, and Eai); however, there appears to be dose-response threshold for carotid β-stiffness index and TACi (i.e. no significant difference among the exercise trained groups). This finding emphasizes the clinical importance of a lesser amount (2-3 times/wk) of weekly aerobic exercise on vascular stiffness and blood pressures.

According to previous large epidemiological studies, the reference values of PWV, carotid distensibility coefficient and central blood pressure in a healthy 70 year old were approximately 10.4 m/s, 13.8, and 114 mmHg, respectively (Herbert *et al.*, 2014; Cunha *et al.*, 2015; Engelen *et al.*, 2015). Thus, the sedentary subjects in this current study are comparable in terms of arterial stiffness and blood pressures to what has been previously reported in the general population, supporting the generalizability of the present findings.

Study limitations

First, this study did not invasively examine the structural and functional adaptations associated with lifelong exercise training frequency; only non-invasive techniques were employed. While invasive assessment of vascular tissue by biopsy would provide the most compelling evidence of structural remodeling, this is not practical in healthy individuals. Second, factors (dietary intake, non-exercise physical activity levels, social background, educational levels, and economic status) not assessed in the current study may influence adherence to exercise training over a lifetime, and consequently arterial compliance. Moreover, small group differences in BP and BMI may have influenced the current findings even though these effects were not statistically significant and these variables were within normal ranges; therefore, we cannot exclude the possibility that the improved arterial compliance demonstrated in our trained subjects is achieved by more than exercise training alone. Accordingly, we also performed ANCOVA including BMI and central blood pressure as covariates and obtained similar results (Supplemental Table2), indicating that effects of modifying these risk factors per se are likely to be small. Third, group allocation was based on lifelong exercise frequency, thus limiting any conclusions based on other components of an exercise training program including intensity, duration or mode, all of which may have a profound impact on the vascular

adaptations to exercise training. Fourth, this study was not designed to focus on gender difference of exercise effects. Thus, future studies are required to properly address gender differences. Our preliminary analysis did not show any significant interactions for gender and exercise frequency, while borderline significance was observed in the carotid artery β-stiffness index and distensibility coefficient (P=0.077 and P=0.079, two-way ANOVA, Supplemental Table1). Fifth, our subjects were non-obese, normotensive, and were carefully screened for CV disease; therefore, it is unclear whether these current results are applicable to a broader population of patients with greater amounts of co-morbidities and CV disease risk factors. Sixth, we must acknowledge that there were some small differences in probability observed in quantifying the changes in central PWV among groups between the Doppler and SphygmoCor devices. Considering that both methods show similar directionality and magnitude, albeit it with different statistical probabilities, we are reasonably confident that the conclusion is correct.

Lastly, the Student-Newman-Keuls multiple comparison test was used for post-hoc analysis in the present study. Since this test does not limit the chance of a Type I error at 5%, some positive results need to be carefully interpreted given the subject number in the present study.

Conclusions

In summary, these current findings suggest that > 4-5 weekly sessions of committed lifelong exercise is associated with more “youthful” levels of central artery compliance in the elderly. A lesser frequency of lifelong exercise (2-3 sessions/wk) is associated with improved carotid artery compliance and decreased left ventricular afterload. Irrespective of frequency, lifelong exercise training does not significantly influence peripheral arterial stiffness.

Competing interests.

There are no competing interests.

Author contributions.

The experiments were performed in the Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital Dallas & the University of Texas Southwestern Medical Center at Dallas, Texas.

SS and BDL contributed to conception or design of the work, acquisition, analysis or interpretation of data for the work, and drafting the work or revising it critically for important intellectual content. NF, JLH, GCR, and PSB contributed to acquisition, analysis or interpretation of data for the work and drafting the work or revising it critically for important intellectual content

All authors approved the final version of the manuscript, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Table 1

	Sedentary Subjects (Q1)	Casual Exercisers (Q2)	Committed Exercisers (Q3)	Competitive Exercisers (Q4)	ANOVA /X^2	Post Hoc Analysis						Effect Size
						Q1 vs. Q2	Q1 vs. Q3	Q1 vs. Q4	Q2 vs. Q3	Q2 vs. Q4	Q3 vs. Q4	partial μ^2
Subjects (gender, m/f)	27 (15/12)	25 (18/7)	25 (20/5)	25 (17/8)	0.286							
Age, yrs	70 ± 6	70 ± 6	68 ± 6	70 ± 4	0.486							0.024
Height, cm	169 ± 10	174 ± 10	173 ± 8	171 ± 10	0.327							0.034
Weight, kg	75 ± 11	76 ± 14	73 ± 11	66 ± 12	0.015	0.741	0.72	0.023	0.777	0.019	0.024	0.100
Bodyfat, %	32 ± 8	30 ± 7	29 ± 5	22 ± 7	<0.001	0.23	0.209	<0.001	0.61	<0.001	0.002	0.225
Body Mass Index, kg/m2	25.9 ± 2.5	25.0 ± 2.9	24.3 ± 2.9	22.2 ± 2.4	<0.001	0.183	0.084	<0.001	0.424	0.002	0.006	0.214
Body Surface Area, m2	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.8 ± 0.2	0.058							0.073
Max VO2, ml/kg/min	23.7 ± 4.9	25.8 ± 4.8	32.0 ± 5.8	39.5 ± 5.3	<0.001	0.145	<0.001	<0.001	<0.001	<0.001	<0.001	0.594
Ambulatory SBP, mmHg	125 ± 8	124 ± 7	125 ± 8	122 ± 11	0.639							0.019
Ambulatory DBP, mmHg	73 ± 6	71 ± 6	74 ± 7	74 ± 6	0.406							0.033

Max VO2, maximal oxygen consumption; Ambulatory SBP, average systolic blood pressure of 24-hour ambulatory blood pressures; Ambulatory DBP, average diastolic blood pressure of 24-hour ambulatory blood pressures; ANOVA, analysis of variance; X^2,chi-square test; Post Hoc Analysis was performed by the Student-Newman-Keuls.

Table 2

	Sedentary Subjects (Q1)	Casual Exercisers (Q2)	Committed Exercisers (Q3)	Competitive Exercisers (Q4)	ANOVA	Post Hoc Analysis						Effect Size
						Q1 vs. Q2	Q1 vs. Q3	Q1 vs. Q4	Q2 vs. Q3	Q2 vs. Q4	Q3 vs. Q4	partial μ^2
Cuff SBP, mmHg	126 ± 16	117 ± 12	119 ± 13	121 ± 15	0.083							0.066
Cuff DBP, mmHg	74 ± 8	71 ± 7	69 ± 8	71 ± 9	0.172							0.049
HR, bpm	66 ± 11	62 ± 7	58 ± 9	56 ± 7	<0.001	0.088	0.004	<0.001	0.129	0.063	0.452	0.165
SV, ml	76 ± 24	80 ± 17	85 ± 18	89 ± 20	0.083							0.066
SVi, ml/m2	40 ± 10	42 ± 7	45 ± 9	50 ± 8	<0.001	0.454	0.08	<0.001	0.165	0,002	0.037	0.180
Central SP, mmHg	115 ± 12	106 ± 10	106 ± 13	110 ± 11	0.011	0.015	0.015	0.082	0.822	0.408	0.293	0.108
Central DP, mmHg	71 ± 10	66 ± 6	67 ± 9	69 ± 8	0.077							0.067
Central MP, mmHg	90 ± 11	82 ± 7	83 ± 9	86 ± 8	0.009	0.013	0.016	0.124	0.784	0.287	0.217	0.110
Central TACi, ml/m2/mmHg	0.88 ± 0.33	1.01 ± 0.23	1.11 ± 0.33	1.09 ± 0.22	0.014	0.089	0.018	0.022	0.42	0.336	0.769	0.102
Central Eai, mmHg/ml/m2	3.06 ± 0.83	2.58 ± 0.43	2.44 ± 0.60	2.23 ± 0.44	<0.001	0.005	<0.001	<0.001	0.406	0.108	0.23	0.217
Central TPRi, dynes•sec•cm-5	2869 ± 614	2606 ± 472	2625 ± 569	2525 ± 623	0.159							0.051

Cuff SBP, systolic blood pressure measured by arm cuff; Cuff DBP, diastolic blood pressure measured by arm cuff; HR, heart rate; SV, storke volume; SVi, stroke volume index; Central SP, central systolic pressure reonstructed from radial pressure waveform; Central DP, central diastolic pressure reonstructed from radial pressure waveform; Central MP, central diastolic pressure reonstructed from radial pressure waveform; Central TACi, total arterial compliance index calculated from reconstructed central blood pressure; Central Eai, effective arterial elastance index calculated from reconstructed central blood pressure; Central TPRi, total peripheral resistance index calculated from reconstructed central blood pressure

Table 3

	Sedentary Subjects (Q1)	Casual Exercisers (Q2)	Committed Exercisers (Q3)	Competitive Exercisers (Q4)	ANOVA	Post Hoc Analysis						Effect Size
						Q1 vs. Q2	Q1 vs. Q3	Q1 vs. Q4	Q2 vs. Q3	Q2 vs. Q4	Q3 vs. Q4	partial μ^2
The Modelflow Aortic Age, yrs	67 ± 14	66 ± 15	56 ± 20	43 ± 14	<0.001	0.854	0.066	<0.001	0.044	<0.001	0.005	0.258
Central PWV, m/s	10.6 ± 3.6	9.8 ± 1.9	8.4 ± 1.8	7.8 ± 1.1	<0.001	0.219	0.004	<0.001	0.038	0.009	0.354	0.191
U-Peripheral PWV, m/s	9.2 ± 1.8	9.6 ± 1.5	9.5 ± 1.9	9.0 ± 1.5	0.575							0.021
L-Peripheral PWV, m/s	10.8 ± 1.3	10.5 ± 1.6	10.3 ± 1.3	10.5 ± 1.4	0.82							0.018
Central PWV (Sphy), m/s	8.7 ± 1.6	8.9 ± 1.7	8.1 ± 1.2	7.9 ± 1.1	0.171							0.076
Peripheral PWV (Sphy), m/s	7.7 ± 1.4	7.9 ± 1.2	7.8 ± 1.5	8.2 ± 0.9	0.73							0.020
PWA-P (Central), mmHg	12 ± 6	9 ± 4	12 ± 7	13 ± 4	0.265							0.061
PWA-I (Central), unit	26 ± 9	23 ± 10	27 ± 11	29 ± 9	0.387							0.047
PWI @ HR75 (Central), unit	21 ± 9	17 ± 9	18 ± 11	21 ± 9	0.443							0.041
PWA-P (Carotid), mmHg	9 ± 6	5 ± 8	8 ± 7	9 ± 3	0.194							0.072
PWA-I (Carotid), unit	21 ± 13	13 ± 17	19 ± 16	21 ± 6	0.277							0.059
PWI @ HR75 (Carotid), unit	15 ± 11	6 ± 16	9 ± 16	11 ± 7	0.232							0.065
Carotid Systolic Area, mm2	49 ± 11	45 ± 7	48 ± 8	42 ± 7	0.017	0.142	0.745	0.03	0.113	0.363	0.036	0.101
Carotid Diastolic Area, mm2	45 ± 9	40 ± 7	44 ± 8	38 ± 6	0.005	0.062	0.582	0.009	0.082	0.344	0.022	0.126
Carotid Beta Stiffness	7.0 ± 2.7	5.6 ± 2.1	5.9 ± 2.0	5.1 ± 1.3	0.014	0.047	0.052	0.009	0.657	0.41	0.414	0.106
Carotid Distensibility Coefficient	13.5 ± 5.0	18.4 ± 7.2	16.7 ± 4.4	17.8 ± 4.0	0.008	0.008	0.034	0.015	0.495	0.663	0.487	0.117
Ascending Systolic Area, mm2	957 ± 213	977 ± 192	1022 ± 179	928 ± 219	0.449							0.030
Ascending Diastolic Area, mm2	911 ± 211	927 ± 197	959 ± 179	863 ± 215	0.443							0.030
Ascending Beta Stiffness, unit	13.5 ± 8.1	13.7 ± 8.8	10.6 ± 7.5	9.2 ± 5.7	0.125							0.063
Ascending Strain, %	5.2 ± 2.9	5.9 ± 4.6	6.9 ± 4.4	7.9 ± 4.4	0.17							0.056
Descending Systolic Area, mm2	579 ± 104	577 ± 97	616 ± 130	584 ± 115	0.608							0.021
Descending Diastolic Area, mm2	538 ± 114	534 ± 94	570 ± 130	537 ± 116	0.69							0.017
Descending Beta Stiffness, unit	10.4 ± 8.2	7.4 ± 3.5	8.0 ± 5.2	7.5 ± 4.8	0.28							0.043
Descending Strain, %	8.5 ± 5.7	8.2 ± 3.6	8.7 ± 4.7	9.3 ± 4.4	0.866							0.008

PWV, pulse wave velocity; U-Peripheral, upper limb peripheral; L-Peripheral; lower limb peripheral; (Sphy), estimated by SphygmoCor; PWA-P, pulse wave analysis augmentation pressure; PWA-I, pulse wave analysis augmentation index; PWI@HR75, augmenation index at heart rate of 75 beats per minute; ANOVA, analysis of variance; Post Hoc Analysis was performed by the Student-Newman-Keuls.

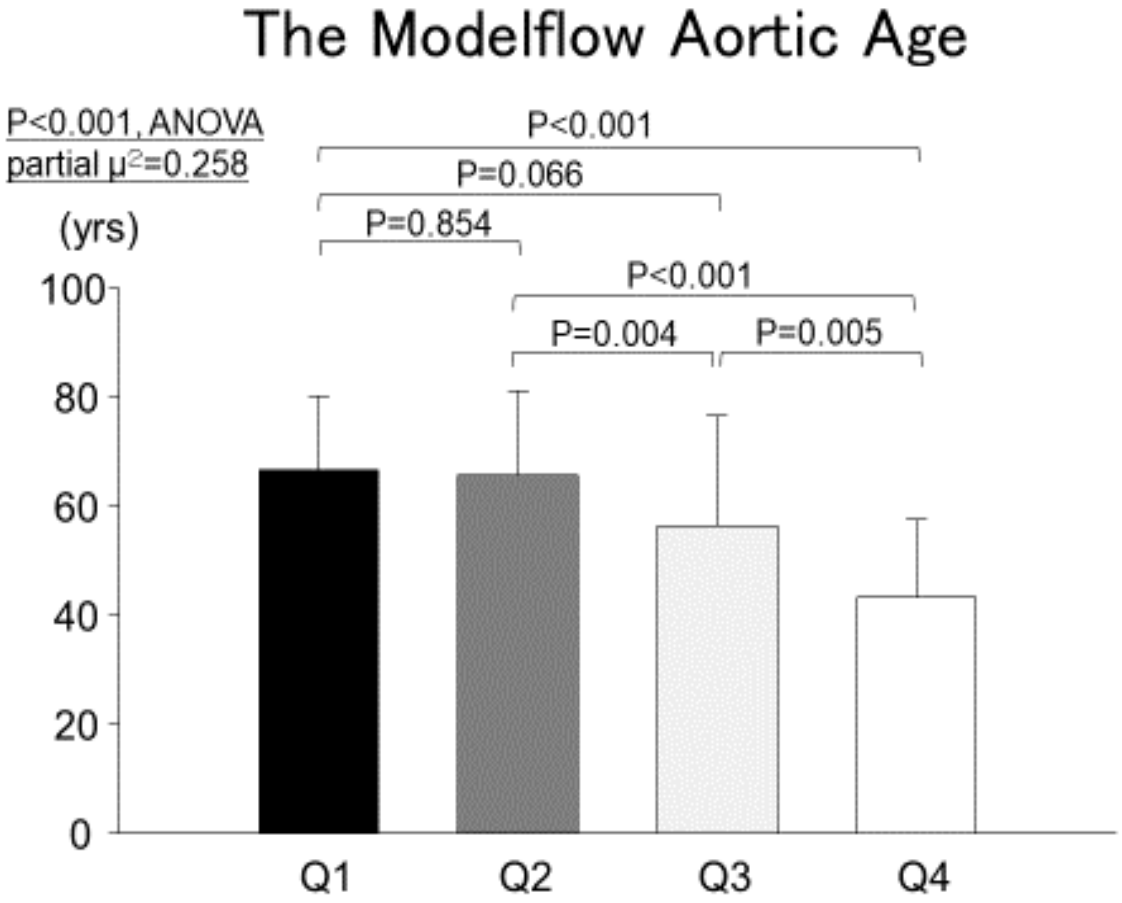

Figure 1: The Modelflow aortic age for sedentary subjects (Q1 n=27), casual exercisers (Q2 n=25) committed exercisers (Q3 n=25) and competitive exercisers (Q4 n=25). P values are derived from post-hoc analysis (Student-Newman-Keuls method).

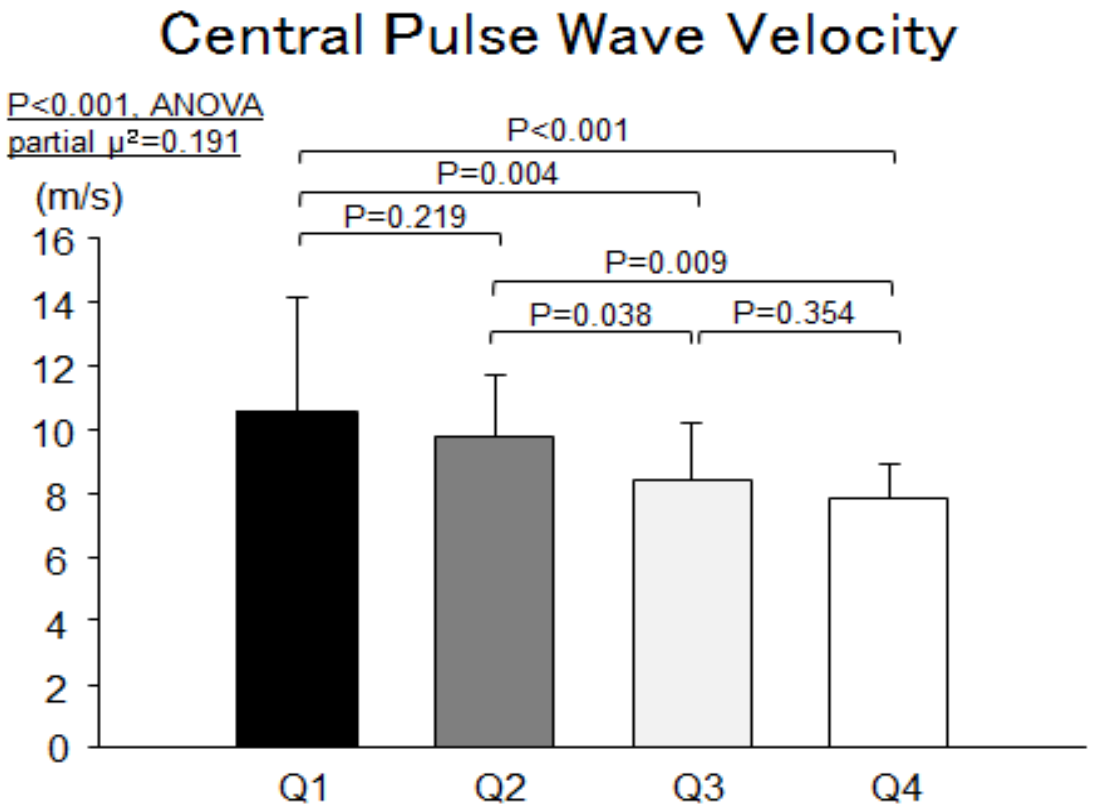

Figure 2: Central pulse wave velocity for sedentary subjects (Q1 n=27), casual exercisers (Q2 n=25) committed exercisers (Q3 n=25) and competitive exercisers (Q4 n=25). P values are derived from post-hoc analysis (Student-Newman-Keuls method).

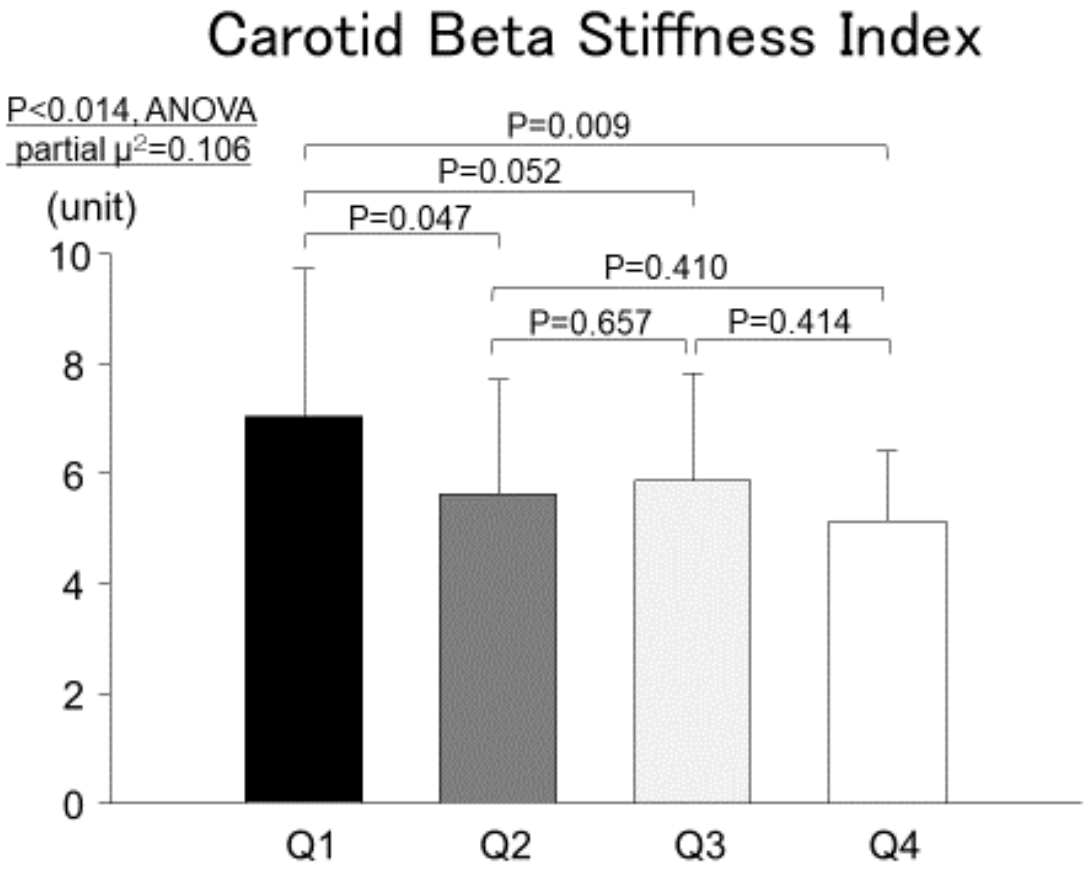

Figure 3: Carotid β-stiffness index for sedentary subjects (Q1 n=27), casual exercisers (Q2 n=25) committed exercisers (Q3 n=25) and competitive exercisers (Q4 n=25). P values are derived from post-hoc analysis (Student-Newman-Keuls method).