The Thesis Committee for Jisok Lim
Certifies that this is the approved version of the following thesis:

Impact of Blood Pressure Perturbations on Arterial Stiffness

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

Hirofumi Tanaka

Robert Matthew Brothers

Impact of Blood Pressure Perturbations on Arterial Stiffness

by

Jisok Lim, B.S.

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Abstract

Impact of Blood Pressure Perturbations on Arterial Stiffness

Jisok Lim, M.S. Kin

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Supervisor: Hirofumi Tanaka

Although the associations between chronic levels of arterial stiffness and blood pressure (BP) have been fairly well studied, it is not clear if and how much arterial stiffness is influenced by acute changes in BP. The primary aim of this study was to determine magnitudes of BP-dependence of various measures of arterial stiffness during acute BP perturbations. Fifty apparently healthy subjects, including 25 young (20-40 years) and 25 older adults (60-80 years), were studied. A variety of BP perturbations, including head-up tilt, head-down tilt, mental stress, isometric handgrip exercise, and cold pressor test, were used in order to encompass blood pressure changes induced by physical, mental, and/or physiological stimuli. Arterial stiffness measures included cardio-ankle vascular index (CAVI), carotid-femoral pulse wave velocity (cfPWV), brachial-ankle pulse wave velocity (baPWV), arterial compliance (AC), elastic modulus (EM), arterial distensibility (AD), beta-stiffness index, and young's modulus (YM). When each index of arterial stiffness was plotted with mean blood pressure, all the

arterial stiffness indices, including CAVI (r=0.50), cfPWV (r=0.51), baPWV (r=0.61), AC (r=-0.42), EM (r=0.52), AD (r=-0.32), β-stiffness index (r=0.19), and YM (r=0.35) were related to mean BP (all P<0.01). Changes in CAVI, cfPWV, baPWV, and elastic modulus were significantly associated with changes in mean BP in the pooled conditions. Changes in AC, AD, β-stiffness index, and YM were not significantly related to changes in mean BP. In conclusion, this study demonstrated that blood pressure changes in response to a various forms of pressor stimuli were associated with the corresponding changes in arterial stiffness indices and that the strengths of associations with BP varied widely depending on what arterial stiffness indices were examined.

Table of Contents

List of Tables

List of Figures

CHAPTER 1

INTRODUCTION

1.1 Background

Arterial stiffness plays an important role in pathogenesis of cardiovascular disease (CVD) and is an important independent risk factor for CVD (36, 49). As the changes in arterial stiffness can be detected before the appearance of clinically apparent and overt vascular disease, arterial stiffness can be used as an early marker for subclinical vascular dysfunction (25). Arterial stiffness can be measured using various techniques and methodologies (41). Each arterial stiffness parameter reflects somewhat different aspect of arterial elasticity and may be affected by behavioral, physiological, and extraneous factors to a different degree. Among these factors, dependence of arterial stiffness on blood pressure (BP) has been a topic of much discussion among investigators working in the field of arterial stiffness.

Arterial stiffness has been implicated as a primary cause of age-related elevation in arterial BP and described as a physiological mechanism underlying hypotensive effects of lifestyle interventions and antihypertensive medications (17, 38). On the other hand, some have questioned the utility of arterial stiffness above and beyond the traditional brachial BP measurement as changes in arterial stiffness are often accompanied by the corresponding changes in BP (24). Most of the available literature focusing on the blood pressure dependency on arterial stiffness deal with basal levels of blood pressure or chronic changes with pharmacological or non-pharmacological interventions (53, 54, 70). Currently, it is not clear

what extent acute changes in blood pressure are accompanied by the corresponding changes in arterial stiffness.

With this information as background, the primary aim of the present study was to determine the effect of acute BP perturbation on various measures of arterial stiffness. There are a number of BP perturbation maneuvers that have been utilized in research settings (51, 63), and these maneuvers use different forms of stress (psychological, physical, and mechanical) to elicit pressor responses. In order to comprehensively address the stated aim, we used 5 different BP perturbations. Additionally, because the effects of BP perturbations might be different in young and older adults (6), two groups of adults that differ widely in age were studied. The working hypothesis was that the magnitude of changes in arterial stiffness parameters induced by acute changes in BP would be different among various measures of arterial stiffness.

1.2 Statement of purpose

The purpose of the present investigation was to determine if there is a different effect of acute BP perturbation on various measures of arterial stiffness in young and older adults. The specific objectives of the study were to;

1. Determine the effect of acute BP perturbation on various measures of arterial stiffness.
2. Determine the effect of acute BP perturbation on various measures of arterial stiffness among young and older adults.

1.3 Hypothesis

In the current study, we tested the following hypotheses;

1. The magnitude of changes in arterial stiffness parameters induced by acute changes in BP would be different among various measures of arterial stiffness.
2. The magnitude of changes in arterial stiffness parameters induced by acute changes in BP would be different among various measures of arterial stiffness in young and older adults.

CHAPTER 2

LITERATURE REVIEW

This literature review summarizes the relevant research that describes the effect of acute changes in BP on various measures of arterial stiffness. The review includes arterial stiffness and variety of arterial stiffness indices such as pulse wave velocity, cardio-ankle vascular index, arterial compliance, arterial distensibility, elastic modulus, Young's modulus, and β stiffness index.

2.1 Arterial stiffness

Cardiovascular diseases (CVD) are well-known to be the most fatal diseases in the US and one-third of annual deaths were due to an onset of numerous independent risk factors of CVD (46). Although recently there has been an increase in concerns regarding the pathogenesis of CVD, CVD related deaths constantly has been a number one cause of death in the US (15, 37). Early prevention and detection of CVD have shown improvements during the last few decades that contributed to an enormous decrease in the mortality rate from CVD (15, 37). However, CVD still remains the most prevalent cause of death in the US (46). Recently, arterial stiffness is well accepted as a surrogate end point for CVD (24) and several epidemiological studies have emphasized the crucial role of arterial stiffness to pathogenesis of CVD (36, 49). On account of the fact that proceeding ill effects of arterial stiffness can be identified before the onset of clinically apparent and overt vascular disease, arterial stiffness can be used as an early marker for early subclinical vascular dysfunction (25).

Arterial stiffness is a reduction in arterial function to expand and recoil the artery in response to pressure changes (4). Arterial stiffness can be evaluated in a noninvasive manner by numerous methodologies (44). The study of arterial pulse transmission serves as the fundamental concept of the most common method in assessing arterial stiffness (3). Propagation of pressure wave throughout the arterial tree is initially generated by the ejection of blood from left ventricle into the aorta (50). As the forward pressure wave, incident waveform, travels through the arterial tree, it generates a reflected waveform at structurally and functionally discontinued locations of the arteries that travels back towards the ascending aorta (50). As a result, incident and reflected pressure waves collide with each other along the arterial circuit and forms the actual pressure wave. As arteries become stiffer, the pulse wave propagates at a higher speed through the arteries that leads to an increase velocity of the reflected wave (20, 40). In consequence of the elevated pulse wave velocity (PWV), the reflected wave and forward wave sums up at a premature period which causes an increase systolic blood pressure (BP) and pulse pressure (30). Therefore, augmented systolic BP, pulse pressure and PWV contributes to an increase onset of CVD (18, 36).

Age-related stiffening of the arterial walls have been well demonstrated by numerous previous studies (33-35) and the association between age and arterial stiffness was more strongly related to population with CVD (35). Arterial stiffness has been previously illustrated by a study that compliance of the artery decreases and BP increases with advancing age (33). This age related stiffening of the artery can be explained by a combination of structural and functional modifications. As arteries age, proportion of collagen to elastin increase in the intima-media wall that cause a reduction in elastic property of the artery and become stiffer (39, 40, 48). In a previous study, the ratio of collagen to elastin related with arterial stiffness and all-cause

mortality has been well established (57). In addition, the smooth muscle contraction of the artery increases due to an elevated baseline vasoconstrictor tone that leads to arterial stiffness among the elderly population (52). Central arterial stiffness is contributed in part by an age related decrease in sympathetic baroreflex sensitivity; decreased sympathetic baroreflex sensitivity cause an exaggerated sympathetic activation, thereby, leads to an elevated prevalence of hypertension among the elderly population (42). Therefore, arteries of young population has an elevated ability to deal with cardiac pulsation whereas older arteries become rigid and lose compliance that leads to an increased velocity of BP waveform propagation through an arterial tree (20).

Recently, the relationship between arterial stiffness and CVD has been better understood and serious attention has been the focus towards precise measurement of arterial stiffness. Several indices of arterial stiffness have been widely used in research and clinical settings such as PWV, cardio-ankle vascular index (CAVI), arterial compliance, elastic modulus, arterial distensibility, beta-stiffness index, and Young's modulus etc. However, the utility of some indices are questioned by researchers due to the lack of comprehensive understanding and interpretation of the measurements.

2.2 Pulse wave velocity

PWV is the most prevalently used index of arterial stiffness measurement and its' capability of predicting CVD events has been supported by numerous previous epidemiological studies (23). The measurement of PWV is considered as the most simple, non-invasive, robust and reproducible method to detect arterial stiffness (24). Even though no one arterial stiffness

index has been proved superior, PWV is the most dominantly used index of arterial stiffness (24).

The measurement of PWV is the velocity of the pressure wave transmission along the arterial tree and it is obtained by dividing the distance by transit time between two separate locations (9). Among the various types of PWV, carotid-femoral PWV (cfPWV), which is widely used in research and clinical settings, has been considered as the "gold standard" measurement of central arterial stiffness. The cfPWV has been demonstrated in several epidemiological studies that cfPWV is an independent predictive value for CVD. In the Framingham Heart Study, central arterial stiffness was evaluated as cfPWV and such measurement was reported as superior to carotid-radial PWV, augmentation index, central pulse pressure and pulse pressure amplification (36). Furthermore, cfPWV in patients with end-stage renal disease also showed a superiority to brachial artery and femorotibial stiffness (43).

The lack of interest directed toward brachial-ankle pulse wave velocity (baPWV) due to its predominant usage in Asian countries and cfPWV has been represented as an indicator of central arterial stiffness in major epidemiological studies. The measurement of baPWV is obtained by placing an oscillometric pressure sensor cuffs on four extremities. However, due to its' sites of measurement on brachial and ankle, the arterial pathway that pressure wave travels through includes not only elastic (central) but also muscular arteries (peripheral) (72). Thus, it is illogical to conclude that baPWV solely represents the central arterial stiffness rather than the peripheral arterial stiffness as same as cfPWV. Although the controversy remains on whether baPWV predicts aortic stiffness, a previous study reported a strong positive relationship between cfPWV and baPWV that indicated both measurements of arterial stiffness as a strong predictive indicator of central arterial stiffness (65). However, constantly higher values of baPWV

compared with cfPWV suggested that relatively small segment of baPWV may be influenced by peripheral arterial stiffness (59). Therefore, baPWV primarily demonstrates central arterial stiffness as well as a small portion of peripheral arterial stiffness.

One of the controversial limitations of PWV in the measurement of arterial stiffness is that the magnitude of PWV depends largely on the arterial blood pressure (60). Various cross-sectional studies demonstrated that cfPWV is highly dependent on BP (32). Also, it has been found that arterial stiffness measurement increases followed by an increase in loading pressure without undergoing any changes on the arterial structure (2). In this regard, based on the previous findings, we are able to conclude that cfPWV not only reflects stiffness of the arteries but also the pressure changes that occurs during the time of measurement. In addition, because the pulse pressure is measured by air pressure, measurements obtained by generating pressure on the arterial wall may influence results (71). Another possibility that induces unreliable measurement in arterial stiffness can be contributed by an increase in PWV during inspiration due to the slight elevation in BP comparing with expiration (1).

2.3 Cardio-ankle vascular index

Taking the issues above into consideration, a cardio-ankle vascular index (CAVI) was suggested as an arterial stiffness and arteriosclerosis indicator of numerous arteries in the human body, which is independent of arterial blood pressure (54, 71). Hayashi et al. originally proposed CAVI based upon the β-stiffness index (13). CAVI was claimed as a BP independent marker of arterial stiffness based on the fact that β-stiffness index, which is known to be a BP independent measurement of arterial stiffness, was incorporated in the CAVI calculation. Although there were controversial questions regarding the validity of applying β-stiffness index to CAVI equation, a

group of investigators validated the application by demonstrating a positive correlation between β-stiffness index and CAVI ($r=0.67$, $P<0.01$) (62). CAVI is obtained by the distance from the brachial level to the ankle level and the time delay between the closing of the aortic valve to the change in arterial pressure wave at the time of measurement (71). The measurement of CAVI is equipped with ECG electrodes on both wrists, phonocardiography placed on the sternum, and 4 blood pressure cuffs on all four extremities which require minimal techniques to obtain the measurement comparing with others.

CAVI has been studied under several clinical experiments that provide evidence on its' BP independent characteristic. Previous reports showed that CAVI is less dependent on BP compared with PWV (16, 21, 53, 62). An experimental comparative study (53) demonstrated the BP dependency on CAVI and baPWV by administrating acute BP modifying medications such as β1-adrenoceptor blocker, metoprolol. The metoprolol decreased both systolic and diastolic BP significantly following by a significant decrease in baPWV (13.9 m/sec to 12.5 m/sec, $P<0.05$). However, CAVI remained the same as before drug administration (8.2 to 8.2, $P=0.45$). In addition, a group of investigators reported a BP dependency on CAVI and baPWV among non-diabetic and diabetic subjects by applying exercise stress test (16). Significant positive linear relationship was observed between baPWV and systolic BP in both group ($r=0.41$, $P<0.001$). However, CAVI was not dependent on BP changes, which is in line with previous studies ($r=0.17$, $P=0.18$). Results of these studies explicitly demonstrated that CAVI is independent of blood pressure during measurement regardless of disease status.

2.4 Arterial compliance & distensibility

Arterial compliance is defined as the absolute change in diameter during systole that explains the buffering ability of an artery (41). A decrease in arterial compliance might lead to a greater risk of cardiac hypertrophy induced by an increase in cardiac afterload (45). Arterial distensibility is defined as the relative change in diameter during systole and diastole that demonstrates the elasticity of the arterial wall (41). A decrease in arterial distensibility might be suspected to have a higher risk of arterial wall damage that leads to atherosclerotic disease (11). Along with arterial compliance and distensibility, elastic modulus, Young's modulus and β stiffness index can also be obtained by ultrasound equipment acquiring diameter, pressure and wall thickness of the measuring artery. However, limitations of this methodology have been emphasized by investigators such as limited resolution of image, operator dependence and reproducibility (31).

Several previous studies reported that arterial compliance and distensibility significantly decreased in hypertension and borderline hypertension group (56, 67, 68). This can be explained by arterial components responding differently to a variety of BP levels. During low BP, elastins located in media layer of the artery primarily functions to coordinate the mechanical behavior and maintain distensibility of the arterial wall (69). Whereas, during higher BP, mainly collagens are recruited from the arterial media that cause a lower extensibility of the wall and provide structural support (12). Therefore, an increase in BP will lead to a greater collagen recruitment in the artery that induces an increase in stiffness of the artery, thereby, causing the arterial compliance and distensibility to decrease.

2.5 Elastic modulus & Young's modulus

Elastic modulus is an arterial pressure change required for a theoretical 100% diameter change from resting diameter. Young's modulus also defines the elastic behavior of the arterial wall and additionally takes into consideration of wall thickness to elastic modulus. Due to the implication of BP changes in the equation of elastic modulus and Young's modulus, both arterial stiffness parameters have been well-known to be BP dependent demonstrated by previous studies. Hirai et al. reported a significant decrease in elastic modulus according to a nitroprusside-induced decrease in BP (14). In a comparative study, elastic modulus elicited a greater significant association with mean BP than β stiffness index and arterial compliance index in both normotensive and hypertensive group (47). In addition, elastic modulus and β stiffness index were compared further in a clinical study (22). Elastic modulus and β stiffness index were measured in the distal abdominal aorta in resting and isometric exercise condition (resting mean BP: 81 mmHg, isometric exercise mean BP: 122 mmHg). After the exercise intervention, elastic modulus increased significantly by 91% whereas β stiffness index increased by 27%, but did not reach the significant level. Elastic modulus also has been reported to exponentially increase with higher BP (69).

2.6 β stiffness index

β stiffness index is defined as ratio of logarithmic change in pressure to change in diameter (19). β stiffness index was developed in order to provide a BP independent measure of arterial stiffness by implicating logarithmic calculation to pressure change unlike elastic modulus and Young's modulus. Due to β stiffness index's pressure-independent characteristic, it recently

became a crucial basis of the CAVI calculation to support the notion that CAVI is a new BP independent measurement of arterial stiffness.

A previous study reported that β stiffness index showed no significant relationship to acute 40 mmHg alteration in systolic BP induced by nitroprusside (14). Lehmann et al. (27) have also showed that β stiffness index was not significantly associated with BP in normal, healthy subjects. In a more recent study, β stiffness index showed an independent relationship with age (β=0.355, $P<0.001$) and smoking history (β=0.151, $P<0.01$) but not with mean BP (47). In addition, the transformation of β-stiffness index into cfPWV showed an enormously less BP dependency compared to cfPWV (70). However, several studies observed a significant BP dependence of β stiffness index among unhealthy populations (28, 29). β stiffness index was strongly associated with mean BP in elderly stroke patients ($r=0.41$, $P<0.005$) and patients with vascular disease and/or diabetes mellitus ($r=0.34$, $P<0.001$). Therefore, based on previous studies, β stiffness index can't generally assume to be a BP independent measurement of arterial stiffness.

CHAPTER 3

METHODOLOGY

3.1 Subjects

A total of 50 subjects (45±3 yrs) were studied. Half of the subjects were young (20-40 yrs) and the other half older (60-80 yrs). Subjects were recruited from the city of Austin and the surrounding community using flyers and e-mails to various organizations and information sharing. Subjects with overt heart disease, diabetes, or other cardiovascular problems as assessed by medical history questionnaire were excluded from the study participation. Additional exclusions from the study included pregnancy, chronic smoking, a recent illness or surgery, or any medical intervention in the 12 hours before any of the study sessions. Subjects were instructed to abstain from strenuous exercise for 12 hours, caffeine consumption for 6 hours, and food for 4 hours prior to the experimental trial. The Institutional Review Board at University of Texas at Austin approved this study. Written informed consent was obtained from all subjects.

3.2 Experimental design

The measurements were performed on two separate visits lasting ~2 hours under comfortable laboratory conditions. The two testing sessions were scheduled at the same time of the day to avoid any potential diurnal effects. In the first testing session, head up tilt, mental stress, and cold pressor test were performed. In the second testing session, head down tilt, isometric handgrip exercise, and cold pressor test were conducted. Each pressor test was separated by at least 10-15 minutes. Cold pressor test was performed on both sessions to accommodate all the arterial stiffness tests (to avoid the measurement of arterial pressure and

waveform on the limb that the cold stress was applied). Selection of sides between the days was randomized. Cold pressor test was always conducted as the final perturbation for each testing visit to minimize the long-lasting residual effects that could influence other vascular reactivity tests. Isometric handgrip test was performed on both sides of the body during the second testing visit and the order of sides being tested was randomized. Importantly, there were no significant differences in pressor responses between the two testing sessions for either isometric handgrip exercise or cold pressor test demonstrating the stability of the pressor responses.

Head-up tilt and Head-down tilt

Head-up and head-down tilt was conducted as previously described (63). Subjects were in a supine position on a tilt table with two Velcro straps placed over the chest and thighs and tilted to 70 degrees. For head-down tilt, the subjects were in a supine position on a tilt table and tilted to 200 degrees and instructed to remain still. The straps prevented subjects from sliding down the table, and ensured subjects were not bearing weight on any surface during the testing period; this prevented muscular contraction of the lower body, which could affect hemodynamics and pressor responses.

Mental stress test

The Stroop Test (58) is a cognitive task inducing psychological stress that can be easily performed in a laboratory setting. A slide show contained randomly-colored words of color names and was programmed to proceed at a rate of one slide per second to produce a sufficient psychological stress. The entire slide show was composed of two types of slides: (a) congruent color-word slide: equivalent printed color and words of a color (i.e. the word “red” appeared in red font on the slides); (b) incongruent color-word slide: inequivalent printed color and words of

a color (i.e. the word “red” appeared in green font on the slide). To increase the stressfulness of the test, subjects were instructed to comply with the speed of the slide show and to respond according to the slide type presented. Subjects were provided with a wireless mouse in each hand and instructed to click the mouse in their right hands whenever a congruent color-word slide was presented and with the left hand whenever an incongruent color-word slide was presented. The Stroop test proceeded for 2-3 minutes while measurements were obtained. The number of correct answers was not quantified.

Isometric handgrip exercise

Subjects performed a 2-3 minute isometric handgrip exercise (HDM-915; Lode Instruments, Groningen, The Netherlands) at 40% of maximal voluntary contraction (MVC). During the test, the force exerted by subjects was shown on a visual display, and the subjects were instructed to hold 40% of their MVC throughout the testing period. Subjects were asked to breathe normally, avoid a Valsalva maneuver (7), and contract only the flexors of the hand and to avoid recruiting additional musculature of the upper arm and shoulder.

Cold pressor test

Subjects submerged their foot in ice water (2-5 ^{o}C) for 2-3 minute (8). The foot was selected, rather than the hand, in order to stimulate maximum hemodynamic and sympathetic responses (51). Subjects were instructed to breathe normally, avoid a Valsalva maneuver (7), and relax as much as possible.

3.3 Measurements

The following arterial stiffness indices were measured in the present study.

Cardio-ankle vascular index

Unilateral brachial BP, ankle BP, and heart rate were measured simultaneously by using oscillometric pressure sensor cuffs, electrocardiograms, and phonocardiograms (VaSera VS-1000, Fukuda Denshi, Tokyo, Japan). CAVI was calculated according to the stiffness parameter β, which is known to be a BP-independent arterial stiffness into an equation (61). CAVI is a relatively new measure, and its reliability has not been well established. Accordingly, the measurements of CAVI were taken three times each by two different investigators at the beginning of each testing day to obtain inter- and intra-observer reliability.

Pulse wave velocity

Both carotid-femoral pulse wave velocity (cfPWV) and brachial-ankle pulse wave velocity (baPWV) was simultaneously measured by the vascular testing device (Colin VP-2000, Colin Medical, San Antonio, TX). Arterial applanation tonometry sensors incorporating an array of 12 micropiezoresistive transducers were placed on carotid and femoral artery to acquire pulse waves (5). In addition, oscillometric pressure sensor cuffs were placed unilaterally on arm and ankle, an electrocardiogram sensors were attached to both wrists and a phonocardiogram were placed above the sternum. baPWV was obtained from the oscillometric pressure sensor cuffs. cfPWV was obtained from distance divided by the time delay of the pulse wave. Distance traveled by pulse waves was calculated by the measurement of body surface from carotid to femoral artery with a segmometer specifically constructed for PWV studies.

Carotid artery compliance

The simultaneous measurement of ultrasound imaging of the common carotid artery and applanation tonometrically-obtained arterial pressure waveforms from the contralateral artery

allows noninvasive determination of arterial compliance (64). Common carotid artery diameter was measured from the images derived from an ultrasound machine equipped with a high-resolution linear array transducer (Philips iE33 Ultrasound System, Bothel, WA). Carotid artery diameter was analyzed using image analysis software (Brachial Analyzer, Medical Imaging Applications, Coralville, IA), and arterial pressure waveforms were analyzed using a waveform analysis software (WinDq 2000, Dataq Instruments, Akron, OH). Arterial compliance, elastic modulus, arterial distensibility, β-stiffness index, and Young's elastic modulus were subsequently calculated.

3.4 Statistical analysis

The intraclass correlation coefficients (ICC) were obtained to determine inter- and intra-observer reliability of the CAVI. Additionally, coefficients of variation were calculated. Analyses of variance (ANOVA) were used to evaluate the group differences. If a significant F-value was detected, a post-hoc test using LSD was performed to identify the significant group differences. Correlational and regressional analyses were performed to determine the relations between changes in arterial stiffness indices and mean BP induced by various vascular reactivity tests. All data were presented as means±SEM. Statistical significance was set a priori at $p<0.05$. SPSS statistics software version 22 (IBM, Chicago, IL) was used for all statistical analyses.

CHAPTER 4

RESULTS

4.1 Subjects

Table 1 presents selected subjects' physical characteristics. A total of 50 subjects, including 25 young and 25 older adults, were studied in this study. There were no significant differences in height, body mass, and body mass index between young and older individuals.

4.2 CAVI reliability tests

Table 2 illustrates the reliability of CAVI measurements. Coefficients of variation of intra-observer and inter-observer reliability were 3.4 and 2.4%. The intra- and inter-observer reliability test showed that ICCs were 0.99 and 0.98.

4.3 Interrelationships among arterial stiffness indices during resting

Table 3 shows relations between various measures of arterial stiffness indices obtained at baseline. CAVI, cfPWV, baPWV, arterial compliance and elastic modulus were significantly related to each other. Arterial distensibility was associated with baPWV, arterial compliance, and elastic modulus. β-stiffness index was not significantly related to any of the arterial stiffness indices, including CAVI.

4.4 Arterial stiffness indices during vascular reactivity tests

Table 4 and Figure 1 show changes in arterial stiffness indices to various vascular reactivity tests. Various pressor tests produced variable degree of changes in arterial stiffness indices. As shown in Table 5, changes in CAVI, cfPWV, baPWV, and elastic modulus were significantly associated with changes in mean BP in the pooled conditions. Changes in arterial compliance, arterial distensibility, β-stiffness index, and Young's modulus were not significantly related to changes in mean BP. When each index of arterial stiffness was plotted with mean blood pressure, all the arterial stiffness indices, including CAVI (r=0.50), cfPWV (r=0.51), baPWV (r=0.61), arterial compliance (r=-0.42), elastic modulus (r=0.52), arterial distensibility (r=-0.32), β-stiffness index (r=0.19), and Young's modulus (r=0.35) were related to mean BP (all $P<0.01$) (Appendix A).

4.5 Changes in arterial stiffness indices during vascular reactivity tests stratified by age and sex

In order to determine if the associations between blood pressure and arterial stiffness indices are modulated by age and sex, we stratified the data by age and sex and presented in Table 6. In general, the strengths of associations were greater in males than in females. For CAVI, cfPWV, and baPWV, young subjects had stronger association with changes in mean BP than in older subjects.

Table 1. Selected subject characteristics

	Total (n = 50)	**Young (n = 25)**	**Older (n = 25)**
Age (yrs)	45.4 ± 2.7	27.1 ± 0.6	63.8 ± 0.9*
Sex (M/F)	29 / 21	17 / 8	12 / 13
Height (cm)	172 ± 1	174 ± 2	170 ± 2
Body mass (kg)	75.9 ± 2.3	78.6 ± 3.4	73.3 ± 3
Body mass index (kg/m²)	25.6 ± 0.7	25.7 ± 1.0	25.4 ± 1.0

Data are mean±SEM. *P<0.01 vs. Young.

Table 2. Intra- and inter-observer reliability tests of cardio-ankle vascular index (CAVI)

	CV	ICC	95% Confidence Interval	
			Minimum	Maximum
Intra-observer reliability	3.4 %	0.99*	0.98	0.99
Inter-observer reliability	2.4 %	0.98*	0.98	0.99

CV=coefficient of variation, ICC=intraclass correlation coefficient. *P<0.05.

Table 3. Interrelationships among different measure of arterial stiffness indices at baseline

	CAVI	cfPWV	baPWV	AC	EM	AD	β-SI	YM
CAVI		.71	.84	-.33	.46	NS	NS	NS
cfPWV			.66	-.34	.42	NS	NS	NS
baPWV				-.41	.57	-.29	NS	NS
AC					-.82	.96	NS	-.69
EM						-.73	NS	.75
AD							NS	-.68
β-SI								NS
YM								

Data are Pearson correlation coefficients. CAVI=cardio-ankle vascular index, cfPWV=carotid-femoral pulse wave velocity, baPWV=brachial-ankle pulse wave velocity, AC=arterial compliance, EM=elastic modulus, AD=arterial distensibility, β-SI=β-stiffness index, YM=Young's modulus.

Table 4. Arterial stiffness indices during various vascular reactivity tests

	Baseline	Head-Up Tilt	Head-Down Tilt	Mental Stress	Isometric Handgrip	Cold Pressor Test
CAVI (AU)	7.3 ± .22	8.83 ± .28 *	6.68 ± .26 †	7.49 ± .25 †‡	8.62 ± .25 *‡§	8.39 ± .31 *‡§
cfPWV (cm/sec)	927 ± 27	1049 ± 45 *	909 ± 31 †	1000 ± 35	1092 ± 36 *‡	1146 ± 52 *‡§
baPWV (cm/sec)	1202 ± 22	1593 ± 39 *	1071 ± 30 *†	1294 ± 38 †‡	1363 ± 30 *†‡	1380 ± 35 *†‡
Arterial compliance (cm/mmHg)	.0101 ± .0005	.0106 ± .0006	.0105 ± .0006	.0096 ± .0006	.0095 ± .0006	.0095 ± .0005
Elastic modulus (mmHg)	395 ± 21	364 ± 20	380 ± 19	452 ± 36 †	472 ± 30 *†‡	451 ± 28 †
Arterial distensibility ($mmHg^{-1}$)	.036 ± .002	.037 ± .002	.038 ± .002	.035 ± .002	.037 ± .003	.035 ± .002
β-stiffness index (AU)	.29 ± .06	.35 ± .02	.33 ± .02	.37 ± .02	.38 ± .05	.29 ± .04
Young's modulus (mmHg/cm)	821 ± 46	757 ± 51	767 ± 40	926 ± 84 †‡	939 ± 58 †‡	920 ± 52 †

Data are mean±SEM. CAVI=cardio-ankle vascular index, cfPWV=carotid-femoral pulse wave velocity, baPWV=brachial-ankle pulse wave velocity. *P < 0.05 vs. baseline, †P < 0.05 vs. head-up tilt, ‡P < 0.05 vs. head-down tilt, §P < 0.05 vs. mental stress.

Table 5. Relations between changes in arterial stiffness indices and mean blood pressure (BP) during vascular reactivity tests

	Δ Mean Blood Pressure (mmHg)					
	Head-Up Tilt	**Head-Down Tilt**	**Mental Stress**	**Isometric Handgrip**	**Cold Pressor Test**	**POOLED**
Δ CAVI (AU)	NS	NS	NS	.49*	.31*	.34*
Δ cfPWV (cm/sec)	NS	.31*	.34*	.35*	.37*	.36*
Δ baPWV (cm/sec)	.32*	NS	.71*	.43*	.69*	.38*
Δ Arterial compliance (cm/mmHg)	NS	NS	NS	NS	NS	NS
Δ Elastic modulus (mmHg)	NS	NS	NS	NS	NS	.20*
Δ Arterial distensibility ($mmHg^{-1}$)	NS	NS	NS	NS	NS	NS
Δ β-stiffness index (AU)	.32*	NS	NS	NS	NS	NS
Δ Young's modulus (mmHg/cm)	NS	NS	NS	NS	NS	NS

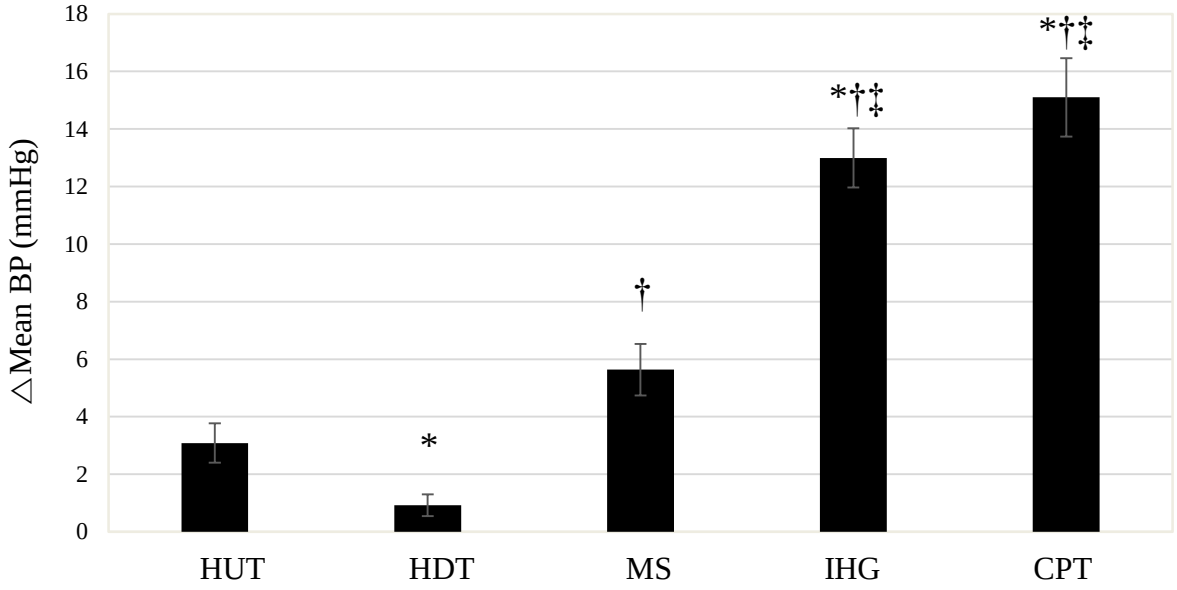
Data are Pearson correlation coefficients. *P<0.05. CAVI=cardio-ankle vascular index; cfPWV=carotid-femoral pulse wave velocity; baPWV=brachial-ankle pulse wave velocity.

Table 6. Associations between changes in arterial stiffness indices and mean blood pressure (BP) stratified by age and sex

	Δ Mean BP (mmHg)			
	MALE	**FEMALE**	**YOUNG (20-40 yrs)**	**OLDER (60-80 yrs)**
Δ CAVI (AU)	.42*	.26*	.45*	.22*
Δ cfPWV (cm/sec)	.44*	.29*	.56*	.23*
Δ baPWV (cm/sec)	.44*	.26*	.45*	.31*
Δ Arterial compliance (cm/mmHg)	NS	NS	NS	NS
Δ Elastic modulus (mmHg)	.30*	NS	NS	.27*
Δ Arterial distensibility ($mmHg^{-1}$)	NS	NS	NS	NS
Δ β-stiffness index (AU)	NS	NS	-.28*	NS
Δ Young's modulus (mmHg/cm)	NS	NS	NS	.22*

Data are Pearson correlation coefficients. *P<0.05. CAVI=cardio-ankle vascular index, cfPWV=carotid-femoral pulse wave velocity, baPWV=brachial-ankle pulse wave velocity.

Figure 1. Changes in mean blood pressure (BP) during vascular reactivity tests



Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test. *P<0.05 vs. HUT, †P<0.05 vs. HDT, ‡P<0.05 vs. MS.

CHAPTER 5

DISCUSSION

The most salient finding of the present study is that all of the arterial stiffness indices examined had some degree of blood pressure dependency to a various forms of pressor tasks. This was true for indices of arterial stiffness that are thought to be blood pressure independent; β-stiffness index and CAVI.

One of the strengths of the present study is the use of various types of pressor tests employed. These maneuvers use different forms of stress to elicit blood pressure responses. For instance, mental stress is psychological, cold pressor test is physical, and isometric handgrip is mechanical. In order to comprehensively evaluate the effects of acute changes in blood pressure on arterial stiffness, we utilized 5 different blood pressure maneuvers. Each test produced a various degree of pressor responses, with the most profound increase in blood pressure observed during the cold pressor test. In return, we also observed corresponding changes in most of the arterial stiffness indices during each maneuver.

When various levels of blood pressure induced by pressor tests were plotted against arterial stiffness, all of the arterial stiffness indices examined in the present study demonstrated significant associations with blood pressure. The strongest association was obtained with baPWV ($r=0.61$) and the lowest with β-stiffness index ($r=0.19$). When the data were analyzed by plotting changes in blood pressure and the corresponding changes in arterial stiffness, CAVI, cfPWV, baPWV, and elastic modulus showed significant

associations with changes in blood pressure in the pooled population whereas no such relations were observed for arterial compliance, arterial distensibility, β-stiffness index, and Young's modulus. These results suggest that some measures of arterial stiffness are affected by acute changes in blood pressure than others and that the magnitude of BP-dependency varies widely among various measures of arterial stiffness.

CAVI has been proposed as an arterial stiffness indicator that is independent of BP (54). The original study from Japan has reported no association between CAVI and blood pressure (r=0.01-18) whereas baPWV was significantly associated with blood pressure (r=0.34-0.46) (54). Since then a number of investigators have confirmed the observation that CAVI was less dependent on BP compared with other arterial stiffness indices (16, 21, 62). CAVI was originally developed by incorporating β-stiffness index, which is thought to be a BP independent measurement of arterial stiffness (62). Indeed in a recent study, the incorporation of β-stiffness index substantially reduced the BP dependency on PWV (70). In marked contrast to the previous studies, the present study demonstrated that CAVI was dependent on mean BP whether it is expressed as absolute levels of blood pressure or changes in blood pressure. Interestingly, CAVI was not associated with β-stiffness index. It should be noted that the previous studies utilized chronic or basal blood pressure whereas the present study used blood pressure that was changed with acute BP perturbations.

As expected, lowest associations with blood pressure were observed in β-stiffness index though its BP dependency appeared in several different analyses. The original assumption involved in β-stiffness index is a simple exponential relation between

the intraluminal pressure and the distension of arteries (13). However, the exponential relation was constructed in a limited blood pressure range obtained in a healthy normal population, and the validity has been questioned as to if it can be applicable to other populations (26). Indeed highly significant associations between blood pressure and β-stiffness index have been reported in a variety of patient populations (26).

Prevalence of hypertension increases markedly with advancing age in both men and women (10). Men are generally at greater risk for developing hypertension than age-matched women (10). Accordingly, we stratified the data for age (young and older) and sex (men and women) and performed separate statistical analyses. In general, the associations between changes in arterial stiffness and blood pressure appear larger for men than for women and for young than for older in many of the arterial stiffness indices. These results are consistent with previous studies reporting that blood pressure reactivity is modulated by both age and sex (55, 66).

The simplest premise of the present study is that any changes in arterial stiffness would be attributed to changes in blood pressure induced by pressor tests. But these changes in arterial stiffness and blood pressure could be independent. Sympathoexcitatory stimuli induced by blood pressure reactivity tests can act on the smooth muscle cells surrounding the large elastic arteries and stiffen arteries. The same vasoconstrictor tone can stimulate smooth muscle cells on the peripheral muscular arteries and increase vascular resistance and increase blood pressure via the Ohm's law. Thus, it should be noted that changes in arterial stiffness may not be epiphenomenon of blood pressure changes induced by various pressor tests.

CHAPTER 6

LIMITATIONS

There were several limitations that should be taken into consideration. First, because only apparently healthy subjects were studied in the present study, the results may not be extraporated to patient populations. Second, vascular reactivity tests were neither measured simultaneously nor randomized in this study. The present protocol provided substantial procedural challenges in placing all the measurement devices on a given subject. In spite of a number of pilot studies conducted, it was not possible to accommodate everything in one testing session. Additionally, some vascular reactivity tests such as CPT induces a prolonged BP changes and had to be placed at the end of the testing session. However, in an attempt to establish the reliability of pressor responses, we performed IHG or CPT twice in different testing sessions and found that there were no significant differences in pressor responses between the two testing sessions.

CHAPTER 7

CONCLUSION

In conclusion, the results of the present study indicate that blood pressure changes in response to various forms of pressor stimuli were associated with the corresponding changes in arterial stiffness indices examined and that the strengths of associations varied widely depending on what arterial stiffness indices were examined.

Appendix A: Relationship between Arterial Stiffness Indices and Mean Blood Pressure

Figure 1. Relationship between cardio-ankle vascular index (CAVI) and mean blood pressure (BP)

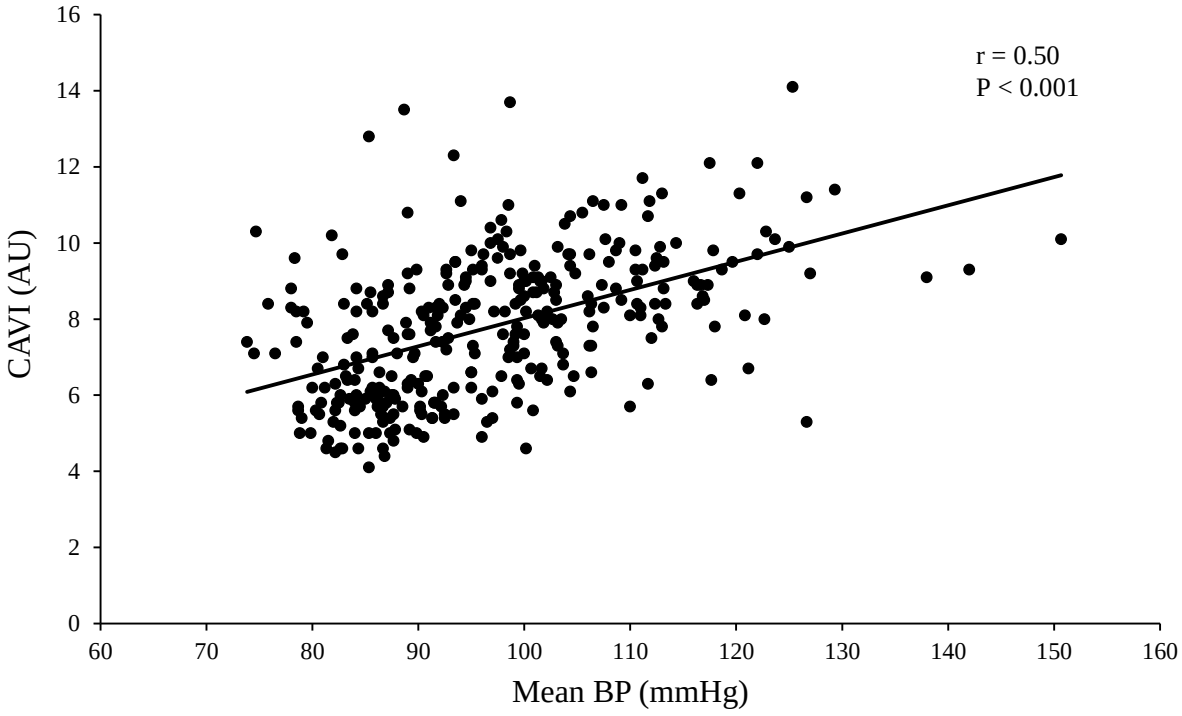


Figure 2. Relationship between carotid-femoral pulse wave velocity (cfPWV) and mean blood pressure (BP)

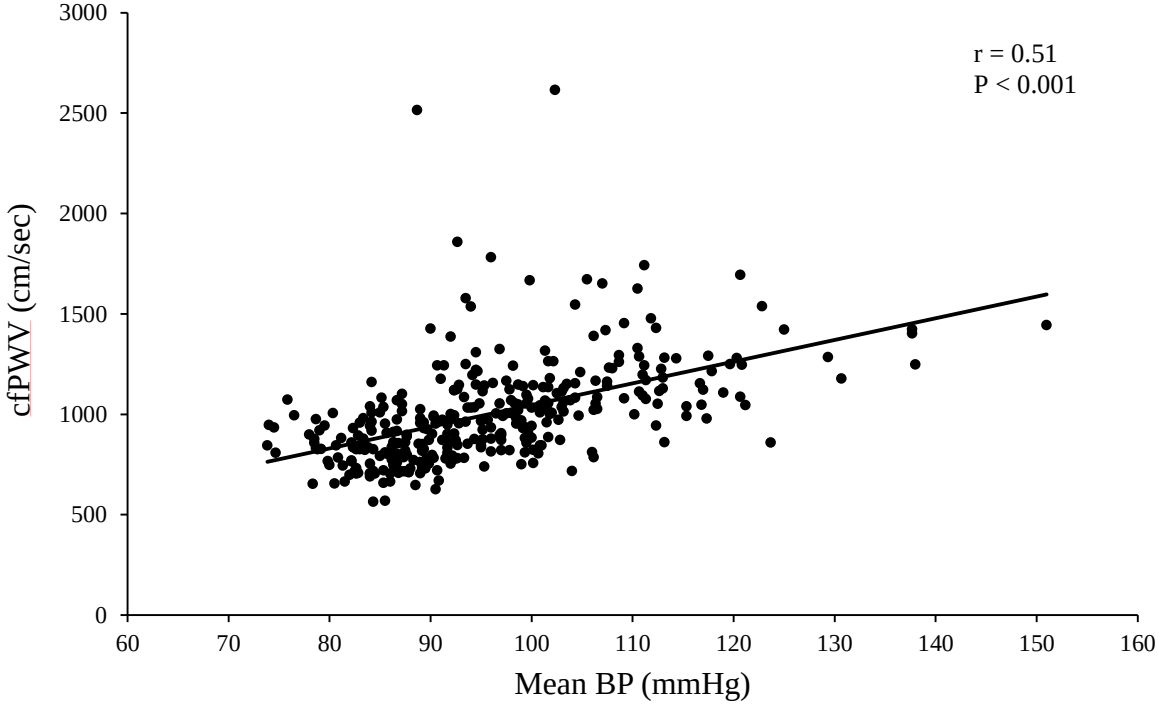


Figure 3. Relationship between brachial-ankle pulse wave velocity (baPWV) and mean blood pressure (BP)

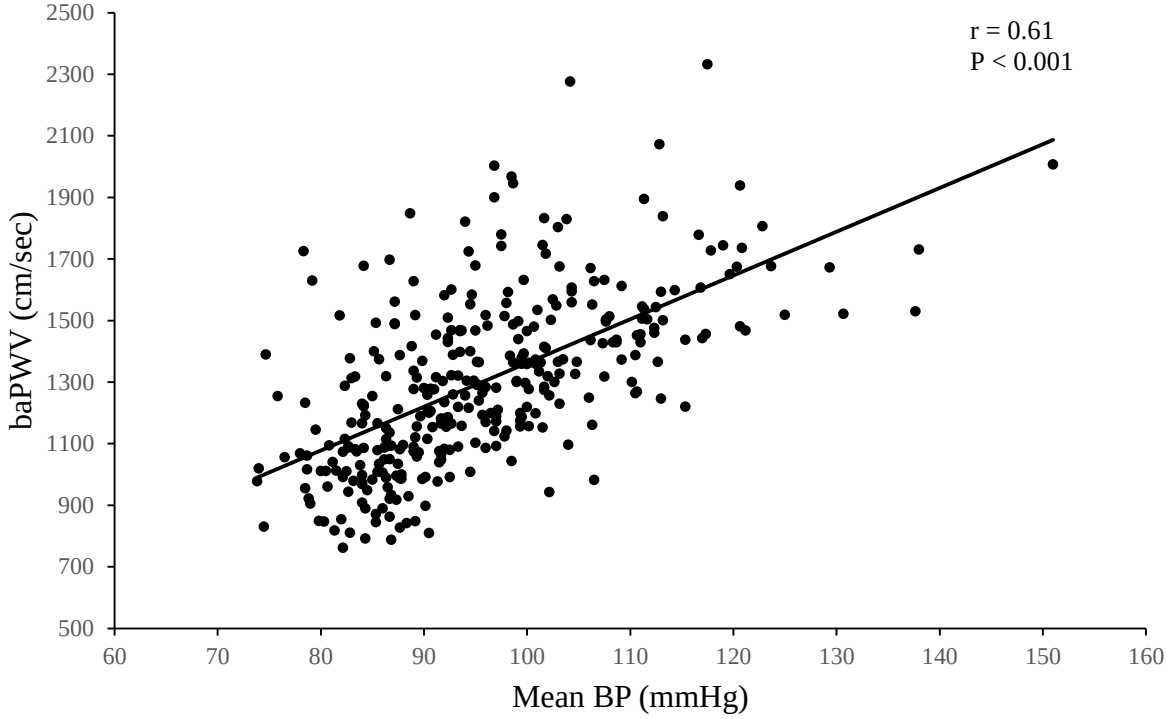


Figure 4. Relationship between arterial compliance and mean blood pressure (BP)

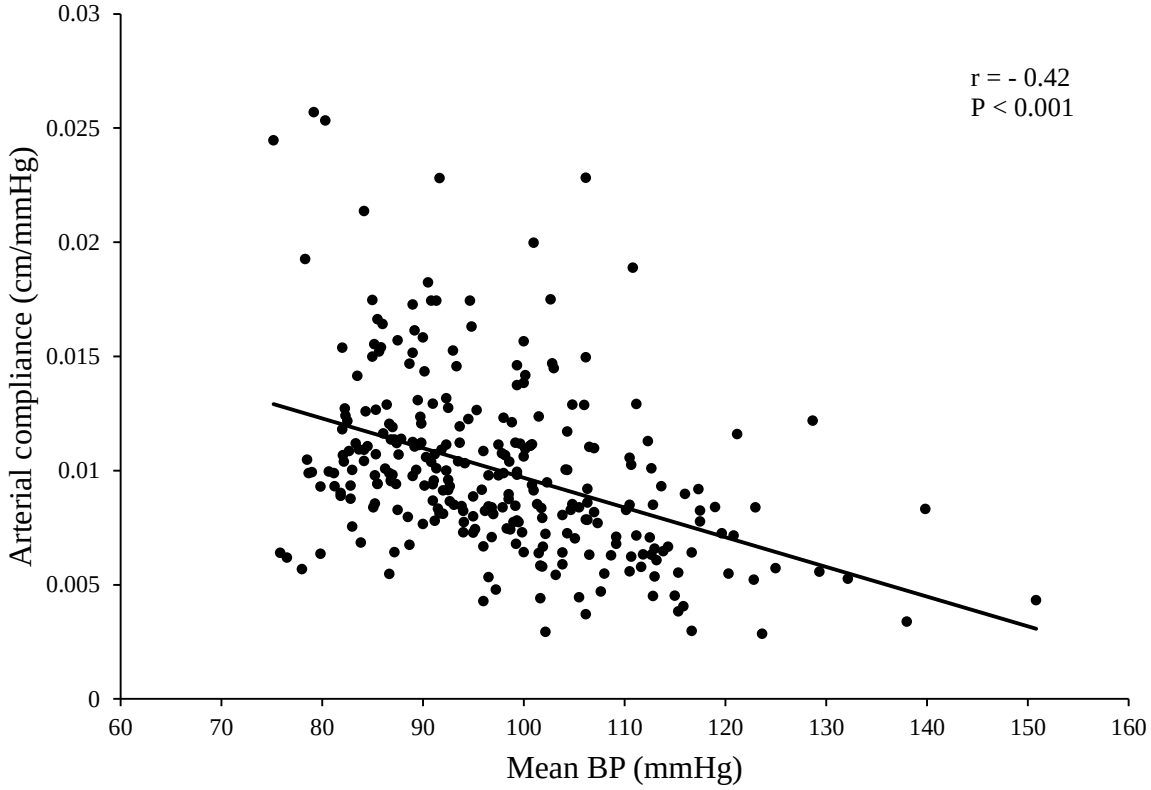


Figure 5. Relationship between elastic modulus and mean blood pressure (BP)

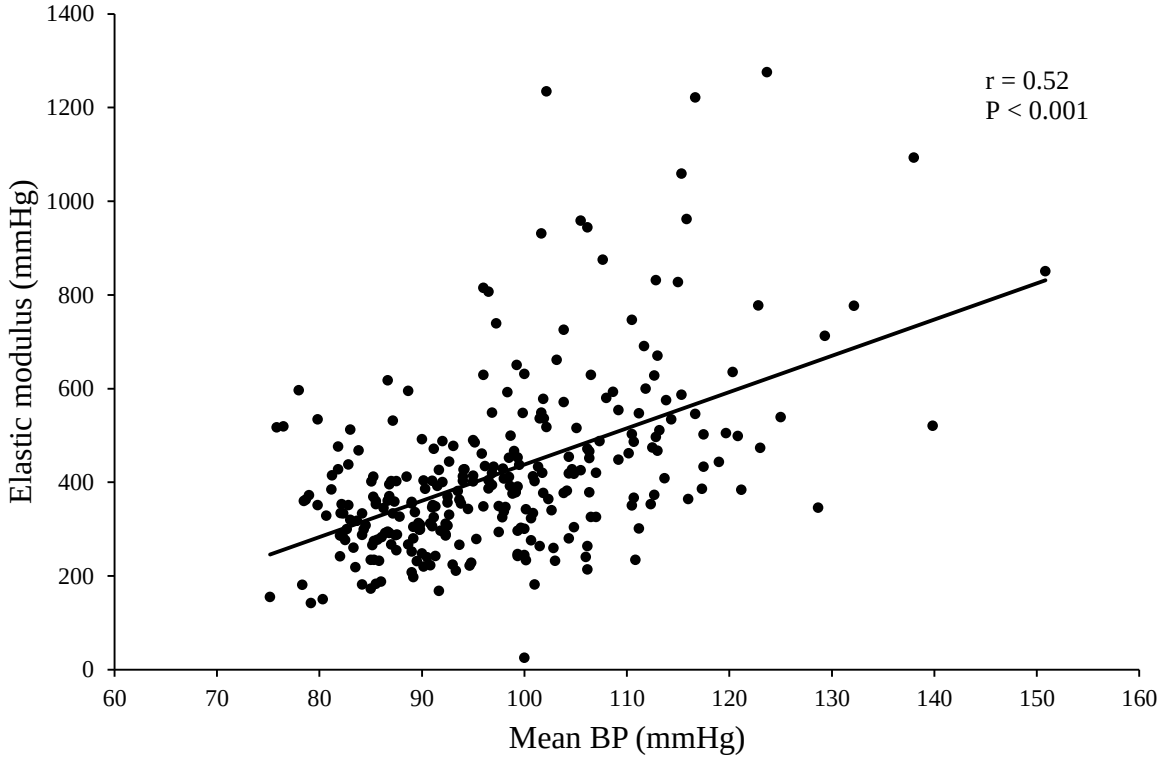


Figure 6. Relationship between arterial distensibility and mean blood pressure (BP)

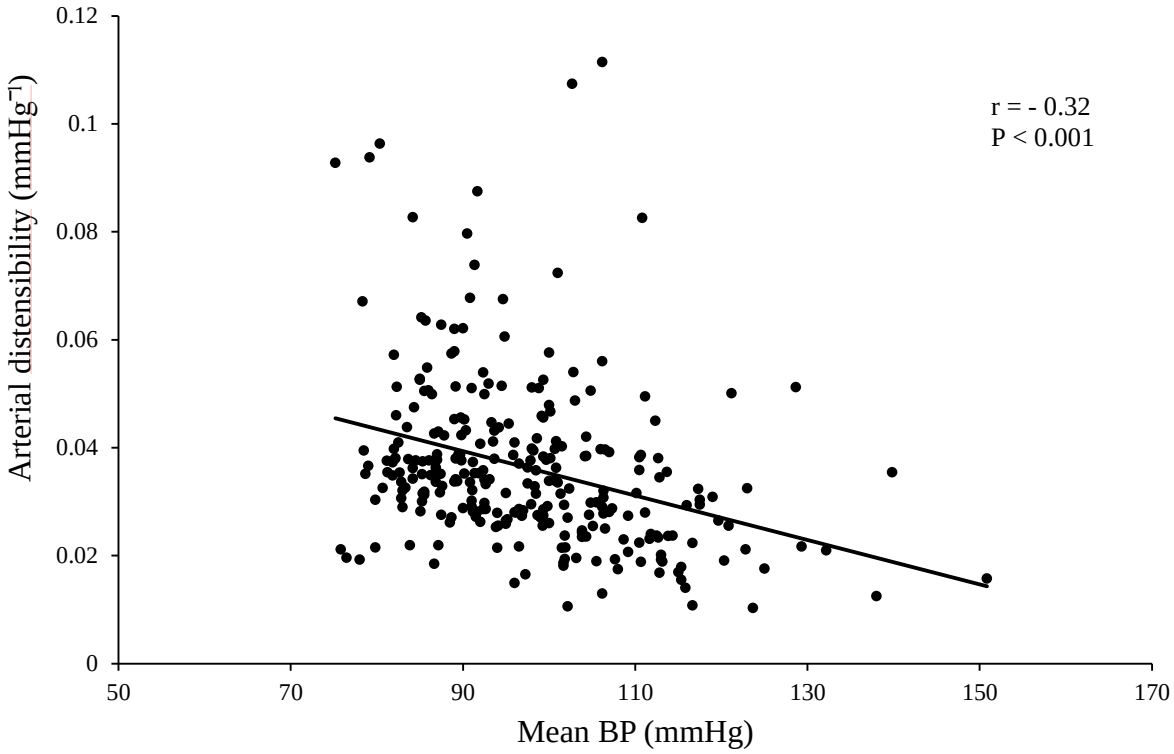


Figure 7. Relationship between β-stiffness index and mean blood pressure (BP)

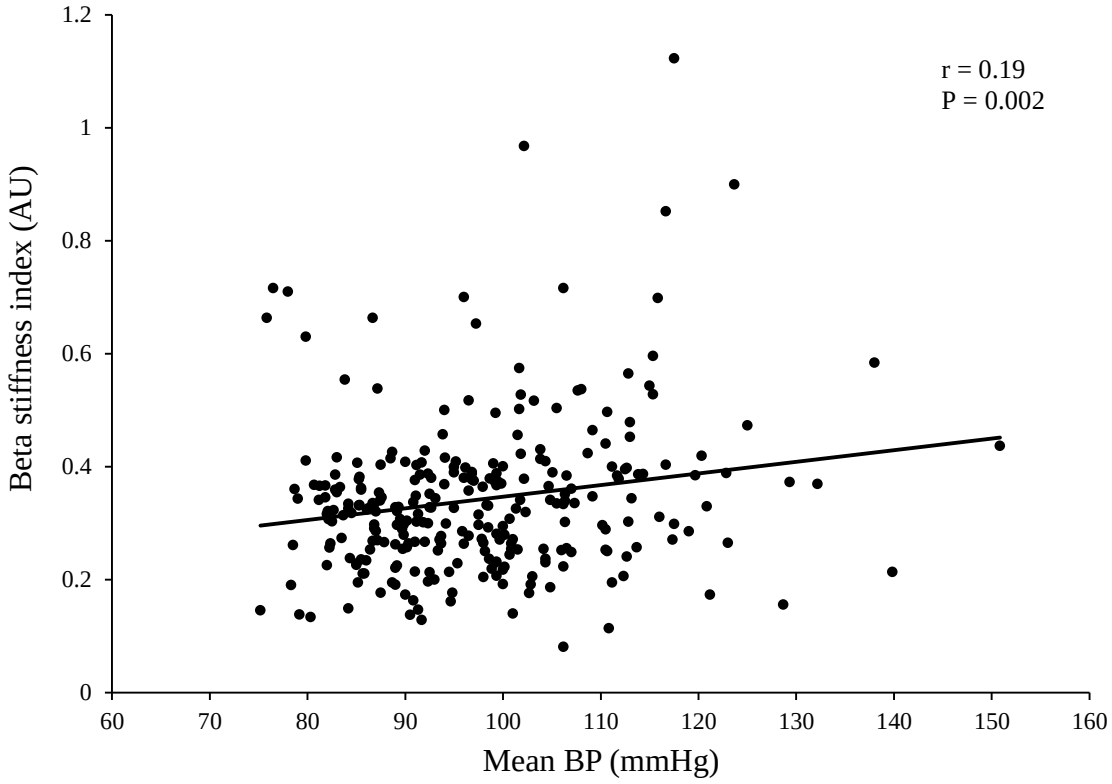
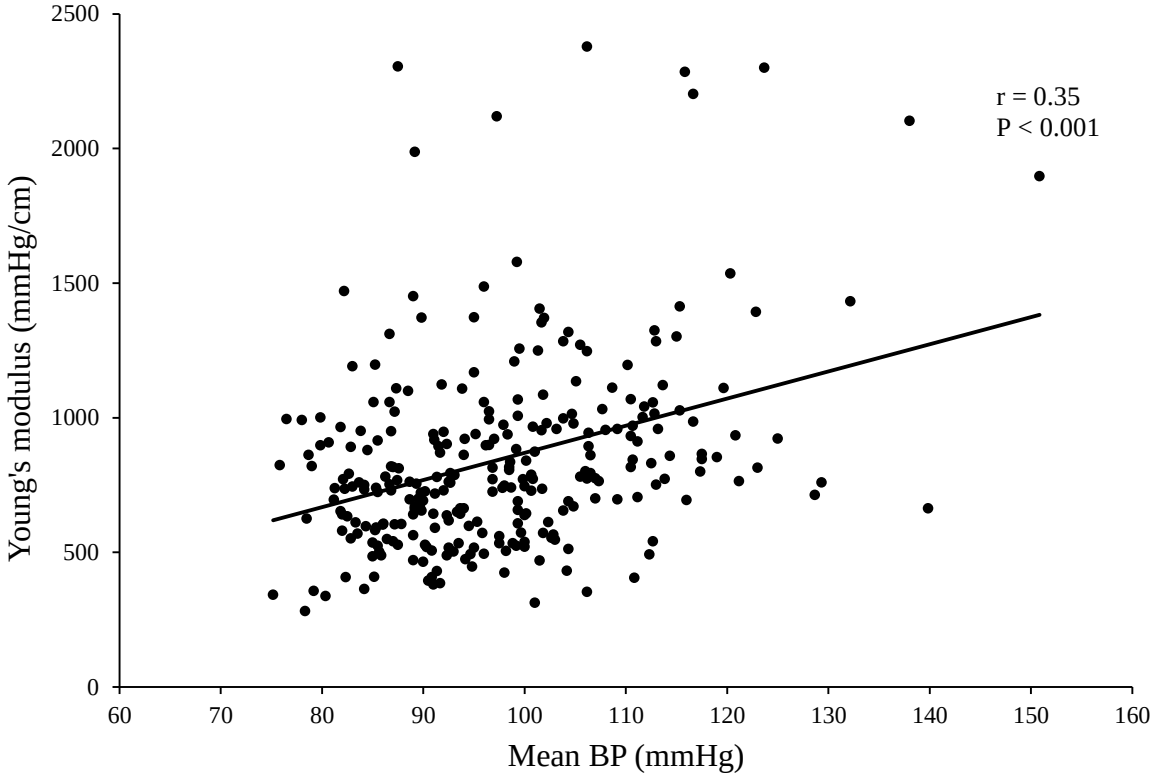
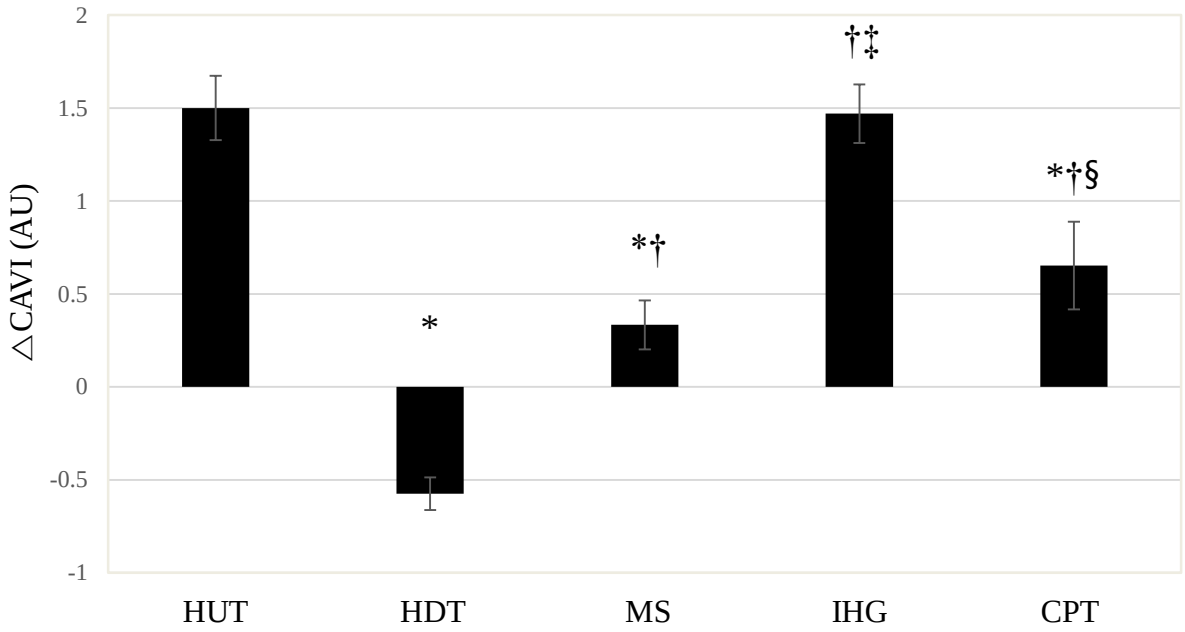


Figure 8. Relationship between young's modulus and mean blood pressure (BP)



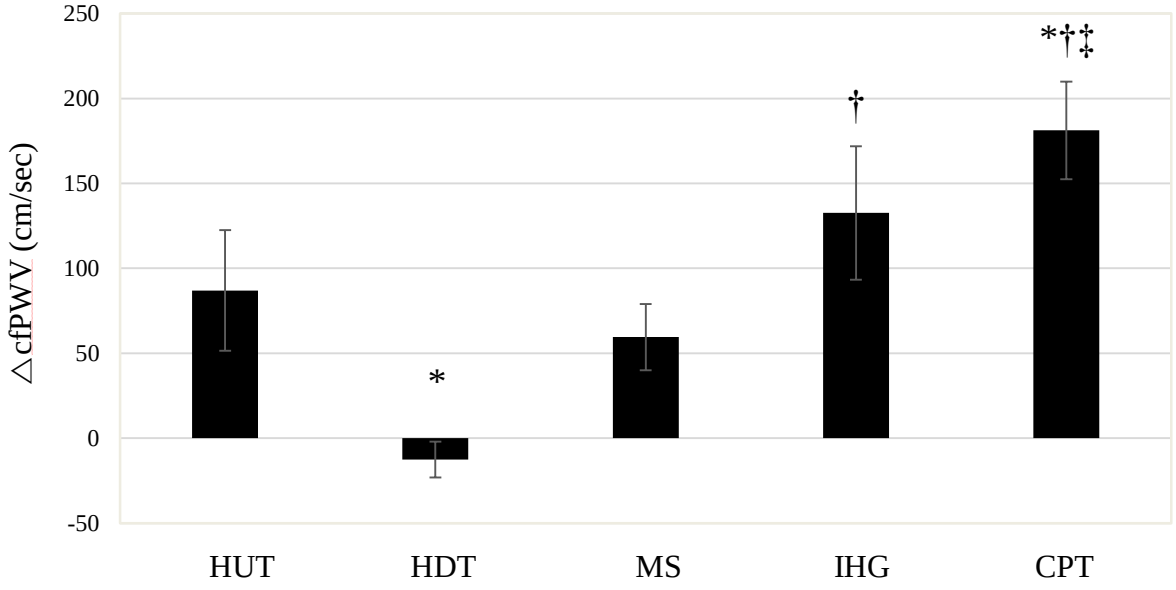
Appendix B: Changes in Arterial Stiffness Indices during Various Vascular Reactivity Tests

Figure 1. Changes in cardio-ankle vascular index (CAVI) during vascular reactivity tests



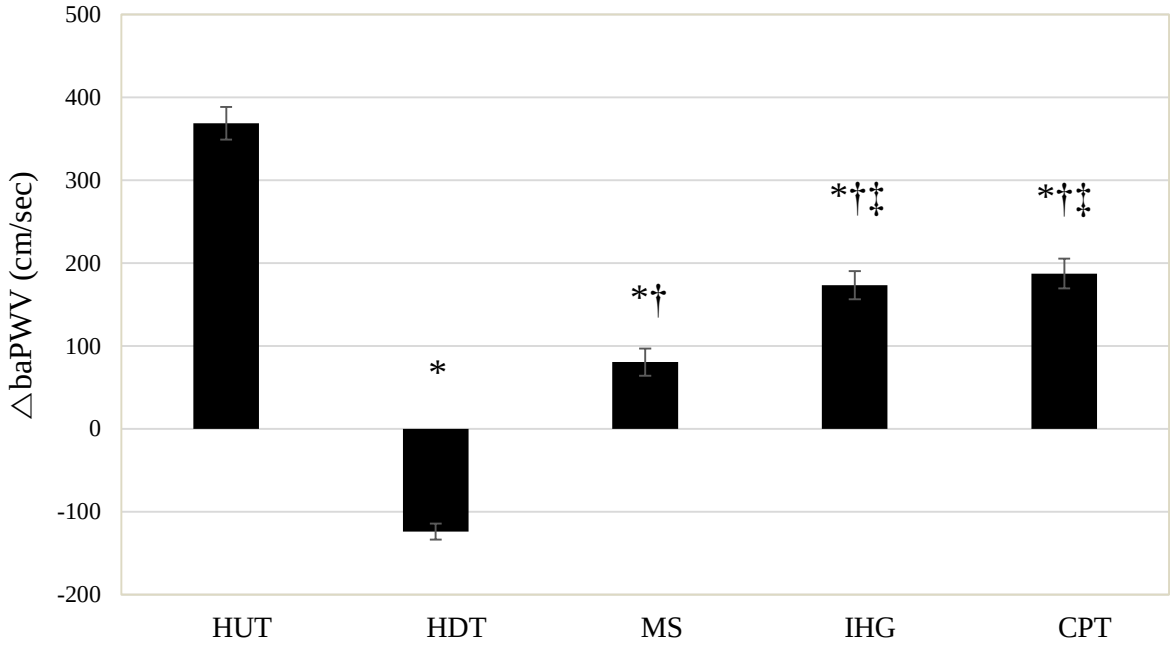
Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test, CAVI=cardio-ankle vascular index. *P<0.05 vs. HUT, †P<0.05 vs. HDT, ‡P<0.05 vs. MS, §P<0.05 vs. IHG.

Figure 2. Changes in carotid-femoral pulse wave velocity (cfPWV) during vascular reactivity tests



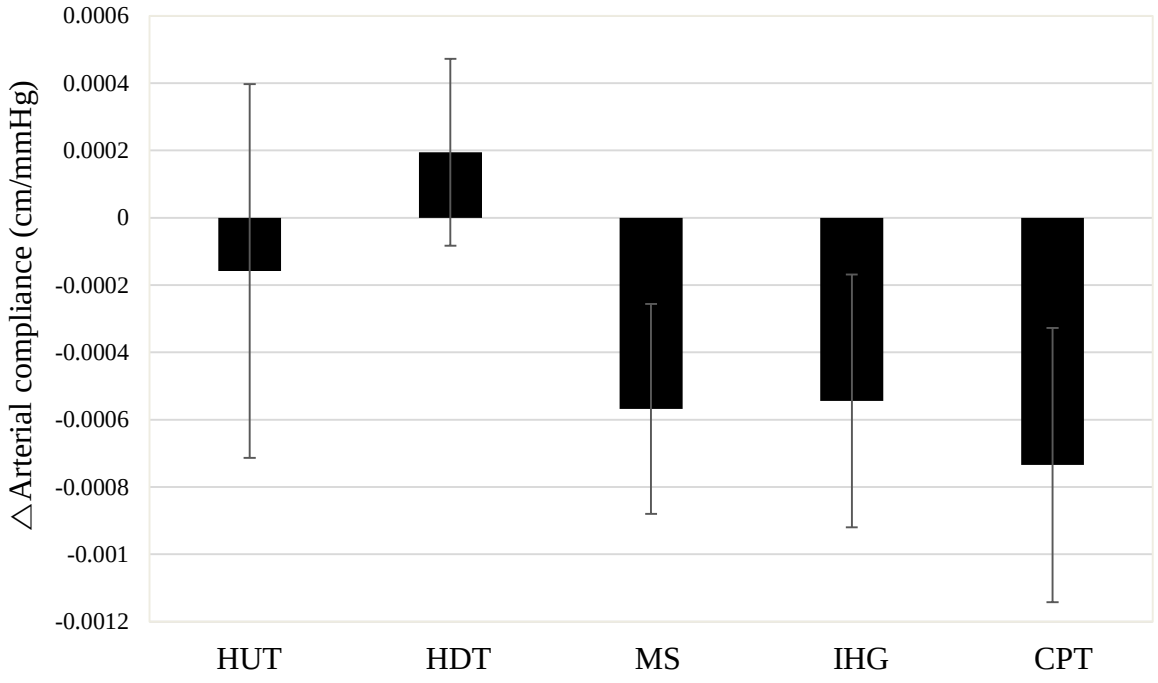
Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test, cfPWV=carotid-femoral pulse wave velocity. *P<0.05 vs. HUT, †P<0.05 vs. HDT, ‡P<0.05 vs. MS.

Figure 3. Changes in brachial-ankle pulse wave velocity (baPWV) during vascular reactivity tests



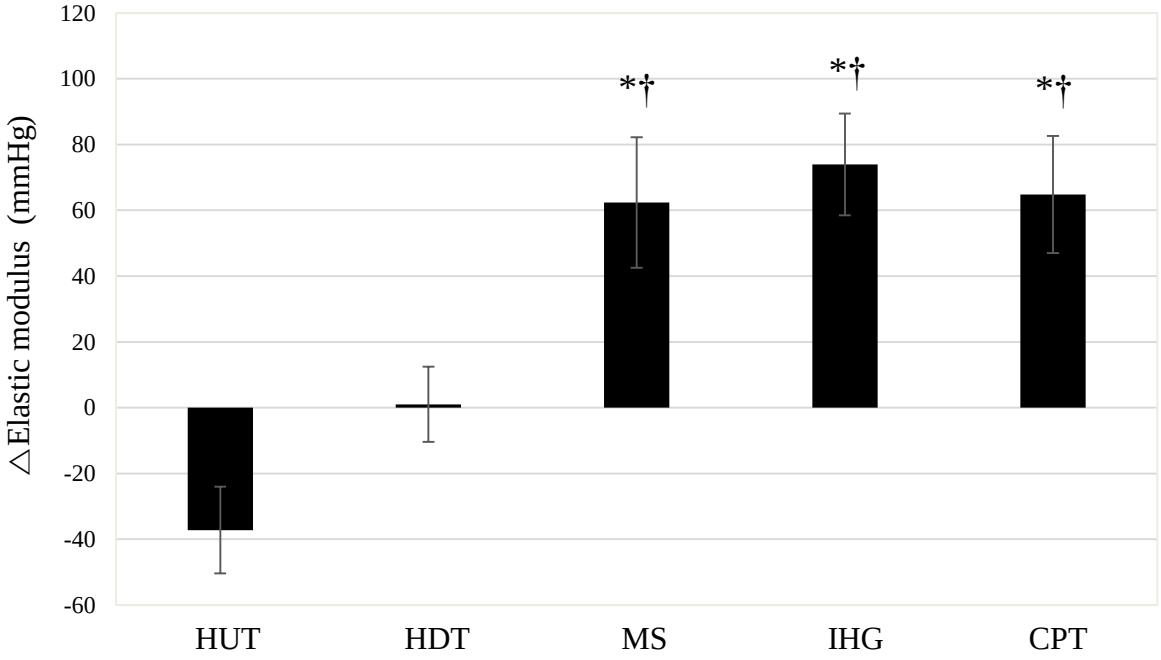
Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test, baPWV=brachial-ankle pulse wave velocity. *P<0.05 vs. HUT, †P<0.05 vs. HDT, ‡P<0.05 vs. MS.

Figure 4. Changes in arterial compliance during vascular reactivity tests



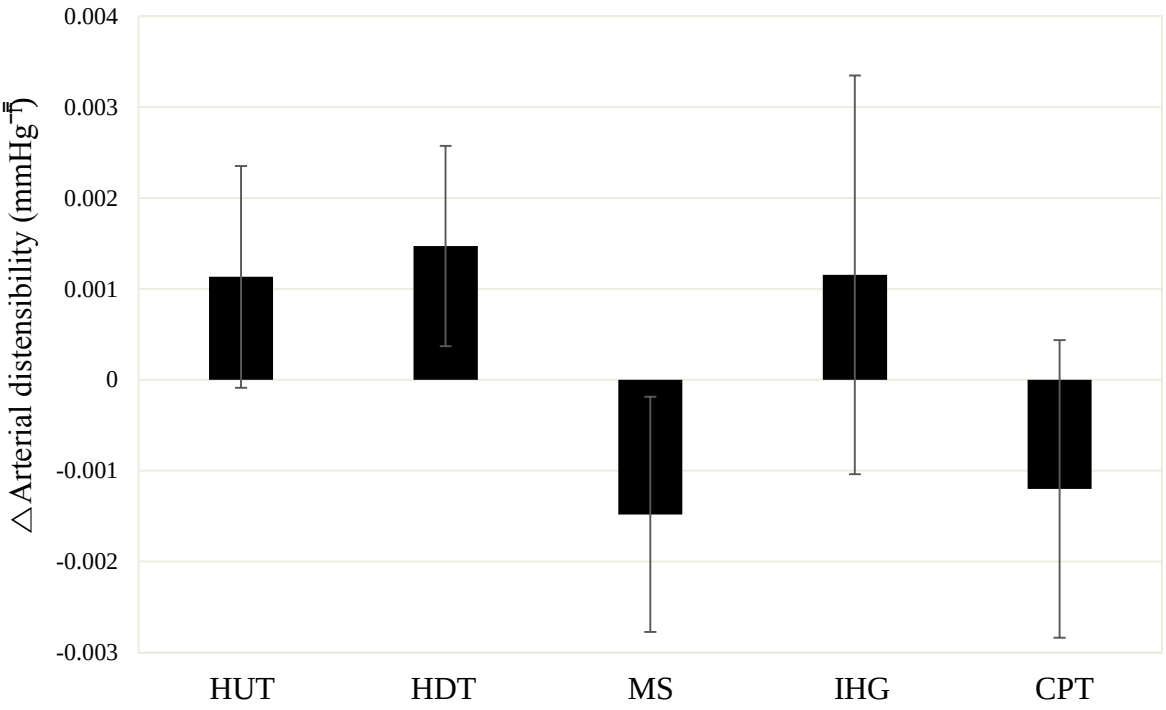
Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test.

Figure 5. Changes in elastic modulus during vascular reactivity tests



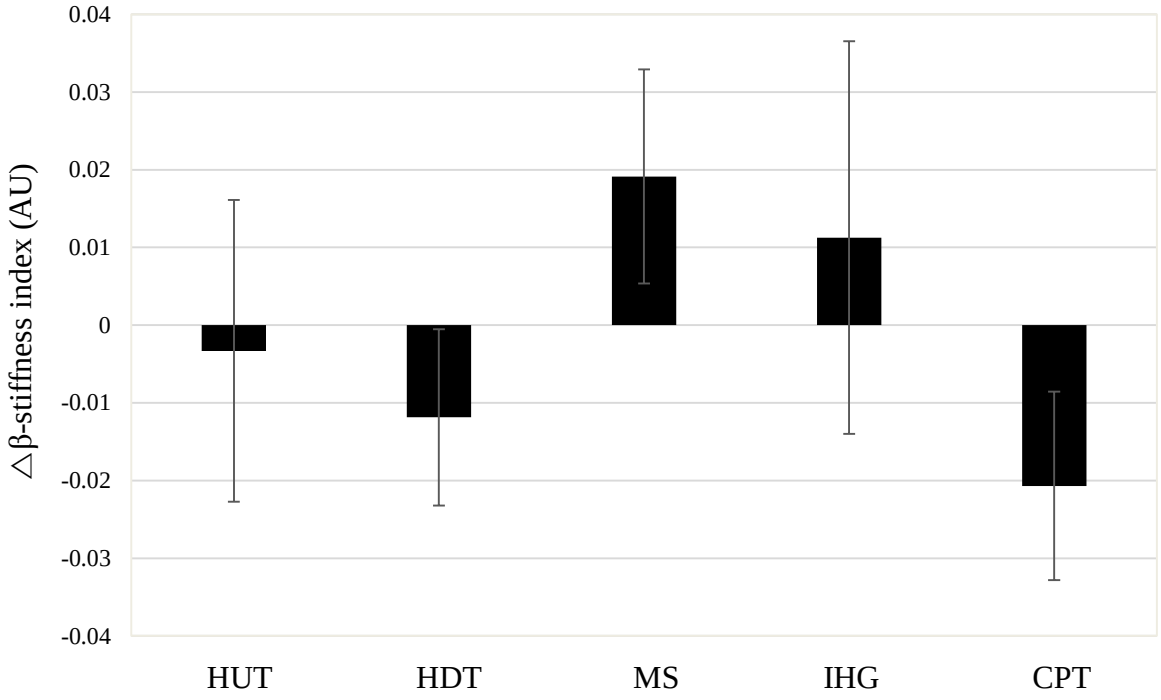
Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test. *P<0.05 vs. HUT, †P<0.05 vs. HDT.

Figure 6. Changes in arterial distensibility during vascular reactivity tests



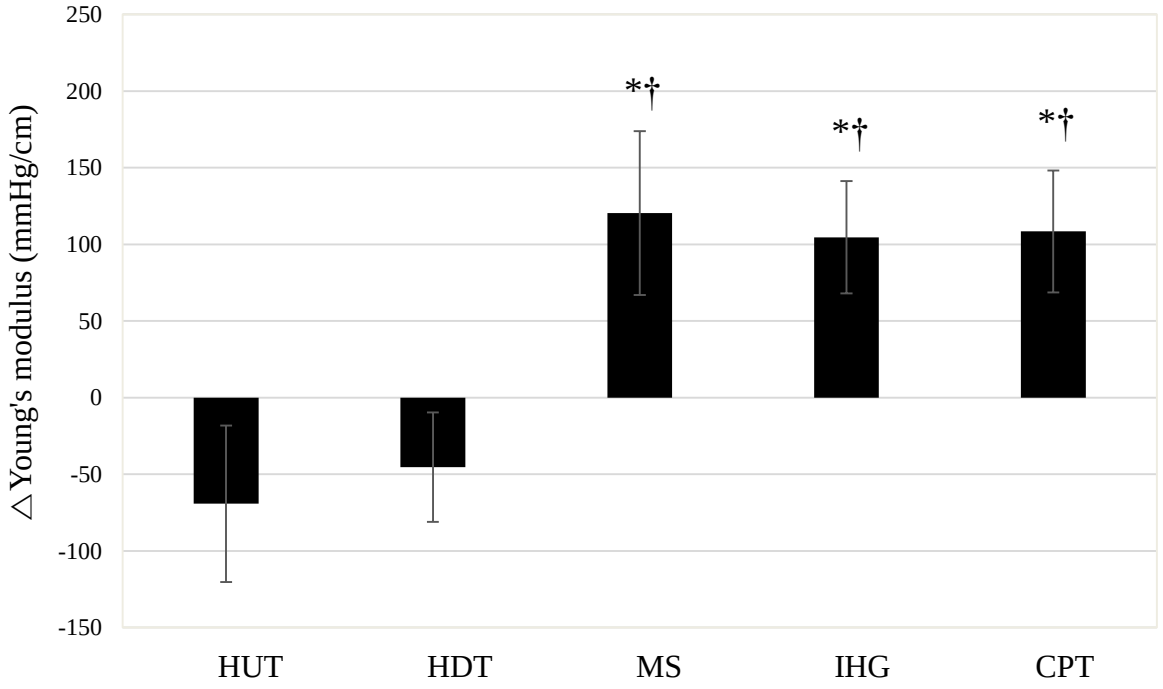
Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test.

Figure 7. Changes in β-stiffness index during vascular reactivity tests



Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test.

Figure 8. Changes in Young's modulus during vascular reactivity tests



Data are △mean ± SEM. HUT=head-up tilt, HDT=head-down tilt, MS=mental stress, IHG=isometric hand grip, CPT=cold pressor test. *P<0.05 vs. HUT, †P<0.05 vs. HDT.

Appendix C: Informed Consent Form

Title: Impact of Blood Pressure Perturbations on Arterial Stiffness

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. The person performing the research will answer any of your questions. Read the information below and ask any questions you might have before deciding whether or not to take part. If you decide to be involved in this study, this form will be used to record your consent.

Purpose of the Study

You have been asked to participate in a research study about a variety of methods to measure how hard or how stiff your blood vessels are. The purpose of this study is to determine if and how much changes in blood pressure affect measures of arterial stiffness.

What will you be asked to do?

If you agree to participate in this study, you will be asked to fill out the Research Health Questionnaire to determine if you are eligible to participate. Exclusion from the study may be due to a) pregnancy, b) a recent illness, recent surgery, or any medical intervention in the 48 hours before any of the study sessions, and c) personal history of diabetes, heart disease, or other cardiovascular problems. This study will take a total of 4 hours (Two 2-hour testing sessions) and will include approximately 50 study participants.

You will be scheduled for 2 testing sessions, about a week apart. For the 4 hours prior to the sessions, you will be asked to fast (not eat or drink anything except water). On each testing session day, blood pressure cuffs will be placed on your arms and legs and sticky patches will be placed on your skin to monitor your heart rates. Additionally, we well place transducers (devices to sense your pulse) on your neck and groin (noninvasively on your skin).

During the tests, we will ask you to perform the following activities.

-Perform handgrip exercise for 1 minute

-Place a hand in ice water for 1 minute

-Do math calculations as fast as possible for 4 minutes

-Lay down on the bed that will be tilted

What are the risks involved in this study?

Every effort has been made by the investigators to keep the risk and discomfort involved in the study to a minimum. You will be screened at the beginning of the study to determine whether you could participate in the study safely. The potential risks associated with this study include a slight risk of fainting, lightheadedness, and mild discomfort with the application of blood pressure cuffs and placement of a hand in ice water.

What are the possible benefits of this study?

You still receive no direct benefit from participating in this study other than possible contribution to science that could result in better screening of heart disease.

Do you have to participate?

No, your participation is voluntary. You may decide not to participate at all or, if you start the study, you may withdraw at any time. Withdrawal or refusing to participate will not affect your relationship with The University of Texas at Austin (University) in anyway.

If you would like to participate, please submit the signed informed consent to the investigator. You will receive a copy of this form.

Will there be any compensation?

You will receive $100.00 for completion of the entire study. Payments will occur at the end of the study period. If you quit after the completion of 1 session, you will be compensated for $40.00. You will be responsible for any taxes assessed on the compensation.

What if you are injured because of the study?

The University has no program or plan to provide treatment for research related injury or payment in the event of a medical problem. In the event of a research related injury, please contact the principal investigator. The University has no program or plan for continuing medical care and/or hospitalization for research-related injuries or for financial compensation. If injuries occur as a result of study activity, eligible University students may be treated at the usual level of care with the usual cost for services at the Student Health Center, but the University has no program or plan to provide payment in the event of a medical problem.

How will our privacy and confidentiality be protected if you participate in this research study?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Your responses will not be linked to your name in any written or verbal report of this research project. Any data that are collected form or about you will be labeled with a code. The data will be kept for a maximum of 5 years.

If it becomes necessary for the Institutional Review Board to review the study records, information that can be linked to you will be protected to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate it with you, or with your participation in any study.

Whom to contact with questions about the study?

Prior, during or after your participation you can contact the researcher Hirofumi Tanaka at 512-232-4801 or send an email to htanaka@austin.utexas.edu for any questions or if your feel that you have been harmed.

This study has been reviewed and approved by The University Institutional Review Board and the study number is 2014-02-0015.

Whom to contact with questions concerning your rights as a research participant?

For questions about your rights or any dissatisfaction with any part of this study, you can contact, anonymously if you wish, the Institutional Review Board by phone at (512) 471-8871 or email at orsc@uts.cc.utexas.edu.

Participation

If you agree to participate, please return the signed informed consent to the investigator.

Signature

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

_____

Printed Name

_____ _____

Signature Date

As a representative of this study, I have explained the purpose, procedures, benefits, and the risks involved in this research study.

_____

Printed Name of Person obtaining consent

_____ _____

Signature of Person obtaining consent Date

Appendix D: Health Research Questionnaire

Personal Information
Today's Date _____ Subject ID _____

Please circle the highest grade in school you have completed:

Elementary school	1	2	3	4	5	6	7	8
High school	9	10	11	12				
College/Post Grad	13	14	15	16	17	18	19	20+

What is your marital status? ☐ Single ☐ Married; ☐ Widowed
☐ Divorced; Separated

Ethnic Background: ☐ Hispanic or Latino ☐ Not Hispanic or Latino

Race:
☐ White ☐ American Indian/Alaskan Native
☐ Pacific Islander ☐ Black or African American ☐ Asian

Symptoms or Signs Suggestive of Disease
Check appropriate box:

Yes	**No**		
☐	☐	1.	Have you experienced unusual pain or discomfort in your check, neck, jaw, arms or other areas that may be due to heart problems?
☐	☐	2.	Have you experienced unusual fatigue or shortness of breath at rest, during usual activities, or during mild-to-moderate exercise (e.g., climbing stairs, carrying groceries, brisk walking, cycling)?
☐	☐	3.	When you stand up, or sometimes during the night while you are sleeping, do you have difficulty breathing?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you suffer from swelling of the ankles (ankle edema)?
☐	☐	6.	Have you experienced an unusual and rapid throbbing or fluttering of the heart?
☐	☐	7.	Have you experienced severe pain in your leg muscles during walking?
☐	☐	8.	Has a doctor told you that you have a heart murmur?

Chronic Disease Risk Factors
Check appropriate box:

Yes	No		
☐	☐	9a.	Are you a male over age 45 years or a female over age 55 years?
☐	☐		b. Are you a female who has experienced premature menopause?
☐	☐		c. If you answered "yes" to 9b, are you on estrogen replacement therapy?
☐	☐	10.	Has your father or brother had a heart attack or died suddenly of heart disease before the age of 55; has your mother or sister experienced these heart problems before the age of 65?
Yes	**No**		
☐	☐	11.	Are you a current cigarette smoker?
☐	☐	12.	Has a doctor told you that you have high blood pressure (more than 140/90 mm Hg) or a heart condition?
☐	☐	13.	Is your total serum cholesterol greater than 200 mg/dl, or has a doctor told you that your cholesterol is at a high risk-level?
☐	☐	14.	Do you have diabetes mellitus?
☐	☐	15.	Are you physically inactive and sedentary (little physical activity on the job or during leisure time)?
☐	☐	16.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐	☐	17.	During the past year, would you say that you have experienced enough stress, strain, and pressure to have a significant effect on your health?
☐	☐	18.	Do you eat foods nearly every day that are high in fat and cholesterol such as fatty meats, cheese, fried foods, butter, whole milk, or eggs?
☐	☐	19.	Do you weigh 30 or more pounds than you should?
☐	☐	20.	Do you know of any other reason you should not do physical activity?

Medical History

21. *Please check which of the following conditions you have had or now have. Also check medical conditions in your family (father, mother, brother(s), or sister(s)). Check as many as apply.*

Self	Family	Medical Condition	Self	Family	Medical Condition
☐	☐	Coronary heart disease, heart attack; by-pass surgery	☐	☐	Major injury/fracture to foot, leg, knee
☐	☐	Arrhythmias	☐	☐	Major injury to back or neck
☐	☐	Angina	☐	☐	Major injury/fracture to hip or shoulder
☐	☐	High blood pressure	☐	☐	Rheumatoid Arthritis
☐	☐	Peripheral vascular disease	☐	☐	Osteoarthritis
☐	☐	Phlebitis or emboli	☐	☐	Gout
☐	☐	Other heart problems	☐	☐	Osteoporosis

☐	☐	Stroke	☐	☐	Fibromyalgia
☐	☐	Asthma	☐	☐	Diabetes mellitus
☐	☐	Bronchitis	☐	☐	Kidney disease
☐	☐	COPD (emphysema)	☐	☐	Cataracts
☐	☐	Lung cancer	☐	☐	Glaucoma
☐	☐	Breast cancer	☐	☐	Hearing loss
☐	☐	Prostate cancer	☐	☐	Depression
☐	☐	Skin cancer	☐	☐	Anxiety, phobias
☐	☐	Colorectal cancer	☐	☐	Eating disorders
☐	☐	Other cancer. Specify:	☐	☐	Sleeping problems
☐	☐	Gallstones/gallbladder disease	☐	☐	Substance abuse problems (alcohol, other drugs, etc.)
☐	☐	Liver disease (cirrhosis)	☐	☐	Chronic Fatigue Syndrome
☐	☐	Hepatitis	☐	☐	Thyroid problems
☐	☐	Anemia (low iron)	☐	☐	Hysterectomy
☐	☐	Stomach/duodenal ulcer	☐	☐	Problems with menstruation
☐	☐	Rectal growth or bleeding	☐	☐	Post-menopausal (date:)
☐	☐	Crohne's disease	☐	☐	Raynaud's disease
☐	☐	Irritable bowel syndrome	☐	☐	Allergies
☐	☐	Marfan's syndrome			

Any other health problems. Please specify and include information on any recent illnesses, hospitalizations, or surgical procedures.

22. *Please check any of the following medications you take regularly and give the name of the medication.*

Medication	**Name of Medication**
☐ Heart medicine	_____
☐ Blood pressure medicine	_____
☐ Blood cholesterol medicine	_____
☐ Hormones	_____
☐ Birth control medicine	_____
☐ Medicine for breathing/lungs	_____
☐ Insulin	_____
☐ Other medicine for diabetes	_____
☐ Arthritis medicine	_____
☐ Medicine for depression	_____

☐ Medicine for anxiety _____
☐ Thyroid medicine _____
☐ Medicine for ulcers _____
☐ Painkiller medicine _____
☐ Allergy medicine _____
☐ Other (please specify) _____
☐ Do you have any drug allergies? _____
☐ Dietary supplements (please specify) _____

Body Weight

23. What is the most you have ever weighed? _____ pounds
24. Are you now trying to:

☐ Lose weight ☐ Gain weight ☐ Stay about the same ☐ Not trying to do anything

Stress

25. During the past month, how would you rate your overall level of stress?

☐ Very high ☐ High ☐ Moderate ☐ Low

26. In the past year, how much effect has stress had on your health?

☐ A lot ☐ Some ☐ Hardly any or none

27. On average, how many hours of sleep do you get in a 24-hour period?

☐ Less than 5 ☐ 5-6.9 ☐ 7-9 ☐ More than 9

Substance Use

28. How would you describe your cigarette smoking habits?

☐ Never smoked

☐ Used to smoke. How many years has it been since you smoked? _____ years

☐ Still smoke. How many cigarettes a day do you smoke on average? ___cigarettes/day

29. How many alcoholic drinks do you consume? (A "drink" is a glass of wine, a wine cooler, a 16oz bottle/12oz can of beer, a shot glass of liquor, or a mixed drink).

☐ Never use alcohol ☐ Less than 1 per week ☐ 1-6 per week
☐ 1 per day ☐ 2-3 per day ☐ More than 3 per day

30. In one sitting, how many drinks do you typically consume?

_____

31. How many cups (8 ounces) of coffee do you drink per day? _____
32. How many ounces of sodas containing caffeine do you drink per day? _____

Physical Fitness, Physical Activity/Exercise

33. Considering a **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your **free time** (write on each line the appropriate number).

a) **STRENUOUS EXERCISE (HEART BEATS RAPIDLY)** **Times Per Week**

(i.e. running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling) _____

b) **MODERATE EXERCISE (NOT EXHAUSTING)**
(i.e. fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

_____

c) **MILD EXERCISE (MINIMAL EFFORT)**
(i.e. yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)

_____

34. Considering a 7-Day period (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)

☐ OFTEN ☐ SOMETIMES ☐ NEVER/RARELY

35. How long have you exercised or played sports regularly?

☐ I do not exercise regularly ☐ Less than 1 year ☐ 1-2 years
☐ 2-5 years ☐ 5-10 years ☐ More than 10 years

Occupational Health

36. Please describe your main job title and duties.

_____

37. How much hard physical work is required on your job?

☐ A great deal ☐ A moderate amount ☐ A little ☐ None

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