EFFECTS OF AGE ON THE ARTERIAL SYSTEM AND THE HEART

by

Julia Wong

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at

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"The attainment of old age is easy and only the inquiry about it difficult and so much the rather, because it is corrupted with false opinions and vaine reports"

Francis Bacon

DALHOUSIE UNIVERSITY

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Effects of age on the arterial system and the heart"

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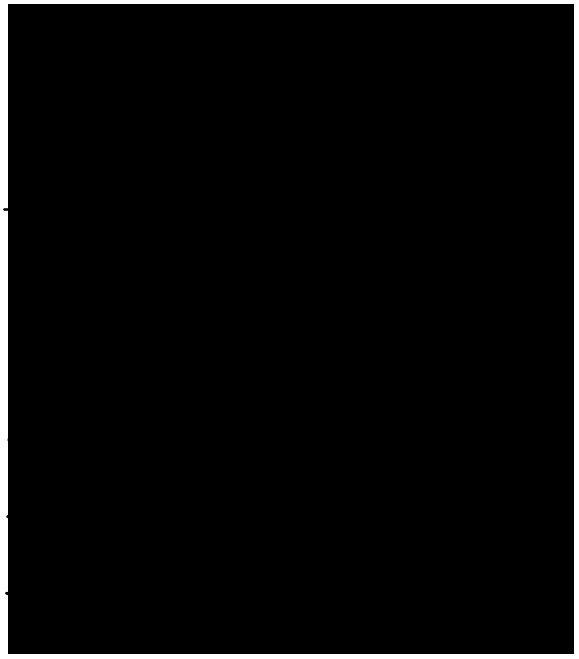
Research Supervisor

Examining Committee



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Abstract

The main purpose of the present investigation was to examine the effects of age on blood pressure, arterial vasculature, and the heart. The pulse transit time and wave reflections were used as proxies for arterial stiffness, and the electrocardiographic estimate of the left ventricular mass index as the end point of age effects on the heart. Data were drawn from one of the five Yugoslavian male cohorts (Velika Krsna, n=511), and one of the two Japanese male cohorts (Tanushimaru, n=508) of the Seven Countries Study. The current study employed an epidemiological rather than clinical approach to investigate the age effects because the epidemiological approach could capture the widest possible spectrum of the aging phenomena and offer a powerful tool to discover associations among the biological and environmental variables related to cardiovascular aging. The clinical approach examines the relationship between age and variables of interest as they exist in a defined population at one particular time.

Several linear regression models were introduced to assess the age effects on blood pressure, pulse transit time, pressure wave contour, and the left ventricular mass index. A small upward trend due to cohort-period effect, and a modest age increase were observed in blood pressure of the Yugoslavian cohort (systolic: 0.8 mmHg/year, diastolic: 0.2 mmHg/year, pulse pressure: 0.7 mmHg/year). The Japanese cohort showed a downward trend owing to cohort-period effect, but a steep upward age trend in blood pressure (systolic: 1.7 mmHg/year, diastolic: 0.6 mmHg/year, pulse pressure: 1.0 mmHg/year). Population stroke mortality is associated with blood pressure trends ascribable to cohort-period effect, and cohort stroke mortality is associated with blood pressure age trends, thus supporting the method of separating these two blood pressure trends.

The pulse transit time was inversely correlated with age in the Yugoslavian ($r=-0.38$, $p<0.001$), but not Japanese cohorts. Blood pressures and heart rate were statistically significant determinants of pulse transit time in both cohorts. An analysis of the pooled sample indicated an inverse relationship between timing of wave reflections and age ($r=-0.23$, $p<0.01$). These findings suggest that increased arterial stiffness can be triggered by degenerative age changes in the arterial walls, or by augmented distending blood pressure.

Age has a differential effect on the cardiac mass in these two cohorts; age and pulse pressure correlated significantly with the left ventricular mass index in the Japanese cohort, whereas body mass index and the systolic pressure load, but not age, were statistically significant determinants of the cardiac mass in the Yugoslavian cohort. These analyses suggest that increased cardiac mass is not a universal aging phenomenon. Other factors besides age participate in the development and/or maintenance of cardiac hypertrophy. In conclusion, findings of the present investigation indicate that the vascular and cardiac responses to aging do not appear to be uniform in these two ethnically different populations.

List of Abbreviations, Symbols, and Glossaries

aVR	- augmented unipolar limb leads devised by Goldberg
b/min	- beats per minute
BMI	- body mass index
ΔBMI	- changes of the body mass index
BP	- blood pressure
bpm	- beats per minute
C	- compliance of the arteries
c	- pulse wave velocity
Ca^{2+}	- calcium ion
cm	- centimeter, unit of length
CV	- curvature, also means coefficient of variation; the meaning is obvious from the context
Characteristic impedance	- relates pressure to flow at the entrance of an infinitely long uniform vessel, uninfluenced by wave reflection
D	- differential
Di	- distance measured, used in Cook's D influence statistic
d	- distance between two surface recording sites
dP/dV	- change in pressure per unit change in volume
DBP	- diastolic blood pressure
DPL	- diastoloc pressure load
E	- elastic modulus, expressing the magnitude of the stress required to produce a given strain
Ep	- the pressure-strain elastic modulus, an index of arterial stiffness
ECG	- electrocardiography
ET1	- endothelin 1
F-ratio	- the statistic obtained in several statistical tests in which variation attributable to different sources is compared
g	- gram, unit of weight
m^2	- squared meter
g/m^2	- gram per squared meter
h	- wall thickness
HR	- heart rate
HRmax	- maximum heart rate

Hz	- hertz, unit of frequency
impedance modulus	- expresses the relationship between the pulsatile pressure and flow in an artery
ips	- inches per second
IP_3	- inositol-1, 4, 5-triphosphate
IT1	- a variable in the algorithm for marking the rapid systolic upstroke in the carotid and femoral pulse
IT1M	- a variable used in the algorithm for marking the point where the pulse signals attains a maximum
kg/m^2	- kilogram per squared meter
LNDIST	- log-transformed acromial sitting height
LNPTT	- log-transformed pulse transit time
LOGBMI	- log-transformed body mass index
LOGDPL	- log-transformed distolic pressure load
LOGPP	- log-transformed pulse pressure
LOGSPL	- log-transformed systolic pressure load
LOGTPL	- log-transformed total pressure load
$LVMI_{ECG}$	- electrocardiographic estimate of the left ventricular mass index
LV	- left ventricle
mg/dl	- milligram per deciliter
min	- minute
ml/min	- millilter per minute
m/s	- meter per second
min/m^2	- minute per squared meter
mm	- millimeter
mm Hg	- millimeter of mercury, unit of pressure
M/V	- ratio between left ventricular mass and left ventricular volume
modulus	- expresses the ratio of the amplitudes of corresponding pressure and flow harmonics at any frequency
N, n	- sample size
NS	- statistically nonsignificant
P	- pressure, transmural pressure
p	- probability that the null hypothesis is true
Pb	- backward pressure wave

Pf	- forward pressure wave
Pi	- inflection point, representing the foot of the reflected wave occurred in systole before the closure of the aortic valve
Ppk	- peak systolic pressure
PP	- pulse pressure, calculated as the difference between the systolic and diastolic blood pressure
ΔP/PP	- the ratio of the height of systolic peak above the inflection point to the pulse pressure; a quantitative index of the contribution of wave reflections to the pressure wave contour
Ps	- systolic shoulder
phase	- a component of impedance, it is the delay between the pressure and flow
PTT	- pulse transit time
PWV	- pulse wave velocity
Q,R,S	- Q, R, and S, waves of the ECG
QONCAR	- time interval from onset of the Q-wave to rapid systolic upstroke in the carotid pressure wave
QONFEM	- time interval from onset of Q-wave to rapid systolic upstroke in the femoral pressure wave
QRS complex	- deflection in the ECG that represents depolarization of the ventricle
r	- radius of the vessel
Δr	- change of the radius of the vessel
R^2	- squared multiple correlation coefficient
RR	- length of time between consecutive QRS waves in the electrocardiogram
RWINT	- timing of the wave reflections
SAS	- a statistical analysis computer software
SC	- serum cholesterol concentrations
SD	- standard deviation
SEM	- the standard deviation of a theoretical sample means
SMC	- number of cigarettes smoked per day
SPINT	- interval from onset of rapid systolic upstroke to the peak systolic pressure
SPL	- systolic pressure load

TPL	- total pressure load
mM	- micromole, unit of measurement of the amount of a substance in body fluid
V	- volume
V1-V6	- precordial leads, unipolar leads devised by Wilson
vascular impedance	- the relationship of pressure to flow at frequencies which are multiples of the heart rate
yr	- year
Zc	- characteristic impedance
α	- alpha-adrenergic receptor
β	- beta-adrenergic receptor
Δ	- (delta), denotes change in a variable
χ^2	- (chi squared), a statistical distribution
%	- percent
/	- denotes division
<	- less than
$\leq$	- equal to or less than
>	- greater than
$\geq$	- equal to or greater than
Δf	- the difference between the phases of upstream and downstream harmonics
$\overline{X}$	- mean, a descriptive statistic that is a measure of central tendency
Δx	- distance between two points
σ^2	- variance, a measure of variability
f	- frequency of a pressure wave
ρ	- density of blood
m	- milli

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Chapter 1

Introduction and Literature Review

1.1 Introduction

1.1.1 Basic aim of the study

The present study sets out to examine the phenomenon of biological aging as it relates to the systemic arterial tree and the heart. Biological aging, as a characteristic of any individual, necessitates the passage of time, and produces profound, progressive changes in structure and bodily function. These changes result from the interactions of life-style, nutrition, state of physical conditioning, and disease, all of which are superimposed on the "aging process". The use of an epidemiological approach to investigate these progressive aging changes offers the broadest picture of phenomena and interactions.

In the past, biological aging has often been studied in experiments on animals, with the implication that an observation in one species could be generalized to all forms of life. In view of the fact that various species have very different cell populations, chemical composition, organ system structure and mechanisms of death, it has recently become clear that it is quite risky to assume that animals with distinct phylogenetic relationships age by similar mechanisms. Because of the complexity, diversity, and particular chronology of age-related changes in human beings, no other species can provide a faithful replica of all aspects of human aging. Serious questions arise about the relevance of data from experiments in animals to the actual interplay of biological aging and exposures to environmental factors. The quantitative equivalents of the variables that promote the structural and functional changes in humans cannot be specified for other species. These age-related alterations in humans concern quantitative influences that operate over many years and must be evaluated in humans themselves.

The utilization of the clinical approach to the study of biological aging also has serious limitations. The clinical approach is primarily concerned with influences pertaining

to aging changes that can be drawn from the examination of one or more selected groups of individuals. This approach has been referred to as epidemiology applied to inadequate numbers [1]. Furthermore, it examines the relationship between age and other characteristics or variables of interest as they exist in a defined population (usually diseased) at one particular time.

Difficulties associated with the experimental and clinical approach to the study of biological aging can be minimized with the application of epidemiological approach. It starts with the study of persons at a certain age, who are followed for a number of years to allow for the determination of changes in the characteristics of interest. This approach involves unselected general populations; studying large groups provides power to minimize sampling errors, even in the face of relatively poor-quality data.

1.1.2 Objectives of the study

There are four major objectives of this study, namely:

1. To describe the true blood pressure trend with aging in two ethnically different populations comprising only male subjects.

2. To assess the effects of age on the vasculature of the systemic arterial tree, after considering blood pressure changes with age in these male populations.

3. To evaluate the effects of age on the heart, after considering the blood pressure age trend in these male populations.

4. To examine the simultaneous interactions between blood pressure aging trend and the proxies for age effects on the arterial vasculature and the heart.

These objectives will be developed with reference to the literature on blood pressure-age relationships, age effects on the arterial vasculature and the heart, and from my data of blood pressure, pulse waves, and the left ventricular mass index. The materials for the present investigation are drawn from the Seven Countries Study of coronary heart disease.

Although its design and results have been reported in major monographs of the study [1,2,3], for the purpose of orientation to the present study, a review of the methods and major findings of the Seven Countries Study is useful, and will be given in Section 1.2. A literature review of blood pressure-age relationships, and the age-related structural and functional changes in the major arteries and the heart will be presented in Section 1.3. To meet the first objective, I analyzed the 10-year follow-up blood pressure data to determine that part of changes in blood pressure that is due to biological aging per se. This analysis will be carried out in Chapter 3. This analysis is important to the present study because the true blood pressure aging trend can be determined only by analyzing the 10-year follow-up blood pressure. The cross-sectional data of pulse transit time, wave reflections and left ventricular mass index allow me to depict the status of these variables at a fixed point in time, but do not permit me to investigate their changes with age. The second objective is attained by a detailed analysis of the effects of age on the elastic properties of the large arteries, after considering the true blood pressure trend with aging. The pulse transit time and the wave reflections are used as proxies for these effects. This analysis is performed in Chapter 5. In meeting the third objective, a thorough assessment of the effects of age on the heart is required. In this assessment, the electrocardiographic estimate of the left ventricular mass index (LVMI) is used as the end point of age effects on the heart. This assessment is carried out in Chapter 6. To meet the fourth objective, I examine how the blood pressure aging trend interacts simultaneously with the pulse transit time, wave reflections, and the left ventricular mass index. This examination is carried out in Chapter 7.

To achieve the study objectives, I analyzed data collected in connection with the Seven Countries Study which is a 25-year prospective study of the natural history, epidemiology and etiology of coronary heart disease. Although data of the 5-year follow-up blood pressure, resting heart rate, and other risk factors were missing for the Japanese cohort, the Seven-Country data set contained the necessary information for the present

study. In spite of its limitations, I used this data set rather than collect my own data for the following reasons.

First, this data set contains 10-year follow-up blood pressure measurements which were recorded at a time when antihypertensive treatment was not prevalent. Thus the aging (or pathological) change in the arterial blood pressure could be observed without interference by drug treatment of elevated blood pressure.

Second, the follow-up data allow me to investigate the change of blood pressure in an aging cohort, and relate it to other facets of aging in the cardiovascular system.

Third, the Seven-Country data are chosen instead of data from other prospective studies such as the Framingham study, for reasons of sample representativeness and heterogeneity. In the Seven Countries Study, for all 16 cohorts combined, 90.4% of men invited appeared for examination. In view of this high response rate, it seems unreasonable to suggest that the men found to be free of coronary heart disease and cardiovascular disease might not be representative of all men similarly free of these disease in the eligible sample. In contrast, though a random roster of a small town in Framingham was used for eligibility to enter the study, about a third of the men eligible did not come into the study; they selected themselves out of it [4]. This raises questions on sample selection bias and representativeness. In addition, the American population samples in the Framingham Study are characterized by a relatively high degree of homogeneity in certain respects of mode of life, so that they throw little light on the effect of differences in mode of life, particularly in relationship to blood pressure. The Seven Countries Study, on the other hand, consists of groups of men in two ethnically diverse cultures. An examination of the Japanese and Yugoslavian populations permits me to study the effects of diverse life-style and economic conditions on the cardiovascular system. The literature has reported considerable international variations for mortality from cardiovascular disease. Studies of mortality trends among the Seven Countries study cohorts [5] have indicated that age, serum cholesterol, systolic blood pressure and cigarette smoking habits were very important risk factors for

coronary deaths for the U.S., northern and southern European cohorts, but not for the Japanese cohorts of the Seven Countries Study. For all-cause deaths, age, blood pressure and cigarette smoking were highly significant risk factors in America, northern and southern Europe, except Japan [5]. Differences in cardiovascular mortality trends between cultures are most likely related to changes in risk factors over time. Kromhout et al. [6] observed large changes in average serum cholesterol, blood pressure and the prevalence of smoking in the Serbian cohorts during a 25-year period. Systolic blood pressure and serum cholesterol levels increased, but no major secular trend in smoking was observed in these cohorts. The increase in these major risk factors during the 25-year period were found to be consistent with changes in cardiovascular disease mortality observed in Yugoslavia during the period 1970-1984. Similar changes in these major factors have not been found in the Japanese cohorts. In investigating the 25-year mortality experience in the two Japanese cohorts of the Seven Countries Study, Toshima et al. [7] observed no significant 10-year decline in mean levels in smoking habit, body mass index, heart rate, and serum cholesterol, but a large and significant rise in blood pressure. Age and blood pressure, but not serum cholesterol or smoking were significant predictors of coronary heart disease and stroke mortality in these cohorts. Findings of these studies clearly show that the cardiovascular experience of the Yugoslavian and Japanese populations is different. In view of these differences in mortality trends and risk factor changes, it would be of interest to examine how age affects the arterial vasculature and the heart in these two populations.

Fourth, the pulse wave tracings and the electrocardiographic (ECG) recordings using the Frank-lead system, were acquired simultaneously with blood pressure measurements during the 5-year follow-up in the Velika Krsna cohort of Yugoslavia and the 10-year follow-up in the Tanushimaru cohort of Japan. These electrocardiographic recordings were recorded in a form that permitted computer processing of the signals.

Fifth, the Seven-Country data set also contains information on mortality data collected during 25 years of follow-up. Availability of these data provides me with the

opportunity to examine the outcomes of the 10-year blood pressure changes in these two cohorts.

1.2 Background

1.2.1 Aim of the Seven Countries Study of coronary heart disease

The Seven Countries Study was initiated in 1957, as an outgrowth of Key's earlier explorations on the epidemiology of coronary heart disease in the early 50s in Italy and Spain [8,9]. The purpose of the Seven Countries Study was to relate differences in incidence among cohorts to the general characteristics of men in the cohorts, including their lifestyle. Specifically, it was designed to answer three major questions. 1. Does the incidence of coronary heart disease and of death in the various cohorts differ from that in the United States? 2. Does the incidence vary among cohorts? 3. To what extent can differences in incidence among the cohorts be explained by a multivariate analysis of characteristics of those cohorts as indicated by the variables recorded for the subjects?

1.2.2 Recruitment of the cohorts

The Seven Countries Study on cardiovascular diseases was initiated in 1957 and enrolled 16 cohorts of men aged 40-59, in United States of America (one cohort), Finland (two cohorts), the Netherlands (one cohort), Italy (three cohorts), Yugoslavia (five cohorts), Greece (two cohorts), and Japan (two cohorts), for a total of 12,763 men. East and West Finland, Crevalcore and Montegiorgio in Italy, Dalmatia, Slavonia, Velika Krsna and Zrenjanin in Yugoslavia, Corfu and Crete in Greece and Tanushimaru and Ushibuka in Japan represented mainly rural communities. One cohort in the United States of America (US Railroad) and one in Italy (Rome Railroad) were samples of railroad employees in specified work activities, and locations. Zutphen in the Netherlands was a 4/9 statistical sample of men in the small town of Zutphen, and Belgrade was a sample of faculty members at the University in the same city.

The exclusion of women in the Seven Countries Study negates the male-female differences in cardiovascular disease. Literature has reported gender differences for level of blood pressure [10] and for the pattern of development of hypertension [11]. Joffres et al.'s investigation [12] showed that systolic blood pressures of men gradually increase with age, whereas in women, the younger age groups have lower systolic blood pressures than men, but increase sharply in the 40-50 age group, to reach a slightly higher level than men in the 65-74 age group. In most industrialized countries, the largest sex differential for mortality is due to coronary heart disease. The sex ratio is remarkably consistent, ranging from 2.5 to 4.5 in countries with very different diets, habits, and heart disease rates [13]. These observations suggest an intrinsic gender-mediated difference that could be explained by differences in sex hormones. Estrogen has been implicated as the mediator of these gender differences in cardiovascular mortality rates.

1.2.3 Methods and procedures

The procedure and methods used in the entry examinations of all cohorts have been described in detail [1]. The measurements made during the initial examinations included anthropometry, blood pressure, serum cholesterol, 12-lead electrocardiography (ECG), and respiratory function for all cohorts. In addition, life-style data such as occupation, physical activity, and smoking were also obtained.

1.2.3.1 Anthropometry

Anthropometric and coronary heart disease history data collection consisted of a clinic interview and a physical examination. During the interview, the nurse or medical technician completed the London School of Hygiene Cardiovascular Questionnaire [14] on each subject. Anthropometric measurements were obtained. These included standing and sitting height, and weight. Standing height was measured as the distance from the sole of the foot to the top of the head, without shoes and to the nearest millimeter (mm). The

subjects stood erect, with both the heels and the scapulae in contact with the wall to which a steel tape, graduated in mm was affixed. The sitting height was measured to the nearest mm with the subjects seated in erect position, on a firm stool with horizontal surface. The subject's back was in contact with the wall at the regions of both scapulae and buttocks. It was determined as the distance from the seat surface to the top of the head. Body weight was measured with men wearing light undergarments. The measurements were made to the nearest 0.5 kg.

Subcutaneous skinfold thickness measured with calipers exerted a constant pressure of 10 gm/mm^2 and a jaw face of 20 mm^2, were recorded at two sites, on the upper arm and below the scapula. Two measurements were made and their mean was used for analysis. The triceps skinfold was measured on the back of the unclothed pendant right arm at a level midway between the tip of the acromion and the tip of the olecronen. The skinfold was lifted parallel to the long axis of the arm and measurement made to the nearest 0.5 mm. The subscapular skinfold was measured to the nearest 0.5 mm on the unclothed chest below the tip of right scapula with the subject standing in a relaxed position.

The biacromial diameter was measured with obstetric caliper at the maximum diameter between the acromial processes, with the subject standing erect against a wall, and shoulders relaxed. The measurement was taken in cm. The bicristal diameter was measured at the maximal diameter between the external margins of the iliac crests, while the arm circumference was measured with a snugly applied flexible tape, to the nearest mm on the unclothed right arm, relaxed and pendant, at a midpoint between the tip of the acromion and the tip of the olecronen.

1.2.3.2 Blood pressure measurements

In the Seven Countries Study, indirect blood pressure measurements were made using the mercury sphygmomanometer, with wrap-around arm cuffs, bladder size 12×23 cm. Two physicians using their personal stethoscope, took alternate subjects for

measurements of blood pressure. Formal training and testing of observers was limited to an explanation of the principles involved in standard measurement, a demonstration of technique, and supervision. Subjects were instructed to avoid smoking, eating, and vigorous exercise, for at least one half hour before measurement was made in a room at a temperature which would not induce sweating or shivering. Arterial blood pressure was measured in the right arm after at least 5 minute rest in a supine position at the end of the physical examination. The mercury in the sphygmomanometer was allowed to return to zero between readings. The fifth phase of the Korotkoff sounds was recorded as the diastolic blood pressure. Supine blood pressure was measured twice, and the average of these two measurements was used in the analyses.

1.2.3.3 Serum cholesterol

Blood samples were drawn, with a minimum of stasis from a vein in the antecubital fossa of the arm. The subjects were not required to be in a basal, fasting state. After being allowed to clot at least 30 minutes, the blood was centrifuged and serum was removed. One tenth of a milliliter portions were measured onto the filter paper, which was hung up in room air until dry (1 to 3 hours) before being packed in envelopes and sent by letter mail to the central laboratories in Minnesota or in Naples for analysis by the method of Abell-Kendall [15] as modified by Anderson and Keys [16].

The method of analysis of serum cholesterol included hydrolysis of the cholesterol esters with alcoholic KOH. After hydrolysis total cholesterol was extracted from the alcoholic solution by shaking with petroleum ether. Upon evaporation of the solvent by warm air, fresh Liebermann-Burchard reagent was added, and the color was developed and read under specified conditions of time and temperature. Blanks and reference standards were processed in the same way and were included in each batch of analyses. This method applied to either fresh serum or to serum dried on filter paper, yielded results that tended to be 1-2% higher than those obtained with the most careful estimation using digitonin

precipitation. Exceptions to the practice of making all analyses on dried serum were the Zutphen series, in which analyses of fresh serum were made locally in addition to the analyses in Minnesota. In Tanushimaru and Ushibuka, fresh serum was analyzed after saponification, by the method of color development with ferric ion intensification [17,18]. A few analyses made on dried serum samples sent from Japan to Minnesota indicated only fair agreement so the cholesterol values of the Japanese cohorts may not be perfectly comparable with the other series [1].

The accuracy of the cholesterol measurements over time as measured in Minnesota was tested in blind samples supplied by the Lipid Standardization Laboratory of the Center for Disease Control (CDC), Atlanta, Georgia. The accuracy of the cholesterol measurements in the Minnesota laboratories was satisfactory according to the criteria used by the CDC. The comparability of the results from the laboratories in Minnesota, Naples, Japan, and the Netherlands has been checked for many years by the frequent exchange of duplicates.

1.2.3.4 Electrocardiogram and resting heart rate

Multichannel, direct-writing, research type instruments were used in all areas. Two-, three- and four-channel models of the Elema, Siemens, Schwarzer, Phillips and Sanborn machines were used. Calibration and paper speed were controlled, and records made routinely at 25 mm/sec speed [2].

The procedure was standard in all areas and consisted of a supine resting record made of leads 1, 11, 111, aVR, aVL, aVF, V1, V2, V3, and V4, V5, V6 after at least 30 minute avoidance of eating, heavy activity or smoking. The skin was prepared by cleaning with a fat solvent before electrode jelly or paste was applied.

The subjects performed a single step test (12" or 30 cm in height), mounting and descending to a metronome count, 20 ascents per minute, for 3 minutes. This was immediately followed by recording of supine ECG, leads 1, 11, aVL, aVF, V3-6. In those

with any suggestion of early change, a 3- to 4- minute recovery record was made. Resting heart rate was calculated from the resting electrocardiogram. One to six per cent of men in most areas were excluded from a standard exercise test because of manifest heart disease. The electrocardiogram was coded according to the Minnesota code [3], and positive findings were verified by an independent coder. In Tanushimaru and Velika Krsna, the special ECG and pulse wave recordings on magnetic tapes were made during the 5- and 10-year follow-up. These procedures are described in detail in Chapter 2.3.

Deaths were ascertained yearly in each area. In addition to obtaining copies of death certificate and other documents, a member of the area team interviewed the relatives and friends of the deceased subjects. Attributed cause of death proposed by the local team was reviewed at the central quarters.

1.2.3.5 Respiratory function

Measurements of respiratory function were included in the initial examinations for ten cohorts, i.e., US railroad men, East and West Finland, Crevalcore, Montegiorgio, Crete, Corfu, Velika Krsna, Zrenjanin and Belgrade. The vital capacity was recorded for all 10 cohorts, and the forced expiratory volume measured in eight of those cohorts. At the 5-year reexaminations, the respiratory measurements were reported in the same ten cohorts, with the addition of three more cohorts: Dalmatia, Slavonia, and Zutphen.

1.2.3.6 Life-style data

Information about smoking habits was collected using a standardized questionnaire developed for the Seven Countries Study [7] (Appendix A). Subjects were asked about the type, frequency, duration, and amounts of cigarettes and/or cigars smoked. Data on smoking habits were collected on entry to study, and during the 5- and 10-year follow-up examinations in both cohorts.

All men in the Seven Countries Study were classified at entry according to their habitual physical activity. Class 1 men were bedridden; Class 2 sedentary, engaging in little exercise; Class 3 moderately active during a substantial part of the day; and Class 4 performed hard physical work much of the time. Classification was based on the responses to questions about the occupation and usual activities. The list of occupations into which the men were classified has been published [3]. This classification system was intended to identify the general character of the occupation and to suggest the probable socio-economic status of the subjects as indicated by their occupation.

The same measurements following the same technique and procedures were made at the 5- and 10-year follow-up examinations. Information on the prevalence of chronic diseases was collected in year 0, years 5 and 10. Information on mortality and causes of death was collected during 25-years of follow-up.

1.2.4 Statistical methods

All data were stored in IBM or Remington-Rand Hollorith punch cards for analysis with counter-sorter machines and electronic computers at the University of Minnesota.

For statistical analysis, the men in each cohort were grouped into 5-year age classes (40-44, 45-49, 50-54, and 55-59), and the complete distribution of values was obtained at each age- and area-specific group for a number of variables.

Prevalence and incidence rates of coronary heart disease were age standardized by single years of age to the age distribution of all men 40 through 59 at the entry examinations. These rates were calculated as cases per 10,000 men at entry or at risk in follow-up, respectively. Standard errors and confidence limits were calculated for all rates. Chi-square tests were made in a number of analyses, to reduce the influences of confounding variables. Fisher's exact method for the 2×2 table was used when indicated.

Multivariate analyses were employed in the evaluation of the significance of the characteristics recorded at entry. In some cases a summary Chi-square with one degree of

freedom was calculated according to Mantel and Haenzel, but Walker and Duncan's solution of the multiple logistic equation was the standard method. The analyses concentrated on all men with no evidence of cardiovascular disease at entry.

1.2.5 Major findings

1.2.5.1 The blood pressure-age relationships

Initially, the Seven Countries Study was pointed to coronary heart disease and its risk factors. With that focus, the relationship between blood pressure and age was examined from the standpoint of its contribution to the incidence and mortality of coronary and "noncoronary" heart diseases. Therefore, there was no systematic and organized evaluation of how blood pressure changed as the cohort aged in the first 5-year follow-up. But in subsequent analyses, there is some evidence indicating the blood pressure-age relationships.

In the 10-year follow-up [4], systolic blood pressure was found to increase with age. For all ages and areas combined, the 10-year change in systolic blood pressure averaged 8.3 mm Hg. The relationship of diastolic blood pressure to age and to the years of follow-up was quite different from the systolic blood pressure. In none of the areas was there any significant relationship between the diastolic blood pressure and age at entry. During 10 years, the average diastolic blood pressure rose significantly in Croatia, Italy, and Serbia, but decreased in Finland and Greece.

The blood pressure-age relationships were also reported in several publications concerning 15-, 20-, and 25-year coronary and cerebrovascular deaths and their risk factors. Tervahauta et al. [19] studied the association between blood pressure and 15-year blood pressure change with future coronary risk among elderly men in the Finnish cohorts of the Seven Countries Study. They noted a mean change of 9.5 mm Hg in systolic blood pressure, 4.7 mm Hg in diastolic pressure, and 4.8 mm Hg in pulse pressure, between

1969-1974 to 1984. Men who experienced a decline in diastolic pressure of ≥ 4 mm Hg from initial levels of ≥ 90 mm Hg had a higher risk of myocardial infarction than men with a change of ≤ 4 mm Hg. For a decline of ≥ 10 mm Hg in systolic blood pressure from levels of ≥ 160 mm Hg, the risk of coronary heart disease was also high. In an investigation of incidence and prediction of a first major cerebrovascular event in two rural cohorts in Italy, Menotti et al. [20] observed an increase in systolic blood pressure from 143.2 mm Hg at entry to 148.6 mm Hg at the 5-year follow-up to 152.6 mm Hg at the 10-year follow-up, representing a 6.2 % increase over a 10-year period. Similarly, the diastolic pressure rose from 85.3 mm Hg at entry to 89.7 mm Hg at 5-year follow-up to 94.1 mm Hg at 10-year follow-up, representing a 9.4% increase during 10 years. Both age and systolic blood pressure demonstrated a direct, significant association with the first major cerebrovascular event over the 25 years of follow-up. Kromhout et al. [6] examined changes in major risk factors for cardiovascular diseases over 25 years in the Serbian cohorts of the Seven Countries Study. The average systolic blood pressure level increased with age, with the largest increase in Zrenjanin and the smallest in Belgrade. In men aged 50-59 an increase of 2-3% was observed in all three cohorts between 1962-64 and 1972-74. The increase reached statistical significance in Velika Krsna only. In men aged 65-69 systolic blood pressure increased by 7% in Zrenjanin between 1973 and 1988, but no significant differences were observed in Velika Krsna and Belgrade. Similar blood pressure-age relationships were also found in the Japanese cohorts [7]; systolic pressure rose from 135 mm Hg in year 0 (entry to study) to 140 mm Hg in year 10 (10-year follow-up), and diastolic blood pressure increased from 76 in year 0 to 79.2 mm Hg in year 10. Systolic blood pressure predicted coronary heart disease, stroke and all-cause mortality. Changes in systolic blood pressure during the first 10 years of follow-up was directly related to deaths between 10 and 25 years of follow-up.

It is well documented that blood pressure is influenced by other factors besides age [21,22]. This aspect of blood pressure-age relationships has never been reported in any of

the published studies emerged from the Seven-Country data set. All analyses were based on combined age and secular trend effect. Furthermore, the status of the arterial vasculature and the heart associated with aging of the blood pressure has not been investigated in those studies.

1.2.5.2 Mortality of coronary heart disease and other diseases

During ten years (entry to 10-year follow-up), 1507 men died; coronary heart disease accounted for 410 cases (27%), cancer and other neoplasms 354 cases (23%), infectious diseases, mostly tuberculosis 72 cases (5%), violence 117 cases (8%), and other causes 554 cases (37%) [4].

Among 12,095 men free of evidence of cardiovascular disease at entry, the 10-year age-standardized coronary mortality rates were less than 75 per 10,000 for the men of Crete, Japan, and Croatia. In contrast, the age-standardized mortality rates from coronary heart disease of 250 or more per 10,000 were recorded for East and West Finland, the American railroad men, Zutphen and Belgrade. The age-standardized coronary mortality rates of the Finns and Dutch were significantly higher than those of the Italians, Greeks, Yugoslavs, and Japanese. The coronary mortality rates of the American railroad men were significantly higher than those of the Italian railroad men.

The all-cause death rates of the Finns and Dutch were significantly higher than those of the Italians and Greeks, but did not differ from the Yugoslavs. The rates of the rural Europeans were significantly higher than the rates for the urban Europeans and the Japanese in all-causes deaths.

During 15 years (entry to 15-year follow-up), important differences among cohorts in death rates, especially from coronary heart disease, were noted [5]. The average yearly age-standardized mortality rate in the first 10 years was 12.8 per 1000, and for the next 5 years, it was 19.5 per 1000. The corresponding figures for age-standardized coronary mortality rate were 2.76 and 7.61; mortality from coronary heart disease increased much

more than the mortality from the total of all other causes. The rate of changes in death rates was different among cohorts. However, the number of coronary deaths in the Japanese cohorts remained to be smaller than the number predicted by the logistic coefficients.

Menotti et al. [23] reported the 20-year stroke mortality in 12 of the original 16 cohorts. The differences in the age-standardized stroke mortality rates between cohorts were relatively large with a more than threefold ratio between the extremes in Zrenjanin (78 per 1000) and Zutphen (22 per 1000). When the national pools were inspected, the highest death rates were located in Yugoslavia and Japan, followed by Italy, Finland, Greece, and Netherlands. The geographical trends for coronary heart disease were different, with Finland having the highest death rates, and Japan the lowest.

1.2.5.3 Risk factors for coronary heart disease and other noncoronary heart diseases

Among the risk factors examined in the Seven Countries Study, age and blood pressure were the two important, independent risk factors for the incidence and mortality rates of coronary heart disease. The 10-year incidence rate varied greatly among the cohorts, especially for coronary heart disease. The slope of the regression of incidence rate of coronary heart disease on age was somewhat less steep than the slope of mortality rate on age.

For middle-aged men free of coronary heart disease at entry, the probability of death in 10 years (all-causes) increased by 15 to 22% with each increase of 10 mm Hg of systolic blood pressure, over the range of 125 to 165 mm Hg, in the different cohorts. The risk of developing coronary heart disease in 10 years was related to the entry blood pressure to much the same extent. The 10-year age-standardized coronary death rates of the cohorts were correlated with the median systolic blood pressure of the cohorts at entry ($r=0.64$). This one measurement of blood pressure at the start accounts for 41% of the variance in coronary death rate. Taking either mortality or the incidence of coronary heart

disease as end points, no significant relationship of incidence to entry blood pressures was found over roughly the lower two-thirds of the distribution of the blood pressures in any of the regions. In the upper third of the blood pressure distribution, the incidence rate tended to rise sharply. The data suggest a curvilinear relationship between blood pressure and incidence of coronary heart disease.

The 10-year rate of death from coronary heart disease in the cohorts was highly correlated with the median cholesterol values of the cohorts (r=0.80), accounting for 64% of the variance of the cohorts in the coronary death rate. Comparisons of the experience of individual men within the cohorts indicated that the incidence of coronary death was importantly related to the entry cholesterol concentration. At the upper end of the cholesterol distribution, the likelihood of becoming a coronary victim increased sharply with the cholesterol level.

The cohort average for cigarettes smoked per day varied from 7.1 (Montegiorgio, Italy) to 14.8 (Tanushimaru, Japan). In Finland, the Netherlands and Yugoslavia, the 10-year incidence of death was roughly twice as great for men who smoked as those who did not smoke. Cigarette smoking habit was not a very important risk factor for 10-year death in Italy and Greece. In Japan, the 10-year death rate was unrelated to smoking habits.

In southern Europe and in Japan, there was a substantial increase in dietary fat from animal sources during the years of follow-up [3]. In the United States, the general trend was for the substitution of vegetable for animal fat and a reduction of cholesterol in the diet [3]. However results indicated that habitual diets, along with body mass index or fatness, resting pulse rate, and respiratory function, showed no significant relationship to the incidence of coronary heart disease and deaths in almost all areas.

The importance of these risk factors to coronary and noncoronary deaths was validated in the 15- and 20-year mortality studies [5,23]. For deaths in the third 5-year follow-up, age and smoking habits of men at entry were significant risk factors for both coronary and noncoronary deaths in northern and southern Europe and in the United

States. Systolic blood pressure at entry was also an important risk factor for coronary deaths in both areas of Europe. The concentration of serum cholesterol at entry was a significant risk factor for late coronary death in northern and southern Europe but not for the Americans.

In summary, the Seven Countries Study concerns relationships of the follow-up incidence of disease and death to the characteristics of men at the entry examinations. It identified three major risk factors for coronary heart disease: age, blood pressure, and serum cholesterol, and also provided some evidence of blood pressure-age relationships. But blood pressure aging trends and the mechanism(s) responsible for blood pressure change with age have never been addressed in studies using the Seven-Country data set.

The present study is completely different from the objectives of the Seven Countries Study, in that it determines the true blood pressure trend with aging, and its relationships to the vascular and cardiac status.

1.3 Literature Review

The main interest of the present study is in the primary changes of the aorta, the major arteries and the heart due to aging per se. The selective literature review that follows, is limited mainly to those papers which address the age-related structural and functional changes in the vasculature of the arterial system and the heart.

1.3.1 Age-related structural changes in the arterial system and the heart

1.3.1.1 Systemic arterial tree

The systemic arterial tree consists of the aorta and its major branches, each of which sequentially branches until the arteriolar level is reached. The aging process affects the intimal, medial, and the adventitial layer of the blood vessel differently as well as each region of the vascular tree. The aorta and the large arteries change in a very characteristic way with age. These alterations are believed to have a significant influence on the functioning of the heart.

In the arterial system, the intima is a continuous monolayer of endothelial cells, lining the luminal surface of all arteries. Since it is the immediate interface between the blood stream and the underlying arterial wall, it is continuously subjected to shearing forces related to flow, and to normal forces related to blood pressure. In addition, the intimal layer is also directly exposed to circulating cells and plasma components. With aging, certain histological changes in the different layers of the arterial vessel wall have been observed. Studies of human arteries by Cotton and Wartmen [24], Guard and Bhende [25] and Movat et al. [26] demonstrate that the endothelial cells become more irregular in their shape and size with advancing age. These changes are found to be most prominent in the aorta. Milch [27] reported the thickening of the subendothelial layer with an increase in connective tissue content. There is increasing thinning, fraying, and fragmentation of individual elastic laminae. In the very late stages of aging, calcification and lipid deposition have been observed in close proximity to the internal elastic membrane [27]. The changes associated with endothelial aging may perhaps account for a decrease in the endothelium-dependent relaxation of the vascular smooth muscle associated with aging [28]. It has been speculated that this reduction is primarily initiated from an altered modulatory role of the endothelial cells [28].

Intimal fibrocellular thickening appears to increase with age [26]. In humans, the aorta and the coronary arteries tend to develop relatively thick intimas associated with

advanced age [29]. Bertelsen [30] investigated the age-related alterations in macroscopically normal aortic and pulmonic tissue, to determine a possible relationship between lipid deposit and calcification in elastic tissue and their mutual relation to ground substance and fibrils. Human vascular tissue from 114 subjects aged 0-90 years were obtained from autopsy. Results show that uniform intimal thickening increased constantly with age, with rupturing of the internal elastic membrane. The proliferating intima was subjected to a lipid accumulation. The increasing proliferation of the intima was accompanied by accumulation of Periodic Acid-Schiff's staining-positive substance. The functional significance of these intimal changes is apparent. Intimal proliferation, and later fibrosis of the major arteries, may lead to increased stiffness of the arteries.

The most conspicuous age-related changes in the media are thickening with elastin fragmentation and calcification. Wolinsky [31] compared the effects of long-term (16 months) to short-term (2.5 months) hypertension and to concurrent aging changes, on the thoracic aorta of male rats. Results indicate that the aortic diameter of the old normotensive rats (19 months) was significantly greater than that of the young normotensive rats (5 months). No significant difference in aortic diameter was found between the young and old hypertensive rats. Wall thickness increased significantly with age in both hypertensive and normotensive rats. There were also sharp increases in the absolute amount of both elastin and collagen in the aging hypertensive and normotensive rats. Wolinsky's data [31] suggest that different mechanisms may be responsible for the aortic morphological changes observed in aging and in hypertensive vessels. Aging is accompanied by a considerable accumulation of fibrous proteins, leading to a two-to-threefold increase in wall thickness. Acute hypertension is characterized by a disproportionate increase in wall thickness in noncollagenous alkali-soluble proteins, attributable to the smooth muscle component of the vessel wall. In chronic hypertension, the continued increase in wall thickness and medial area reflects considerable accumulation of elastin and collagen.

Similar age-related changes in the aortic media have been observed in other species. Maruffo and Malinor [32] reported a progressive increase in thickness, collagen content, and the number of fibroblasts in the aging aortic media of monkeys. The increased synthesis of the major connective tissue components, elastin and collagen by smooth muscle cells is thought to contribute appreciably to wall thickening [31].

Age-related medial changes have also been reported in the human aorta. Bertelsen [30] reported that the elastic fibers in the media of the aging aorta were found gradually ruptured from each other, and the collagen fragments increased with age both in length and in number. In addition, the ground substance increased constantly with age; this process began from birth.

Besides the structural changes in the elastin, widespread and progressive calcification in human aorta specimens has been reported [33,34]. By the fourth decade of life, 98% of the aortas examined showed significant medial calcification. Similar age-related changes in the aortic media have also been observed in other species. In rats, the media thickens from 60 μm at one week of age to 120 μm at 3 years of age [35].

Functionally, these age-associated changes in the aortic media result in a gradually increasing vascular stiffness, with the considerable reduction in aortic distensibility frequently observed in the elderly [36].

Age-related changes in the adventitia are less specific and less studied than in the intima and the media. However, there is experimental evidence [32] indicating that lymphocytes, collagen fibers, and fatty tissues, increase in number with age. Although the functional significance of these adventitial changes is not fully known, it is possible that increased collagen fibers and fatty tissues may also be involved in the process of augmenting arterial stiffening.

Changes with age are not confined to the large arteries; they may involve the microvasculature as well as the arteriolar bed. Hyaline thickening, similar to that seen in younger hypertensive patients, may occur in the smaller arteries of various organs, and may

contribute to the elevated peripheral vascular resistance found in the aged [37]. Hassler [38] observed a number of deformities of small arteries, including spiraling and looping, with age. In a study of age alterations in the human lingual artery, Semba [39] reported that the most frequent intimal change was diffuse fibrous thickening. In addition, the internal elastic lamina regressed with increasing age. The thickness of the media increased until the third decade when it became fairly constant. The relative luminal radius remained constant until the seventh decade and then showed a distinct decrease. Thickening of the basement membrane with increasing age has been reported in other studies. Jordon and Perely [40] and Kilo et al. [41] reported significant thickening of capillary basement membrane from the quadriceps femoris of nondiabetic and diabetic subjects, and concluded that it is an aging phenomenon. An ultrastructural study of human conjunctival capillaries showed a diminution of endothelial pores and thickening of the basement membrane with increasing age [42]. Similarly, Leuenberger [43] reported an age-related thickening of the retinal capillary basement membranes in rats and mice.

In summary, aging appears to affect the large and small arteries in a similar fashion. The intima thickens due to cell proliferation and the formation of the connective tissue. Changes in the shape and size of endothelial cells, associated with aging, enhance membrane permeability, thus facilitating the smooth muscle cells' migration to the intima [44]. Modified smooth muscle cells enter the intima from the media, and synthesize connective tissue proteins, collagen and elastin. There is a progressive fibrosis in the media, with an increase in the number of fibroblasts. The degeneration of elastic fibers is associated with an increase in collagenous material and ground substance, and often with a deposition of calcium. Changes in the load-bearing media contribute to arterial wall stiffness, with significant functional implications.

1.3.1.2 The heart

Age-related changes in cardiac anatomy have been demonstrated on examinations of chest X-rays. Oberman et al. [45] reported a distinct age-related rise of the heart diameter to about 50 years, after which the heart diameter becomes stable. They also observed a significant increase with age in the cardiothoracic ratio. The longitudinal increment of the cardiothoracic ratio from 0.405 to 0.427 was noted over a 17-year follow-up of 66 rigorously screened, healthy men from the Baltimore Longitudinal Study on Aging [46]. This modest increase was attributed to a smaller increase in cardiac diameter and a decrease in chest diameter with advancing age.

Myocardial structures change characteristically during aging. Olivetti et al. [47] studied the effect of age on the structural properties of the myocardium in 54 hearts from individuals, aged 17 to 90 years, who died from causes other than cardiovascular disease. The ventricular weight decreased with age, and the number of myocytes was reduced as a function of age. This cellular adaptation was paralleled by an increase in myocyte cell volume. The combination of these two events resulted in the preservation of the ventricular wall thickness with age. Olivetti et al. concluded from this investigation that aging of the heart leads to myocyte loss and reactive cellular hypertrophy of the remaining myocytes. Investigating the morphological characteristics of individual myocytes isolated from rats, Fraticelli et al. [48] noted that the average ventricular myocyte length increased progressively with age, but the average sarcomere spacing did not increase significantly from adulthood (6-9 months old) to senescence (24-25 months old). The number of sarcomeres in series, however, increased with age. The senescent ventricular myocytes showed an age-related prolongation in the time-to-peak-shortening, and an increase in contraction duration. The absolute amplitude of peak myocyte shortening during a twitch in cells from hearts of old rats, was greater than in the younger ones. Since the number of sarcomeres in series increases with age, a similar absolute amplitude of myocyte shortening during a twitch in all ages could be accomplished with less shortening of each sarcomere in

the older versus younger myocyte. Thus, the series addition of sarcomeres can be interpreted as an adaptive mechanism of aging cells to maintain a given extent of myocyte shortening.

The effect of age on the heart weight has been examined in a number of investigations. Reiner et al. [49] concluded from their study of normal hearts, freed from epicardial fat, that age per se does not influence myocardial weight; the apparent age-related increase in heart size is a function of the amount of epicardial fat. Linzbach and Akuamoa-Boateng [50] reviewed the heart weights in an autopsy series of 7112 patients, including both normal subjects and persons with cardiovascular disease. They reported an average increase of heart weight of 1 gm to 1.5 gm/year between ages 30 and 90, but the actual weight of the heart was not given in their study. In a series of patients undergoing endomyocardial biopsy, Unverferth [51] showed that this age-related increase in left ventricular weight is due to cellular hypertrophy rather than hyperplasia. These inconsistent findings may be attributable to the differences in sampling and measurement methods. In Linzbach and Akuamoa-Boateng's study [51], hearts from individuals with cardiovascular disease, which as noted increases sharply with age, were included with those from normal individuals. Thus it is possible that the observed increase in cardiac mass with age is related, at least in part, to cardiovascular pathology, and may not be an age-related phenomenon in the normal population. In addition, changes occur in cadavers that can affect the heart size. These changes do not allow for a valid comparison of the heart weight measured at autopsy with that determined in living organisms.

A considerable body of evidence has accumulated from large population studies as well as from prospective epidemiological data, which shows that cardiac enlargement may occur and be directly related to aging in humans. Gerstenblith et al. [52] demonstrated echocardiographically, an age-related increase in the left ventricular posterior wall thickness, corrected for body surface area, in 62 (aged 25 to 84) carefully screened, healthy, participants in the National Institute on Aging Volunteer Longitudinal Program. The average

diastolic wall thickness increased significantly from 4.3 mm/m^2 for the 25-44 age group to 5.7 mm/m^2 for the 65-84 age group. Systolic wall thickness for these age groups was also increased significantly with age (7.6 mm/m^2 versus 10 mm/m^2). It is not known if this increase in wall thickness is due to an increase in cardiac fat, connective tissue content, number of myocardial fibers, or a combination of all these components.

Changes in the properties of heart connective tissue have been observed in different regions of the myocardium. Lenkiewicz et al. [53] studied the muscle and collagen fibers in 40 human hearts from male patients ranging in age from 13 to 92 years. Results indicate a small, but significant, increase with age for collagen gel in regions in which the muscle fibers were predominantly transverse, that is, in the subendocardial zones. However, there was no significant age-related increase in the total proportion of collagen in the interventricular septum. The characteristic pattern of augmentation in collagen with age reported by Lenkiewicz et al. [53] may partially explain the increase in ventricular wall stiffness associated with advanced age. Augmented ventricular wall stiffness may account for the reduced left ventricular early diastolic filling associated with aging [54].

A review of the literature related to structural changes in the systemic arterial system and the heart, indicates that most of these changes have been observed in multiple species and in many of the populations studied. These structural alterations can have important functional implications in the cardiovascular system. The following review of literature will address this point.

1.4.1 Age-related functional changes in the arterial system and the heart

1.4.1.1 The relationship between wall stress, tension, and distensibility

The relationship between tension and pressure in blood vessels is given by the law of Laplace, and is expressed as

$$T = P \cdot r \qquad (1.1)$$

where T = tension in the vessel wall, P = transmural pressure, and r = radius of the vessel. In this simple derivation, the walls of the vessel are assumed to be infinitely thin.

Equation (1.1) can be rewritten as

$$\Delta P = \frac{\Delta T}{\Delta r} \qquad (1.2)$$

where ΔP = change in pressure, ΔT = change in vessel wall tension, Δr = change in the radius of the blood vessel.

Distensibility of an artery is defined as the relative increase in arterial volume ($\Delta V/V$) for a given increase in pressure (ΔP), and is given by the equation

$$D = \frac{\Delta V}{V \cdot \Delta P} \qquad (1.3)$$

where D = arterial distensibility, ΔV = change in arterial volume, V = arterial volume, ΔP = change in arterial pressure.

Since it is practically impossible to accurately determine $\Delta V/V$ and V non-invasively in man, it is assumed that the increase in volume is caused by distension of the artery, i.e., an increase in the cross-sectional area. Hence equation 1.3 can be re-written as

$$D = \frac{2\pi r \cdot \Delta r}{\pi r^2 \cdot \Delta P} \qquad (1.4)$$

and can be simplified as

$$D = \frac{2\Delta r}{r \cdot \Delta P} \qquad (1.5)$$

where r = radius of an artery, Δr = change in the radius of an artery.

From the above equations, it is evident that the distensibility of an artery is directly proportional to a change in its internal radius, and inversely proportional to a change in pressure. This pressure-dependent characteristic of distensibility is due to the biphasic elastin and collagen composition of the arteries. Tension at low distending pressure is borne by the elastin fibers, and at high pressure predominantly by the less extensible collagen fibers. Thus the distensibility of the artery decreases when the blood pressure increases.

With increasing age, the elastic tissue breaks down, and its task is taken over by the much less extensible collagen tissue. As the internal pressure increases, the wall tension of

an artery increases accordingly. But because the young arteries are endowed with considerable elasticity, the increase in tension is compensated for by an augmentation in the arterial diameter. However, with advancing age, the vessel diameter increases only slightly with internal pressure, reflecting the net result of a loss of elastic tissue and a concomitant increase in collagenous fibers. Figure 1.1 shows the mean volume-elasticity curves of the thoracic aorta at five age groups, redrawn from data presented by Hallock and Benson [55].

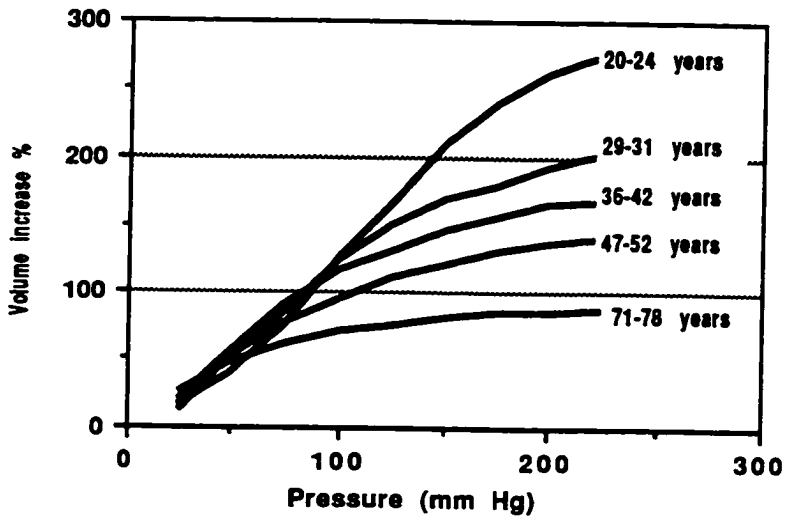


Figure 1.1 Mean volume-elasticity curves of thoracic aortas at five age groups, redrawn from data presented by Hallock and Benson [55].

1.4.1.2 Aging and the mechanical properties of the systemic arterial tree

Earlier studies on the mechanical property changes with age were primarily descriptive and qualitative. One of the earliest reports concerned with age-related changes in the mechanical properties of the arterial system is that by Roy [56]. He studied the effect of age on the elasticity of the arterial wall, using strips of human aorta obtained from autopsy, ranging in age from 2 to 71 years old. He concluded from his experiments that with advancing age, the elasticity of arterial segments decreased.

Hallock and Benson [55] measured the pressure-volume relation in segments of the human aorta from five age groups (see Figure 1.1). The aortic segments were stripped of all of the dense fibrous tissue constituting the adventitia, in order to ligate the intercostal vessels tightly. The results show a progressive loss of elasticity with age. The pressure-volume curves of the 20-24 and 28-31 year old were S-shaped, while curves of the remaining age groups showed the effect of the diminished arterial elasticity associated with advancing years. By age 75, the aortic elasticity was 25% of younger specimens. However, these results may not accurately represent the pressure-volume relation in living persons. The aorta in the living man is surrounded by a considerable amount of dense fibrous tissue. In all probability, this tends to increase the rigidity of the arterial wall somewhat. Although the exact effect of this coating of fibrous tissue on aortic elasticity is unclear, it is likely that the stripping off of this coating may produce results which may underestimate the aging effect on the elasticity of the aorta.

The increasing stiffness of the human aorta with age has been confirmed in a number of investigations. Bader [57] observed a marked increase in the elastic modulus of thoracic aorta with age, from 10 kg/cm^2 at age 20 to 42.5 kg/cm^2 at age 85. The elastic modulus is directly related to stiffness: the greater the elastic modulus, the stiffer the vessel. The tangential wall stress at 100 mm Hg, decreased linearly with age from about 2.5 kg/cm^2 at age 20 to about 1.2 kg/cm^2 at age 85. The internal radius as well as the inside volume at zero pressure increased at higher ages. According to Bader, the ratio of internal radius to wall thickness (r_i/t) increased with rising pressure, but the older the aorta, the lower the ratio at a given pressure. This means that the aortas become more and more rigid with age, and the relative volume increase is less with rising pressure. The pressoreceptors in the wall of the elastic vessels respond to wall stress by either increasing the blood pressure progressively in order to keep the same wall stress, or by adapting their sensitivity to a lower wall stress throughout life, so that it will be kept constant. Bader speculated that weakness of the smooth muscles or a failure of the pressoreceptors to adapt themselves to

lower wall stress at higher ages, may be the cause of essential hypertension in some individuals.

In their work on autopsied specimens of iliac arteries from 63 individuals, of ages from birth to 91 years, Roach and Burton [58] observed an increase in the arterial wall thickness from birth to age 20, but no significant change thereafter. Arterial elasticity decreased with age, by a factor of 2 or 3 between the ages 30 and 90. They argued that increased medial thickness could not account for the reduced elasticity, since no aging trend in wall thickness was noted after the period of growth (age 20) in this population. Their finding of an increased modulus at zero stretch in aged iliac artery was interpreted to reflect a large number of collagenous fibers with shorter unstretched lengths since there was no change in elastin content. They postulated that the large collagen content found in the aging iliac artery was due to increased cross-linking of collagen fibers. Nakashima and Tanikawa [59] also observed a steady decrease with age in the distensibility of autopsied human aortas. The circumferential distensibility was about 90% of the original value in subjects under 20 years old, and about 20% in those above 80 years old. The original value was determined at the beginning of the experiment with a distending pressure at zero mm Hg. This progressive decrease in circumferential distensibility was also present in longitudinal distensibility. The researchers did not provide the original value of the circumferential distensibility in the paper. Using Gore's atherosclerotic indexes [60] to determine the role of atherosclerosis in aortic distensibility, Nakashima and Tanikawa [59] observed that the maximal aortic distensibility of Japanese aortas with a high atherosclerotic index (severe atherosclerosis) was similar to that of the aortas with a low atherosclerotic index (mild atherosclerosis). They concluded that this reduced distensibility was apparently not related to the degree of atherosclerosis, but aging itself seemed to be the major factor.

A number of clinical investigations into the mechanical properties of aging aortas, using direct and indirect methods of measurement, have been reported. Abboud and Huston [61] studied the effects of aging on the arterial rigidity of normotensive control subjects and

patients with clinical evidence of arteriosclerotic vascular disease. Arterial rigidity was determined as the ratio of change in pulse pressure to the change in diastolic pressure following the inhalation of amyl nitrite, a vasodilator. Intra-arterial blood pressure tracings were obtained before and after the inhalation of amyl nitrite. A strong positive correlation of the arterial rigidity index (fall in pulse pressure × 100/fall in diastolic pressure) with age was found in the normotensive subjects. Of the 25 patients showing signs and symptoms of arteriosclerotic vascular disease, 23 (92%) had arterial rigidity index that exceeded the upper limit of normal for their age group. These results suggest that age and degenerative arterial disease contribute to the arterial rigidity.

Compliance is the ratio of change in volume (ΔV) resulting from a change in transmural distending pressure (ΔP), or $\Delta V/\Delta P$, the slope of the pressure-volume relationship. Such a relationship is curvilinear due to the nonlinear distensible characteristics of arteries [55]. Thus compliance decreases with increasing pressure, and is expressed by the equation

$$C = \frac{\Delta V}{\Delta P} \qquad (1.6)$$

where C = arterial compliance, ΔV = change in arterial volume, ΔP = change in arterial pressure.

Arterial distensibility, on the other hand, is expressed as the fractional increase in volume for each millimeter of mercury rise in pressure, in accordance with the following equation:

$$D = \frac{\Delta V}{V \cdot \Delta P} = \frac{C}{V} \qquad (1.7)$$

where D = arterial distensibility, V = arterial volume, ΔV = change in arterial volume, ΔP = change in arterial pressure.

Since the slope of the pressure-volume is not fixed and changes with increased pressure, this parameter is difficult to determine in humans [62]. Indirect indices of the arterial pressure-volume curves have been developed, these include the ratio of change in

pulse pressure and stroke volume [63], exponential analysis of diastolic blood pressure decay [64], pulse wave velocity [65,66], and characteristic impedance [67].

The effects of age on the mechanical properties of the ascending thoracic aorta and right pulmonary artery were studied angiographically in 43 patients during routine cardiac catheterization by Gozna et al. [64]. In the pulmonary artery, the pressure-strain elastic modulus (Ep), an index of arterial stiffness, was found to increase linearly with age, with the rate of change of Ep being 2.6 g/cm^2 per year in the pulmonary aorta, and 16.0 g/cm^2 per year for the ascending aorta.

The pressure pulse generated by ventricular ejection is propagated throughout the arterial tree at a speed determined by the elastic and geometric properties of the wall and the density of the blood. For large arteries, pulse wave velocity is given by the equation [69]:

$$c = \sqrt{\frac{\Delta P \cdot V}{\Delta V \cdot \rho}} \qquad (1.8)$$

where c = pulse wave velocity; ΔP = change in arterial pressure, ΔV = change in arterial volume, $\Delta P \cdot V / \Delta V$ = relative volume elasticity of the vessel segment, ρ = blood density, V = arterial volume.

From this equation, compliance (C) can be expressed as :

$$C = \frac{\Delta V}{\Delta P} = \frac{V}{\rho c^2} \qquad (1.9)$$

Since V can be expressed in terms of radius per unit length, then

$$C = \frac{\pi r^2}{\rho c^2} \qquad (1.10)$$

where r is the inner radius of the artery.

In clinical investigations, pulse wave velocity is a widely used index for the study of arterial stiffness; it is the rate of propagation of pressure waves along arteries, and is inversely related to compliance or distensibility. It has been shown that the velocity with which the pressure waves travel along an artery, is influenced primarily by the arterial wall

elasticity in normotensive subjects [70]; the stiffer the arterial walls, the faster the pulse waves travel. The inverse relationship between pulse transit time and aortic distensibility has been validated by Tarazi et al. [63].

Replotting a series of Roy's [56] volume-pressure curves in the isolated strips of femoral and carotid arteries of healthy rabbits and cats, Bramwell and Hill [69] calculated the velocity of the pulse wave at various pressures. They reported that the velocity was constant as the pressure rose from a low value of 20 mm Hg to about 80 mm Hg. Thereafter, it increased slowly at first, and then more rapidly. At high pressures (120 mm Hg to 160 mm Hg), the velocity was considerably increased.

To determine whether the increase in pulse wave velocity that occurs with aging is due to degeneration of the arterial wall or atherosclerosis, Avolio et al. [71] measured the pulse wave velocity by means of non-invasive transcutaneous Doppler techniques, in the aorta, right arm, and right leg of 480 normal subjects, aged 3 to 89 years in urban Beijing, China. The rationale for choosing these three arterial segments for measuring the pulse wave velocity was not given in the paper. Serum cholesterol was measured to determine the prevalence of hyperliplidemia in the community studied. Hyperlipidemia was used as indirect evidence for the presence of atherosclerosis in this community. The aortic pulse wave velocity increased significantly and substantially with age, representing an increment of 134% between birth and age 90. Pulse wave velocity values in the right arm and leg were similar; they were higher than in the aorta at the younger age, but increased to a lesser degree with increasing age. No significant difference was found in serum cholesterol between the second and ninth decade. It was concluded that aging and not concomitant atherosclerosis, was the dominant factor associated with increased pulse wave velocity. Compared to the pulse wave velocity of the other racial groups, Avolio et al. [71] reported that the aortic pulse wave velocity in the Chinese subjects was markedly higher than in subjects from Germany, United States of America, Canada, Israel, England, and France.

The Chinese subjects' pulse wave velocity was high at a younger age, and increased to a greater degree with increasing age.

From the point of view of large arteries, hypertension can be looked upon as an accelerated form of aging. Similar degenerative changes are seen in the walls of the large arteries with hypertension, but at an earlier age [32,72]. Hypertension induces major changes in the arterial intima and media. These changes are thought to be in many ways adaptive to the increased stress from arterial blood pressure [73], and are responsible for the increase in arterial stiffness, pulse pressure, and the rate of rise of pressure.

Merillon et al. [72] calculated the pulse wave velocity from pressure/diameter changes in the ascending aorta, and showed that this increased in normotensive subjects from 4 m/sec at age 25 to about 8 m/sec in subjects at age 50 and about 10 m/sec in persons in the late 60s. This represents an increase of some 150% over 40 years. In investigating pulse wave transmission in patients with sustained essential hypertension, Pannier et al. [74] observed a positive and strong correlation between carotid-femoral pulse wave velocity, with age and blood pressure. At any age, this correlation was higher in hypertensive than in normotensive subjects. But the brachioradial pulse wave velocity was not correlated with age. Although age is the most important factor contributing to increased pulse wave velocity in the human population, this significant contribution is not manifested in all parts of the arterial system, such as in the brachial artery. It was surmised that this may be related to the differences in proportions of collagen, elastin, and arterial smooth muscle in the central and peripheral large arteries. A more recent study of the consequences of aging and hypertension on the left ventricle and aorta [75] confirms previous reports of an age-related augmentation of pulse wave velocity.

Arterial distensibility has been observed to be reduced in hypertensive patients, particularly in elderly hypertensives. Simon et al. [76] reported a significantly higher pulse wave velocity, greater arterial diameter, and lower arterial compliance in hypertensive patients, when compared to age-matched normotensive subjects. Similarly, Pasierski et al.

[77] reported a significant reduction in arterial distensibility in elderly patients with isolated systolic hypertension. Gribbin et al. [78] and Levenson et al. [62] observed a decrease in arterial distensibility with elevated distending pressure in sustained hypertension. The relationship between arterial distensibility and distending pressure did not change with age, implying that elevated blood pressure, and not increased rigidity of the arterial wall, might be responsible for a reduced arterial distensibility in hypertension.

1.4.1.3. Age-related changes in the pulse wave contour

The pressure waves generated in the ascending aorta by blood ejected from the ventricle, propagate to other arteries throughout the body. The normal aortic pulse contour of an adult is characterized by the appearance of three distinctive features: an early systolic shoulder, a late systolic shoulder, and the diastolic wave [79]. There is no consensus in the literature on what is responsible for the normal contour of the pressure wave. However, most do agree that the early systolic shoulder is due to the arrival of the impulse generated by ventricular ejection. But different explanations have been given for the late systolic shoulder and the diastolic wave [79]. The late systolic shoulder has been attributed to the summation of the forward wave with the reflected wave returning to the heart from the upper part of the body [80]. The diastolic wave is the secondary wave separated from the late systolic shoulder by a broad, smooth trough, attributable to the return of reflected waves to the heart from the lower part of the body. Figure 1.2 is an example of the aortic pulse wave.

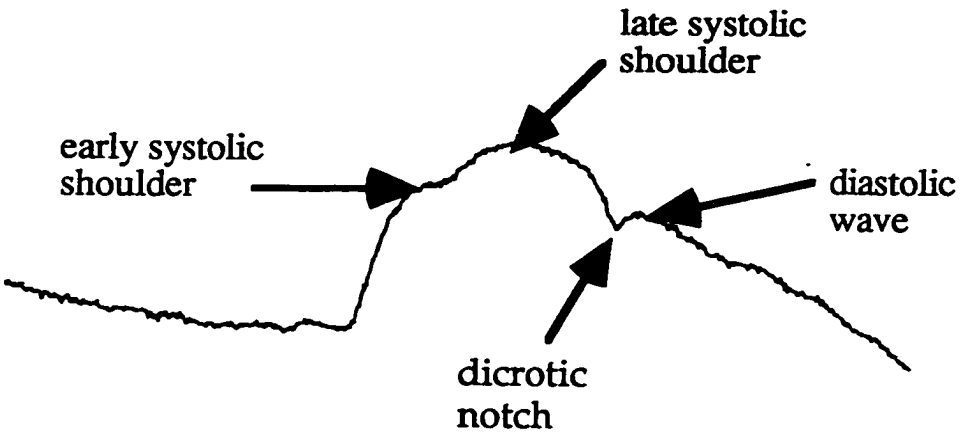


Figure 1.2 An example of the aortic pulse wave, showing the early systolic shoulder, the late systolic shoulder and the diastolic wave. The diastolic wave is separated from the late systolic shoulder by the dicrotic notch, and is attributable to the return of reflected waves to the heart from the lower part of the body.

Aging changes in the arterial pressure wave contour are more obvious in man than in experimental animals [80]. Yin et al. [81] studied beagle dogs over a 10-year span and found little or no change in arterial hemodynamics or pressure wave contour. It is speculated that the lack of apparent change may be related to relative and absolute age, as the period of 10 years which accounts for the full life span of a dog, is just 1/8 of the life span for man [80]. Therefore, a 10-year span is insufficient to allow for the progressive stiffening of the arterial wall which may take decades to develop in man. Arterial stiffness is responsible for increased pulse wave velocity and a subsequent altered pulse wave contour.

Age-related changes in the arterial pulse contour were first described by Mahomed in his work published in 1872, and likened to that seen with hypertension, as cited in O'Rourke et al.'s book on the arterial pulse [82]. Mahomed correctly recognized the pressure pulse in these two states as one with an augmented late systolic shoulder and with little or no diastolic fluctuation. He detected these characteristic features of the pressure wave by palpation and confirmed them by the use of a sphygmograph. Mahomed's findings

of the typical radial pulse contours with hypertension and aging were confirmed over 100 years later [82].

Murgo et al. [83] and Nichols et al. [84] also reported changes with age, in the pressure wave contours of the ascending aorta. They identified three types of waveforms according to their shape. Murgo et al. [85] observed characteristic age-related changes in pulse contour, and related them to the impedance spectral patterns. They studied the relationships between the shape of the ascending aortic pressure contour and input impedance in 18 patients who underwent elective cardiac catheterization. The ascending aortic flow velocity and pressure were recorded simultaneously from a multisensor catheter. Pulse wave velocity was determined from the left ventricular pressure, obtained from another pressor sensor located at the catheter tip. Using the electrocardiographic RR interval as the fundamental period, they calculated the Fourier series of pressure and flow for the first 10 harmonics. For each patient, impedance moduli and phases from a minimum of 15 beats during a steady state were averaged. The impedance spectra for the waveform of the older individuals showed an oscillatory behavior that suggests considerable reflection in the arterial system. The impedance plots derived from the waveform of the younger persons demonstrate flatter spectra, indicating smaller or more diffuse reflections.

Kelly et al. [86] demonstrated age-related alterations in the carotid, radial, and femoral pressure waveforms. Pulse amplitude was found to increase with advancing age in all sites, with the carotid wave showing the greatest increase in amplitude (91.3%) from the first to eighth decade. In the carotid pulse, after the third decade of life, the late systolic peak becomes dominant, adding to the initial pressure rise. The augmentation index, defined as the ratio of the height of the peak above the shoulder of the wave to the pulse pressure, increased from 1.6% to 24.1% from the first to eighth decade, suggesting that augmentation of the pressure pulse from wave reflections accounted for 24% of the carotid pulse pressure. These changes in pulse wave contour are attributed to a higher pulse wave velocity,

resulting from increased arterial stiffness with age, and progressively earlier wave reflection.

Using data derived from both the impedance spectra and the pressure waveforms, Murgo et al. [85] estimated the location in the arterial system from which wave reflections arise. In most of their patients who were catheterized for various clinical indications, the distance to the effective reflection site was between 44 and 48 cm from the ascending aorta. This distance approximates the region of the terminal abdominal aorta and bifurcation into iliac and femoral arteries in the adult population. Reviewing data of published studies [87,88], O'Rourke and Yagninuma [89] concluded that in mammals, there is a single, functionally discrete reflecting site representing the result of all individual reflecting sites at the periphery of the body. This site appears to be in the region of the abdominal aorta or renal arteries.

1.4.1.4 Aging and arterial blood pressure

Mean arterial blood pressure is the product of cardiac output and total peripheral resistance. Age-related changes in one or both determinants would result in elevated blood pressure. In terms of aging, the central question is whether blood pressure changes in progressive and irreversible ways in every individual.

The overall relationship between age and blood pressure has been remarkably consistent in most community-based surveys conducted in developed countries [90,91,92]. These cross-sectional studies show that the older the population, the higher the blood pressure. The Framingham data [91] indicate that systolic pressures continue to rise into the seventh decade in men, after which they reach a plateau. Diastolic pressures peak sooner in men, between the ages of 50-56, and decline precipitously thereafter, until late 70's. In women, both the systolic and diastolic pressures initially are lower than in men of the same age, and rise more steeply with age until about age 50, when they equal those of men, then progressively increase to surpass those in men.

An age-related increase in blood pressure has been confirmed by several prospective studies. Harlan et al. [93], in a 30-year follow-up study of blood pressure in a white male cohort, reported a symmetrical frequency distribution of systolic blood pressure in the young population (mean SBP = 120.58±8.60 mm Hg), but such distribution curve skewed to the higher values as the population aged (mean SBP = 123.80±14.53 mm Hg). The systolic blood pressure increased with age, but some aging individuals have blood pressures comparable to those in young adults. In determining changes in blood pressure from 25-30 to 50-54 years of age, Harris [94] observed generalized rises in blood pressure with age. But a small number of individuals showed little or no change in their blood pressure over the 25-year period of observation.

A longitudinal survey investigating the relations between blood pressure and age was conducted in two Welsh populations aged 10 to 83, over a period of 10 years [95]. Data analyses show that in these populations, the rate of increase was highly significantly related to the original pressure, but only indirectly related to age. The higher the initial pressure, the greater the increase during the period of observation.

Docks and Fukashima [96] conducted a 14-year survey to examine blood pressure as well as demographic and medical follow-up data in a Japanese cohort, between the period 1958-72. They observed a slow upward trend of systolic blood pressure with age, except in the oldest group. In men, the systolic blood pressure values of the younger age groups (<30 to 50-59 years) rose from 118 to 138 mm Hg, while the systolic pressure of the oldest group (60 years and over) declined from 150 to 145 mm Hg during the 14-year observation period. Similar aging trend in diastolic pressure was observed. The initial blood pressure values were found to be predictive of subsequent pressure elevation. For example, in the 40-49 age range, 75% of systolic normotensive males, and 80% of females, were still normotensive 14 years later, with these percentages significantly less in older age groups.

Aging of blood pressure is not a biological necessity. In many "unacculturated" societies, little, if any, relationship seems to exist between age and blood pressure

[97,98,99,100]. In a cross-sectional study of 152 bushmen in Botswana, aged 15 to 83 years, Trunswell et al. [101] reported that the systolic and diastolic pressures of men showed a slight but definite downward trend with increasing age throughout adult life. In women, there was a slight increase in systolic pressure in the second half of life, but their diastolic pressures decreased slightly after the menopause [101]. Comparing Londoners with natives of the Fiji and Gilbert Islands, Maddocks [99] reported the same increase in the mean systolic blood pressures in all groups until the fifth decade. However, neither Pacific population showed the steeper rise of pressures in later years found in Londoners. Mean diastolic pressures changed little with age in Fijians and Gilbertese, but rose in Londoners.

Most of the published population surveys have shown that blood pressure rises with age. An increase in arterial rigidity, body weight, and abnormal carbohydrate metabolism have been implicated in blood pressure elevation associated with advancing age. However, none of these surveys uses blood pressure values that have been corrected for the effects of biological, social, and behavioral factors in their analysis. Without this correction, one cannot conclude that changes in blood pressure with age is attributed to biological aging per se.

1.4.1.5. Aging and the resting heart rate

Cardiac output is the product of the heart rate and stroke volume, and for many years it has been believed that aging is associated with an obligatory decline in resting cardiac function [102]. The effect of aging on the resting heart rate has been determined in animal as well as in human studies. Rathbaum et al. [103] reported a higher resting heart rate in the 24-month-old-rats, compared to that in the 12-month-old animals, but this age difference was abolished by propanolol.

The effect of age on resting heart rate in humans has been determined in a number of investigations using human subjects, and have produced equivocal results. In a cross-sectional study of 67 hospitalized patients between 19 and 86 years old, with no obvious

cardiovascular disease, Brandfonbrener et al. [104] reported an age-related decline in resting heart rate. In contrast, Strandell [105] demonstrated that resting heart rate did not change significantly with age. In the Baltimore Longitudinal Study of Aging (BLSA) population, Rodeheffer and Gerstenblith [102] observed a slight, statistically nonsignificant trend towards lower heart rate in older subjects. The conflicting finding of Brandfonbrener et al. and that of Rodeheffer et al. is most likely due to the fact that the BLSA participants were free of significant coronary disease, and were not physically deconditioned.

Current evidence indicates that resting heart rate is not related to age, but the intrinsic heart rate, that is, in the presence of both sympathetic and parasympathetic blockade, is diminished with age. Using propanolol and atropine to block the sympathetic and vagal stimuli to the heart in a group of 311 normal volunteer subjects aged 15 to 70 years, Jose [106] demonstrated a significant decrease in resting heart rate, which he called the intrinsic heart rate, with advancing age. In normal adults aged 20 to 30 years, the intrinsic heart rate is 104 beats per minute, as compared with 92 beats per minute in the older men aged 45 to 55 years.

1.4.1.6 Effect of age on the ventricular-vascular interaction

The profound influence of changes in the vascular system on the performance of the ventricle has been clearly elucidated by Urschel et al. [107]. One of the best-known examples of the prolonged effect of ventricular-vascular interaction, is the development of left ventricular hypertrophy in response to chronic arterial hypertension. Indeed, a number of studies report that increased aortic stiffness and concentric left ventricular hypertrophy are common consequences of aging and hypertension [108,109,110].

In the past, hemodynamic factors have been considered as the primary determinants of ventricular hypertrophy. An increase in wall stress generated by augmented hemodynamic workloads is regarded as the primary stimulus of cardiac hypertrophy. Linzbach [111] concluded from his observation in autopsy data that an important factor

controlling the increased cardiac mass is the systolic force or tension generated by the myocardial fibers. The augmented wall stress induces the addition of sarcomeres in parallel, resulting in an increased wall thickness. Since systolic pressure is one of the most important components of end-systolic stress, its contribution to the development of increased cardiac mass has been under close scrutiny.

Studies of the relationships between arterial blood pressure and the left ventricular mass in normotensives and hypertensives have produced inconclusive and inconsistent results. An early autopsy study [112] showed a close correlation between heart weight and systolic pressures in 138 patients with essential hypertension. However, clinical studies revealed discrepancies between the level of casual cuff blood pressure and the degree of left ventricular hypertrophy in hypertensive subjects [113,114,115,116], although the association was stronger with the 24-hour average blood pressure. Bauwens et al. [117] reported a significant correlation between echocardiographically derived left ventricular mass and mean ambulatory systolic and diastolic pressures among 36 hypertensive patients. Similarly, Drayer et al. [118] observed different relationships between the 24-hour ambulatory blood pressures and left ventricular mass in two groups of hypertensive patients, one with left ventricular hypertrophy, and the other without left ventricular hypertrophy. No statistically significant relationship was observed between the 24-hour average systolic and diastolic blood pressure and left ventricular mass for the group as a whole. But subgroup analyses showed a significant, positive relationship between the mean 24-hour systolic and diastolic pressure and the left ventricular mass in hypertensive patients without hypertrophy. In contrast, a significant negative correlation was observed between the 24-hour mean diastolic pressure and left ventricular mass in patients with hypertrophy. These results indicate that blood pressure is one of the important factors in the development of left ventricular hypertrophy in hypertensive patients without cardiac hypertrophy. In hypertensive patients with left ventricular hypertrophy, factors other than blood pressure must be involved in the regulation of the cardiac mass.

In recent years, there has appeared increasing evidence suggesting the contributory role of arterial distensibility in the development and/or maintenance of cardiac hypertrophy in aging and hypertension. The deleterious effect of aging on the ventricular-vascular interaction has been investigated in several clinical studies. Merillon et al. [72] examined the interactions between the physical properties of the arterial system and the left ventricular performance, as a function of age in normotensive controls and in hypertensive patients. Their study demonstrated a close correlation between the degree of concentric cardiac hypertrophy, evaluated by the ratio of left ventricular mass to left ventricular end-diastolic volume, and the characteristic impedance of the ascending aorta in both groups. In that study, characteristic impedance was calculated from the relationship:

$$Z_C = \frac{\rho c}{\pi r^2} \qquad (1.11)$$

where Z_C = characteristic impedance, ρ = blood density taken as a constant, c = pulse wave velocity, r^2 = aortic radius squared.

Nichols et al. [84] demonstrated a definite increase in the pulsatile (characteristic impedance and reflections) and nonpulsatile (peripheral resistance) components of the left ventricular load with age. The increase in the characteristic impedance was reported to be 137% over the age range of 20 to 60 years. Results of the study also showed an increase in the fluctuations of the impedance modulus with advancing age. The investigators attributed these changes to increased arterial stiffness with age, and this may account for the mild left ventricular hypertrophy and prolonged relaxation seen in the elderly population. Prolonged relaxation is defined as delayed relaxation of the ventricular muscle during the cardiac cycle. Yin et al. [119] hypothesized that the prolonged ventricular relaxation may be a secondary phenomenon related to an age-associated increase in the left ventricular mass. The extended relaxation may be attributable to a prolongation of the cytosolic calcium transient, due to a reduced rate of calcium sequestration by the sarcoplasmic recticulum. The action potential is

prolonged, leading to delayed relaxation and a subsequent slower velocity of early diastolic left ventricular filling [120].

Bouthier et al. [108] hypothesized that the development and regression of cardiac hypertrophy in hypertension do not depend exclusively on the level of blood pressure, but are modulated by a number of factors, one of which is the state of the large arteries. They reported strong correlations between cardiac hypertrophy and arterial distensibility, using the ratio between left ventricular mass and left ventricular volume (M/V) and pulse wave velocity as representative indices. Using Doppler echocardiography, Dahan et al. [75] studied the consequences of age and hypertension on the left ventricular geometry and the physical properties of the aorta, as well as on the ventricular-vascular interaction. They observed a significant and positive correlation between pulse wave velocity (an index of arterial stiffness), left ventricular mass, and age, in normotensive, but not in hypertensive subjects. The left ventricular hypertrophy was significantly correlated with pulse wave velocity in both groups. These findings support previous reports that aging and hypertension have similar effects on the physical properties of the large arteries and the geometry of the left ventricle.

The work of Isnard et al. [127] lends further support to the hypothesis that a reduced arterial distensibility contributes to an increased left ventricular stress and mass. They have successfully shown that the aortic arch elastic modulus (an index of stiffness) was significantly associated with cardiac mass, even after adjustment for mean blood pressure. This finding suggests that increased aortic stiffness might influence the degree of cardiac hypertrophy, and that both age and blood pressure act independently as factors that alter the arterial wall of subjects with sustained essential hypertension.

A lack of correlation between blood pressure and myocardial hypertrophy demonstrated in both experimental animal models [122,123] and in clinical studies in humans [113,116,124], has led to the speculation that factors other than blood pressure might be responsible for the modulation of myocardial hypertrophy. Sens et al. [125] using

an *in vitro* assay system to study isolated myocytes from hypertrophied rat hearts, have successfully shown the existence of a soluble factor that stimulated protein synthesis. They speculated that this factor may play a key role in the modulation of myocardial structure during the development or regression of cardiac hypertrophy in hypertension. Nair et al. [126] showed that among the early changes in the mature Syrian hamster with cardiomyopathy, is an increase in DNA dependent RNA polymerase 1 and adenyl cyclase activity. The increase in adenyl cyclase activity persisted even after the development of cardiac hypertrophy and failure. These preliminary data present neither new nor conclusive results, but they provide some insight into the relationship between RNA polymerase, adenyl cyclase, and cyclic AMP in the genesis of increased cardiac mass. If augmented adenyl cyclase activity can indeed increase the myocardial cyclic AMP level, then one may speculate that changes in histone phosphorylation would affect the degree of depression of DNA, and this in turn would involve RNA transcription.

An adrenergic influence that contributes to cardiac hypertrophy was suggested more than seven decades ago. In 1912, Stewart [127] produced cardiac hypertrophy in rabbits by intravenous injection of adrenaline. Recent experimental evidence supports a direct association between left ventricular mass or thickness and noradrenaline. Laks et al. [128] provided the first clear evidence that noradrenaline in subhypertensive doses could directly induce ventricular hypertrophy in conscious dogs. Noradrenaline appears to be important for the induction of myocardial cellular growth. Sens and Tarazi [129] have demonstrated adrenaline- and noradrenaline-induced myocardial cell hypertrophy. *In vitro* experiments have also indicated that both of these catecholamines promoted protein synthesis [130] and that β-adrenergic blockade prevented catecholamine-induced hypertrophy. Thus, current data indicate that molecular mechanisms, in addition to the hemodynamic factors, may contribute to the development and/or maintenance of cardiac hypertrophy.

1.4 Summary of the literature review

It is clear from the literature review, the vasculature of the systemic arterial tree and the heart undergo structural and functional changes associated with increasing age. These changes are summarized as follows:

1. Definite histologic changes occur in aging in the systemic vasculature; these consist of a loss of endothelial uniformity, fragmentation, and degeneration of elastin with replacement by and proliferation of collagen.
2. In general, the vessel wall becomes less extensible, and the diameter and wall thickness of the aorta and the large arteries increase with advancing age.
3. The mechanical properties of the aorta and large arteries also change with age. The arterial distensibility has been observed to decrease with increasing age.
4. The maximum heart rate (HR_{max}) attained during exercise declines with increasing age.
5. The vascular smooth muscle appears to be less responsive to β-adrenergic stimulation with advancing age.

Chapter 2

Methods for Data Collection and Data Analysis

2.1 Introduction

In the following sections, the two cohorts selected for the present investigation, recording procedures, digitization and analysis of the computerized electrocardiographic (ECG) signals, pulse wave tracings, pulse wave contours and statistical analysis, will be described.

2.2 Material

The data consisting of blood pressures, total serum cholesterol levels, and the anthropometric measurements for this study were obtained from the Seven Countries Study. I received these data from Dr. A. Menotti of the Laboratory of Epidemiology and Biostatistics, National Institute of Health, Rome, Italy. Dr. Menotti is the custodian of the Seven Countries Study data. The computerized electrocardiographic signals and the pulse wave tracings were recorded in Dr. Wolf's laboratory, as one of the collaborators in the Seven Countries Study.

One of the study cohorts (Velika Krsna) included in the present study, is from a former province of Yugoslavia, now known as Slovenia, the other (Tanushimaru) from Kyushu, the southern most island of Japan. They will be referred to as the Yugoslavian and Japanese cohorts thereafter. These cohorts were chosen for reason of data availability. Recordings of the carotid-femoral pulse wave and the computerized electrocardiographic (ECG) signals were considered as special procedures in the Seven Countries Study. Among the 16 cohorts in the Seven Countries Study, only men of the Velika Krsna and Tanushimaru cohorts had undergone these special procedures in 1965 and 1968,

respectively. Logistically, it was impossible to make measurements of the pulse wave and special lead ECGs on all men of these two cohorts. Consequently, these measurements were made on 293 men.

Velika Krsna is a typical farming village of the older type. As elsewhere in the former Yugoslavia, registry of the population in Velika Krsna provided a roster of all men aged 40 through 59. Among a total of 571 men in the roster 552 (97%) were examined in the fall of 1962. The records of 41 men were removed due to error in age; the study cohort consisted of 511 men (93%). Tanushimaru is a farming village in southern Japan. At the time of subject recruitment, the population of Tanushimaru tended to be stable in residence. Every Japanese adult must be locally registered; good local registries were maintained of births, deaths, residents, and voters. Consequently, it was easy to establish a reliable roster of all men aged 40-59 in a given locality. In Tanushimaru, all the men on the roster were examined. The record of one man was removed because of an error in age. Consequently, the Tanushimaru cohort comprised 508 men (99.8% of the initial roster).

2.3 Recording procedures

The procedures and methods for collecting data of anthropometry, blood pressure, serum cholesterol, and life-style during the initial, 5- and 10-year follow-up examinations, have been described in detail in Chapter 1.2. In the present study, I analyzed data of blood pressure, serum cholesterol, standing and sitting height that were obtained during the first 10 years of the study in the Yugoslavian and Japanese cohorts. In addition, data of the pulse wave and the Frank-lead electrocardiogram recorded in Dr. Wolf's laboratory were also used in the analyses. The following is a time line for the procedures used for collecting data in these two study cohorts.

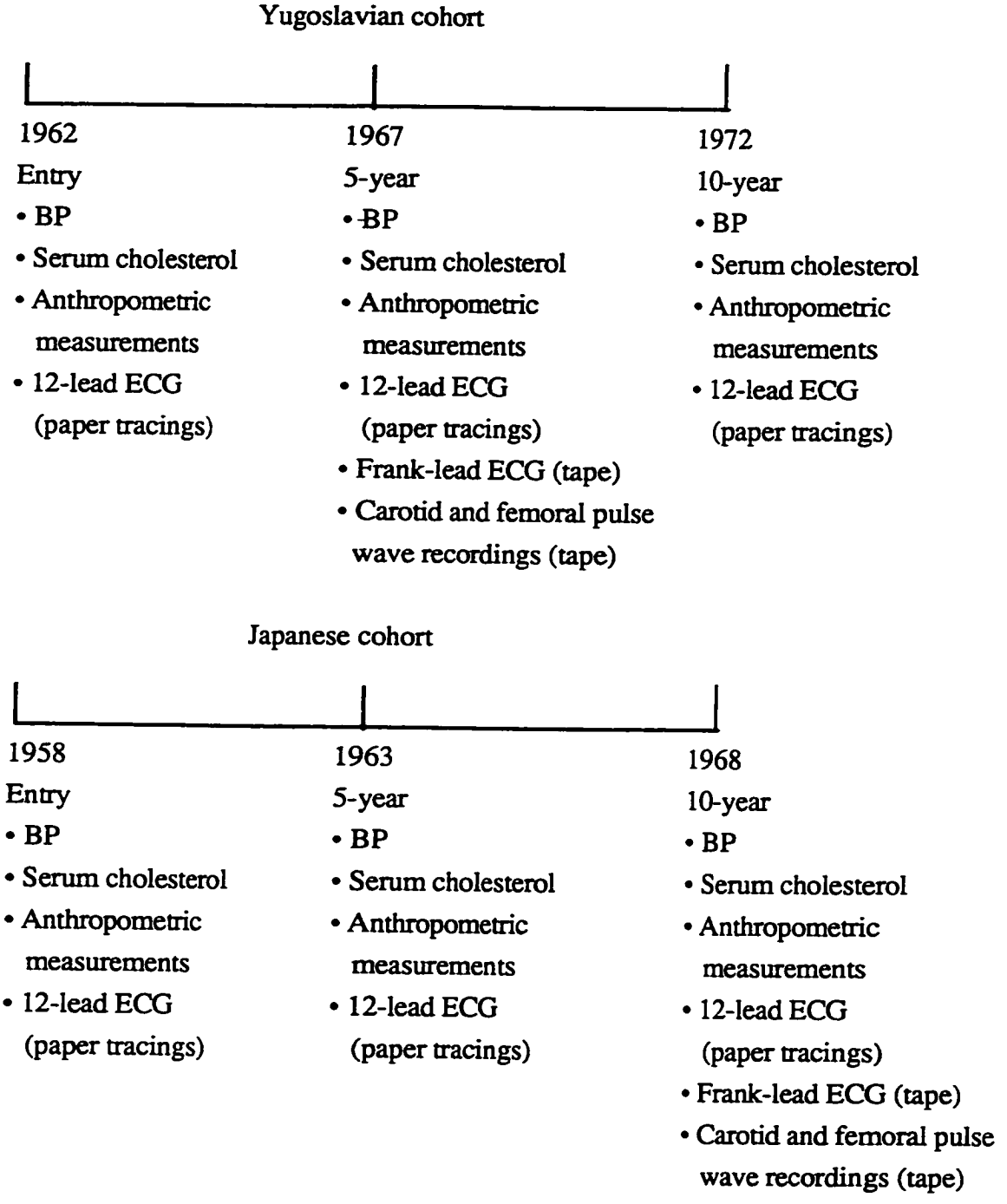


Figure 2.1 A sketch of the time line for data collection in the Yugoslavian and Japanese male cohorts.

2.3.1 Blood pressure measurements

The entry blood pressures were measured in the Yugoslavian cohort in the fall of 1962, and in the Japanese cohort in the spring of 1958. These measurements were repeated during the 5- and 10- year follow-up survey.

2.3.2 Electrocardiographic measurements

The computerized electrocardiogram (ECG) recordings using the Frank-lead system were made on magnetic tapes to allow for computer analysis, during the 5-year follow-up in the Yugoslavian cohort, and the 10-year follow-up in the Japanese cohort. Supine ECG records were made with placement of electrodes at a level corresponding to the electrical center of the heart according to the Frank-lead system [131]. Electrode A and I are positioned in the left and right midaxillary lines. Electrode E and M are positioned on the sternum and spine, respectively. Electrode C is positioned so that an angle of 45 degrees is produced with respect to the center of the thorax. Electrode H is usually placed on the back of the neck, and electrode F is positioned in the left foot (Figure 2.2). This special lead arrangement permitted the Frank vectorcardiograms to be made. A transformation from Frank to standard 12 leads was then performed by computer, by a linear combination of the Frank XYZ signals, using weighting coefficients calculated by statistical means [132].

The Frank XYZ leads have become the most commonly used vector lead system. If one accepts that the Frank system accurately measures all components of cardiac electrical activity, then a transformation of Frank XYZ leads to standard 12 leads is feasible. The 12-lead signals are necessary to use the NOVACODE module of the DALECG program to estimate the left ventricular mass index [133]. The NOVACODE module takes the 12-lead ECG measurements to calculate the left ventricular mass indexed for body surface from an established multivariate model [133]. The NOVACODE module of the DALECG program will be described in detail in section 2.4.1.2.

Orkin et al. [134,135] also used standard 12-lead ECGs and orthogonal lead signal-averaged ECGs to evaluate the accuracy of the time-voltage integral of the QRS complex for the identification of left ventricular hypertrophy. Findings of their investigations suggest that the use of time-voltage integral of QRS complex can improve the accuracy of ECG methods for identification of cardiac hypertrophy.

In the computerized electrocardiographic recordings, six simultaneous signals were recorded from the 6 lead-off points of the Frank system. The Frank neck electrode H was used as the common reference. Signals from electrode E were recorded on channel 1 of the Ampex tape recorder, electrode A on channel 2, electrode I on channel 3, electrode M on channel 4, electrode C on channel 5, and electrode F on channel 6. These signals were recorded as the potential differentials between index electrode and electrode H.

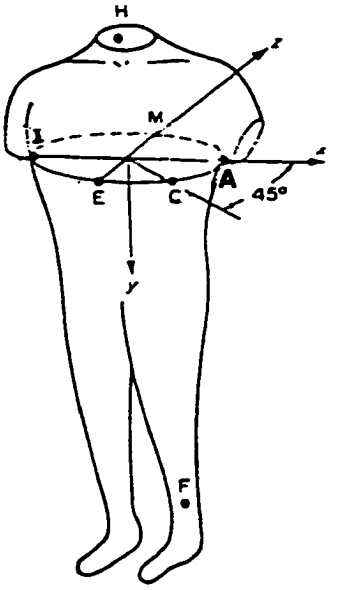


Figure 2.2 A sketch of the Frank electrode placement. A, I, E, M, C, H, and F are the 7 Frank electrodes used for recording vector cardiograms [131].

2.3.3 Pulse wave recordings

The carotid and femoral pulse waveforms were recorded in Dr. Wolf's laboratory on a 7-channel Ampex tape recorder (model FR-1300), with six different tape speeds. The speed of 1 7/8 inches per second (ips) was used to record pulse wave tracings of Yugoslavian men, and 7 1/2 ips of Japanese men. A Sanborn Apexcardiograph displacement transducer was placed over the external carotid and affixed with a strap around the subject's neck. An identical transducer was placed over the femoral artery, and was affixed with a specially designed device that exerted sufficient pressure to permit good waveform recording. The femoral and the carotid pulse waves were recorded simultaneously for approximately 20 seconds on channel 1 and 2, respectively, and the ECG signals on the remaining four channels. The right side pulse was recorded first, followed by the left side pulse waves. Pulse wave records were made during the 5-year follow up in Yugoslavia and during the 10-year follow up in Japan. Figure 2.3 shows a 5-second "raw" pulse wave signals along with the simultaneously recorded ECG signals from a male subject in the Yugoslavian cohort.

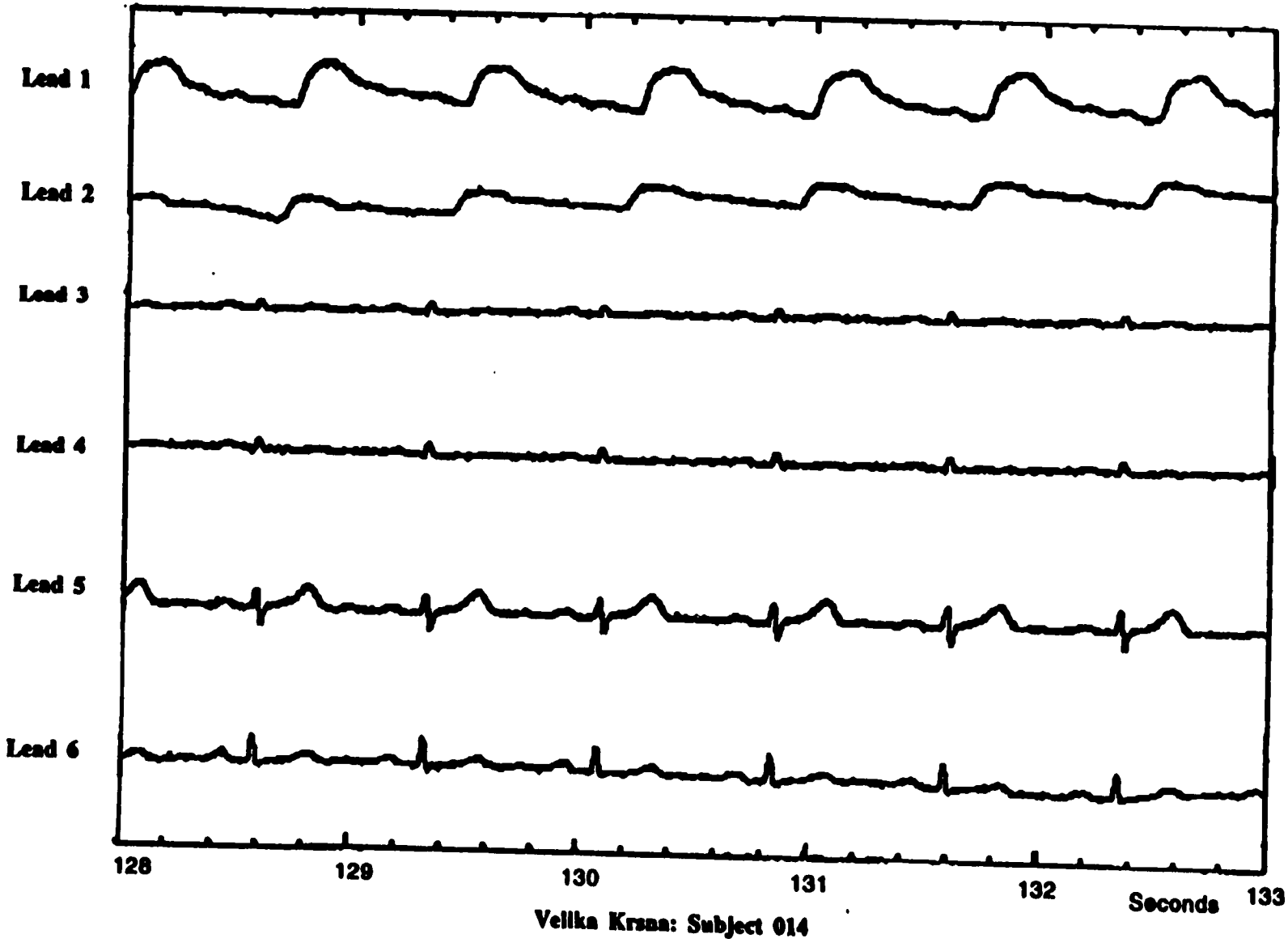


Figure 2.3 A 5-second "raw" pulse wave signals from a subject in the Yugoslavian cohort. Lead 1 records the femoral pulse wave signals, and lead 2 records the carotid pulse wave signals. Leads 3-6 record the simultaneously recorded ECG signals.

2.4. Digitization, processing, and analysis procedure

2.4.1 Digitization and processing of the ECG signals, and estimation of the left ventricular mass index

The procedure for preliminary data handling of the ECG signals is based on a modified version of the Dalhousie ECG program (DALECG), version 3.0. Figure 2.4 shows the major processing steps. This program is capable of handling six signals (four ECG leads and two arterial pulse). All of the data reported in this thesis used the six signal version and will be described in detail. The major steps up to and including estimation of the left ventricular mass index follows the principal logic of the Dalhousie ECG program which has been described elsewhere [133,136].

2.4.1.1 Sampling

The six signals from the analog tape are sampled simultaneously at a rate of 500 samples per second per signal. Prior to sampling, the signals are filtered with a low-pass filter with a cutoff frequency of 125 Hz and an attenuation slope of 24 db per octave. This is done to avoid aliasing which would introduce additional noise into the system. The sampled data are stored on disk for subsequent processing.

2.4.1.2 Digitization and signal averaging process

I digitized the ECG signals with a PC computer (Model Compaq 386), with a DT 2821 data translator at a rate of 500 samples per second per signal, with a 12-bit resolution. The digitized signals were displayed on the computer screen for the purpose of selecting 10-second segment of the tracings which contain signals of the best quality and with the least noise.

Processing of the selected segment of digitized ECG signals was done with the DALECG program that occurs in an interactive mode. When a data file containing digitized signals is presented for analysis, the program offers several processing options for

selection: the presented lead groups to be analyzed, the number of seconds of samples to be skipped, and the number of seconds to be analyzed, printed or plotted output for debugging purposes, one-or-two second averaging windows to be used, standard deviation cycles to be produced for each averaged cycle produced, and the naming of lead groups and/or leads. After an initial scanning of the input signal to identify QRS complexes, the program assigns each complex to a beat family. Family assignment is performed in the following manner. The candidate beat is time-aligned with the family template by calculating the covariance between one of the family's high-amplitude leads, and that same lead in the candidate beat. Similarity tests are performed on each lead used for family assignment, by locating in each comparison lead of the family template the top 10 consistently detected rises and falls. Each subsequent beat is compared with the templates for all existing families, and is either added to one of these families' accumulators or used to start a new family. After all detected beats have been assigned to families, one family is selected as the representative family for detailed analysis in which the global onsets and offsets are determined for P, QRS, and T waves.

Random noise levels in the ECG signals, movements and artifacts are factors which cause inconsistencies in measurements. These factors do not correlate with the ECG signals and can be reduced by the time coherent averaging technique [137]. This technique helps to improve the signal-to-noise ratio for almost any noise pattern without distorting the signal. It reduces the noise levels in the signals in proportion to the square root of the number of segments of QRS complexes.

The measurement program uses a large number of variables and arrays which are grouped by function into FORTRAN COMMON areas. Each COMMON area has a single occurrence in memory, but some COMMON areas have multiple occurrences, for example, one for each lead-group, or one for each family. The additional occurrences are held in the "swap" file on disk with only the current occurrence occupying the COMMON area in

memory. Upon the completion of the program, all COMMON areas in memory are flushed to the "swap" file and this file is considered to be the output of the measurement program.

The NOVACODE module of the DALECG program has the following characteristic features: a modernized version of the Minnesota Code, supplemented by several newer ECG classification schemes suitable for computer coding of the rest and exercise ECGs, and improved statistical models for enhanced detection of myocardial infarction (MI), and for quantification of the left ventricular mass index. The NOVACODE module estimates the left ventricular mass indexed to body surface area (g/m2) based on an established multivariate model using echocardiographic measurements as a standard [136]. Wolf et al. [137] reported a moderate correlation between echocardiographic and ECG estimates of left ventricular mass index ($R^2 = 0.58$ for men and $R^2 = 0.42$ for women). The regression equation used for estimating the left ventricular mass index of men is :

$$\text{Left ventricular mass index } (g/m^2) = -36.4+0.010R\times(V5)+0.020\times S(V1)+0.028\times S^{\ddagger}III +0.182Tneg(V6)-0.148Tpos(aVR)+1.049\times QRS \quad (2.1)$$

Where $S^{\ddagger}$ = S or Q or QRS amplitude, whichever is larger. Amplitudes (absolute values) are in microvolts and QRS duration in milliseconds.

I submitted the averaged ECG complex from each subject held in the "swap" file, to the NOVACODE module of the DALECG program for estimation of the left ventricular mass index using the above regression equation. These measurements were used later for analyzing the effect of age on the cardiac mass in the Yugoslavian and Japanese cohorts.

2.4.2 Processing of the pulse wave signals

I also digitized the pulse wave signals in a manner similar to that used in digitizing the ECG signals (see Figure 2.4). It was necessary to average the pulse wave signals in order to reduce the noise levels, thus allowing me to make the precise measurements possible.

The digitized pulse wave signals were processed with the DALECGPULSE program, a modified version of the computer program developed at Dalhousie University for automatic measurement of the principal systolic time intervals from carotid pulse-wave and heart sound recordings made simultaneously [139]. This computer program has the built-in characteristic design to allow the carrying of ECG and non-ECG (pulse wave) signals. The pulse wave signals, however, must be synchronized with the heart beat. The DALECGPULSE program uses the ECG signals to develop the time-averaged pulse wave complex. For the purpose of this thesis, a separate algorithm was written to mark the rapid systolic upstroke in the carotid and femoral pulse. It defines IT1 (a variable) as the first point at least 200 milliseconds from the start of the pulse wave signal where the signal has a positive value. Because some of the recorded signals have a high level of noise, the algorithm smoothes them by using a center weighted five-point smoother, with the following weights: -3/35, 12/35, 17/35, -3/35 for S(t minus 2), S(t minus 1), S(t), S(t+1), and S(t+2) respectively. It is assumed that the sampling interval is two milliseconds, so that smoothing range is from t minus 4 milliseconds to t+4 milliseconds. This smoother is applied three times. The algorithm scans the signal from IT1 to the end, setting ITIM to the point where the signal attains a maximum. If the value of the maximum is equal or less than zero, then it will report "NO UPSTROKE" and stop. It also scans backwards from ITIM, finding the first point I where the signal is less than +100 microvolts, and the signal values of the points 4 milliseconds and 2 milliseconds preceding the Ith point are equal to or greater than the signal values at point I. It then defines STP as the S(I) minus 9 microvolts. I must also have the signal values 24 milliseconds and 22 milliseconds preceding I, both equal to or greater than STP minus 13 microvolts, and the signal values 12 milliseconds and 10 milliseconds preceding I, both equal to or greater than STP. If no such point I can be found before searching IT1, then set I equal to IT1. The upstroke is defined as I + 4 milliseconds.

After the analysis has been completed, the averaged signals are displayed on the screen of the computer terminal with vertical lines indicating the various time points. Figure

2.5 shows an example of such a display. I have the option of rejecting the run and re-trying the analysis on a fresh signal, making on-the-spot corrections, or accepting the analysis results. These corrections may involve the remarking of the beginning of rapid systolic upstroke of the carotid or femoral pulse wave, or both. Figure 2.6 depicts the unedited and edited signals of the carotid and femoral pulse. The data for the averaged complex along with the measurement results are copied to digital magnetic tape for further analysis.

The DALECGPULSE performs better with the Japanese pulse wave tracings, less than 35% of them require on-the-spot correction of one or all the measurements. On the other hand, more than half of the Yugoslavian tracings require on-the-spot corrections. This may be due to the inferior quality of the pulse wave tracings I observed in the Yugoslavian cohort; the pulse wave signals were very noisy. The presence of excessive noise in the signals may affect the performance of the program.

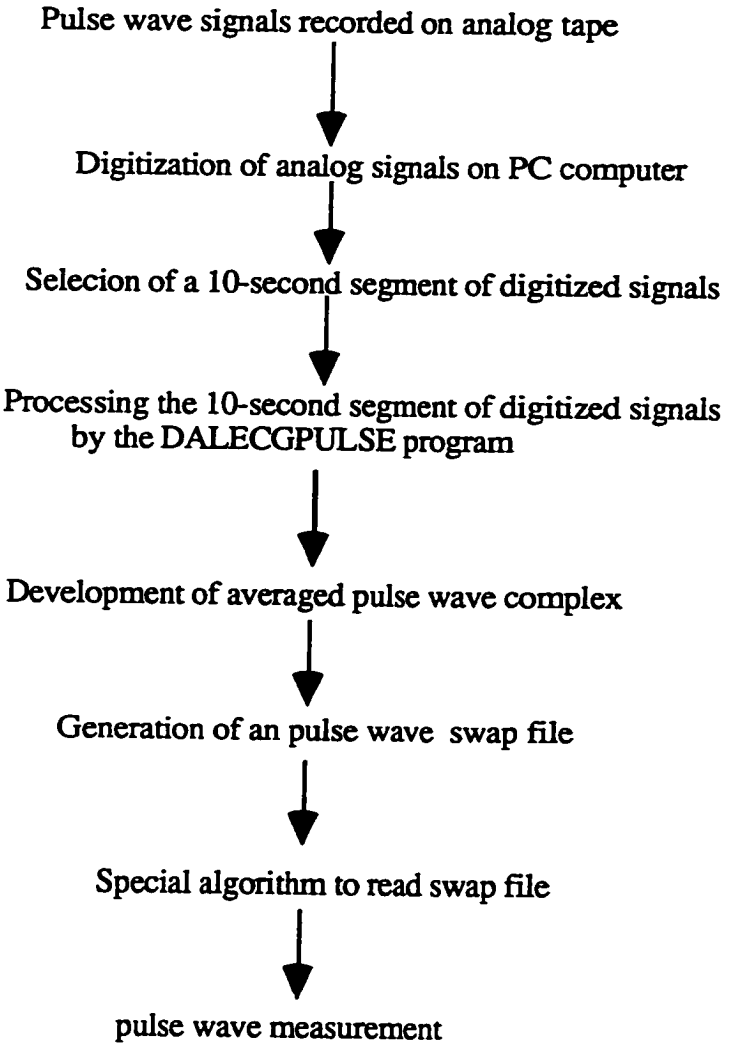


Figure 2.4 Processing steps of the pulse wave signals.

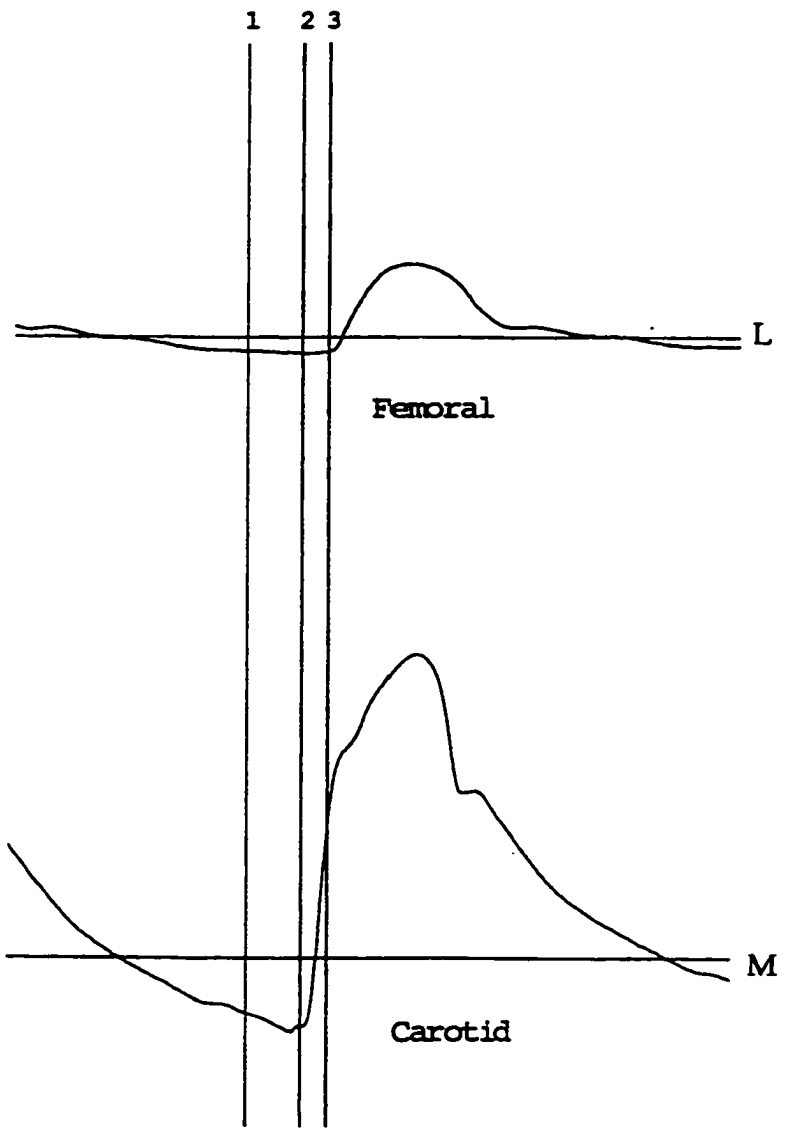


Figure 2.5 Computer display presented to the operator by the DALECGPULSE program. The time points identified by the program are indicated by the vertical lines on the plotted signals. Vertical line 1 represents the point of onset of Q-wave in the ECG that is not displayed, line 2 indicates the point of onset of rapid systolic upstroke of the carotid pulse, and line 3 the point of onset of rapid upstroke of the femoral pulse wave. Signal L records the femoral pulse wave signals on channel 1 of the Ampex tape recorder. Signal M records the carotid pulse wave signals on channel 2 of the Ampex tape recorder. The amplitudes shown in this graph have been dynamically scaled to allow for a clear display of the pulse wave signals. They do not represent the true amplitudes.

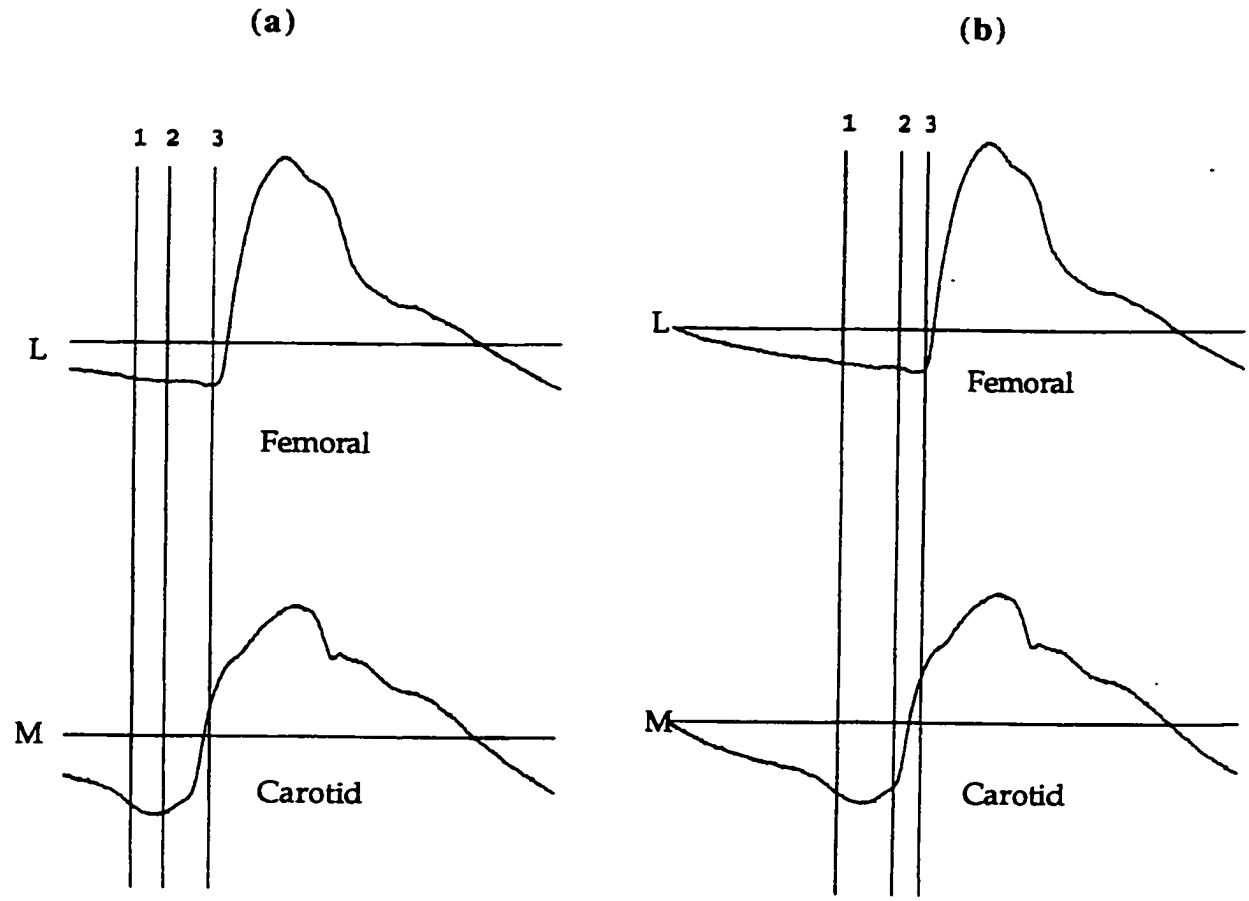


Figure 2.6 (a) The unedited signals of one subject's carotid and femoral pulse wave; (b) The same signals have been edited by the computer operator. The time points identified by the program are indicated by the vertical lines on the plotted signals. Vertical line 1 represents the point of onset of Q-wave in the ECG that is not displayed. Line 2 indicates the point of onset of rapid systolic upstroke of the carotid pulse, and line 3 the point of onset of rapid upstroke of the femoral pulse. Signal L records the femoral pulse wave signals on channel 1 of the Ampex tape recorder. Signal M records the carotid pulse wave signals on channel 2 of the Ampex tape recorder. The amplitudes shown on this graph have been dynamically scaled to allow for a clear display of the pulse wave signals. They do not represent the true amplitudes.

2.5 Analysis of pulse wave contour

2.5.1 Computer simulated pressure waves

The measured pulse waves can be separated into their forward (incident) and backward (reflected) component [140]. The measured pulse wave is equal to the sum of the forward and backward waves. The forward waves travel from the heart toward the periphery, and the backward waves are composite waves, with the many individual waves returning from different locations, lumped into these waves [140].

Wave reflection is a major determinant of the arterial pulse wave contour, and is influenced by two factors: 1. the arrival time of the backward waves, and 2. the amplitude of these waves. To demonstrate the interactive effects of these two factors on the pulse wave contour, I used the computer to simulate a series of pulse waves with various arrival times and amplitudes of the reflected waves. Figure 2.7 presents a few of these waves.

In my series of simulated waves, early systolic shoulder of type A waveform appears when the backward waves (P_b) return to the heart 0.07 second after the forward waves (P_f) travel toward the periphery. The amplitude of the backward waves is 18% of the forward wave (Figure 2.7a). This early systolic shoulder becomes prominent when the arrival time of the backward waves is 0.09 seconds, and the amplitude increases to 30% of the forward wave (Figure 2.7c). The combination of an arrival time of 0.105 seconds and an amplitude of 17% of the forward wave, produces a type D wave contour (Figure 2.7b). Type C waveform is generated when the reflected wave returns to the heart 0.13 seconds after the initial forward wave, and the amplitude of the backward wave is 20% of the forward wave (Figure 2.8d).

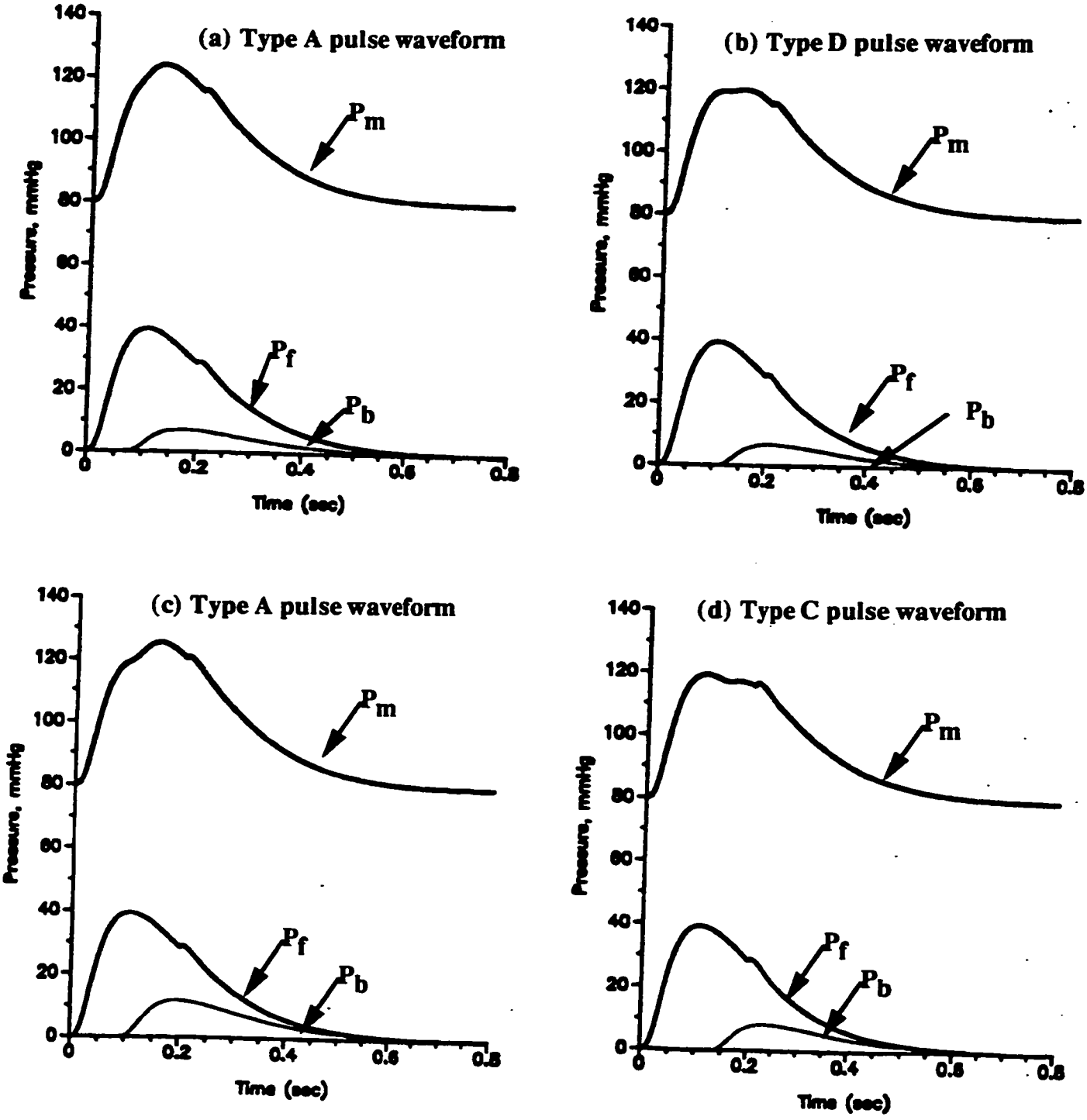


Figure 2.7 A series of computer simulated pulse waves; (a) shows the appearance of the early systolic shoulder of type A pulse waveform, (b) shows a type D pulse waveform where the inflection point is superimposed on the early systolic shoulder, (c) presents a type A waveform in which the early systolic shoulder becomes prominent, and (d) presents a type C pulse wave contour. P_m = simulated measured pulse wave, P_f = simulated forward wave, and P_b = simulated backward wave.

2.5.2 Algorithm for analyzing the pulse wave contour

Kelly et al. [86] designed a computer algorithm with which they identified the two distinct contour features automatically, from the time derivatives of the pressure wave: the beginning of the pressure wave upstroke and the shoulder of the pressure wave. They defined this shoulder as the first concavity on the upstroke of the wave that separates the initial pressure rise from the late systolic peak. The algorithm uses the fourth derivative and identifies the first zero crossing, in a direction from above to below the zero line, as the beginning of the systolic upstroke. The second zero crossing in the fourth derivative identifies the shoulder as the beginning of the second wave.

Based on Kelly et al.'s algorithm, a computer program was written for the present investigation to plot the first, second, third, and fourth time derivative, and to determine in the fourth derivative, if the first zero crossing accurately detected the beginning of the pressure wave upstroke. In addition, I also wanted to find out if the second zero crossing in the same direction corresponded to the shoulder, as described by Kelly et al. [141]. In a trial run of 12 pulse waves, Kelly's algorithm as implemented by myself could accurately detect these two contour features in only one waveform. In the remaining waves, neither the first nor the second zero crossing in the fourth derivative, correctly identified the beginning of the systolic upstroke and the shoulder. Several revisions to the computer algorithm had to be made before a usable program was produced. Now, this program automatically, objectively, and consistently identified the pulse wave upstroke, the inflection point (the onset of the return of reflected waves), the peak of systolic pressure, and the systolic shoulder of the pressure wave which separates the initial pressure rise from the systolic peak. The carotid upstroke was identified in the manner described in section 2.4. The inflection point was detected at the negative to positive zero crossing of the second difference. The absolute late systolic shoulder was the highest value in the averaged data in a window which started at the onset of the rapid systolic upstroke and ended at the onset+400 msec. To locate this point,

the algorithm searched for a positive to negative zero crossing in the first difference. Figure 2.8 illustrates how these points are identified by the computer algorithm.

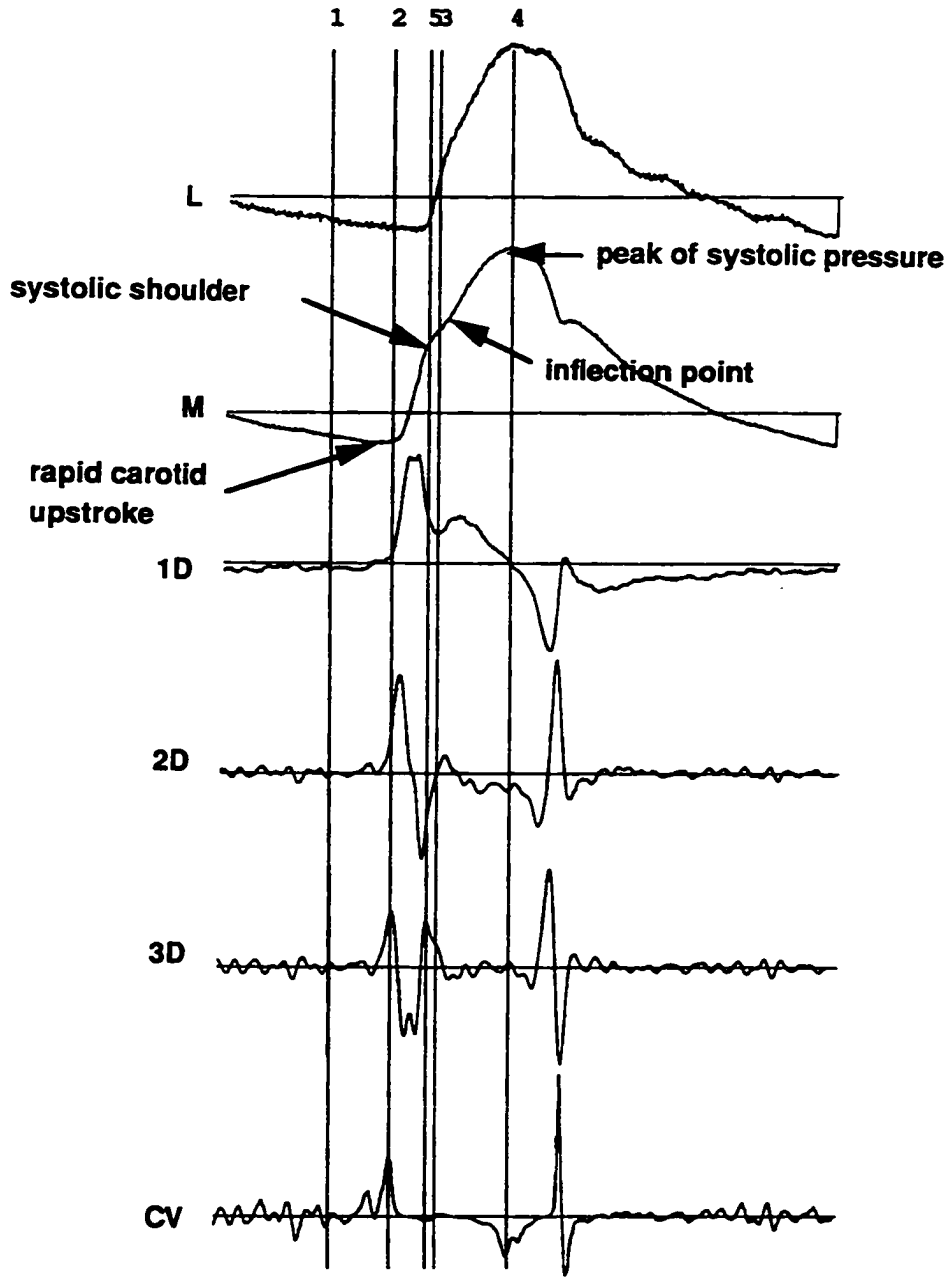


Figure 2.8 A program output of the carotid pulse waveform, showing how the computer algorithm identifies the carotid systolic upstroke, the systolic shoulder, the inflection point, and the peak of systolic pressure. Signal L records the femoral pulse wave signals on channel 1 of the Ampex tape recorder. Signal M records carotid pulse wave signals on channel 2 of the Ampex tape recorder. Signal 1D is the first difference, signal 2D the second difference, signal 3D the third difference, and CV the curvature. The amplitudes shown on this graph have been dynamically scaled to allow for a clear display of the pulse wave signals. They do not represent the true amplitudes.

Since the early systolic shoulder of the pulse wave consisted of neither apparent peaks nor valleys, my algorithm was unable to detect this point, although its location was visually obvious to me. Consequently, I felt that using the curvature would be the best way to detect the shoulder in my pulse waves. The curvature was calculated according to the following equation [142]:

$$c = y''/(1+y'^{**}2)^{**}(3/2) \qquad (2.2)$$

where $y' = dY/dX$, and $y'' = d^2Y/dX^2$.

The equation for curvature [142] is converted from its differential form to a difference form to be applicable to a sampled signal. It involves introducing a scaling factor that depends on the sample interval and the units of signal amplitude per unit of sampled value. The scaling factor for my data was determined by trial-and-error to be:

sf= (# of samples between pulse wave peak and onset)*2.4/(sample numbers at peak - sample numbers at onset)

$c= sf*y''/(1+(sf*y')^2)^{3/2}$

y' and y" are calculated from consecutive amplitude differences, applying a low-pass filter with zero lag at each step.

I encountered two different patterns of the systolic shoulder placement; in one pattern, the shoulder was placed before the inflection point, while in the other, it was located after the inflection point (Figure 2.9). In view of this variation, two strategies were used for detecting the shoulder: 1. at the minimum curvature in the window before the inflection point, and 2. at the minimum curvature in the window after the inflection point. The placement of the window was determined by the location of the absolute late systolic peak, i.e. point 4.

Figure 2.10 is a sample of the program output, showing the time points of the following: Q onset, beginning of the pressure wave upstroke, the inflection point, the absolute late systolic peak and the systolic shoulder.

The computer algorithm accurately identified the carotid systolic upstroke and the peak of systolic pressure in all pulse waves. However, it did not perform with 100% accuracy in marking the inflection point as well as the systolic shoulder. Of 583 pulse waves obtained in the two cohorts, the program accurately identified these two points in 560 waves (96%). An editing program was used to visually identify the inflection point and the systolic shoulder in the remaining 23 waves (4%). Figure 2.11 shows an example of an unedited and edited pulse waveform.

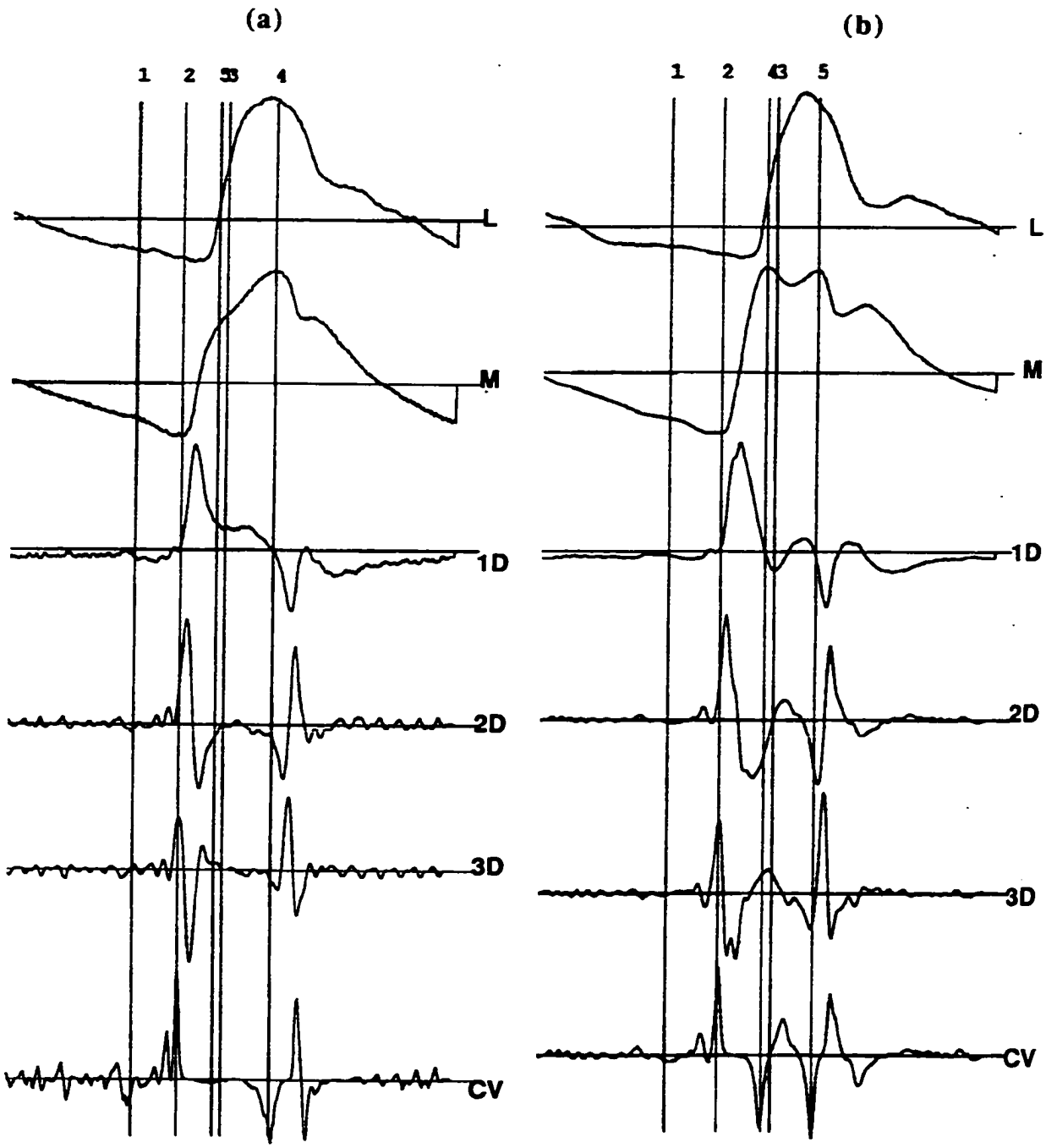


Figure 2.9 A program output of the carotid pulse waveform (signal M) analysis, showing (a) the placement of the systolic shoulder before the inflection point (3), and (b) the placement of the systolic shoulder after the inflection point (3).Signal L records the femoral pulse wave signals on channel 1 of the Ampex tape recorder. Signal M records the carotid pulse wave signals on channel 2 of the Ampex tape recorder. Signal 1D is the first difference, signal 2D the second difference, signal 3D the third difference, and CV the curvature. The amplitudes shown on this graph have been dynamically scaled to allow for a clear display of the pulse wave signals. They do not represent the true amplitudes.

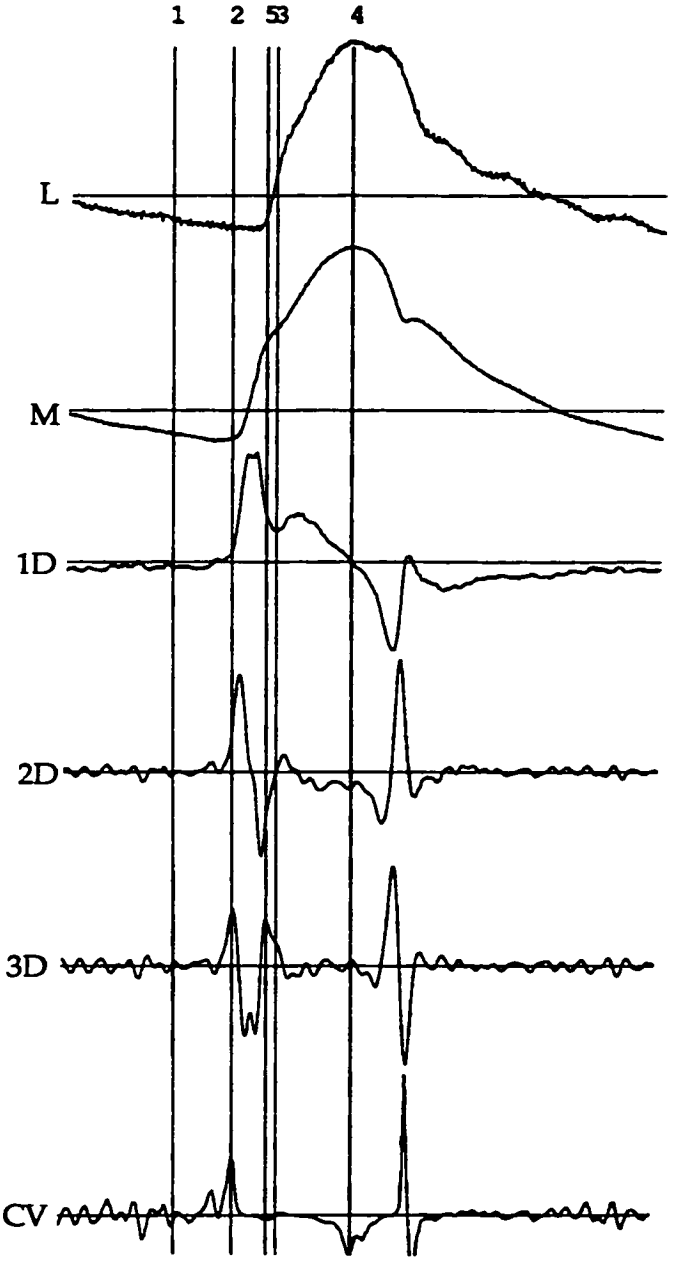


Figure 2.10 A program output of the carotid pulse waveform analysis, showing the time points. The line 1 indicates the point of onset of Q-wave in the of the ECG that is not displayed. Line 2 represents the beginning of the rapid systolic upstroke, line 3 the inflection point, line 4 the peak of systolic pressure, and line 5 the systolic shoulder. Signal L records the femoral pulse wave signals on channel 1 of the Ampex tape recorder. Signal M records the carotid pulse signals on channel 2 of the Ampex tape recorder. Signal 1D is the first difference, signal 2D the second difference, signal 3D the third difference, and CV the curvature. The amplitudes shown on this graph have been dynamically scaled to allow for a clear display of the pulse wave signals. They do not represent the true amplitudes.

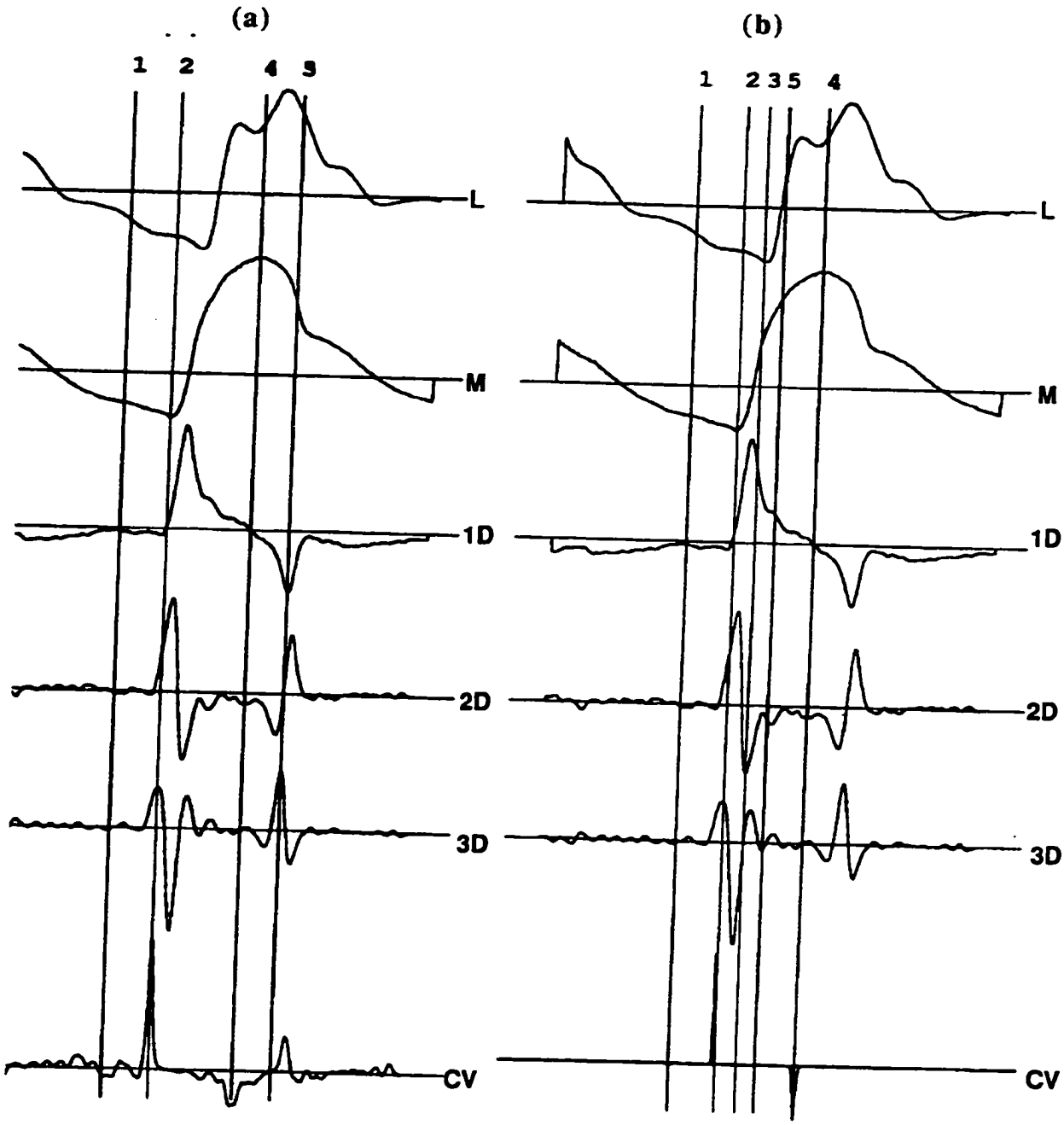


Figure 2.11 A program output of (a) an unedited and (b) edited carotid pulse waveform analysis, showing the various time points. The line 1 indicates the point of onset of Q-wave in the ECG that is not displayed. Line 2 represents the beginning of the rapid systolic upstroke, line 3 the inflection point, line 4 the peak of systolic pressure, and line 5 the systolic shoulder. Signal L records the femoral pulse wave signals on channel 1 of the Ampex tape recorder. Signal M records the carotid pulse wave signals on channel 2 of the Ampex tape recorder. Signal 1D is the first difference, signal 2D the second difference, signal 3D the third difference, and CV the curvature. The amplitudes shown on this graph have been dynamically scaled to allow for a clear display of the pulse wave signals. They do not represent the true amplitudes.

2.6 Statistical Analysis

Descriptive statistics (means, standard deviations, standard errors, and range) were calculated according to standard statistical methods [143]. The primary objective of the present investigation was to determine the effects of age on several physiological variables related to the arterial tree and the heart, namely : blood pressure, the pulse transit time, wave reflections, and the cardiac mass. To attain this objective, multiple regression analyses were performed using the least-squares method. This statistic is chosen because of its capability to simultaneously study the effects of age on those dependent variables of interest. The application of analysis of variance is inappropriate because this statistic tests the mean differences among groups, but cannot test the contribution of age to blood pressure change, the status of the vascular wall, or the left ventricular mass index. The univariate regression analysis method studies one dependent variable at a time. A significant effect that only becomes apparent when all the dependent variables are studied simultaneously may not be discovered from the univariate analyses alone.

The multiple regression analysis statistic is founded on the assumption that the data were normally distributed. The application of this statistic necessitates data transformation if the variables are not normally distributed. In the present investigation, the Shapiro-Wilk test was used to test for normality of the data distribution. To determine whether to reject the null hypothesis that the data values are a random sample from a normal distribution, it is only necessary to examine the probability associated with the test statistic (Prob<W) [144]. For the purpose of this thesis, a Prob<W of less than 0.3 was chosen to reject the null hypothesis of normality. The W statistic is the ratio of the best estimation of the variance to the usual corrected sum of squares estimation of the variance. Data which were not distributed normally, were transformed by either the logarithmic or exponential transformation. The logarithmic and exponential method of data transformation are two commonly used methods for stabilizing variances, producing relationships and providing more nearly normal distribution, a requirement of many statistical tests. The choice of

logarithmic or exponential transformation was guided by the value of the W statistic. If manipulation of the input data with the logarithmic method produces a value of the W statistic greater than 0.3, then the data are considered to have a normal distribution. If, on the other hand, this transformation failed to produce a higher than 0.3 probability, the exponential transformation was used. Data transformation was carried out before the analyses. Both paired and unpaired differences in means were assessed by the Student's t test. A p value < 0.05 was considered significant in single variable comparisons. Bonferroni correction for multiple variable analyses was used to determine the level of significance. The statistical analyses were carried out using the SAS (Statistical Analysis Software) program package.

Cook's D influence statistic was used to isolate peculiarities in the data. This statistical measure, developed by Cook [145] is based on the confidence ellipsoids for judging the contribution of each data point to the determination of the least squares estimate of β, the regression coefficient of the unknown parameters. Cook stated that the influence of the observations in the linear regression be judged by using the distance measure (Di). A large value of the Di indicates that the associated observation has a strong influence on the estimate of β. Cook's D measures the change to the estimates that results from deleting each influential observation. Outliers or observations with a large value of the Di were removed from the linear regression model.

Chapter 3

Aging Trends in Blood Pressure

3.1 Introduction

Follow-up blood pressure data available for the study of age trends in free-living populations are usually subject to a number of potentially biasing influences. The most common one is that the protocol is not precisely replicated at each follow-up measurement. But even with exact protocol replication, other factors besides aging can influence the study cohort to alter the blood pressure trends. Examples are change in life style or socioeconomic status or change in body weight which is also associated with blood pressure changes. Furthermore, the cohort may change its profile through a loss of individuals to follow-up.

For my study of blood pressure aging I used the follow-up blood pressure data of the Japanese and Yugoslavian cohorts of the Seven Countries Study [3]. The objective of the present investigation was to determine the part of the change in blood pressure observed during the 10-year observation period that was due to biological aging per se. This involved: 1. the estimation of bias caused by a loss to follow-up and, 2. the separation of blood pressure trends due to aging and those due to the cohort effect. For the purpose of this investigation, I defined cohort effect as the sum of all factors that were not measured by the study protocol but cause a particular age group to have different blood pressure values in different observation years. Thus, changes in body mass index (BMI) are excluded from this effect, since they have been measured by the Seven Countries Study. I further defined that the cohort effect influences all members of a cohort equally in a particular observation year. Using the data from Table 3.1 as an example, I demonstrated that the trends due to the cohort effect and possible body weight changes, account for the changes in systolic pressure for the 50-54 year age group from 133.34 mm Hg in 1962 (95% confidence interval:

130.15 and 136.53 mm Hg) to 128.67 mm Hg in 1967 (95% confidence interval: 124.93 and 132.41 mm Hg) to 136.78 mm Hg in 1972 (95% confidence interval: 133.04 and 140.52 mm Hg). Without the cohort effect, one would expect this age group to have identical blood pressure values, except for changes due to body mass index or random fluctuations. In Section 3.2, I presented the blood pressure data of the Seven Countries Study; in Section 3.3, I assessed the differences in cross-sectional and longitudinal data, and in Section 3.4, I explored the cohort effect.

3.2 Blood pressure data for the Yugoslavian and Japanese cohorts of the Seven Countries Study

Tables 3.1 and 3.2 present the means and standard errors of blood pressure of 4 male birth cohorts of Yugoslavia and Japan. Birth cohorts were defined as 5-year age groups at the time of entry to the study, that is, those born between 1918-1922 are in birth cohort 1, 1913-1917 in birth cohort 2, etc.

Table 3.1 The mean values and standard errors of (a) systolic and (b) diastolic blood pressure of four age groups of men in the Yugoslavian cohort.

(a) Systolic

Age at time of measurement	Entry (1962)	5-yr (1967)	10-yr (1972)
40-44	124.51 (±1.11) n=139	–	
45-49	128.89 (±1.74) n=84	122.83 (±1.91) n=127	
50-54	133.34 (±1.63) n=136	128.67 (±1.91) n=78	136.78 (±1.90) n=126
55-59	137.27 (±1.71) n=152	133.34 (±1.70) n=126	144.25 (±2.65) n=77
60-64		133.75 (±1.62) n=131	146.96 (±2.01) n=113
65-69			148.94 (±1.99) n=119

(b) Diastolic

40-44	79.58 (±0.81) n=139		
45-49	81.31 (±0.91) n=84	79.06 (±0.93) n=127	
50-54	82.42 (±0.84) n=136	81.08 (±1.31) n=78	84.70 (±1.12) n=126
55-59	83.72(±0.94) n=152	81.22 (±0.81) n=126	84.62 (±1.41) n=77
60-64		81.38 (±0.96) n=131	83.76 (±0.99) n=113
65-69			83.30 (±1.15) n=119

Table 3.2 The mean values and standard errors of (a) systolic and (b) diastolic blood pressure of four age groups of men in the Japanese cohort.

(a) Systolic

Age at time of measurement	Entry (1958)	10-yr (1968)
40-44	121.49 (±1.79) n=92	
45-49	128.00 (±1.83) n=120	
50-54	132.88 (±1.83) n=139	124.77 (±1.99) n=87
55-59	144.04 (±2.3) n=157	129.98 (±2.42) n=104
60-64		141.51 (±2.61) n=120
65-69		146.71 (±2.88) n=123

(b) Diastolic

40-44	66.42 (±1.28) n=92	
45-49	72.08 (±1.10) n=120	
50-54	74.56 (±1.15) n=139	72.22 (±1.23) n=87
55-59	78.45 (±1.20) n=157	75.77 (±1.20) n=104
60-64		78.59 (±1.23) n=120
65-69		79.24 (±1.27) n=123

Two observations may be made on these data. First, for each birth cohort of Yugoslavian men living in Velika Krsna, the systolic and diastolic blood pressure decreased in the 5-year but increased in the 10-year follow-up examinations. For the Japanese cohort, systolic and diastolic blood pressure increased as they aged. Second, change in blood pressure with age is different when viewed cross-sectionally at a particular examination year as compared to a longitudinal view for a particular birth cohort in both study populations.

To explain the first observation, three probable factors may be considered. The first factor relates to the issue of representativeness of the study sample. Non-random sampling of the population is one of the major sources in measurement error [146]. In the present study, non-random sampling is not likely to be a factor that could account for the unusual blood pressure profile of Yugoslavian men. Ninety-seven percent (n=522) of all Yugoslavian men living in Velika Krsna, aged 40-59, were recruited in the study. Due to an error in age, records of 41 men were removed; the final sample consisted of 511 men, representing 89% of the total male population of Velika Krsna in the fall of 1962. In Japan, all men living in Tanushimaru who were eligible for entry to the study, were examined. It would appear that these samples were probably representative of the two cohorts being studied. Thus non-random sampling can be ruled out as an explanation for the first observation.

The difference in the blood pressure aging profile of the Yugoslavian and Japanese cohorts could also be due to a bias introduced by the persons who were lost to follow-up. The group that was followed up 5 and 10 years later can consist of subjects whose entry blood pressure distribution was different from that of the persons who did not have a follow-up blood pressure measurement. For reason of uniformity in the analysis of the two study cohorts, I focused only on the 10-year follow-up, which was common to the two groups. Four hundred and thirty-five men (85.1%) in the Yugoslavian cohort, and 434 men (85.4%) in the Japanese cohort were re-examined 10 years later. Table 3.3 presents the frequency distribution, by age groups, of the follow-up and the non-follow-up groups in the

two cohorts. The loss to the follow-up was due to death or simply not showing up for re-measurements. Tables 3.4 and 3.5 present the mortality status of the non-follow-up group in both cohorts, and the cause of death for subjects lost to the 10-year follow-up.

Table 3.3 Frequency distribution of the follow-up and non-follow-up groups of men in the (a) Yugoslavian and (b) Japanese cohorts.

(a) Yugoslavia

Age group	Follow-up (n)	Non-follow-up (n)
40-44	126	13
45-49	77	7
50-54	113	23
55-59	119	33
Total	435	76

(b) Japan

Age group	Follow-up (n)	Non-follow-up (n)
40-44	87	5
45-49	104	16
50-54	120	19
55-59	108	31
60-64	15	3
Total	434	74

Table 3.4 Mortality status of male subjects who were lost to the 10-year follow-up in both cohorts.

	Yugoslavia		Japan	
Age group	Alive (n)	Dead (n)	Alive (n)	Dead (n)
40-44	6	7	2	3
45-49	1	6	4	12
50-54	2	21	4	15
55-59	0	33	2	29
60-64	0	0	0	3
Total	9	67	12	62

Table 3.5 Cause of death for male subjects who were lost to the 10-year follow-up in both cohorts.

	Yugoslavia (n=67)			Japan (n=62)		
Age group	Cardiac (n)	Stroke (n)	Others (n)	Cardiac (n)	Stroke (n)	Others (n)
40-44	0	1	6	0	0	3
45-49	1	1	4	0	0	12
50-54	1	4	16	1	1	13
55-59	2	4	27	4	5	20
60-64	0	0	0	0	2	1
Total	4	10	53	5	8	49

Cause of death is coded as follows: cardiac = deaths caused by coronary heart disease with myocardial infarction, severe arrhythmia, or heart failure; stroke = deaths caused by cerebrovascular disease; others = deaths caused by non-cardiac disease, including accidents, neoplasms, pulmonary disease.

The majority of deaths was not cardiovascular-related. In the Yugoslavian male cohort, 6% (age-standardized mortality: 4750 per 100,000 population) and 15% (age-standardized mortality: 4800 per 100,000 population) of the deaths were due to cardiac and cerebrovascular disease. Similarly, in the Japanese male cohort, 8% (age-standardized mortality: 4200 per 100,000 population) of the deaths was related to cardiac disease, while 13% (age-standardized mortality: 4125 per 100,000 population) was due to stroke. These death rates were much lower than the Yugoslavian and Japanese national age-standardized mortalities recorded in 1972 and 1968, respectively. In Yugoslavia, the age-standardized mortality rates from heart disease and stroke were 8633 and 8528 per 100,000 population [147]. In Japan, the age-standardized cardiac and stroke death rates were 8368 and 8478 per 100,000 population [148]. These differences in cardiovascular mortalities between the cohorts and the populations suggest that they were not representative of the Yugoslavian and Japanese populations, and may be related to life-style differences. As discussed in Chapter 2.2, men living in Velika Krsna of Yugoslavia and in Tanushimaru of Japan, were farmers who worked on the land, and whose diet contained little dietary fat [1]. Active life-style and low dietary fat intake can contribute to differences in death rates from cardiovascular disease.

To test whether men with no follow-up were significantly different from those with follow-up, I used regression analysis to compare them, by age group, on baseline variables, including entry systolic and diastolic pressure, serum cholesterol concentrations, body mass index, resting heart rate, and cigarette smoking habits. In Yugoslavia, no statistically significant difference was found between these two groups in all of the variables, except for the body mass index. The follow-up group had significantly higher body mass index than the non-follow-up group. In Japan, no statistically significant difference was observed between the follow-up and non-follow-up group in any of the baseline variables. To determine if the variance between these two groups is sufficiently large to show a statistical

significance, with a p value < 0.01, I used the Student's t test of difference for unequal standard deviations. Table 3.6 summarizes these findings.

Table 3.6 A comparison between the follow-up and the non-follow-up groups of men on selected baseline variables in (a) Yugoslavian and (b) Japanese cohorts.

(a) Yugoslavian study cohort

Variable	Mean values		Coefficient	Expected difference required to show statistical significance	p Value
	Follow-up	Non-follow-up			
SBP (mm Hg)	136.4	138.7	0.2517	7.1511	NS
DBP (mm Hg)	82.0	83.2	0.5945	4.1032	NS
HR (b/min)	69.9	71.0	1.1807	4.0630	NS
BMI (kg/m^2)	22.2	21.2	0.8699	0.8956	0.009*
SC (mg/dl)	160.1	159.8	1.2849	10.2076	NS
SMC (number/day)	3.1	3.6	0.5311	0.9444	NS

(b) Japanese study cohort

Variable	Mean values		Coefficient	Expected difference required to show statistical significance	p value
	Follow-up	Non-follow-up			
SBP (mm Hg)	120.4	128.5	2.6200	10.3491	NS
DBP (mm Hg)	70.1	74.9	3.1524	5.1961	NS
HR (b/min)	60.3	67.2	0.3505	5.2669	NS
BMI (kg/m^2)	21.9	21.5	1.8787	0.7577	NS
SC (mg/dl)	167.5	167.9	1.0780	3.8331	NS
SMC (number/day)	5.0	4.6	0.3935	0.9175	NS

SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = resting heart rate; BMI = body mass index; SC = serum cholesterol levels; SMC = cigarette smoking habit; *p < 0.01.

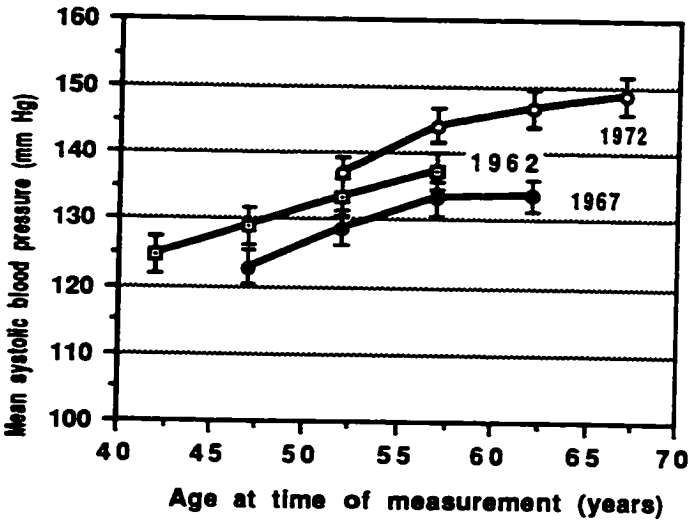
On the basis of these results, I concluded that the non-follow-up group in Yugoslavia and Japan did not consist of those subjects at the high extreme of the blood pressure scale, and thus were more likely to have higher morbidity and mortality rate in coronary heart and cerebrovascular diseases than those in the follow-up group.

The third factor to be considered is related to errors in blood pressure measurement. Keys et al.[1] have reported the evidence leading to the conclusion that in some areas of the Seven Countries Study, the two examining physicians produced different distributions of blood pressure from random samples from the same population. Differences of up to 10 mm Hg in the mean systolic and diastolic pressures of such parallel samples were found to be fairly common [1]. This would appear to be the most likely explanation for the decline in the 5-year follow-up blood pressure in all age groups in Yugoslavia.

3.3 Cross-sectional versus longitudinal blood pressure data

When blood pressure data were analyzed cross-sectionally and longitudinally, different age trends emerged. Figures 3.1 - 3.4 present these age trends.

(a)



(b)

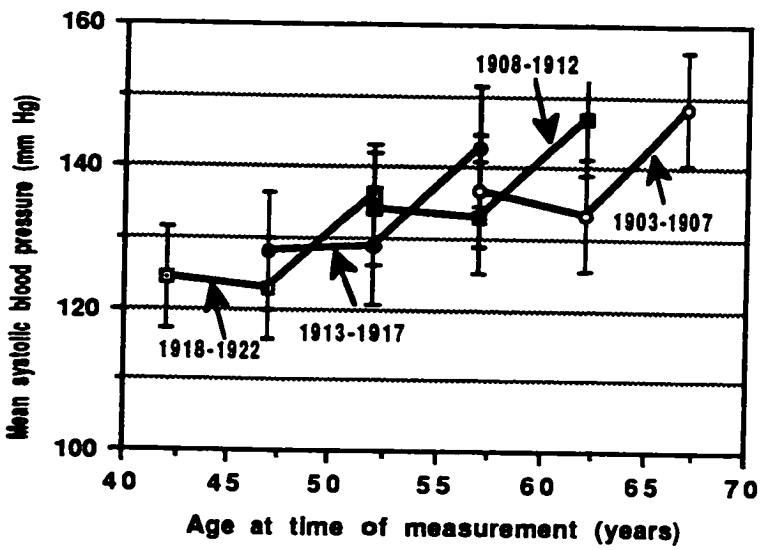
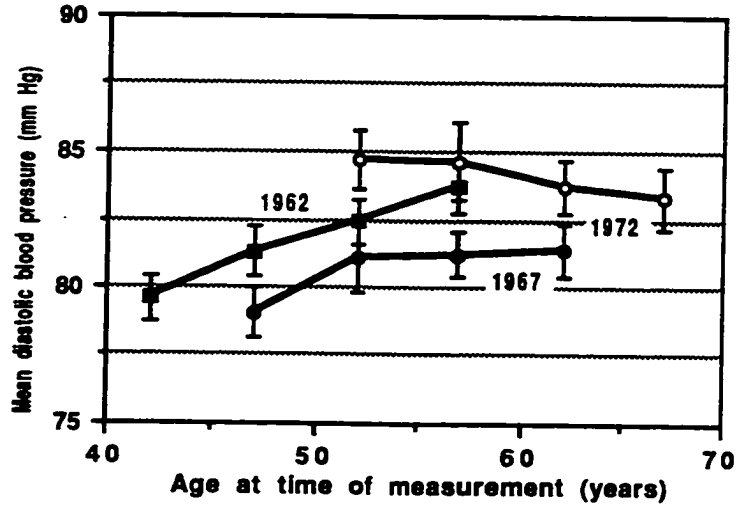


Figure 3.1 Average age trends in systolic blood pressure levels for men in the Yugoslavian cohort, based upon (a) cross-sectional and (b) longitudinal data. The three curves in (a) are labeled with the year of examination. The four curves in (b) are labeled with the year of birth of each cohort. The error bars represent SEM.

(a)



(b)

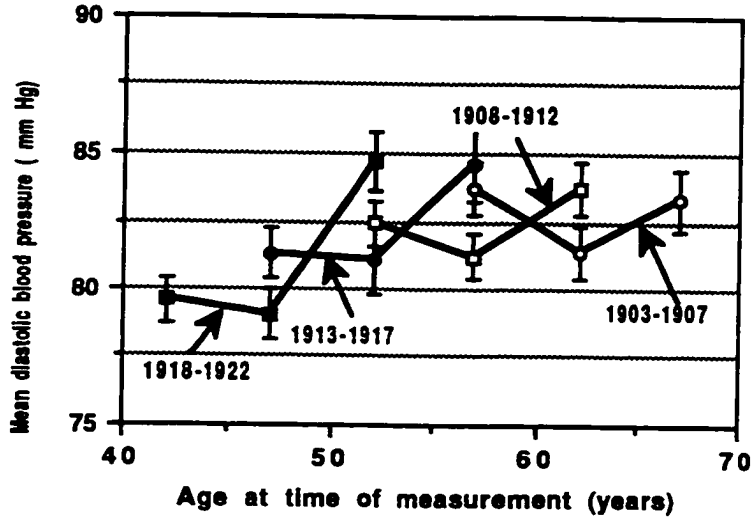
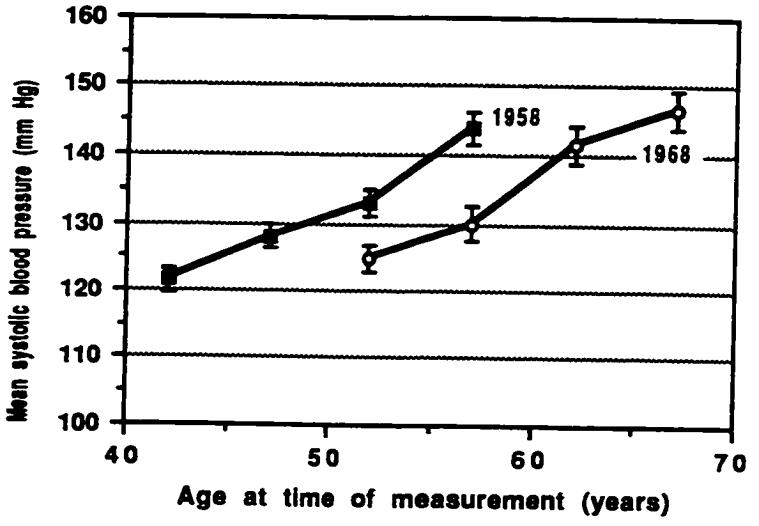


Figure 3.2 Average age trends in diastolic blood pressure levels for men in the Yugoslavian cohort, based upon (a) cross-sectional and (b) longitudinal data. The three curves in (a) are labeled with the year of examination. The four curves in (b) are labeled with the year of birth of each cohort. The error bars represent SEM.

(a)



(b)

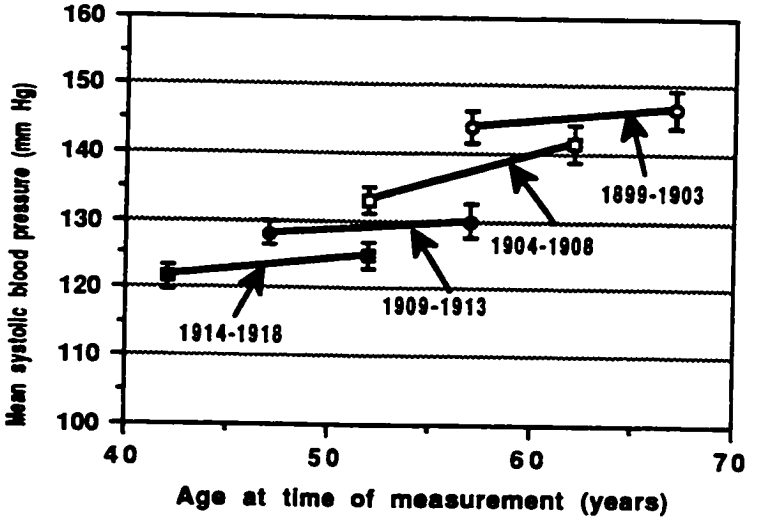
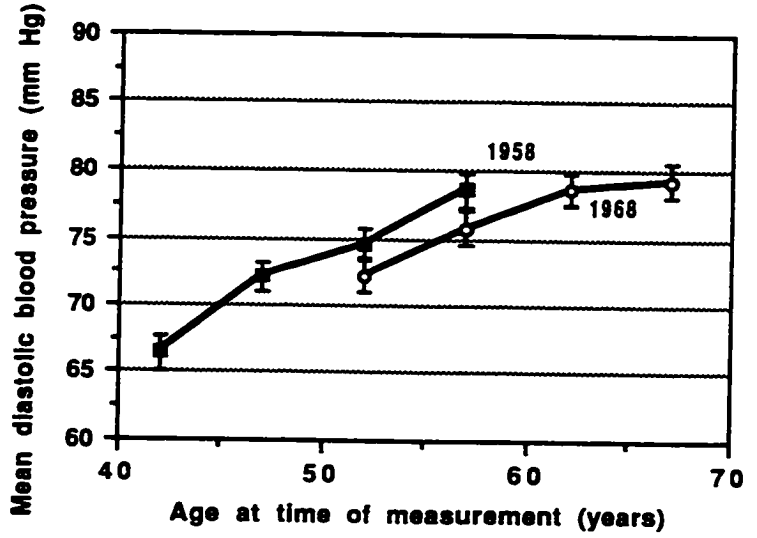


Figure 3.3 Average age trends in systolic blood pressure levels for men in the Japanese cohort, based upon (a) cross-sectional and (b) longitudinal data. The two curves in (a) are labeled with the year of examination. The four curves in (b) are labeled with the year of birth of each cohort. The error bars represent SEM.

(a)



(b)

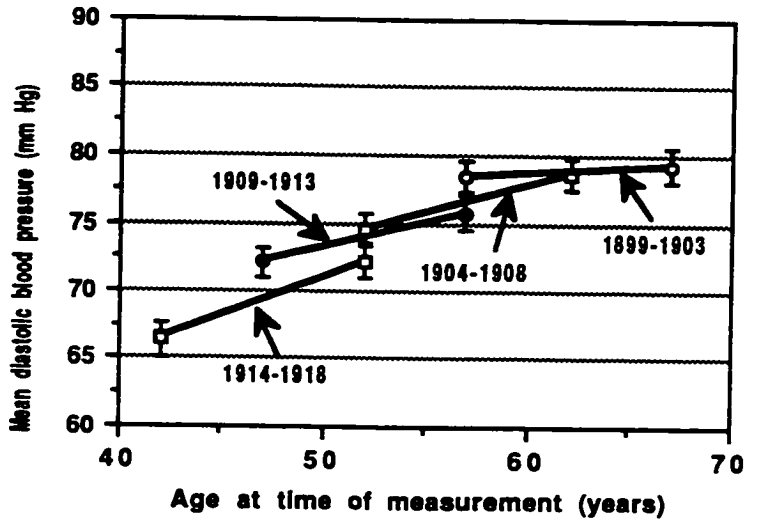


Figure 3.4 Average age trends in diastolic blood pressure levels for men in the Japanese cohort, based upon (a) cross-sectional and (b) longitudinal data. The two curves in (a) are labeled with the year of examination. The four curves in (b) are labeled with the year of birth of each cohort. The error bars represent SEM.

The cross-sectional blood pressure data in Yugoslavia (Table 3.1; Figure 3.1a) show a difference of 6 mm Hg in systolic pressure of men aged 45-49 in 1962 and 1967. On the other hand, longitudinal data (Table 3.1; Figure 3.1b) indicate that the systolic blood pressure of the 1918 -1922 birth cohort was reduced by only 1.68 mm Hg as it aged 5 years later. Similar observations were also made in the Japanese cohort (Table 3.2; Figure 3.2a and 3.2b). If change of blood pressure is primarily the result of aging per se, then, I would expect that the difference in blood pressure obtained cross-sectionally should be the same as that shown in longitudinal data. However, my analysis failed to support this assumption. This difference in blood pressure suggests that biological aging per se cannot explain the blood pressure change observed in my study. Other factors need to be considered.

There is a general belief that the aging process occurs with relative uniformity in all humans. If aging is considered to be a major determinant of systemic blood pressure, it is reasonable to assume that individuals from developed societies would demonstrate similar changes in the blood pressure levels as they age, irrespective of their birth year. Results of Miall and Lovell's prospective study [94] do not support this assumption. Data collected from three surveys over periods of ten years in the Rhonada Fach and eight-and-one-half years in Vale of Glamorgan in South Wales, show that the blood pressure curves of the three surveys do not exactly superimpose. Miall and Lovell speculated that this may be due to secular changes occurring in either the pressures or their determination. Kumanyika et al. [149] recently studied secular trends in blood pressure among 18- to 34-year old white and black adults in two body mass strata in the United States of America, between 1960 and 1980. Body mass index was found to be consistently associated with higher systolic and diastolic blood pressure. A secular increase in diastolic pressure was observed for white men in all four age-body mass index strata, but only in higher body mass index strata (> 25 kg/m^2) for black men. This secular upward trend has not been noted in systolic blood

pressure in either white or black subjects. In their study, Kumanyika et al. did not clearly define the term secular trend.

3.4 Estimation of trends due to the cohort-period effect

3.4.1 Statistical model

The cohort analysis is a commonly used approach to analyze longitudinal data to assess the change in disease frequency over time [150]. This approach involves the collection of data from at least three observation periods, and the entire study usually spans over 20 or more years. It separates empirically three time-related effects that could provide alternative explanations for the observations: age, period, and cohort effects. But this separation is difficult due to an inherent limitation of any cohort analysis: the linear dependency of each time-related variable on the other two [150].

The primary aim of the analysis carried out in this section was to test the hypothesis that factors besides biological aging contribute to blood pressure change over the 10-year observation period. The age effect on blood pressure will be addressed in section 3.5. For the purpose of the present analysis, cohort and period effects on blood pressure were not analyzed separately, and the combined effect was referred to as the cohort-period effect in my study. This decision was based on the following reasons : 1. the linear dependency of period and cohort effects, 2. the possibility that when observing one birth cohort, the period effect can get mixed in the data [150], and 3. insufficient blood pressure measurements for a meaningful analysis of the period effect, particularly in the Japanese cohort. Wu [151] and Mariotti et al. [152] did not separate the period and cohort effects in their investigation of how and the extent to which these effects have on the predictive power of a risk factor of coronary heart disease.

Without the trends due to cohort-period effect, the blood pressure of an age group should remain constant during the follow-up period. The data limit me to a simple model

that assumes a constant effect on all age groups. For the Yugoslavian cohort, initially I compared the cross-sectional mean entry and 5-year systolic and diastolic pressure of the 45-49, 50-54, and 55-59 age groups. This was followed by a comparison of the cross-sectional 5- and 10-year blood pressure of the 50-54, 55-59, and 60-64 age groups. In this analysis, the regression equation was solved with cross-sectional blood pressure as dependent variable, age and body mass index at time of blood pressure measurement, smoking habits, and year of examination as independent variables. Due to a lack of blood pressure measurements obtained during the 5-year follow-up in the Japanese cohort, I compared the cross-sectional mean systolic and diastolic blood pressure obtained on entry and at the 10-year follow-up. For reason of data availability, this analysis was limited to the 50-54 and 55-59 age groups only. Smoking habits did mot correlate statistically significantly with blood pressure, and the inclusion of this covariate failed to improve the R^2 value markedly (from 0.08 to 0.09 in the Yugoslavian cohort, and from 0.09 to 0.10 in the Japanese cohort). As a result, the smoking habits variable was removed from the regression model. The model underlying the analysis has the form:

$$Y_i = \alpha_i + \beta_{1i} + \beta_{2i}$$

where Y_i = blood pressure (either systolic or diastolic) of subject i, α_i = age of subject i at time of blood pressure measurement, β_{1i} = body mass index of subject i at time of blood pressure measurement, and β_{2i} = year of examination for subject i.

3.4.2 Results

Results confirmed the findings reported by Miall and Lovell [95] and by Kumanyika et al. [149] that the cohort-period effect contributes significantly to blood pressure during the 10-year observation period. Tables 3.7 and 3.8 present these findings.

Table 3.7 Multiple regression of blood pressure on cohort -period effect during (a) 5-year follow-up and (b) 10-year follow-up of the 40-60 years old Yugoslavian men.

(a)

SBP			DBP		
Independent variable	Coefficient	p Value	Independent variable	Coefficient	p Value
Age	0.9950	0.0001	Age	0.2478	0.0038
BMI	0.7465	0.0018	BMI	0.6178	0.0001
Yr exam 62	5.2107	0.0002	Yr exam 62	2.5716	0.0008
67	0.0000	.	67	0.0000	.
Intercept	59.5936	0.0001	Intercept	53.4647	0.0001
R^2	0.0854		R^2	0.0544	

(b)

SBP			DBP		
Independent variable	Coefficient	p Value	Independent variable	Coefficient	p Value
Age	0.9013	0.0001	Age	0.0385	0.6922
BMI	0.8988	0.0002	BMI	0.6829	0.0001
Yr exam 67	-10.5425	0.0001	Yr exam 67	-2.8975	0.0007
72	0.0000	.	72	0.0000	.
Intercept	70.7795	0.0001	Intercept	66.7148	0.0001
R^2	0.1035		R^2	0.0598	

Model tested: (a) SBP = age + BMI + yr. exam.
Model tested: (b) DBP = age + BMI + yr. exam.
SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; Yr exam = year of examination; 62 = 1962 (entry to study); 67 = 1967 (5-year follow-up); 72 = 1972 (10-year follow-up).

Table 3.8 Multiple regression of blood pressure on cohort-period effect during the 10-year follow-up of the 50-60 year old Japanese men.

SBP			DBP		
Independent variable	Coefficient	p Value	Independent variable	Coefficient	p Value
Age	1.5865	0.0001	Age	0.6217	0.0067
BMI	1.4840	0.0016	BMI	0.6823	0.0083
Yr exam 58	11.5674	0.0001	Yr exam 58	2.9533	0.0208
68	0.0000	.	68	0.0000	.
Intercept	8.1619	0.7549	Intercept	24.9233	0.0840
R^2	0.0919		R^2	0.0378	

Model tested: (a) SBP = age + BMI + yr. exam.
Model tested: (b) DBP = age + BMI + yr. exam.
SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; Yr exam = year of examination; 58 = 1958 (entry to study); 68 =1968 (10-year follow-up).

In the Yugoslavian cohort, a statistically significant difference was found between the entry and 5-year systolic pressure by +5.21 mm Hg, and between the 5- and 10-year systolic pressure by -10.54 mm Hg. Similar differences were observed between entry and 5-year, and 5-year and 10-year diastolic pressure. In the Japanese cohort, the difference between the entry and 10-year blood pressures was also statistically significant. The entry systolic pressure differed from the 10-year systolic pressure by +11.57 mm Hg, and the entry diastolic pressure differed from the 10-year diastolic pressure by 2.96 mm Hg.

The 5-year decline in blood pressure in the Yugoslavian cohort is the result of period effect, which is most likely due to the measurement errors. Keys [3] noted that in spite of standardization of instructions and equipment for blood pressure measurement, it was obvious at the entry examinations that the recordings were not always strictly comparable, even in the same areas. The two examining physicians produced different distributions of blood pressure from what should have been random samples from the same population. It was reported that at Velika Krsna, compared with Observer B, Observer A tended to read higher values at the lower end of the distribution, but lower values at the upper end, particularly in diastole [1].

In view of the fact that I did not know whether the entry or the 10-year blood pressures were affected by the cohort effect, I decided to choose the 10-year blood pressure values as my reference pressures. The following calculations were carried out to correct for the cohort effect from the measured blood pressures of the two cohorts:

Yugoslavians' systolic pressure:	entry systolic pressure minus 5.2107
	5-year systolic pressure + 10.5425
diastolic pressure:	entry diastolic pressure minus 2.5715
	5-year diastolic pressure + 2.8915
Japanese' systolic pressure:	entry systolic pressure minus 11.5074
diastolic pressure:	entry diastolic pressure minus 2.9533

The data shown in Tables 3.9 and 3.10 result from adjusting the measured blood pressure data at entry and the 5-year follow-up by the cohort effect. The values for a particular age group at different years of examination now differ only to the extent explained by the body mass index changes and random effects.

Table 3.9 (a) The mean values and standard errors of systolic blood pressure of four age groups of men in the Yugoslavian study cohort after correction for the cohort effect. (b) The same data for diastolic blood pressures.

(a) Systolic

Age at time of measurement	Entry (1962)	5-yr (1967)	10-yr (1972)
40-44	129.84(±1.11) n=139		
45-49	134.22 (±1.74) n=84	133.37 (±1.44) n=127	
50-54	138.67(±1.63) n=136	139.21 (±1.91) n=78	136.78 (±1.90) n=126
55-59	142.60 (±1.71) n=152	143.88(±1.70) n=126	144.25 (±2.65) n=77
60-64		144.29 (±1.62) n=131	146.96 (±2.01) n=113
65-69			148.94 (±1.99) n=119

(b) Diastolic

Age at time of measurement	Entry (1962)	5-yr (1967)	10-yr (1972)
40-44	79.90 (±0.81) n=139		
45-49	81.64 (±0.91) n=84	81.95 (±0.93) n=127	
50-54	82.75 (±0.84) n=136	83.97 (±1.31) n=78	84.70 (±1.11) n=126
55-59	84.04(±0.94) n=152	84.12 (±0.81) n=126	84.62 (±1.41) n=77
60-64		84.28 (±0.96) n=131	83.76 3±0.99) n=113
65-69			83.30 (±1.15) n=119

Table 3.10 The mean values and standard errors of (a) systolic and (b) diastolic blood pressure of four age groups of men in the Japanese cohort after correction for the cohort effect.

(a) Systolic

Age at time of measurement	Entry (1958)	10-yr (1968)
40-44	109.92 (±1.79) n=92	
45-49	116.44 (±1.83) n=120	
50-54	121.32 (±1.83) n=139	124.77 (±1.99) n=87
55-59	132.91 (±2.51) n=157	129.98 (±2.42) n=104
60-64		141.51 (±2.62) n=120
65-69		146.53 (±3.10) n=123

(b) Diastolic

Age at time of measurement	Entry (1962)	10-yr (1972)
40-44	63.47 (±1.28) n=92	
45-49	69.13 (±1.10) n=120	
50-54	71.61 (±1.15) n=139	72.22 (±1.23) n=87
55-59	75.86 (±1.31) n=157	75.77 (±1.20) n=104
60-64		78.59 (±1.23) n=120
65-69		79.51 (±1.35) n=123

3.5 Aging trend in blood pressure

3.5.1 Statistical models

The aging trend of blood pressure was examined in terms of how it affected the groups as well as the individuals. Aging of blood pressure in the groups was assessed by regression analysis, with blood pressures corrected for the cohort-period effect, during the 10-year observation period as the dependent variable, the 5-year age group, and the body mass index at time of measurement as the independent variables (Model 1, Table 3.11). To determine the aging of blood pressure in the individuals, I examined how the adjusted blood pressure values changed as the individuals in each birth cohort aged. To achieve this goal, I devised a model with change of blood pressure during the 10-year observation period as dependent variable, age, the 10-year change of body mass index, and entry blood pressure as independent variables (Model 2, Table 3.11). These parameters were included in the model as they have been shown to be significant predictors of blood pressure change in a prospective population study [149]. Body mass index (BMI), defined as weight (kg) divided by height (meters) squared (Wt/Ht^2) more accurately reflects a person's fatness than does weight alone, because the BMI takes height into consideration, and has been found to correlate well to health measures in population studies. The relationships between the BMI and health risks are compiled from epidemiological findings which are population-based. Smoking habits, 10-year change in smoking habits, and interaction terms between age, change of body mass index, and level of blood pressure when observation was made, were entered into the model for detecting their effects on the blood pressure change in the Yugoslavian and Japanese cohorts. The inclusion of these terms improved the squared multiple correlation coefficient (R^2) of the model only marginally. At the same time, the level of statistical significance of dependent variables was either improved marginally or reduced to statistical nonsignificance. In addition, none of these interaction terms was statistically significantly correlated with the dependent variables. For these reasons, I chose

to use the simple regression models, that is, models without any interaction terms to explain the age trends in blood pressure in both cohorts.

Table 3.11 Statistical models used for examining aging trends in blood pressure in the groups (Model 1) and in the individuals (Model 2).

Variables	Model 1	Model 2
Dependent	•BP of the group at time of measurement	•Individual BP change
Independent	•Age group at time of measurement •BMI at time of measurement	•Age at entry •Blood pressure at entry •ΔBMI

ΔBMI = change in the body mass index during the 10-year follow-up.

3.5.2 Results

3.5.2.1 Aging of blood pressure in the groups

Table 3.12 presents the observed mean systolic and diastolic pressure for each 5-year age group, controlling for the body mass index, in the Yugoslavian and Japanese male cohorts.

Table 3.12 The observed mean systolic and diastolic pressure of men in the (a) Yugoslavian and (b) Japanese cohort, controlling for the body mass index.

Age group (years)	SBP (mm Hg)	DBP (mm Hg)
(a) Yugoslavian		
40-44	129.77±1.60	79.84±0.89
45-49	133.31±1.31	81.59±0.73
50-54	137.83±1.03	83.55±0.57
55-59	143.52±1.01	84.28±0.56
60-64	145.79±1.21	84.26±0.18
65-69	149.48±1.74	83.71±0.97
(b) Japanese		
40-44	109.15±2.60	63.09±1.36
45-49	116.45±2.28	69.07±1.20
50-54	121.64±1.67	71.29±0.88
55-59	131.63±1.54	75.59±0.81
60-64	141.88±2.33	78.12±1.22
65-69	147.65±2.82	79.96±1.19

Values are given as Mean±SEM.

On the average, the one-year increase was 0.81 in systolic and 0.17 mm Hg in diastolic blood pressure in the Yugoslavian cohort. The one-year increase in both systolic and diastolic pressure was larger in the Japanese cohort, 1.67 mm Hg in systolic and 0.56 mm Hg in diastolic pressure in this cohort. These differences between the two cohorts were statistically significant ($p = 0.0001$).

3.5.2.2 Aging of blood pressure in the individuals

Using the adjusted blood pressures I determined the change in blood pressure over the 10-year period for each individual. The cumulative distribution of systolic and diastolic blood pressure changes is shown in Figures 3.5 and 3.6. In order to account for the different age distribution in the two study populations, the cumulative distributions of 5-year age groups were aggregated under equal weighting.

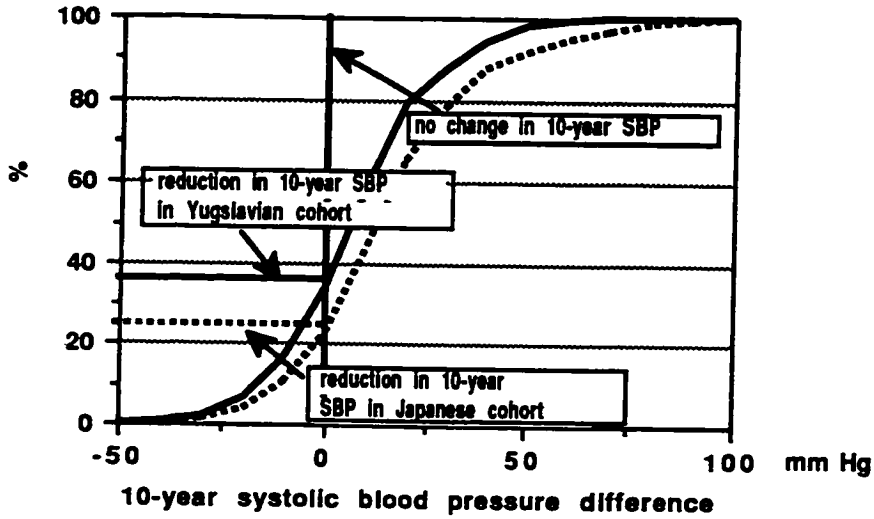


Figure 3.5 Cumulative distribution of 10-year systolic blood pressure differences in the Yugoslavian (solid line) and Japanese (dashed line) male cohorts.

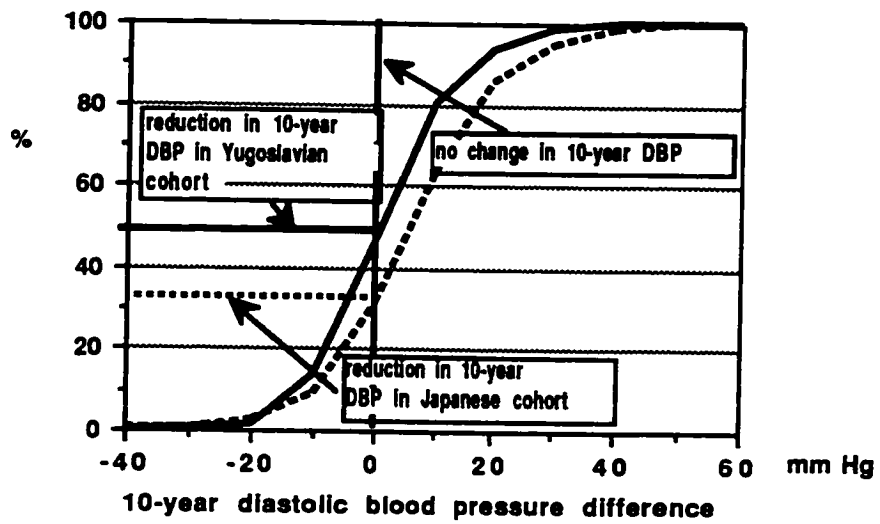


Figure 3.6 Cumulative distribution of 10-year diastolic blood pressure differences in the Yugoslavian (solid line) and Japanese (dashed line) male cohorts.

Figures 3.5 and 3.6 show that in each study population, a fraction of the cohort's blood pressure decreased over the 10-year observation period. Although the Yugoslavian men had significantly higher entry systolic and diastolic blood pressure than the Japanese men (136.71±0.82 mm Hg versus 121.55±1.08 mm Hg for systolic, and 82.18±0.45 mm Hg versus 70.75±0.62 mm Hg for diastolic), a higher percentage of these men (35%) had a reduction in systolic pressure in the 10-year period, than that of Japanese men (23%). Close to 50% of the Yugoslavian men had a decline in diastolic pressure, compared to 35% of the Japanese men whose diastolic pressure decreased over the 10-year period.

I used the multiple regression analysis (see Model 2, Table 3.11) to determine the aging trend of blood pressure in the individuals in both cohorts. Table 3.13 presents the findings.

Table 3.13 Regression analysis of the adjusted blood pressure change on age, controlling for age at entry 10-year change in body mass index, and entry blood pressure of (a) Yugoslavian men, and (b) Japanese men.

(a) Yugoslavian

ΔSBP			ΔDBP		
Independent variable	Coefficient	p Value	Independent variable	Coefficient	p Value
Age at entry	0.3493	0.0214	Age at entry	-0.1547	0.0631
ΔBMI	1.9094	0.0004	ΔBMI	1.4608	0.0001
Entry SBP	- 0.2988	0.0001	Entry DBP	- 0.3728	0.0001
Intercept	30.9071	0.0005	Intercept	40.1137	0.0001
R^2	0.0990		R^2	0.1807	

(b) Japanese

ΔSBP			ΔDBP		
Independent variable	Coefficient	p Value	Independent variable	Coefficient	p Value
Age at entry	0.8902	0.0001	Age at entry	0.2538	0.0124
ΔBMI	3.3781	0.0001	ΔBMI	0.9171	0.0129
Entry SBP	- 0.3188	0.0001	Entry DBP	- 0.5419	0.0001
Intercept	11.7621	0.2616	Intercept	32.2078	0.0001
R^2	0.1376		R^2	0.2957	

Model tested: (a) ΔSBP = age at entry + ΔBMI + entry SBP.
Model tested: (b) ΔDBP = age + ΔBMI + entry DBP.
ΔSBP = the 10-year change of systolic blood pressure; ΔDBP = change of diastolic blood pressure; ΔBMI = the 10-year change of body mass index.

The systolic and diastolic blood pressure change was significantly correlated with age in the Japanese cohort, controlling for change of body mass index, and entry blood pressure. A marginal statistically significant correlation was observed between age, systolic and diastolic blood pressure in the Yugoslavian cohort. The level blood pressure attained when observation was made, was inversely and significantly correlated with the change of blood pressure in both cohorts. The change of body mass index was a significant predictor of the blood pressure. The low R^2 value indicates that most of the variance in the systolic and diastolic pressure change is not explained by the model.

3.6 Relationship of blood pressure change to the attained blood pressure

As shown in Table 3.13, pressure change was inversely correlated with the attained pressure, the level of systolic and diastolic blood pressure at the time when observation was made. The inverse relationship between pressure change and the attained pressure is most likely due to the "regression towards the mean" phenomenon. The following examples illustrate this phenomenon.

According to the regression equation in Table 3.13, the predicted systolic blood pressure change for a 55-year old Japanese with an attained pressure of 130 mm Hg, and no change in the body mass index, would be :

$$\Delta SBP = 11.76 + 0.89\times(55) + 3.38\times(0) - 0.32\times (130) = 19.11 \quad (3.1)$$

If another individual had a pressure of 170 mm Hg, and the other variables remained unchanged, then the predicted systolic pressure change would be:

$$\Delta SBP = 11.76 + 0.89\times(55) + 3.38\times(0) - 0.32\times (170) = 6.31 \quad (3.2)$$

Thus, the initial difference of 40 mm Hg between the two persons has narrowed to 27 mm Hg. Figure 3.7 is a graphic presentation showing how the systolic pressure regresses towards the mean.

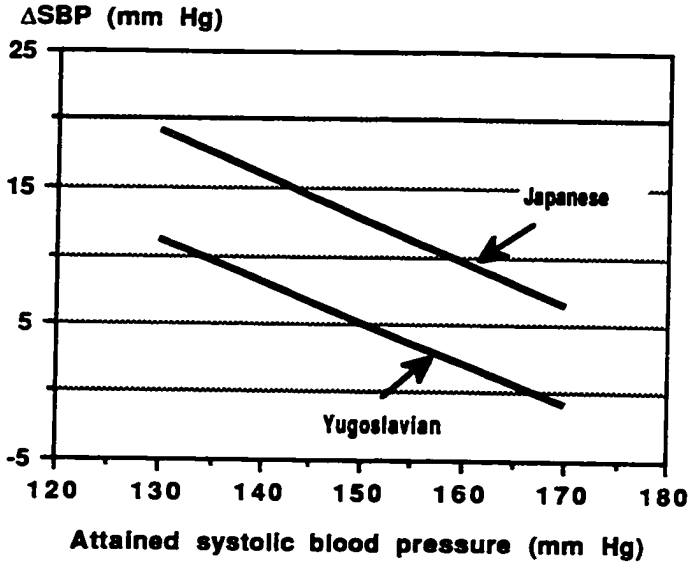


Figure 3.7 A graphic presentation illustrating the regression towards the mean phenomenon. ΔSBP denotes change of systolic blood pressure. Attained systolic blood pressure refers to the level of systolic pressure when the observation was made.

The phenomenon of "regression towards the mean" can also be demonstrated with the tracking of the blood pressure in the two cohorts. The entry and the 10-year systolic pressure values in the Japanese and Yugoslavian cohorts were categorized into quartiles. I tracked the systolic pressures by determining how many subjects in each quartile remained in the same quartile at the 10-year follow-up (Table 3.14).

Table 3.14 Tracking of the systolic pressure of men in the (a) the Japanese and (b) the Yugoslavian cohorts.

Entry SBP (mm Hg)	10-year SBP (mm Hg) Q1	Q2	Q3	Q4
(a) Japanese				
Q1	50.4	28.6	14.3	6.7
Q2	30.6	27.9	27.9	13.6
Q3	12.7	23.6	35.5	28.2
Q4	4.2	19.2	19.2	57.4
(b) Yugoslavian				
Q1	28.6	71.4	0.0	0.0
Q2	6.9	61.1	20.8	11.2
Q3	1.9	40.1	43.0	15.0
Q4	0.0	21.9	27.6	50.5

Values are expressed in percentage. Q1 = first quartile; Q2 = second quartile; Q3 = third quartile; Q4 = fourth quartile; Entry SBP = systolic blood pressure measured on entry to the study; 10-year SBP = systolic blood pressure measured at the 10-year follow-up examination.

In the Japanese cohort, 50% of the subjects whose entry systolic pressure was categorized in the first quartile remained in the same quartile 10 years later. Similarly, 57% of the Japanese men with the entry systolic pressure in the fourth quartile stayed in this quartile in the 10-year follow-up. In the Yugoslavian cohort, similar observations can be made; 28% with the entry systolic pressure in the first quartile, and 50% with the entry systolic pressure in the fourth quartile, stayed in the same respective quartiles 10 years later. There is an overall regression towards the mean.

3.7 Aging trend of the pulse pressure

Thus far, the analyses have shown that both the systolic and diastolic pressure increase with age in the majority of the Yugoslavian and Japanese men. The systolic pressure increases to a greater extent than the diastolic pressure. This difference in the rate of change between the systolic and diastolc pressure can lead to an increase in the pulse pressure, i.e., the difference between systolic and diastolic pressure, with age.

The following analyses were designed to examine the aging trend of the pulse pressure in the cohorts and the individuals; the adjusted blood pressures were used in these analyses. The statistical models for analyzing the aging of blood pressures were used for examining the aging trend of the pulse pressure (see Model 1, Table 3.11).

3.7.1 Results

3.7.1.1 Aging of pulse pressure in the groups

Tables 3.15 and 3.16 present the means and standard errors of the pulse pressure of four male birth cohorts of Yugoslavia and Japan. Table 3.17 summarizes the results of regression analysis of the adjusted pulse pressure on age in each cohort, controlling for the body mass index at the time of measurement.

Table 3.15 The mean values and standard errors of the pulse pressure of four age groups of men in the Yugoslavian cohort.

Age at time of measurement	Entry (1962)	5-yr (1967)	10-yr (1972)
40-44	49.94 (±0.77) n=139		
45-49	52.59 (±1.20) n=84	51.42 (±0.87) n=127	
50-54	55.92 (±1.15) n=136	55.23 (±1.23) n=78	52.08 (±1.12) n=126
55-59	58.56 (±1.15) n=152	59.76 (±1.29) n=126	59.62 (±1.61) n=77
60-64		60.01 (±1.09) n=131	63.19 (±1.54) n=113
65-69			65.64 (±0.73) n=119

Table 3.16 The mean values and standard errors of the pulse pressure of four age groups of men in the Japanese cohort.

Age at time of measurement	Entry (1958)	10-yr (1968)
40-44	46.45 (±1.29) n=92	
45-49	47.31 (±1.33) n=120	
50-54	49.71 (±1.29) n=139	52.55 (±1.47) n=87
55-59	57.05 (±1.64) n=157	54.21 (±1.67) n=104
60-64		62.92 (±1.92) n=120
65-69		66.83 (±2.24) n=123

Table 3.17 Regression analysis of pulse pressure after correction for the cohort-period effect on age, controlling for the body mass index and diastolic pressure in the Yugoslavian and Japanese male cohorts.

Yugoslavia PP			Japan PP		
Independent variable	Coefficient	p Value	Independent variable	Coefficient	p Value
Age	2.9939	0.0001	Age	3.4733	0.0001
BMI	-0.0414	0.7114	BMI	0.3758	0.1052
DBP	0.3564	0.0001	DBP	0.3946	0.0001
Intercept	-6.2371	0.1416	Intercept	-22.2824	0.0021
R^2	0.1891		R^2	0.2067	

Model tested: PP = age group + BMI + DBP.
PP = pulse pressure; age = age at time of measurement; BMI = body mass index at time of measurement; DBP = diastolic blood pressure at time of measurement.

Data presented in Table 3.17 indicate that the pulse pressure increased with age in both cohorts. In the Japanese cohort, the slope of the pulse pressures/age relationship was steeper than that in the Yugoslavian cohort, but this difference did not reach statistical significance. The body mass index at the time of measurement was a statistically significant predictor of the pulse pressure in the Japanese cohort only. Figure 3.8 is a graphic presentation of the observed pulse pressure in different age classes, controlling for the body mass index, in both cohorts.

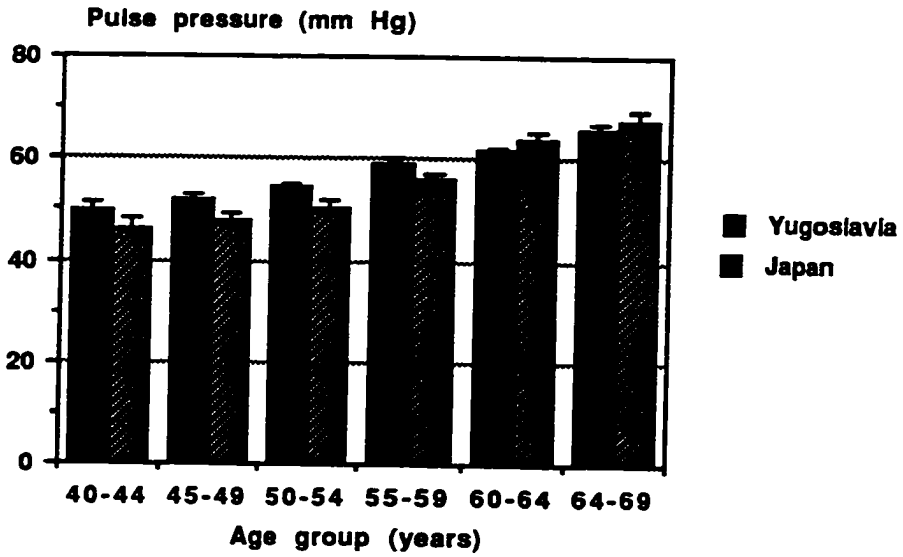


Figure 3.8 Distribution of the observed 10-year pulse pressure of men in the Yugoslavian and Japanese cohorts, controlling for body mass index and diastolic pressure. The error bars represent SEM.

In the Japanese cohort, the pulse pressure of the younger age groups was lower than that of the same age groups in the Yugoslavian cohort. But the Japanese men's pulse pressure rose more steeply with age, at age 60-64, it exceeded the pulse pressure of the Yugoslavian men. This trend reflects the steep rise with age in both the systolic and diastolic blood pressure in this cohort.

3.7.1.2 Aging of the pulse pressure in individuals

Results of regression analysis are presented in Tables 3.18. In the regression model, the pulse pressure change was the dependent variable, and age at entry to study, entry pulse pressure, and the 10-year diastolic pressure change were the independent variables.

Table 3.18 Multiple regression of pulse pressure change after correction for cohort-period effect on age, controlling for 10-year change in body mass index, entry pulse pressure, and 10-year change in diastolic pressure in Yugoslavian and Japanese men.

	Yugoslavian ΔPP			Japan ΔPP	
Independent variable	Coefficient	Pr>F	Independent variable	Coefficient	Pr>F
Age at entry	0.6523	0.0001	Age at entry	0.8416	0.0001
ΔBMI	0.1900	0.6219	ΔBMI	2.0345	0.0003
Entry PP	-0.4667	0.0001	Entry PP	-0.4152	0.0001
Δ DBP	0.1493	0.0009	Δ DBP	0.2199	0.0005
Intercept	-0.1639	0.7595	Intercept	-11.7144	0.0472
R^2	0.1929		R^2	0.1884	

Model tested: PP = age at entry + Δ BMI + entry PP + Δ DBP.
ΔPP = pulse pressure change; ΔBMI = change in the body mass index; PP = pulse pressure at entry to study; Δ DBP = change in pulse pressure.

These results indicate that pulse pressure increases significantly with age in both cohorts. The slope of the ΔPP/age relationship was steeper in the Japanese than in the Yugoslavian cohort (coefficient estimate: 0.8416 versus 0.6523 mm Hg/ year). This can be explained by a steeper rise in systolic pressure than in diastolic pressure in the Japanese men. The 10-year change in the body mass index was a statistically significant predictor of the 10-year pulse pressure change in the Japanese but not the Yugoslavian cohort. The Japanese men's body mass index decreased by 0.5 kg/m^2 (entry BMI = 21.83±0.10; 10-year BMI = 21.37±0.13), whereas the Yugoslavian men's body mass index increased by 0.1 kg/m^2 during the 10-year follow-up (entry BMI = 22.06±0.12; 10-year BMI = 22.35±0.16). This statistically, significantly larger change in the body mass index ($t = 6.12$, $p = 0.0000$) accounts for the significant correlation between the 10-year change in the pulse pressure and body mass index in the Japanese cohort.

3.8 Stroke mortality trends for the population and cohort

Blood pressure, in particular systolic pressure, is an important risk factor for stroke. The purpose of the following analysis was to investigate how blood pressure trends due to age and cohort-period effect, are associated with population and cohort stroke mortality. If my method of separating blood pressure trends due to these two effects is valid, then these trends and stroke mortalities should show a similar direction. In my study, I assumed that trends due to cohort-period effect not only apply to the study population, but also to the whole country; it is therefore legitimate to use country stroke mortality to associate with blood pressure trends due to the cohort-period effect.

The mortality data used in the analysis were those of the first 20 years follow-up in the Yugoslavian and Japanese cohorts. Stroke death rates have been computed per 100,000 population with direct age adjustment, taking the world standard population as reference population. I used the stroke death rates in two time periods, i.e., the first and second 10-year follow-up stroke mortalities (1962-1972 and 1972-1982 in the Yugoslavian cohort, and 1958-1968 and 1968-1978 in the Japanese cohort) in my investigation of the association between cohort blood pressure trends and cohort stroke mortalities. In my analysis, the averaged 10 year mortalities were used instead of the annual stroke deaths due to the fact that there were few annual stroke deaths in either cohort during these time periods. The entry blood pressure in the cohorts predicts the first 10-year stroke mortalities, whereas the 10-year follow-up blood pressure predicts the second 10-year stroke mortalities.

In my investigation of how blood pressure trends due to cohort-period effect are associated with population stroke mortalities, I used the published annual stroke death statistics in 1962 and 1972 in Yugoslavia [153], and in 1958 and 1968 in Japan [154]. I chose these years because of their close proximity to the time periods when blood pressure

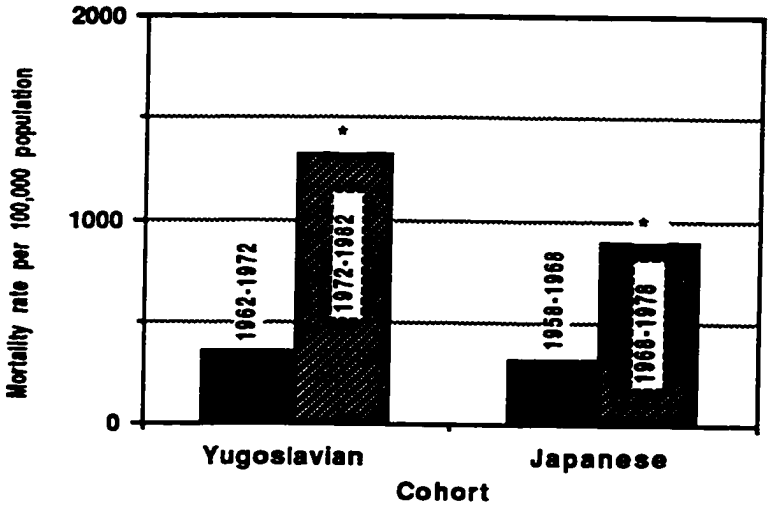
trend due to cohort-period effect analyses in the Yugoslavian and Japanese cohorts were carried out.

The χ^2 statistic was used to determine the differences between the first and second 10-year stroke mortality rates. To test significance between 1958 and 1972 stroke mortalities in Japan, and between 1962 and 1972 stroke mortalities in Yugoslavia, I developed an approximate test based on the normal approximation to the binomial distribution.

3.8.1 Results

Figure 3.9 (a) is a graphic presentation of the first and second 10-year age-standardized stroke mortality rates for the Yugoslavian and Japanese cohorts. Figure 3.9 (b) depicts the age-standardized mortality trends from stroke in the Yugoslavian and Japanese populations of the similar periods.

(a)



(b)

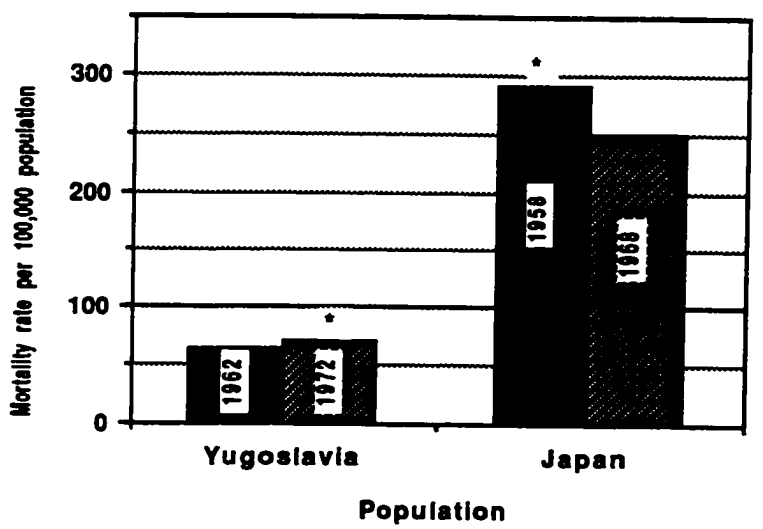


Figure 3.9 (a) The age-standardized first and second 10-year stroke mortality rates for the Yugoslavian and Japanese male cohorts. * $p < 0.05$, comparing the first and second 10-year age-standardized stroke mortality rates. (b) Age-standardized stroke mortality trends for the Yugoslavian and Japanese populations. * $p < 0.05$, comparing the 1962 and 1972 stroke mortalities in Yugoslavia, and the 1958 and 1968 stroke mortalities in Japan. Stroke mortality data for Yugoslavia were obtained from the published World Health Statistics [153]. The stroke mortality data for Japan were obtained from publication by Ushima and Pisa [154].

As presented in Chapter 3.5.2, both cohorts demonstrated a statistically significant upward age trends in blood pressure. These blood pressure age trends are associated with a significant increase in stroke mortalities in the Yugoslavian and Japanese cohorts. The upward blood pressure trends due to cohort-period effect in the Yugoslavian cohort correspond to a moderate increase in population stroke mortalities. The downward blood pressure trends due to cohort-period effect in the Japanese cohort are associated with a significant decrease in the Japanese national stroke mortality rates during the period of 1958-1968.

3.9 Discussion

As discussed in Chapter 1.2.1, the aim of the Seven Countries Study was to explore the relationship between blood pressure trends and cardiovascular events. In this context, it was irrelevant to identify the source of the blood pressure trend, be it cohort or aging. Publications by the Seven Countries investigations [2,11,12,14] have reported that the systolic but not diastolic blood pressure increased with age in all cohorts. However, neither the magnitude, the rate, nor the cohort-period effect on this change has been reported. My analysis of the blood pressure-age relationships confirms the observation that blood pressure indeed rises with age in the Yugoslavian and Japanese cohorts even after correcting for the cohort-period effect. But when I analyzed how the blood pressure changes in each individual during the 10-year observation period, I noted that 35% of Yugoslavian men, and 23% of Japanese men had a decline in systolic pressure values. Similarly, 50% of men in the Yugoslavian cohort and 33% men in the Japanese cohort showed a decrease in the 10-year diastolic blood pressure. Furthermore, my study also demonstrated that the trends due to cohort-period effect play a significant role and have to be considered in the study of aging. Findings of the type described in this study have never been reported before.

The significant trends due to cohort-period effect on blood pressures of the Yugoslavian and Japanese cohorts cannot be explained by either the effects of pharmacological therapy, or a change in the body mass index. No drug treatment was used in these groups during the 10-year observation period, and I had controlled for the change in body mass index in my analyses. Therefore, other factors that exist in my study populations are most likely responsible for the cohort-period effect on blood pressure in these cohorts.

The arterial blood pressure is regulated by physical and physiological factors [155]. The arterial blood volume and the elastic characteristics of the system are the two major physical factors, while the heart rate, stroke volume, cardiac output, and peripheral resistance are the main physiological factors affecting blood pressure. Other physiological factors such as sympathetic nervous system activity, blood glucose, and serum protein have been found to associate significantly with blood pressure increase [16,156]. In addition, family history of hypertension, alcohol consumption, and a lack or abundance of some nutritional components have been cited in the literature as factors thought to be involved in the development of high blood pressure [22,157]. These factors were not measured in my study protocol, but could contribute to trends due to the cohort-period effect in my data.

Results of prospective studies [93,94,95,96] all support the hypothesis that blood pressure rises as age advances in acculturated societies. My analysis of the prospective data from two cohorts of the Seven Countries Study lends further support to this hypothesis. Blood pressure increases significantly with age in both the Yugoslavian and Japanese cohorts, but the magnitude of this increase differs between them. Although the Japanese men's entry systolic blood pressure was significantly lower than that of the Yugoslavian men (121.6±1.1 versus 136.7±0.8 mm Hg, $p = 0.0001$), they demonstrated a steeper slope of systolic pressure change with age, as compared to that of the Yugoslavian men (coefficient estimate : 0.8902 mm Hg/year for the Japanese cohort versus 0.3493 mm Hg/year for the Yugoslavian cohort). Similarly, the entry diastolic blood pressure of the Japanese men was significantly lower than that of the Yugoslavian men. The diastolic

pressure increased with age in both cohorts; however, the slope of change in diastolic pressure was relatively flat. Without correcting for the cohort-period effect, the slope of blood pressure change with age in the Japanese cohort would have been flatter.

Blood pressure increase with age may be explained on the basis of structural changes in arteries and arterioles associated with normal aging. Evidence from the observational studies clearly demonstrates that uniform intimal thickening increases constantly with age, and that the proliferating intima is subjected to lipid accumulation [27]. Medial thickening with elastin fragmentation and calcification also occur with advancing age. These morphological alterations in arterial walls result in stiffening of the arteries, and subsequent augmentation of the blood pressure.

The steep increase in blood pressure despite a low initial level in the Japanese cohort, may be due to a difference in aortic morphology between the Japanese and Yugoslavian populations. Evidence is emerging that indicates that the oriental population tends to have a larger age-related increase in the circumference of the ascending aorta [158] and a significantly higher ratio of radius to medial wall thickness (R/h) [159]. With a higher ratio R/h, the ascending aorta of the oriental population is subjected to a higher wall stress, assuming that the medial wall thickness remains unchanged. This results in a relative increase in wall stiffness and a rise in the arterial blood pressure. This differential morphology in aortic dimensions may also exist in my two study populations. Another plausible explanation for the apparent different blood pressure aging in the Japanese and Yugoslavian cohorts is related to sodium intake. The Intersalt data [160] clearly showed a statistically significant relationship between sodium intake and the slope of blood pressure with age. Since neither urinary sodium excretion nor dietary sodium intake was included in the Seven Countries Study protocol, I was unable to determine if the Japanese men's steep rise in blood pressure with age, was attributable to higher sodium intake. However, Sasaki [161] reported an average daily salt intake of 19.9 gm for farmers in Kyushu. The high dietary sodium intake in many Japanese is the result of their fondness for a traditional diet of

rice, salted pickles, miso and soya sauce, which are produced from fermented beans, cereals and salt. The significant relationship between sodium intake and blood pressure increase with age may offer a reasonable explanation for the steep rise of blood pressure in the Japanese cohort.

Dietary sodium intake has been considered important in the genesis and maintenance of hypertension. This view is predicated on epidemiological observations, results of experiments in animals, and the results from dietary intervention trials. Several mechanisms have been proposed to explain the relationship between salt intake and blood pressure elevation. Guyton [162] suggested that increased cardiac output in response to renal sodium retention results in elevated peripheral resistance, leading to blood pressure increase. Hemodynamic alterations in salt-sensitive individuals is another possible mechanism linking sodium intake to blood pressure elevation. Sullivan and Ratts [163] observed that forearm vascular resistance was greater during salt loading in the salt-sensitive subgroups of both the normotensive and borderline hypertensive groups. These vascular functional changes were associated with responsiveness of the renin-aldosterone system during salt depletion. Skrabel et al. [164] hypothesized that altered α- and β-receptor regulation in salt-sensitive individuals may promote increased vasoconstriction, decreased vasodilatation, and increased renal sodium reabsorption in salt-sensitive persons. There is increasing evidence suggesting an association between salt intake and arterial stiffness that is independent of blood pressure. Avolio et al. [165] reported that the pulse wave velocity, an noninvasive index of arterial stiffness, in low salt subjects (mean intake 44 mmol/day) was significantly lower than the pulse wave velocity of control subjects on a regular diet (mean intake 130-200 mmol/day). Similar observations were reported by Safar et al. [166]. In elderly subjects with systolic hypertension and arteriosclerosis obliteran of the lower limbs, intravenous infusion of isotonic saline caused a significant increase in forearm arterial compliance in parallel with an increase in systolic blood pressure, with no change in diastolic blood pressure. Although the mechanism that connects arterial stiffness with salt intake is

unknown, the peculiar effect of sodium on the arterial wall due to a 'direct' action or to an associated modification of the autonomic nervous system, may explain the observation of increased arterial stiffness with increased salt intake [167].

The cellular mechanisms underlying the relationship between sodium and development of hypertension remain unclear. Levine et al. [168] observed changes in sodium-hydrogen (Na^+ - H^+) exchange activity associated with essential hypertension, and proposed that enhanced Na^+-H^+ exchange may play a role in the pathogenesis of elevated blood pressure in salt-sensitive patients. Overactivity of Na^+ - H^+ exchange enhances proximal tubule Na^+ reabsorption and lead to a defect in renal excretion. Hypertension may be expressed via two effects. The renal defect would require the kidney to be perfused at a pressure level greater than normal to maintain salt balance [169]. An increase in intrathoracic blood volume stimulates the hypothalamus to secrete more of the circulating Na^+ - K^+ ATPase inhibitor, interfering with Na^+ - K^+ transport [170]. Impaired Na^+ - K^+ pump activity gives rise to an increase in intracellular sodium concentration. Since Na^+ - Ca^{2+} exchange helps maintain calcium concentration of the cytosol, an increased intracellular sodium would result from higher intracellular calcium concentration. Elevated levels of intracellular calcium concentration raises the tone and vascular reactivity of the smooth muscles of the arteries and veins so that arterial pressure rises and venous compliance is diminished.

In the present study, pressure change was inversely correlated with the attained pressure. This finding is at variance with the results reported by Miall and Lovell [95] and by Tervahauta et al. [19] that blood pressure changes on average are positively correlated with initial pressures: the higher the pressure, the greater the rate of change. This inverse relationship between pressure change and the attained pressure is most likely due to the "regression towards the mean" phenomenon, as illustrated in Figure 3.7 and Table 3.14. This expression literally means a falling back towards the average for the population. In

repeated measurements of blood pressure, an extremely high value would most likely fall back towards the mean, while the extremely low values would rise closer to the mean.

It is well known that hypertension represents the most potent risk factor for stroke, and is also one of the three major risk factors for ischemic heart disease. In my present study, both cohorts demonstrated a statistically significant increase in age-standardized stroke mortality rates. The upward trend in population stroke mortality corresponds to the slight increase in blood pressure due to cohort-period effect, in the Yugoslavian cohort. The increase in the cohort stroke death rates represents a modest age trend in systolic pressure. The downward trend in blood pressure due to the cohort-period effect in the Japanese cohort supports the decline in the population stroke death rates, and the steep aging trend in blood pressure explains an increase in the cohort stroke mortality rates.

My analysis of the stroke mortality trends for the population and cohort provides major support for the separation of cohort and age effects in the study of blood pressure aging.

3.10 Summary

In order to determine the part of change in the blood pressure during the 10-year observation period that was due to aging per se, I separated the age and the cohort-period effects, and accounted for body weight changes. The major findings of my analyses are as follows. In the Yugoslavian cohort, there was a small upward blood pressure trend due to the cohort-period effect, and a modest age trend in the systolic, diastolic, and pulse pressure. These blood pressure trends were associated with an upward trend in the population and cohort stroke death rate. In the Japanese cohort, I observed a downward trend due to the cohort-period effect, but a steep age trend in systolic, diastolic, and the pulse pressure. Once again, these trends were associated with population decrease but

cohort increase in the stroke mortality rate. This supports my initial decision to consider trends due to the cohort-period effect as phenomena that affect the total cohort equally.

My findings demonstrate a significant aging trend in the arterial blood pressure, corrected for the cohort-period effect in both cohorts. Compared to the entry systolic pressure, the Yugoslavian men's 10-year systolic pressure was increased by 6.9, 10.0, 8.3, and 6.3 mm Hg for the 40-44, 45-49, 50-54, and 59-64 age groups. Similar observations were made in the diastolic pressure, but the increase was of a smaller magnitude. The Japanese men's blood pressure also rose with age during the 10-year follow-up. There was an increase of 14.9, 13.5, 20.2, and 14.5 mm Hg in the systolic pressure, and of 8.8, 6.6, 1.0, and 3.7 mm Hg in the diastolic pressure, for the four respective age groups. The entry arterial blood pressure values of the Yugoslavian men were significantly higher than those of the Japanese men. A higher percentage of the Yugoslavian men's blood pressure was reduced during the 10-year observation period, compared to the Japanese men (systolic: 35% in the Yugoslavian cohort versus 23% in the Japanese cohort; diastolic: 50% in the Yugoslavian cohort versus 33% in the Japanese cohort). To the best of my knowledge, detailed analyses of the effects of age on blood pressure such as those performed in the present investigation, have not been reported by any research groups using the Seven Country data set.

The major limitation of the present work is related to data availability for analysis. Three blood pressure measurements were available for analyzing the age and cohort-period effects in the Yugoslavian cohort, but only two measurements (entry and 10-year) were available for such analysis in the Japanese cohort. In addition, the cohort analysis in both cohorts was restricted to three age groups in the Yugoslavian and two in Japanese cohorts. Such a restriction seriously limits my interpretations of the findings concerning the contributions of cohort-period effect in blood pressure. Further prospective studies with more blood pressure measurements are needed to determine the contribution of other effects besides age to the blood pressure change.

Another limitation of the study is that the two cohorts were composed of men only. Therefore the results are not generalizable to women on whom the age and cohorts effects may have differential influences on their blood pressure. Similar studies including female population should be conducted to fully explore the blood pressure-age relationships.

Chapter 4

Effect of Age on the Resting Heart Rate

4.1 Introduction

The purpose of this investigation was to determine the effect of age on the resting heart rate in the Yugoslavian and Japanese cohorts. This study is important to the next investigation in which I examined the effects of age on the elastic properties of the large arteries (see Chapter 5.1). This is because of the characteristic relationship between heart rate and the frequency of pressure wave. Waves with predominantly high frequency components travel faster than those with low frequency components. Literature has demonstrated a significant association between heart rate and pulse wave velocity, a parameter which gives a direct evaluation of arterial wall stiffness [171]. The determination of the heart rate aging trend in these cohorts will help me decide if this physiological variable should be controlled for in my analysis of the age effects on the status of the arterial vasculature.

The rhythmic discharge of the sinoatrial (SA) node occurs spontaneously, and is under the constant influence of nerves as well as hormones. A large number of parasympathetic and sympathetic fibers end on the SA node. Stimulation of the parasympathetics to the SA node causes slowing of the heart, while stimulation of the sympathetics increases the heart rate. In the resting state, the parasympathetic influence is dominant. The autonomic nervous system function declines with age. Aging is associated with changes in the sympathovagal balance [172,173].

Factors other than the cardiac nerves also can alter heart rate. These factors include the adrenomedullary hormones (circulating catecholamines), temperature, plasma electrolyte concentrations, and other hormones such as the thyroid hormones, insulin, and glucagon. A rise in the concentrations of the catecholamines, particularly epinephrine,

thyroid hormones, insulin, and glucagon, causes an increase in the resting heart rate. Similarly, elevation of temperature causes greatly increased heart rate. The plasma electrolyte concentrations also have an effect on the heart rate, for example, an excess potassium or a deficiency in calcium in the extracellular fluid can slow down the heart rate. However, these are generally of lesser importance; the heart rate is primarily regulated precisely by balancing the slowing effects of parasympathetic discharge against the accelerating effects of sympathetic discharge.

4.2 Statistical models

The statistical models for examining the aging trend of heart rate were similar to those used for assessing aging of blood pressures; they have been described in detail in Chapter 3 (see Table 3.11). Aging of the resting heart rate in the groups was assessed by regression analysis, with resting heart rate as the dependent variable, and the age at the time of measurement as the independent variable. The aging trend of heart rate in the individuals was analyzed by examining how the heart rate changed as the individuals in each study cohort aged. In this analysis, the 10-year change of heart rate was the dependent variable, age and the heart rate on entry to the study were the independent variables. The effects of the cardiac sympathetics and parasympathetics on heart rate were not controlled for in this analysis due to data unavailability.

4.3 Results

4.3.1 Aging of heart rate in the groups

Table 4.1 presents the mean values and standard errors of resting heart rate of four male birth cohorts of Yugoslavia and Japan.

Table 4.1 The mean values and standard errors of resting heart rate of four age groups of men in (a) Yugoslavia, and (b) Japan.

(a) Yugoslavia*

Age at time of measurement	Entry (1962)	10-yr (1972)
40-44	69.18±0.98 (n=139)	
45-49	69.34±1.19 (n=84)	
50-54	72.08±1.03 (n=136)	69.93±1.14 (n=122)
55-59	68.73±1.02 (n=136)	70.80±1.49 (n=76)
60-64		70.53±1.30 (n=113)
65-69		72.28±1.30 (n=116)

(b) Japan

Age at time of measurement	Entry (1958)	10-yr (1968)
40-44	61.39±1.12 (n=92)	
45-49	59.21±0.84 (n=119)	
50-54	60.12±0.90 (n=138)	61.46±1.24 (n=87)
55-59	61.71±1.02 (n=136)	60.53±0.95 (n=105)
60-64		62.74±0.95 (n=125)
65-69		63.08±1.05 (n=125)

*Data of the 5-year resting heart rate in the Yugoslavian cohort were not available.

The resting heart rate-age relationship was assessed by regression analysis. The result indicated that heart rate was not related significantly to age in either cohort. Table 4.2 presents the regression analysis of the resting heart rate on age in the cohorts.

Table 4.2 Regression analysis of the resting heart rate on age in the Yugoslavian and Japanese male cohorts.

	Yugoslavia RHR			Japan RHR	
Independent variable	Coefficient	Pr>F	Independent variable	Coefficient	Pr>F
Age	0.3197	0.2400	Age	0.5947	0.0001
Intercept	66.7692	0.0001	Intercept	55.3469	0.0121
R^2	0.0015		R^2	0.0067	

Model tested: RHR = age.
RHR = resting heart rate.

There is a slight, but statistically nonsignificant trend towards higher heart rate with age in the Yugoslavian cohort. A highly significant correlation was found between resting heart rate and age in the Japanese cohort. The slope of the resting heart rate-age relationship in the Japanese cohort was steeper than that in the Yugoslavian cohort (coefficient estimate: 0.5947 versus 0.3197 bpm/year). But this difference did not reach statistical significance. The low R^2 value in both regression models suggests that most of the variance in the resting heart rate is not explained by age.

Figure 4.1 is a graphic presentation of the observed resting heart rate for each of the 5-year age group in the Yugoslavian and Japanese cohorts.

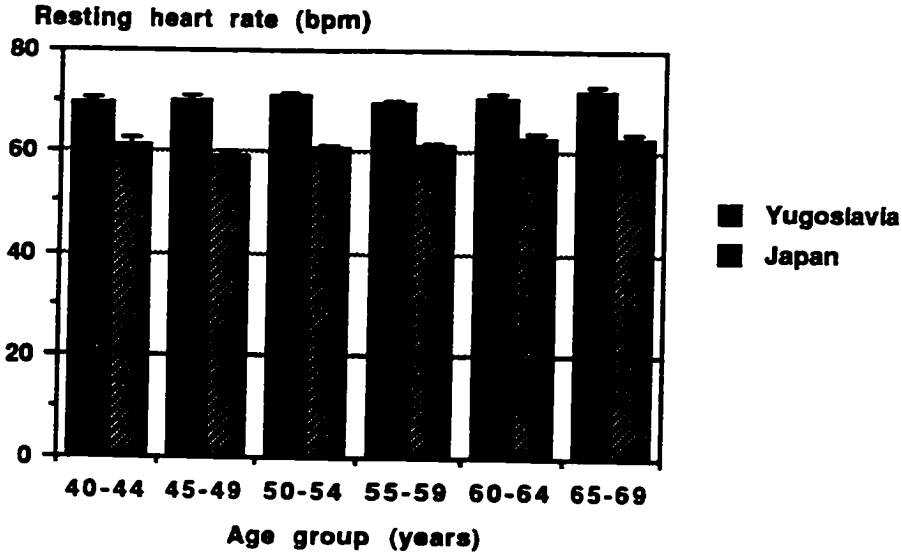


Figure 4.1 Distribution of the observed resting heart rate by the 5-year age groups in the Yugoslavian and Japanese male cohorts. The error bars represent SEM.

As shown in Figure 4.1, the observed resting heart rate of the Yugoslavian cohort was higher than that of the Japanese cohort, for any given age group. An upward age trend was observed in the Japanese, but not the Yugoslavian cohort.

4.3.2 Aging of heart rate in the individuals

The cumulative distribution of the change in resting heart rate over the 10-year period for each individual is shown in Figure 4.2. Similar to the cumulative distribution of 10-year blood pressure change, the distribution of the 5-year age groups were aggregated under equal weighting in order to account for the different age distribution in the two study populations.

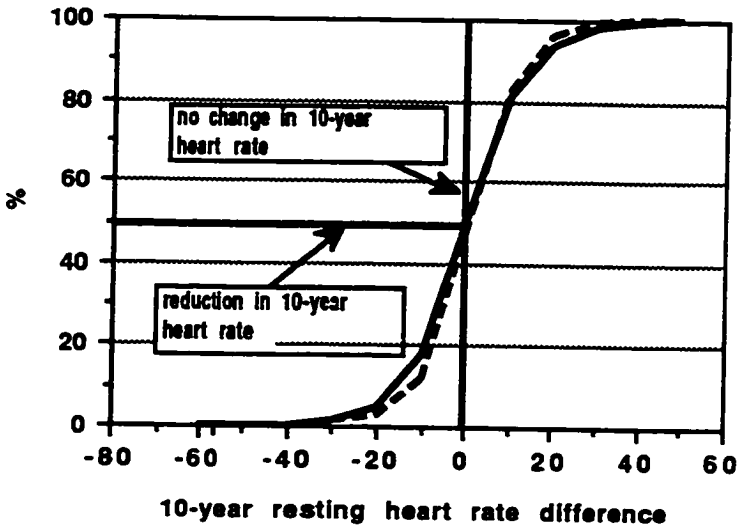


Figure 4.2 Cumulative distribution of 10-year resting heart rate differences in the Yugoslavian (solid line) and Japanese (dashed line) male cohorts.

Individuals in both cohorts showed a similar aging pattern in the resting heart rate. Close to 50% of the study population's resting heart rate either remained unchanged or decreased over the 10-year observation period, and the remaining subjects showed an increase. Table 4.3 summarizes the regression analysis of the aging trend in resting heart rate in individuals of both cohorts.

Table 4.3 Regression analysis of the 10-year change in resting heart rate on age, controlling for entry resting heart rate of the Yugoslavian and Japanese men.

Yugoslavian ΔRHR			Japan ΔRHR		
Independent variable	Coefficient	Pr>F	Independent variable	Coefficient	Pr>F
Age	0.0622	0.4538	Age	0.0322	0.6660
RHR	0.5811	0.0001	RHR	0.5128	0.0001
Intercept	-43.8010	0.0001	Intercept	-32.1411	0.0001
R^2	0.3614		R^2	0.2728	

Model tested: ΔRHR = age + RHR.
ΔRHR = 10-year change in resting heart rate; age = age at time of measurement; RHR = resting heart rate on entry to study.

The analysis failed to detect a statistically significant correlation between change in heart rate and age. The slope of the 10-year heart rate change-age is steeper in the Yugoslavian than in the Japanese cohorts (coefficient estimate: 0.0622 versus 0.0322 bpm/year). Testing of the heterogeneity of these slopes failed to detect a statistically significant difference. The regression results also showed that the 10-year heart rate change is a function of the entry resting heart rate in both cohorts.

4.4 Discussion

The finding of a statistically nonsignificant relationship between resting heart rate and age is consistent with that reported in the Seven Countries Study [3], and in a number of cross-sectional studies [103, 174]. But the finding of a small increase in resting heart rate with age, differs from previous investigations in which decreasing or unchanged trends were demonstrated [100,102,175].

Data of the resting heart rate acquired from the Seven Countries Study for the current investigation was calculated from the resting electrocardiogram. Specific

instructions were adhered to by the examining physicians when performing the electrocardiographic examination. Therefore, the way in which the heart rate was measured, is not likely to play a role in the slight increase of the resting heart rate with age in both cohorts. Resting heart rate, although not related to age, is modulated by autonomic tone, specifically by the relative sympathetic and parasympathetic tone. There is mounting evidence suggesting that sympathetic and vagal-cardiac activity changes reciprocally with increasing age [104,172,176,177]. Using spectral analysis to analyze heart rate variability, Korkushko et al. [174] demonstrated age-related differences in the ratios of sympathetic and parasympathetic influences on heart rates in normal subjects. I speculated that the upward aging trend in resting heart rate observed in my study populations may be related to age-associated changes in the sympathovagal balance. Infants and subjects after the sixth decade have predominant sympathetic chronotropic function, whereas those between these ages have predominant parasympathetic influence [178]. The mechanism responsible for this age-related increase in sympathetic, and a decrease in parasympathetic activity is not fully understood. The autonomic as well as nonautonomic influences, such as temperature, electrolyte concentrations, and other humoral factors, may play a role in this shift.

A statistically significant correlation was found between the 10-year heart rate change and the entry heart rate in both cohorts. This age-independent increase in heart rate is most likely caused by the cohort and period effects. In the Yugoslavian cohort, the body mass index increased during the 10-year follow-up, implying reduced physical activity. Reduced physical activity is associated with a higher resting heart rate. In the Japanese cohort, the body mass index decreased, but heart rate increased during the 10-year observation period. The significant relationship between heart rate change and the entry heart rate in the Japanese men is likely related to factors other than reduced physical activity.

4.5 Summary

In the present investigation, I have found that resting heart rate is not significantly related to age, but there is a trend towards higher heart rate with advancing age in both study cohorts. Similar to aging of blood pressures, only a fraction of the populations demonstrated a decrease in resting heart rate with age. Thus it does not qualify as an aging phenomenon.

The major limitation of the study is a lack of measurements of cardiac sympathetic and parasympathetic activity, therefore, its influences on the resting heart rate cannot be determined. Although resting heart rate is not age-related, but sympathetic and vagal-cardiac activity has been shown to be influenced by age. Since this activity can influence the resting heart rate, follow-up studies with measurements of heart rate, and activity of the sympathetic and parasympathetic nervous system made at different points of time in the life cycle, would permit the study of aging effect on heart rate.

Chapter 5

Age, Pulse Transit Time, and Pulse Wave Contour

5.1 Effects of age on the elastic properties of the large arteries

5.1.1 Introduction

Section 5.1 presents the assessment of the effects of age on the vasculature of the systemic arterial tree.

Vascular properties are determined by the material composition of vascular structure, and the ability of peripheral arteries to dilate [179]. Each of these properties is changed by the aging process. Morphological studies demonstrate increased intimal thickness, elastin fragmentation, calcification, and increased collagen in senescent vessels [31,180]. These structural changes are associated with the stiffening of the proximal vessels, leading to an increase in the pulse wave velocity [65,77,181].

The effects of aging on the cardiovascular system have been well established, and arterial blood pressure has been shown to rise as age advances [92]. In recent years, arterial stiffness, the reciprocal of arterial distensibility, has been reconsidered as being of prime importance in the genesis of systolic hypertension in the aged. There have been several published reports on the significant correlation between arterial distensibility, mean and systolic blood pressure in hypertensives [73,75,76]. Distensibility of the major arteries has been found to correlate negatively and significantly with arterial blood pressure. But it has not been established if a similar relationship between these two variables exists in normotensives and the aged. The purpose of the present study was to examine the influence of age on the elastic properties of the carotid arteries. It was also designed to identify other factors which can influence the pulse transit time. In this study, the pulse transit time was used as a proxy for arterial stiffness.

5.1.2 Method

In the present study, pulse transit time was determined from the time delay between two corresponding points on the proximal (carotid) and distal (femoral) pulse waves. The time delay was measured between the feet of simultaneously recorded pulse waves, with the foot of the wave identified as the point where the sharp systolic upstroke commenced.

The recording, digitizing, and analyzing of the carotid and femoral pulse wave signals have been described in detail in Sections 2.3, 2.4, and 2.5. In studies of the pulse wave velocity, the distance traveled by the pulse is measured over the surface of the body with a tape measure [71,77,81]. This method of measurement provides an indirect estimate of the distance traveled by the pulse; the exact distance, however, would require autopsy data. In the Seven-Country data set, there was no estimated distance between the two recording sites. Consequently, I used the acromial sitting height to substitute for this distance in my calculation of the pulse wave velocity. The acromial sitting height was estimated from the regression model using the standing and sitting height obtained from two sources: an ergonomic study [182] and a pilot study which I conducted to estimate the distance traveled by the pressure wave between the carotid and femoral arteries. The sample in the ergonomic study comprised of 25 Caucasian and 8 Asian men, while the pilot study included 6 Caucasian and 28 Asian men. The acromial standing height was the dependent variable, and the standing and sitting height were the independent variables. Table 5.1 gives the mean, minimum, and maximum vales for the sitting height, acromial sitting height, and standing height of these subjects.

Table 5.1 The mean, minimum, and maximum values for the sitting height, acromial sitting height, and standing height of Caucasian and Asian men in two field studies.

	Caucasian men (n=31)				Asian men (n=36)			
	mean	SEM	min	max	mean	SEM	min	max
sitting ht (cm)	92.48	0.49	85.2	97.5	89.00	0.69	81.3	98.5
acromial sitting ht (cm)	60.22	0.90	44.0	67.4	59.94	0.59	53.0	67.8
standing ht (cm)	176.29	1.06	162.0	190.0	171.64	1.02	161.5	184.2

SEM = standard error of the mean; min = minimum; max = maximum; ht = height.

Regression analysis indicated that the model could account for 51 % (R^2=0.51) of the variance in the acromial sitting height for the Caucasian men, and could explain only 25% (R^2=0.25) of the variance in the acromial sitting height for the Asian men. In view of the low R^2 values for both racial groups, particularly the Asians, and the fact that my estimated acromial sitting height does not accurately represent the arterial length between the recording sites, I decided to examine the relative contribution of the pulse transit time, and the acromial sitting height to the pulse wave velocity. This was done by comparing the magnitude of the variance of these two components. The variance of pulse wave velocity comprises of:

$$\sigma^2 (\text{LNDIST}) + \sigma^2 (\text{LNPTT}) = \sigma^2 (\text{LNPWV}) \qquad (5.1)$$

where σ^2 (LNDIST) = variance of the log-transformed acromial sitting height; σ^2 (LNPTT) = variance of the log-transformed pulse transit time; σ^2 (LNPWV) = variance of log-transformed pulse wave velocity. The calculated variance is 0.0012 for the log-transformed acromial sitting height, and 0.00723 for the log-transformed pulse transit time. Substituting these variances in equation 5.1, I obtain

$$\sigma^2 (\text{LNDIST}) + \sigma^2 (\text{LNPTT}) = 0.0012+0.0723 = 0.0735 \qquad (5.2)$$

The variance of the log-transformed pulse transit time contributes 98% of the variance of pulse wave velocity, while the acromial sitting height contributes only 2% of the variance. This observation, coupled with my uncertainty of the acromial sitting height, and the fact that pulse waves were measured at relatively constant anatomical positions in all subjects, has prompted me to use the pulse transit time as an (inverse) index of the velocity of the pulse wave in my data analysis. O'Rourke et al. [183], in their investigation of pressure wave transit time along the human aorta, also used the pulse transit time as an index of pulse wave velocity.

The recordings of pulse wave signals in the Yugoslavian and Japanese cohorts have been described in detail in Chapter 2.3. One hundred and thirty-two Japanese men, and 161 Yugoslavian men were measured for pulse waves.

Regression models were constructed to analyze the effects of age on the pulse transit time. In these statistical models, concurrent arterial blood pressure, heart rate, height, serum cholesterol concentrations, and cigarette smoking habits were included in the model as covariates. Previous studies have shown that the arterial blood pressure [181], heart rate [171], height [184], serum cholesterol concentrations [185], and smoking [186] significantly influenced the pulse transit time.

Because the elastic modulus of the arterial wall increases with circumferential tension [57], the pulse wave velocity depends on arterial pressure: the higher the pressure, the faster the speed of wave travel. The passive stretching of the collagenous fibers by the elevated blood pressure can lead to augmented stiffness of the arterial wall.

The effect of the heart rate on the pulse wave velocity has not been as extensively studied as the effects of age and arterial pressure. However, Simon et al. [187] and Taquet et al. [171] reported a moderately significant correlation between the heart rate and the pulse wave velocity, and attributed it to some nervous adrenergic influence on the smooth muscle of the aortic wall.

Several studies have shown that atherosclerosis in humans and in experimental animals can lead to an increase in pulse wave velocity [185,188,189]. Atherosclerosis is a disease of the arteries in which fatty tissues called atheromatous plaques develop in the intima and the sublying smooth muscles. In time, the fibroblasts of the plaque eventually deposit extensive amounts of dense connective tissue, and the sclerosis becomes so great that the arteries become stiff and unyielding.

Arterial stiffness is caused by a loss in elastin, an increase in collagen, or biochemical alterations in both. Cigarette smoke is known to injure the arterial smooth muscle cells, thus releasing proteins that stimulate collagen synthesis. In addition, it is postulated that cigarette smoke may also adversely affect the endothelium-derived relaxing factor or prostaglandin 1_2, thus increasing the vessel wall stiffness. This hypothesis is supported by the work of Wollersheim et al. [186] who observed a significant increase in vessel wall stiffness among smokers.

Initially, regression analysis was performed to determine if the relationship between the pulse transit time and age was different between the Yugoslavian and Japanese cohorts. If a difference was detected, I then tested the heterogeneity of the slopes.

The pulse transit time-age relationship was determined by a multiple regression analysis, using the PROC GLM procedure for the total group, and the PROC REG procedure [190] for the individual cohorts. The PROC REG analysis procedure produces the coefficients labeled "standardized estimate", which are the estimates that would be obtained if all the variables were standardized to a zero mean and unit variance prior to performing the regression computations. Standardization of the variables is done by subtracting their means and dividing by their standard deviations. Thus, these coefficients are independent of the scales of measurement of the independent variables and might be useful in ascertaining the relative importance of the effects of the independent variables on the dependent variable.

5.1.3 Results

I compared the pulse transit time of the left and right carotid arteries, to investigate if differences existed between them. In addition, I also compared the pulse transit time, and the interval from the onset of the Q-wave to the carotid and femoral upstroke between the two cohorts. The results of these comparisons are presented in the following section.

5.1.3.1 Descriptive statistics

In the Japanese cohort, four observations on the left and right pulse waves were deleted. Three observations on the left, and one on the right pulse waves in the Yugoslavian cohort were also deleted from the data set. These observations were deleted because they exerted a strong influence on the estimate of the regression coefficient of the unknown parameters.

A regression analysis was performed to test if subjects with the pulse wave tracings were significantly different from those without the tracings on the following baseline variables: age, systolic and diastolic blood pressure, resting heart rate, serum cholesterol, and cigarette smoking habits in both cohorts. There was no statistically significant difference between these two groups in any of these variables in either cohort.

I compared the two cohorts on some demographic, hemodynamic, and anthropometric variables. The comparisons were made on raw data which did not correct for the age difference for five years between the Yugoslavian and Japanese cohorts. Table 5.1 presents comparisons.

Table 5.2 Comparisons of demographic, hemodynamic, and anthropometric characteristics of men in the two populations who had pulse wave tracings.

Variable	Japan N=132	Yugoslavia N=161
Age (year)	59.6±0.5	54.9±0.5*
Height (cm)	160.7±0.5	168.4±0.5*
Weight (kg)	54.8±0.6	63.8±0.9*
BMI (kg/m^2)	21.2±0.1	22.5±0.3*
SBP (mm Hg)	133.5±2.5	139.0±1.5**
DBP (mm Hg)	74.0±1.1	83.4±0.7*
MAP (mm Hg)	93.9±1.5	102.0±0.9*
SC (mg/dl)	148.9±0.1	171.8±0.1*
Cigarette smoking (%)		
non-smokers	16.5	24.2**
smokers	83.5	75.8**
amount smoked	6.4±0.05	6.2±0.05**

SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure, and is calculated as 1/3(SBP-DBP)+DBP; SC = serum cholesterol concentrations; SMC = number of cigarettes smoked per day. Amount of cigarette smoked is coded as follows: 0-1 = none or stopped smoking for at least one year; 4 = smoking 1-4 cigarettes per day; 5 = smoking 5-9 cigarettes per day; 6 = smoking 10-19 cigarettes per day; 7 = smoking 20-29 cigarettes per day; and 8 = smoking > 30 cigarettes per day. Values are expressed as means±SEM or as percentage of the number of each group.
*$p < 0.001$.
**$p < 0.05$.
The p value is for the Student's t test for independent samples.

Compared to the Yugoslavians, the Japanese were significantly older, slimmer, had lower arterial blood pressure, and serum cholesterol concentrations at the time when the pulse wave signals were recorded. But the proportion of cigarette smokers was significantly higher in the Japanese than in Yugoslavian cohorts.

Table 5.2 summarizes the mean values, standard errors of the pulse transit time, interval from the onset of Q-wave to carotid upstroke, and to femoral upstroke in the two cohorts.

Table 5.3 Mean values, standard errors of carotid pulse transit time, interval from onset of Q-wave to carotid upstroke (QONCAR), and to femoral upstroke (QONFEM) of men in the Japanese and Yugoslavian cohort.

Variable	Japan Mean±SEM	Range	Yugoslavia Mean±SEM	Range
Pulse transit time(msec)				
left	54.5±1.22	26-91	62.6±1.29	24-104.0
right	53.3±1.19	20-98	63.6±1.22	20-108.0
QONCAR(msec)				
left	122.9±1.6	81-172.2	125.6±1.6	75.4-204.0
right	123.2±1.8	59-171.9	123.1±1.7	62.1-224.3
QONFEM(msec)				
left	177.5±1.7	135.1-223.1	188.4±1.9	137.4-258.0
right	176.5±1.7	129.2-225.6	186.7±1.9	115.4-284.9

The pulse transit time of the Yugoslavian men was significantly longer than that of the Japanese men, $p < 0.0005$. The interval between the onset of the Q-wave and the carotid upstroke of the left pulse of the Yugoslavian men was significantly longer than that of the Japanese men, $p < 0.05$.
These measurements are raw data, and do not correct for the age difference for 5 years between the two cohorts.

An intra-group comparison using the paired t test, indicated no statistically significant difference between the left and right pulse transit time when data were analyzed as a total group (Mean±SE = -0.14±0.91, t = -0.16, p = 0.88). The interval from the onset of the Q-wave to the carotid upstroke of the left side did not differ significantly from that of the right side in the pooled data (Mean±SE = 1.51±1.01, t = 1.49, p = 0.14). Similar results were observed in the comparison of the interval from the onset of the Q-wave to the femoral upstroke between the left and right pulse waves (Mean±SE = 1.35±0.81, t = 1.76, p = 0.10).

Separate group analysis also showed that in the Japanese cohort, no statistically significant difference was found between the left and right pulse transit time (Mean±SE = 1.02±1.37, t = 0.74, p = 0.46), the interval from the Q onset to the carotid upstroke (Mean±SE = -0.31±1.48, t = -0.21, p = 0.83), and to the femoral upstroke (Mean±SE = 0.70±0.98, t =0.72, p = 0.47). In the Yugoslavian cohort, the pulse transit time (Mean±SE

= -1.11±1.21, t = -0.92, p = 0.36) and the interval from the onset of the Q wave to the femoral upstroke of the left pulse did not differ significantly from that of the right pulse (Mean±SE = 1.88±1.23, t = 1.53, p = 0.13). But the interval from the onset of the Q-wave to the carotid upstroke of the left pulse was significantly longer than that of the right pulse (Mean±SE = 3.01±1.3, t = 2.19, p = 0.03).

The Student's t test for two independent samples was used to compare the pulse transit time of the two cohorts. The transit time of the left and right pulse of the Yugoslavian men was significantly longer than that of the Japanese men (left pulse: t = 4.57, p = 0.0001; right pulse: t = 6.06, p = 0.0001), but no significant difference was observed in the two intervals.

The pulse transit time of the Yugoslavian and Japanese men was compared with that of Avolio et al.'s Chinese and Australian subjects of the same age range [191]. Table 5.3 gives the mean values and standard errors of the pulse transit time of my two study cohorts, as well as those of Avolio et al.'s subjects. Figures 5.1 and 5.2 compare my pulse transit time data of the Japanese and Yugoslavians with Avolio et al.'s Chinese and Australian subjects [191].

Table 5.4 A comparison of the mean and standard error of the pulse transit time of two male cohorts in the present study and Avolio et al.'s study .

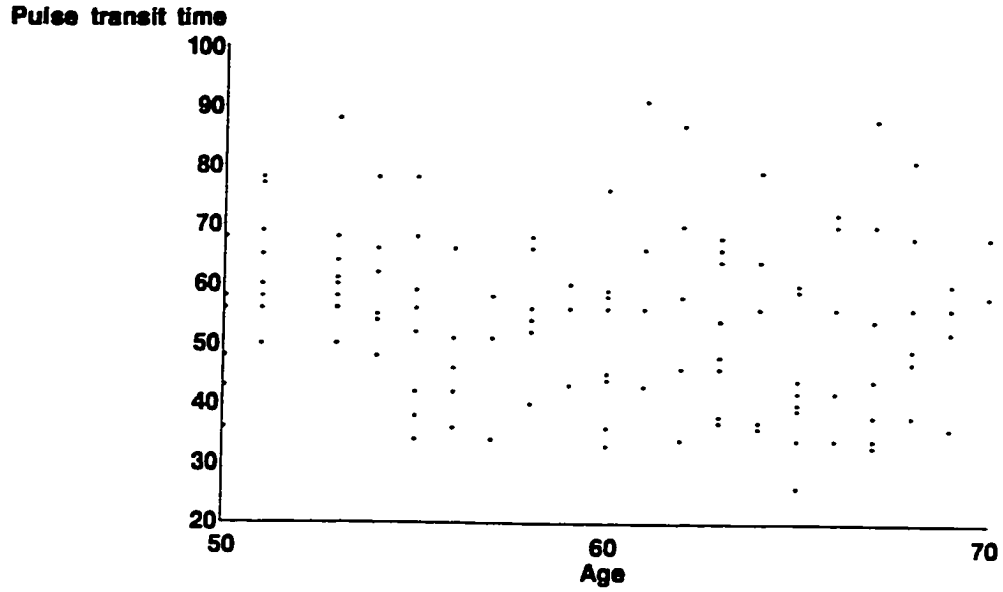
	Asians (aged 50-70 yrs)		**Caucasians** (aged 45-65 yrs)	
	Chinese (Avolio's study)	Japanese (present study)	Australians (Avolio's study)	Yugoslavians (present study)
N	153	130	166	159
Mean (msec.)	54.51	54.44	68.98	62.55
SE	1.07	1.72	1.40	1.29
t value	0.019		3.37	
p value	NS		0.0005	

NS = statistically nonsignificant.

The p value is given for the Student's t test comparing the mean pulse transit time of two cohorts in the present study to that of the Chinese and Australian subjects in Avolio et al.'s study.

In Avolio et al.'s study, the Chinese' pulse transit time was significantly shorter than the Australians' (Mean±SE: 54.51±1.06 versus 68.98±1.40, $t = -8.13$, $p = 0.0001$). Similarly, the pulse transit time of the Japanese men in the present investigation was also shorter than that of the Yugoslavians (Mean±SE: 54.44±1.72 versus 62.55±1.29, $t = -4.51$, $p = 0.0001$). When I compared the pulse transit time of the Chinese with that of the Japanese, no statistically significant difference was found (Mean±SE: 54.51±1.72 versus 54.44±1.72, $t = 0.02$, $p = 0.25$). But a statistically significant difference was observed in the pulse transit time of the Yugoslavians and the Australians (Mean±SE: 62.55±1.29 versus 68.98±1.40, $t = 3.37$, $p = 0.0005$).

(a)



(b)

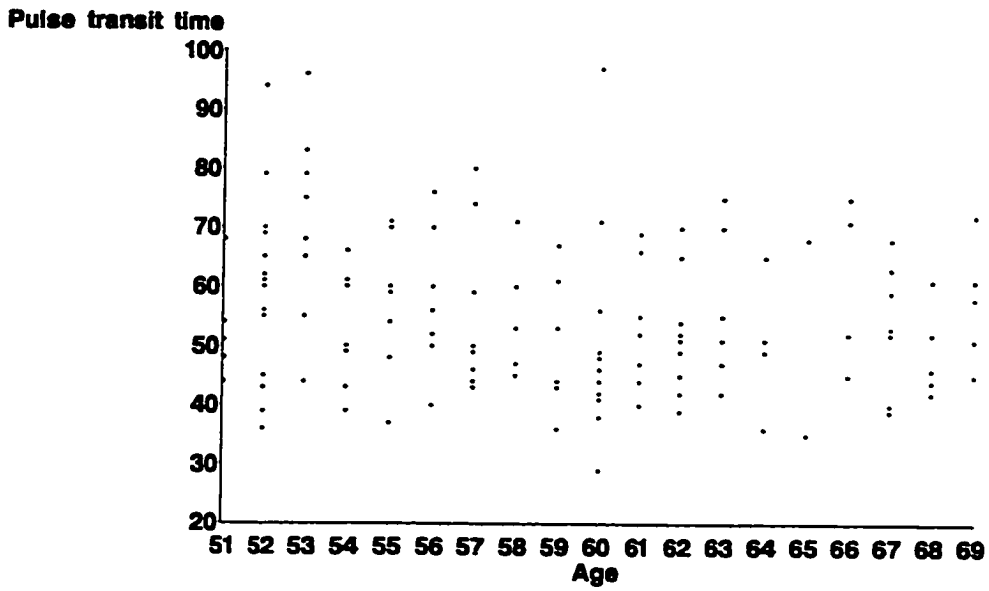
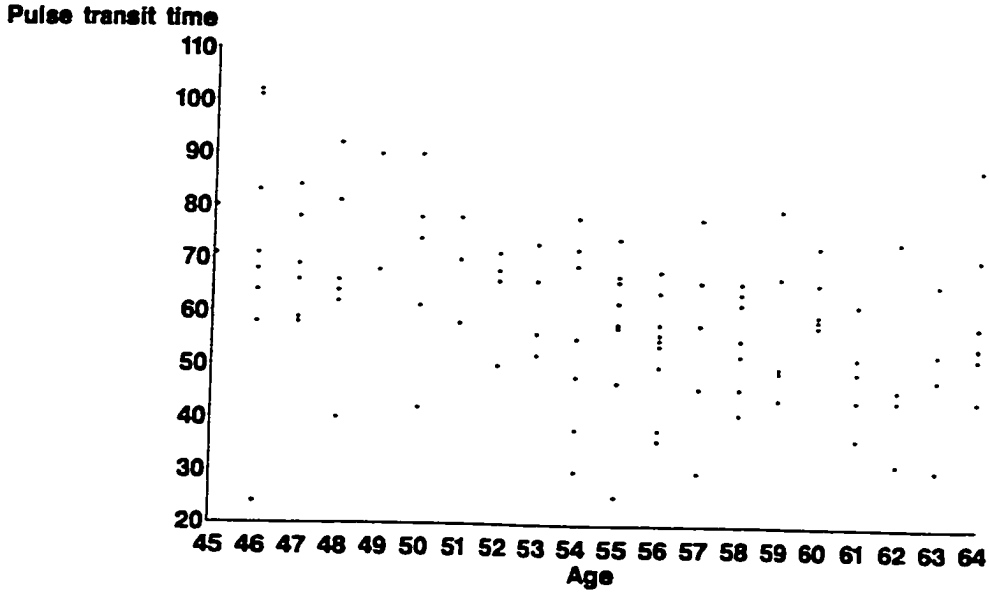


Figure 5.1 Comparison of (a) the pulse transit time distribution of the Japanese men in the present study with (b) the pulse transit time distribution of the Chinese subjects in Avolio et al.'s study [191].

(a)



(b)

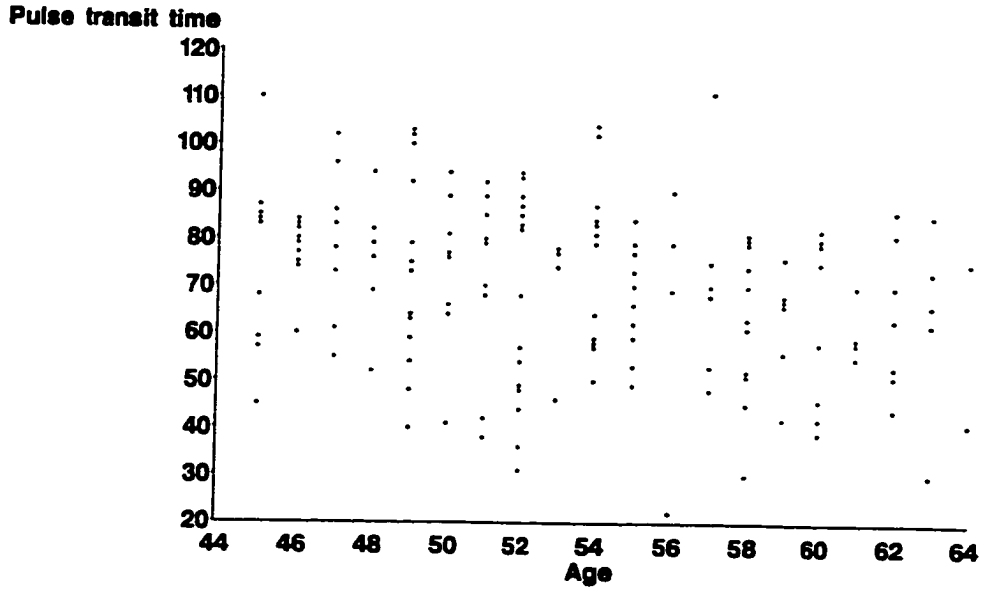


Figure 5.2 Comparison of (a) the pulse transit time distribution of the Yugoslavian men in the present study with (b) the pulse transit time distribution of the Australian subjects in Avolio et al.'s study [191].

My pulse transit time data show a large variability, with a coefficient of variation of 0.2553 in the Japanese cohort, and 0.2595 in the Yugoslavian cohort. However they compare well with those reported by Avolio [191]. For their Chinese subjects aged 50-70, the coefficient of variation is 0.2399, and 0.2617 for Australians aged 40-60 years.

The large variability in the pulse transit time distribution, particularly among the Yugoslavian men, deserves consideration. The following explanations are proposed: 1. There is mounting evidence of a loss of endothelium-dependent relaxation in older people, and in hypertensives [28,192]. It has been speculated that this loss is primarily initiated by an altered modulatory role of endothelial cells. It is possible that some subjects in the Yugoslavian cohort may have dysfunctional endothelial cells, leading to a state of sustained vascular contraction. Reduced arterial lumen in the peripheral arterioles causes elevated vascular resistance, which in turn, results in a chronic increase in blood pressure and arterial wall stiffness. It has been documented that higher heart rate reflects heightened adrenergic activity [193]. Norepinephrine induces contraction of the vascular smooth muscle by binding it to its specific receptors on the smooth muscle cell membrane, to hydrolyze the membrane phospholipid (phosphatidyl inositol 4.5 biphosphate PIP_2) by phospholipase C, which is activated by the GTP-based G proteins. This results in the release of diacylglycerol and inositol-1, 4, 5-triphosphate (IP_3). IP_3 releases Ca^{2+} from the IP_3-sensitive Ca^{2+} store. This Ca^{2+} induces Ca^{2+} release from the IP_3-insensitive store. High norepinephrine concentrations can also depolarize the smooth muscle cell membrane to the level which activates the L-type Ca^{2+} channel. Dysfunctional endothelial cells in aging or hypertension may have reduced the endothelium-dependent relaxing factor (nitric oxide), thus unmasking the vasoconstricting effect of the endothelin 1 (ET1). This speculation is supported by the observation that the concurrent heart rate of the Yugoslavian men was significantly higher than that of the Japanese men (Mean±SD: 74.24±12.66 bpm versus 58.3±8.7 bpm, $t = 13.21$, $p = 0.0001$). The variation in heart rate is correspondingly higher in the Yugoslavians (coefficient of variation = 0.1706) than in the

Japanese (coefficient of variation = 0.1496). Although analysis shown in Chapter 4 indicated no statistically significant aging trend in heart rate in individuals of both cohorts, a trend towards higher heart rate with age was observed. Thus it seems possible that the large variability in the pulse transit time distribution could be related to heightened sympathetic nervous system activity.

2. The relationship between pulse wave velocity and the arterial wall properties is given by Moens-Korteweg's equation [67]:

$$PWV=\frac{\sqrt{E\cdot h}}{2r\rho} \tag{5.3}$$

where E is the elastic modulus, h is the arterial wall thickness, r is the internal radius, and ρ is density of blood.

This equation can be re-written as:

$$PTT=\frac{L}{PWV}=\frac{(L\cdot 2r\rho)}{\sqrt{E\cdot h}} \tag{5.4}$$

where PTT = pulse transit time, and L = distance between two recording sites, and PWV = pulse wave velocity.

The pressure pulse generated by the ventricular ejection is propagated throughout the arterial tree at a speed determined by the elastic properties of the arterial wall, its thickness, the lumen caliber, and the density of the blood. Since blood is contained in a system of elastic conduits, energy propagation occurs predominantly along the wall of the distensible arterial wall, and not through the incompressible blood. The contribution of the blood density to the pulse wave velocity is negligible. The major determinants of the speed of propagation are therefore, the elastic properties of the vessel wall, its thickness, and diameter.

Age is a well known determinant of the caliber of arteries as demonstrated by anatomic reports [194]; the older the subjects, the higher the large artery diameter [195].

The augmentation of the artery diameter is a compensation for the age-related increase in arterial stiffness. However, it has been shown that such increase in the artery diameter occurs up to an age of 60 years [57]; thereafter, the artery diameter remains unchanged, while the vessel stiffness continues to rise.

My data of pulse transit time, an index of arterial stiffness, show a large variability, even at a fixed age. Published aortic dimensions indicate a relatively small variability. Table 5.4 gives the coefficient of variation of the aortic dimensions, derived from the data reported by Dixon et al. [196], Isnard et al.[121], Dahan et al. [75], and Stefanadis et al. [197].

Table 5.5 Values for the coefficient of variation of the aortic dimensions reported in the literature.

Year	Investigator	Age	Measurement Location	Measurement Method	CV
1984	Dixon et al.	40-69	Abd aorta	computed tomography	0.1023
1989	Isnard et al.	20-52	Aortic arch	echocardiography	0.1523
1990	Dahan et al.	21-84	Ascending aorta	echocardiography	0.1414
1990	Stefanadis et al.	40-50	Aortic root	echocardiography	0.1234

CV = coefficient of variation derived from the published data, by using the formula : $CV = \frac{SD}{\overline{X}}$; Abd aorta = abdominal aorta.

The large variability of my data demonstrates that pulse transit time is not a simple function of the age-related differences in aortic dimensions. Other factors that contribute to the speed with which the pressure wave propagates along an artery are the elastic properties of the arterial wall and its thickness. Arterial stiffness, measured by elastic modulus, has been shown to have a comparatively larger variation than the aortic diameter. Derived from data reported by Gozna et at. [68], the coefficient of variation of the elastic modulus is 0.301 for the 20-50 years age group, and 0.3341 for the 20-52 age group in Isnard et al.'s

study [121]. It seems reasonable to speculate that variability in elastic modulus, and to a lesser extent, variability in the aortic diameter account for the large variability of my pulse transit time distribution.

5.1.3.2 Determinants of the pulse transit time

Research studies have consistently shown a downward age trend in the pulse transit time, and that age is a significant determinant of the speed with which the pulse wave propagates along an artery. The following analyses were designed to determine 1. if an age trend was present in the pulse transit time distribution of these cohorts, and 2. the relationship of the pulse transit time to age, controlling for the covariates identified in the method section (5.1.2). These covariates were included in the regression model because they were known to affect the travel time of the pulse wave. Systolic and diastolic blood pressure were entered in the model separately as covariates of the regression analysis, because of a relatively high correlation between these two variables ($r = 0.65$).

An analysis was first performed on the pooled data, with the testing of the heterogeneity of slopes of the pulse transit time-age relationship for the two cohorts. Since a significant difference in the slopes was observed, the data from the individual cohorts were then analyzed. Figures 5.3 and 5.4 present the age trend of the pulse transit time of the pooled sample, and the individual cohorts. Table 5.5 summarizes the regression analysis of the relationship between pulse transit time and age in the pooled sample.

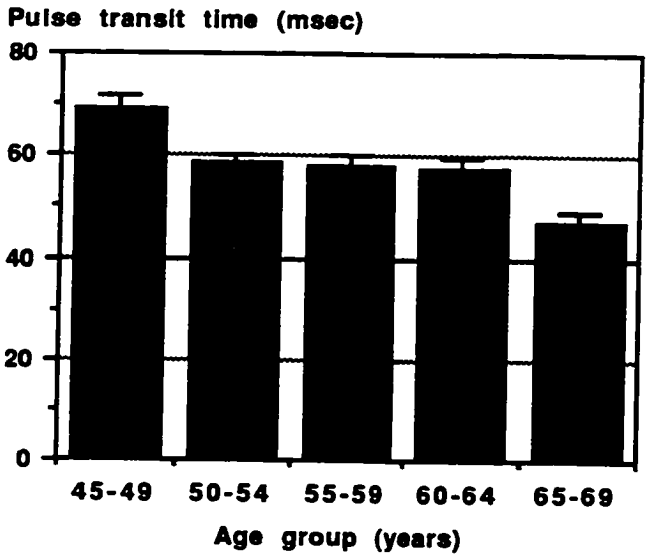
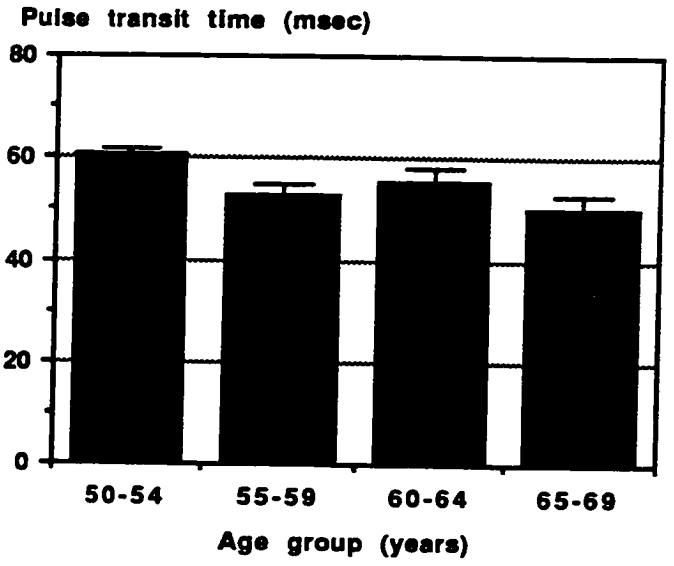


Figure 5.3 Distribution of the pulse transit time by age group in the pooled sample. The error bars represent SEM.

(a)



(b)

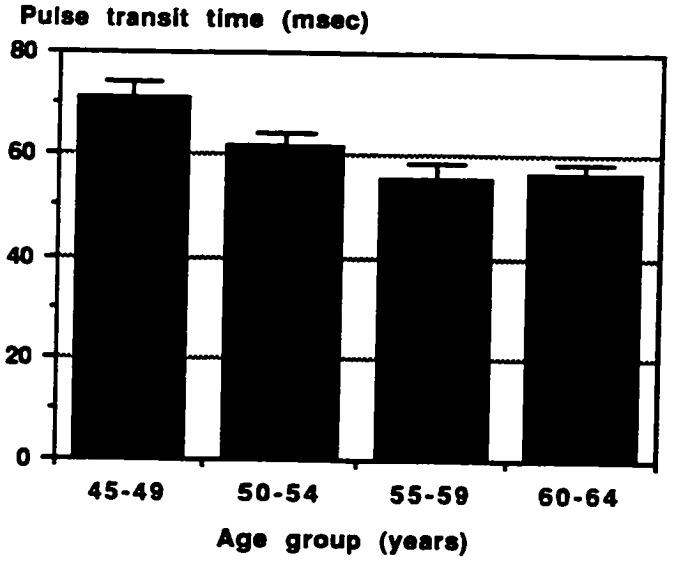


Figure 5.4 Distribution of the pulse transit time by age group in the (a) Japanese and (b) Yugoslavian male cohorts. The error bars represent SEM. These measurements are raw data, and do not correct for the age difference for 5 years between the two cohorts.

The results indicate a downward age trend in the distribution of the pulse transit time in the pooled sample, as well as in individual cohorts. They suggest that the pulse wave propagates faster in older age groups as compared with the younger ones.

Table 5.6 (a) Regression analysis of pulse transit time on age, controlling for systolic blood pressure and various study variables in the pooled sample. (b) The same analysis, controlling for diastolic blood pressure and other covariates.

(a)

Variable		Estimate of coefficient	p Value
Age		0.0026	0.9899
Log-transformed SBP		-22.5190	0.0001
Log-transformed BMI		9.1834	0.2109
Log-transformed SC		8.3314	0.0645
Log-transformed HR		-26.0827	0.0001
Height		0.3828	0.0109
SMC		0.2802	0.3676
Area	12	46.5906	0.0055
	13	0.0000	.
Age*area	12	-0.6868	0.0178
	13	0.0000	.
Intercept		137.2953	0.0041
R^2		0.2928	

(b)

Variable		Estimate of coefficient	p Value
Age		- 0.0201	0.9191
Log-transformed DBP		-27.6513	0.0001
Log-transformed BMI		9.3298	0.1907
Log-transformed SC		10.6493	0.0153
Log-transformed HR		-24.9489	0.0001
Height		0.4147	0.0046
SMC		0.2763	0.3616
Area	12	52.0767	0.0017
	13	0.0000	.
Age*area	12	- 0.7780	0.0065
	13	0.0000	.
Intercept		125.9099	0.0076
R^2		0.2982	

Model tested: (a) Pulse transit time = age + Log SBP + Log BMI +Log SC +Log HR +height +SMC +area + age*area.
Model tested: (b) Pulse transit time = age + Log DBP + Log BMI +Log SC +Log HR +height +SMC +area + age*area.
SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; SC = serum cholesterol concentrations; HR = heart rate; SMC = number of cigarettes smoked per day; Area = study area (area 12 = Yugoslavian cohort, area 13 = Japanese cohort); Age*area = an interaction term between age and the study area .
The regression analysis was performed with the PROC GLM procedure in the SAS system for linear models. The estimate of coefficients are for the parameters (intercept and coefficient), and are not standardized coefficients as those produced with the PROC REG procedure.

An analysis of the pooled data failed to detect a significant correlation between pulse transit time and age. The systolic and diastolic blood pressure, and the concurrent heart rate were significant predictors of the pulse transit time.

The study area had an effect on the pulse transit time at any given age ($p = 0.006$ when controlling for the systolic blood pressure, and $p = 0.002$ when controlling for the diastolic pressure). The test of heterogeneity of the slopes indicated that the pulse transit time-age relationship was significantly different for the two areas. The regression data of the pulse transit time with age for each cohort, and its predicted mean and standard error are summarized in Table 5.6.

Table 5.7 (a) The pulse transit time in each study cohort: observed mean, standard error, and regression on age controlling for systolic blood pressure and other covariates. (b) The same analysis, controlling for diastolic blood pressure and other covariates.

(a)

Study cohort	Observed mean (msec.)	SEM	Regression on age		
			Slope	SEM	p Value
Japan	52.76	1.45	0.0026	0.2091	0.9899
Yugoslavia	59.85	1.63	-0.6841	0.2175	0.0019

(b)

Study cohort	Observed mean (msec.)	SEM	Regression on age		
			Slope	SEM	p Value
Japan	52.34	1.42	-0.0201	0.1998	0.9199
Yugoslavia	59.65	1.62	-0.7981	0.2131	0.0002

Since the pulse transit time-age relationship was significantly different for each cohort, I analyzed the data from Japan and Yugoslavia, to investigate the effect of age on the pulse transit time, controlling for the same covariates as in the regression analysis of the pooled data. Tables 5.6 and 5.7 present the analysis results in the Japanese and Yugoslavian cohorts.

Table 5.8 (a) Regression analysis of the pulse transit time on age, controlling for concurrent systolic blood pressure and other study variables in the Japanese male cohort. (b) The same analysis, controlling for diastolic blood pressure and other covariates.

(a)

Variable	Parameter Estimates	Standardized Estimates	p Value
Age	-0.0966	-0.0438	0.6668
Log-transformed SBP	-24.4758	- 0.3639	0.0001
Log-transformed BMI	11.3437	0.0987	0.2957
Log-transformed SC	15.2856	0.2089	0.0114
Log-transformed HR	-27.4121	- 0.3182	0.0002
Height	0.5584	0.2366	0.0084
SMC	0.1556	0.0313	0.6944
Intercept	77.4051	0.0000	0.2676
R^2	0.3286		

(b)

Variable	Parameter Estimates	Standardized Estimates	p Value
Age	-0.2054	-0.0871	0.3787
Log-transformed DBP	-28.0847	- 0.3224	0.0002
Log-transformed BMI	18.7395	0.1490	0.1050
Log-transformed SC	12.2779	0.1582	0.0567
Log-transformed HR	-35.5212	- 0.3548	0.0001
Height	0.5873	0.2304	0.0102
SMC	0.7427	0.0796	0.3198
Intercept	80.0803	0.0000	0.3007
R^2	0.3233		

Model tested: (a) Pulse transit time = age + Log SBP + Log BMI +Log SC +Log HR +height +SMC.
Model tested: (b) Pulse transit time = age + Log DBP + Log BMI +Log SC +Log HR +height +SMC.
SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; SC = serum cholesterol concentrations; HR = heart rate; SMC = number of cigarettes smoked per day. The coefficients labeled Standardized Estimate are the estimates that would be obtained if all variables were standardized to a zero mean. These coefficients are independent of the scales of measurements of independent variables, and might offer a comparison of the magnitude of the effects of the variables.

Table 5.9 (a) Regression analysis of the pulse transit time on age, controlling for concurrent systolic blood pressure and other study variables in the Yugoslavian male cohort. (b) The same analysis, controlling for diastolic blood pressure and other covariates.

(a)

Variable	Parameter Estimates	Standardized Estimates	p Value
Age	- 0.7817	- 0.3141	0.0006
Log-transformed SBP	-32.9193	- 0.2569	0.0045
Log-transformed BMI	13.0294	0.1261	0.1807
Log-transformed SC	0.0607	0.1487	0.1224
Log-transformed HR	-24.4041	- 0.2850	0.0033
Height	0.2147	0.0892	0.3136
SMC	0.2570	0.0531	0.5656
Intercept	281.4671	0.0000	0.0002
R^2	0.3441		

(b)

Variable	Parameter Estimates	Standardized Estimates	p Value
Age	- 0.8277	- 0.3436	0.0003
Log-transformed DBP	-18.1524	- 0.1309	0.1511
Log-transformed BMI	12.4065	0.1240	0.2055
Log-transformed SC	0.0420	0.1072	0.2819
Log-transformed HR	-23.3887	- 0.2786	0.0051
Height	0.3256	0.1464	0.1126
SMC	0.2771	0.0592	0.5374
Intercept	184.1590	0.0000	0.0179
R^2	0.2758		

Model tested: (a) Pulse transit time = age + Log SBP + Log BMI +Log SC +Log HR +height +SMC.
Model tested: (b) Pulse transit time = age + Log SBP + Log BMI +Log SC +Log HR +height+SMC.
SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; SC = serum cholesterol concentrations; HR = heart rate; SMC = number of cigarettes smoked per day. The coefficients labeled Standardized Estimate are the estimates that would be obtained if all variables were standardized to a zero mean. These coefficients are independent of the scales of measurements of independent variables, and might offer a comparison of the magnitude of the effects of the variables.

Regression analysis of data from the individual cohorts presents somewhat different findings. In the Japanese cohort, the pulse transit time was inversely, but not significantly correlated with age. Concurrent arterial blood pressure (systolic and diastolic) and heart rate were significant predictors of the transit time (Table 5.6). In the Yugoslavian cohort, the pulse transit time was inversely and significantly correlated with age; age exerted an important effect (standardized estimate = -0.3141 when controlling for systolic pressure, and -0.3436 when controlling for diastolic pressure) on the pulse transit time. The systolic blood pressure (standardized estimate = -0.2569), and the concurrent heart rate (standardized estimate = -0.2800 when controlling for systolic pressure, and -0.2786 when controlling for diastolic pressure) were also significant and important factors influencing the pulse transit time (Table 5.8).

To estimate the magnitude of the influence of age and other covariates on the pulse transit time, I performed the univariate regression analysis in each cohort separately, and compared the R^2 value from each analysis. Table 5.9 presents the results.

Table 5.10 Regression coefficients, p value, and R^2 values derived from the univariate regression analysis of pulse transit time on various covariates in the individual cohorts.

Variable	Coefficient	p Value	R^2
Japan			
LGHR	-38.9489	0.0001	0.1741
LGDBP	-32.7519	0.0001	0.1449
LGSBP	-25.0032	0.0001	0.1246
Age	- 0.3605	0.0891	0.0231
Height	0.3470	0.1438	0.0182
SC	9.0246	0.1962	0.0135
LGBMI	3.3759	0.7695	0.0007
SMC	0.0823	0.8693	0.0002
Yugoslavia			
Age	- 1.0145	0.0001	0.1419
LGSBP	-42.4563	0.0004	0.1034
LGHR	-32.0562	0.0001	0.0980
Height	0.5347	0.0201	0.0428
LGBMI	16.1102	0.1258	0.0199
LGDBP	-19.9782	0.1624	0.0166
SMC	- 0.3897	0.4349	0.0053
SC	0.0091	0.8210	0.0005

Models tested: Pulse transit time = age; Pulse transit time = LGSBP; Pulse transit time = LGHR; Pulse transit time = height; Pulse transit time = LGBMI; Pulse transit time = LGDBP; Pulse transit time = SMC; Pulse transit time = SC.
LGSBP = log-transformed systolic blood pressure; LGDBP = log-transformed diastolic blood pressure; LGHR = log-transformed concurrent heart rate; LGBMI = log-transformed body mass index; LGSC = log-transformed serum cholesterol concentrations; SC = serum cholesterol concentrations; SMC = number of cigarettes smoked.

In the Japanese cohort, the concurrent heart rate contributes the most to the pulse transit time ($R^2 = 0.1741$), whereas in the Yugoslavian cohort, 14% of the variability in the pulse transit time can be understood in terms of the variance in age ($R^2 = 0.1419$). The contribution of systolic and diastolic pressure to the pulse transit time is moderate in both cohorts.

5.1.4 Discussion

5.1.4.1 Cohort differences in pulse transit time

The pulse transit time of the Japanese cohort was significantly shorter than that of the Yugoslavian cohort, suggesting stiffer arterial walls and/or smaller arteries. However, the smaller body size of the Japanese men does also contribute to the shorter pulse transit time (see Table 5.1). It has been shown that short stature is associated with a short pulse travel time [184,195] which agrees with my analysis. My finding of a shorter transit time among Japanese men concurs with that of Avolio et al. [65], who compared their pulse wave velocity results with those of the aortic pulse wave velocity studies in German [198], American [55,199], Canadian [68], Israeli [200], French [72], and English [69] subjects. They reported a higher pulse wave velocity in Chinese subjects at a younger age, and a greater age-related increase in pulse wave velocity than in the Caucasian subjects, not controlling for arterial blood pressure.

The contribution of atherosclerosis to increased arterial stiffness, measured by pulse transit time, is not likely in my study. The serum cholesterol concentrations of the Japanese men was significantly lower than that of the Yugoslavian men (Mean±SE: 148.9±1.3 versus 171.8±1.7 mg/dl, $t = 5.89$, $p = 0.0001$). To date, there has been no satisfactory physiological explanation for the difference in the pulse transit time between two ethnically different populations. However, on the basis of Avolio et al.'s results [65,71] as well as mine, it is tempting to speculate that different individuals and genetic pools may have a differential rate of arterial degeneration. It is conceivable that the collagen and elastin fibers

in the walls of the large arteries of Asians may undergo aging changes faster than those experienced by Caucasians. Factors such as diets, physical activities, or genetic differences in arterial morphology, might be responsible for instigating these changes earlier and faster in some racial groups.

5.1.4.2 Determinants of the pulse transit time

The pulse transit time was significantly associated with age in the Yugoslavians, even after controlling for blood pressure, heart rate, serum cholesterol, body mass index, height, and the number of cigarettes smoked per day. Among these covariates, age contributes the most to the pulse transit time ($R^2 = 0.1419$) (see Table 5.9). This finding implies that age-related alterations in the elastic properties of the large arteries are responsible for the shortened pulse transit time in this population. The progressive disintegration of elastic fibers caused by increased circumferential tension with high blood pressure and the fatiguing effects of cyclic mechanical stress [195], the cross-linking of collagen, hypertrophy of smooth muscle cells and deposition of calcium within the media associated with advancing age, all lead to increased stiffness of artery walls. On the other hand, the significant association between pulse transit time and age may be due to the increase in wall thickness associated with advancing age. Arteries have been observed to dilate and lengthen progressively with age [201,202], and the arterial wall thickens. The pulse wave velocity is related to Young's modulus (E) and arterial wall thickness by the Moens-Korteweg equation (equation 5.3). Under normal circumstances, with a constant vasomotor tone, an artery becomes stiffer as it is dilated [187,195], because tension is progressively transferred to the less extensible collagenous elements in its wall [203]. The speed with which the pressure wave travels increases along an artery with augmented wall thickness. In the present study, there are no data on the aortic or carotid diameter and its wall thickness; nevertheless, their possible contributions to the significant relationship between the pulse transit time and age must be considered.

My analysis indicated that heart rate, systolic and diastolic blood pressure have statistically significant effects on the pulse transit time in both the Japanese and Yugoslavian cohorts. This finding is consistent with observations reported by Taquet et al. [171] and Simon et al.[187]. In the Japanese cohort, the 12% and 15% of variability in the pulse transit time can be explained by the systolic and diastolic blood pressure. In the Yugoslavian cohort, the systolic blood pressure accounts for the 10%, while the diastolic pressure accounts for the 2% variability in the pulse transit time. This finding lends support to the hypothesis that the speed of the propagation of the pressure pulse depends on the levels of arterial blood pressure. Taquet et al. [171] demonstrated a strong correlation between the pulse wave velocity and blood pressure ($r = 0.56$, $p = 0.001$), and speculated that this may be due to the passive stretching of collagen fibers related to increased blood pressure.

The contribution of an elevated blood pressure to arterial wall stiffness, is further demonstrated by Gribbin, Pickering and Sleight [204]. In a study of the relationship of pulse wave velocity to blood pressure change, they showed that an acute rise in arterial distending pressure was accompanied by a rise in pulse wave velocity, suggesting that poor distensibility of the large arteries in hypertensives was a consequence of the elevated distending pressure.

Although the pulse transit time correlated significantly with the concurrent heart rate, it explains only 1.3% of the variability in pulse transit time in the pooled sample. But in the Japanese cohort, the concurrent heart rate accounts for 17% of the variance in pulse transit time. This finding is consistent with Taquet et al.'s [171] observation which indicated a low but significant correlation between pulse wave velocity and heart rate ($r = 0.27$). In the present study, the heart rate was inversely and moderately correlated with pulse transit time in the Japanese ($r = -0.44$, $p = 0.0001$) and Yugoslavian ($r = -0.32$, $p = 0.0001$) cohorts. This correlation may reflect some adrenergic influence on the aortic wall. The elastic properties of the large arteries are determined by the passive (elastin and

collagen) and active factors related to neural and hormonal stimuli, both of which can activate the smooth muscle cells of the large arteries [187]. Thus, neural stimuli may increase the smooth muscle tone in the arterial wall, leading to an increased stretch of bioelastomers, and eventual arterial stiffness. In view of the fact that the elastic content of the aorta is high compared to the predominant smooth muscle content of the smaller peripheral arteries, the contribution of the nervous adrenergic influence on the aortic smooth muscle to arterial stiffness in large arteries may be only moderate.

An additional factor that contributes to higher velocity at higher heart rate is the relationship between wave velocity and frequency of the pressure wave. One can decompose the pulse wave by Fourier analysis into a set of sine waves. The relationship of the velocity and the frequency of a sinusoidal wave is given by the equation [205]:

$$c_1 = \frac{2\pi f \Delta x}{\Delta \phi} \tag{5.5}$$

where c_1 = wave velocity, f = frequency in Hz, Δx distance between two points in cm, $\Delta\phi$ = the difference between the phases of the upstream (ϕ_1) and downstream (ϕ_2) harmonics.

As a higher heart rate means more rapid ejection from the left ventricle, the rapidly ejected pressure waves contain greater high frequency components. Since wave velocity is a function of the frequency of the pressure wave, it is possible that waves with predominantly high frequency components travel faster than those with low frequency components. Furthermore, a faster heart rate also means that the basic periodic frequency is higher (the inverse of the RR interval). Again, this results in faster pulse transit time.

A significant, inverse relationship was observed between the interval from the onset of the Q-wave to rapid femoral upstroke and age in the Yugoslavian and Japanese cohorts, controlling for blood pressure, concurrent heart rate, serum cholesterol concentrations, and cigarette smoking. This finding can be explained by the observation that the pulse transit time is age-dependent. The interval from the Q-onset to femoral upstroke comprises the

electromechanical lag (i.e. the duration from depolarization of the ventricle to its contraction) and the pulse travel time. The duration of the electromechanical lag in normal adults is estimated to be 4 msec. [155], and contributes to 2% of the Q-onset to femoral upstroke interval in both cohorts. The significant, inverse relationship between this interval and age is most likely due to the significant inverse relationship between pulse transit time and age.

5.2 Effects of age on the pulse wave contour

5.2.1 Introduction

The aim of the present study was to 1. determine the effects of age on the shape of the carotid pulse wave, and 2. identify the hemodynamic and anthropometric factors related to differences in the pressure wave contour. My study of the pressure wave contour differs from others in two respects. First, unlike previous studies in which the age effects on the shape of the pressure wave configuration were investigated exclusively in Caucasian populations, I examined the age effects on pressure wave contour in two ethnically different populations. Second, pulse wave signals in my study were recorded from the left and right carotid artery, rather than from either the left or right common carotid artery.

Studies of pressure wave contour have shown that the shape of the wave differs in persons of different ages [141,206]. In a large-scale cross-sectional study, Kelly et al. [86] observed characteristic age-related differences in the pressure wave contour of the central and peripheral arteries. In large arteries, these differences are characterized by the early emergence of systolic shoulder, and a progressive increase in the size of the late systolic peak, with a resultant increase in the pulse pressure.

The mechanisms responsible for the characteristic changes in the shape of the pressure wave have not been fully explained. Kelly et al. [86] stated that an altered timing of wave reflection and a progressive stiffening of the vasculature are two important factors in the magnitude and shape of the pressure wave. London et al. [184] concluded from their study that body height is a major determinant of the carotid artery pulse contour and amplitude. O'Rourke and Yaginuma [89], and Avolio [207] emphasized the importance of wave reflections in the determination of the arterial pressure wave contour.

5.2.1 Method

The registration, digitization, and analysis of the carotid pulse wave tracings have been described in Sections 2.3, 2.4, and 2.5.

Five pulse records from Japan and 13 from Yugoslavia were excluded from the analysis due to poor quality. A total of 286 left pulse wave records and 293 right pulse waves were used in the data analysis. Table 5.10 shows a breakdown of the left and right pulse wave records by area.

Table 5.11 Number of the left and right pulse wave records by study cohort.

Cohort	Left pulse wave	Right pulse wave
Japan	128	131
Yugoslavia	158	162
Total	286	293

In their analysis of the pressure wave contour, Murgo et al. [85] used terminology which is somewhat different from that used by O'Rourke [79]. According to Murgo et al. [85], peak systolic pressure represents the late systolic shoulder, and the inflection point represents the return of reflected wave from the periphery before the closure of the aortic valve. In the present study, I adopted Murgo et al.'s terminology [85] in labeling the various points on the pressure waveform.

The contour of the carotid pressure wave was analyzed according to Murgo et al. [85]. Arterial pulse waves in which the peak systolic pressure (Ppk) occurred in a late systole following a well-defined inflection point (Pi), were classified as type A or type B waveforms, depending on the ratio of the height of the peak above the inflection point (Δ P) to the pulse pressure (PP). This ratio of the type B waveforms was less than that of type A waveforms. Because type B waveforms were similar to type A, they were treated as type A contour in the present analysis. Pressure waves in which the peak systolic pressure (Ppk)

occurred in the early systole preceding a well-defined inflection point (Pi) were classified as type C. Waveforms whose systolic shoulder (Ps) and inflection point (Pi) overlap each other and appeared after the systolic peak, were classified as type D. This type of pulse configuration was not described by Murgo et al. [85]. It may be an artifact of the pulse recordings or due to the deficiency of my algorithm to detect the various points on these type of waveforms. Because of the small number of type D waveforms in my study, (Japan: 1.6% left pulse, 0% right pulse; Yugoslavia: 1.9% left pulse, 0.6% right pulse), they were excluded from the analysis. Figure 5.5 presents examples of the three types of pulse waveforms.

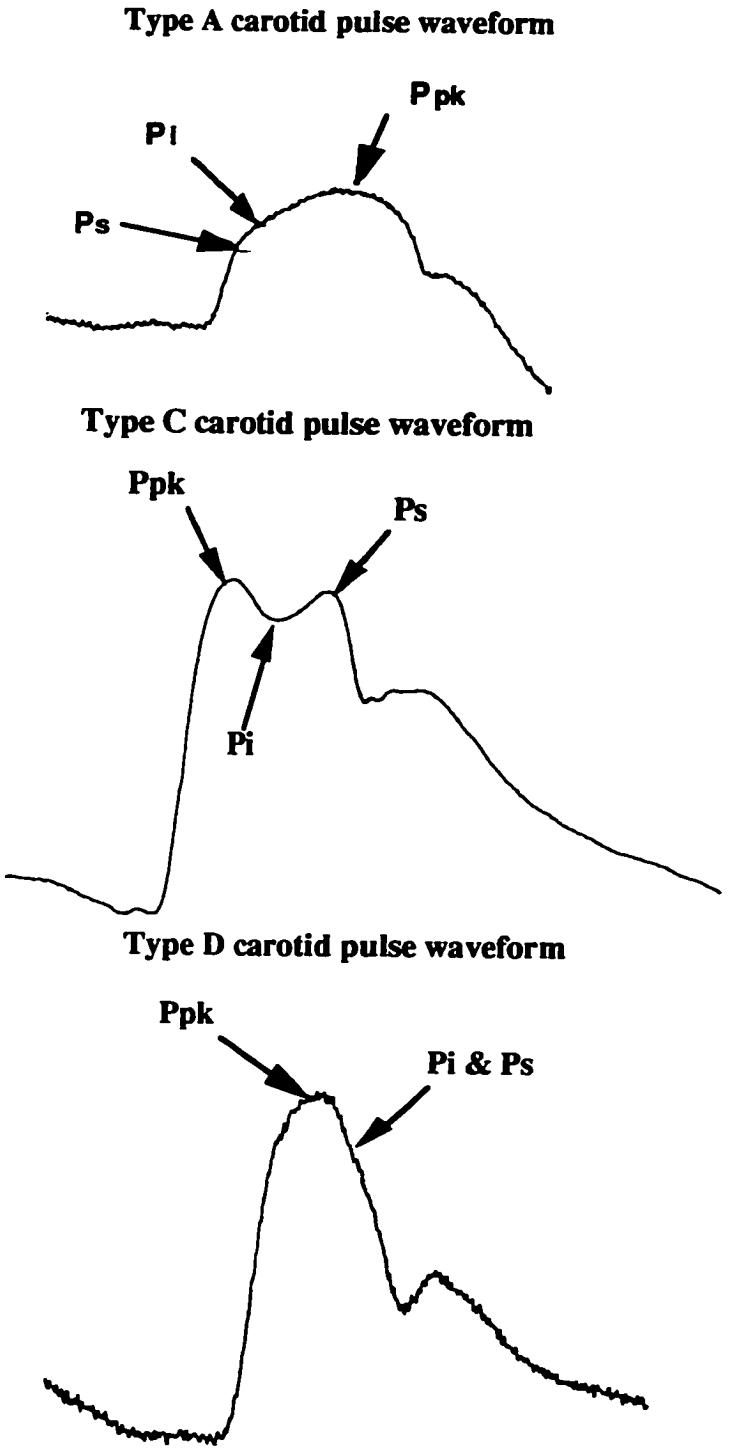


Figure 5.5 Three types of the carotid pressure waveform. Type A and C are those described by Murgo et al. [82]. Type D is not described by Murgo. Pi represents the inflection point, Ps the early systolic shoulder, and Ppk the peak systolic pressure.

Murgo et al. [85] divided the aortic pressure waves in man into three classes, based on the timing and prominence of the "secondary" wave caused by reflections: type A, B, and C. To make classification unambiguous, quantitative criteria have been established, using a ratio that in effect compares the size of the reflected wave to the overall pulse pressure. In type A pulse, the $\Delta P/PP$ ratio is greater than 0.10; this ratio is greater than 0.0 but less than 0.10 in type B, and is less than 0 in type C. A negative slope between peak systolic pressure and inflection point is present in patients with type C pressure waveform. Figures 5.6 and 5.7 illustrate how the ratio $\Delta P/PP$ is calculated for both type A and type C simulated pressure waves.

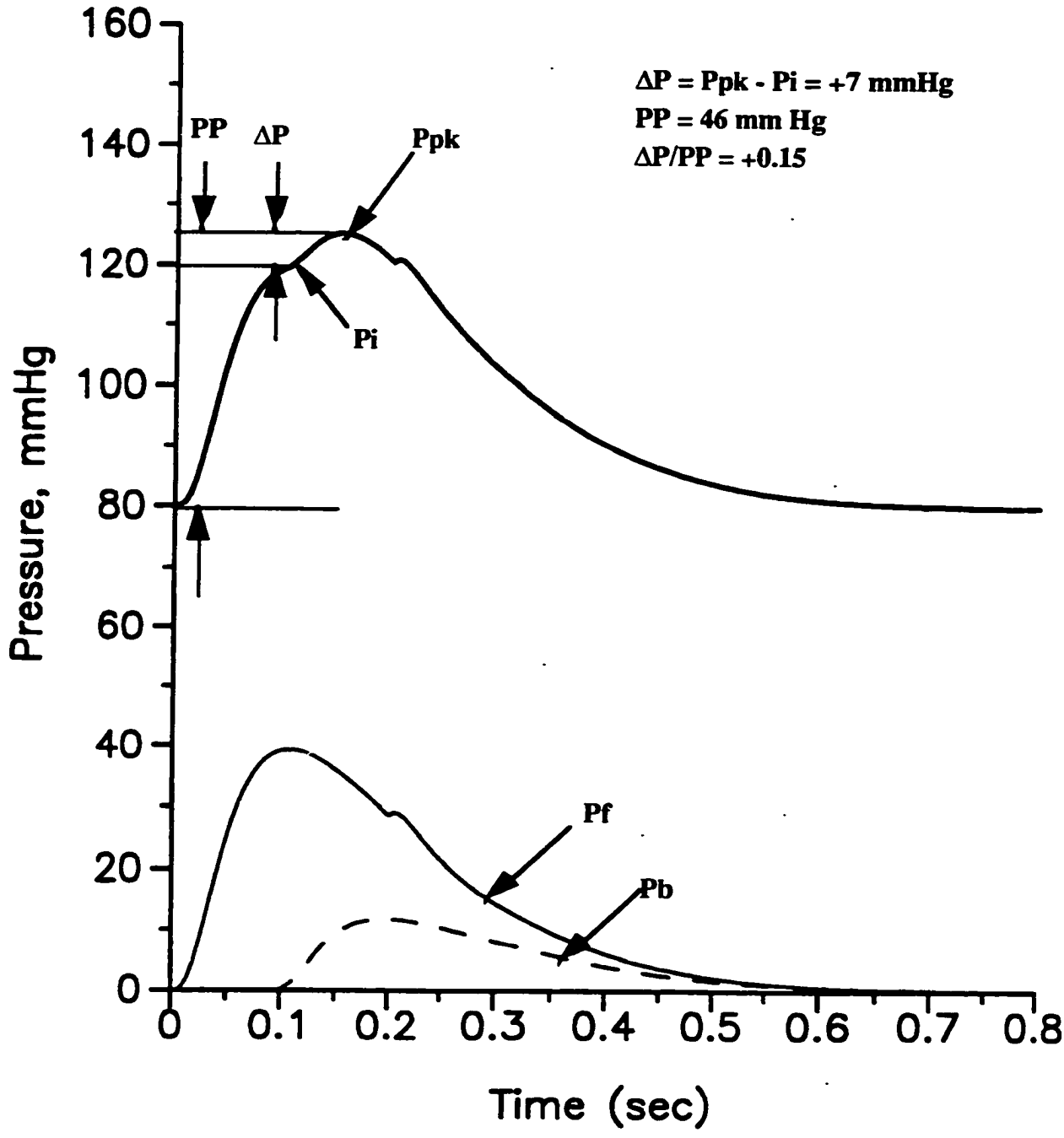


Figure 5.6 An example showing how the ratio ΔP/PP is calculated for type A simulated pressure waveform . ΔP is the height of peak systolic pressure above the inflection point. PP is the pulse pressure. Ppk is the peak of systolic pressure; Pi is the inflection point; Pf represents the simulated forward pressure wave, and Pb is the simulated backward pressure wave.

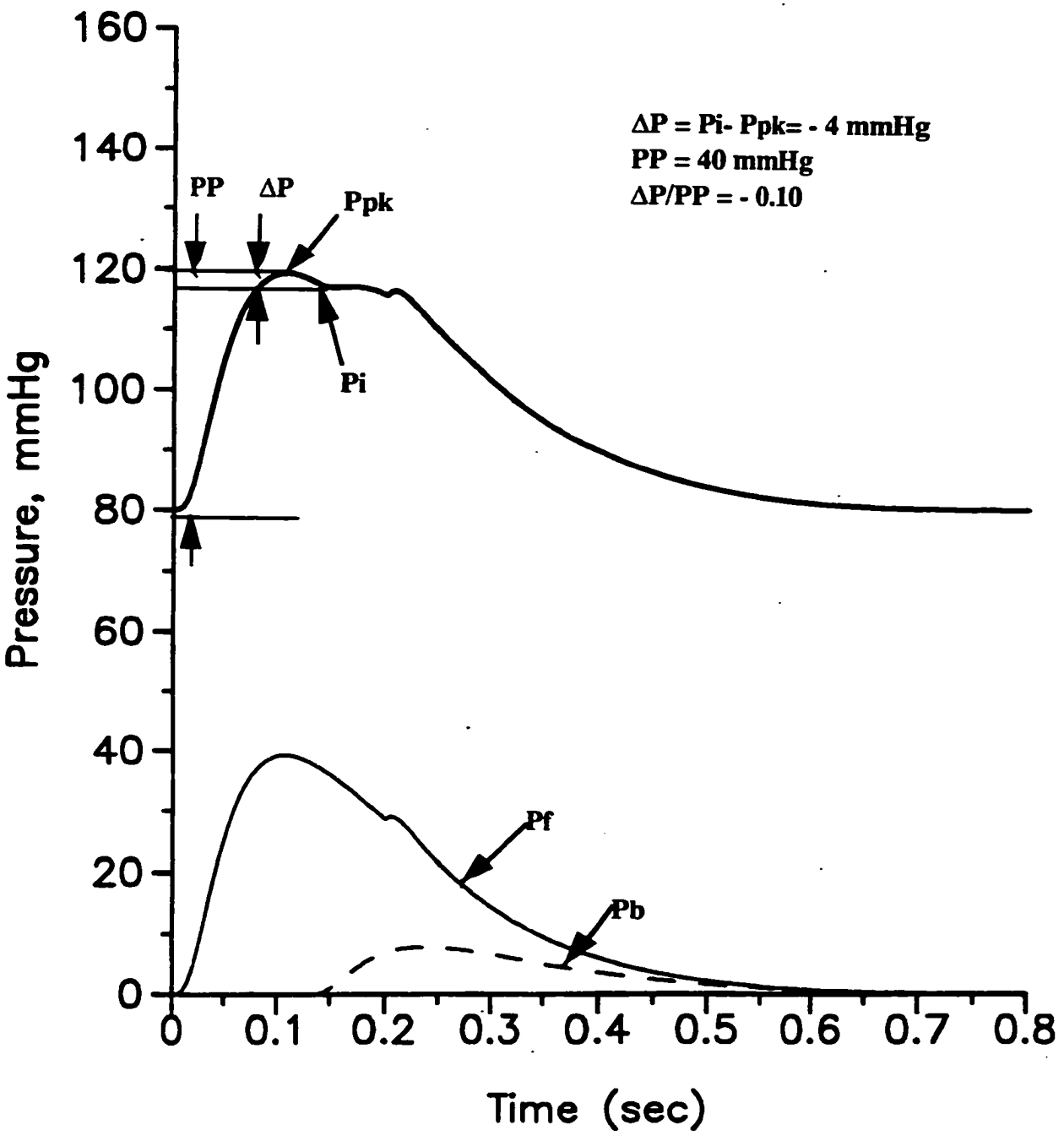


Figure 5.7 An example showing how the ratio ΔP/PP is calculated for type C simulated pressure waveform. ΔP is the height of the peak systolic pressure above the inflection point. PP is the pulse pressure. Ppk is the peak systolic pressure; Pi is the inflection point; Pf represents the simulated forward pressure wave, and Pb is the simulated backward pressure wave.

The measured pressure wave comprises forward and backward components, and the backward wave is the combination of many reflected waves. The identification of the inflection point on the recorded measured pressure wave is uncertain. Therefore I used the simulated type A aortic pressure waves to demonstrate the relationship between the height of the systolic peak above the inflection and the amplitude and timing of wave reflection. The use of simulated pressure waves is advantageous because the accurate location of the inflection point on these waves is possible. The first demonstration involved a series of four simulated type A waveforms that have a constant arrival time (0.08 seconds), but the amplitude of the reflected waves varies from 20% to 50% of the forward wave amplitude (Figure 5.8). The second demonstration also included four type A simulated waveforms, with a constant amplitude (30% of the forward wave amplitude). The arrival time of wave reflection is different: 0.07 second, 0.09 second, 0.10 second, and 0.12 second (Figure 5.9). Figure 5.10 illustrates the relationship of the height of the systolic peak above the inflection point (ΔP) to the amplitude and timing of the wave reflection.

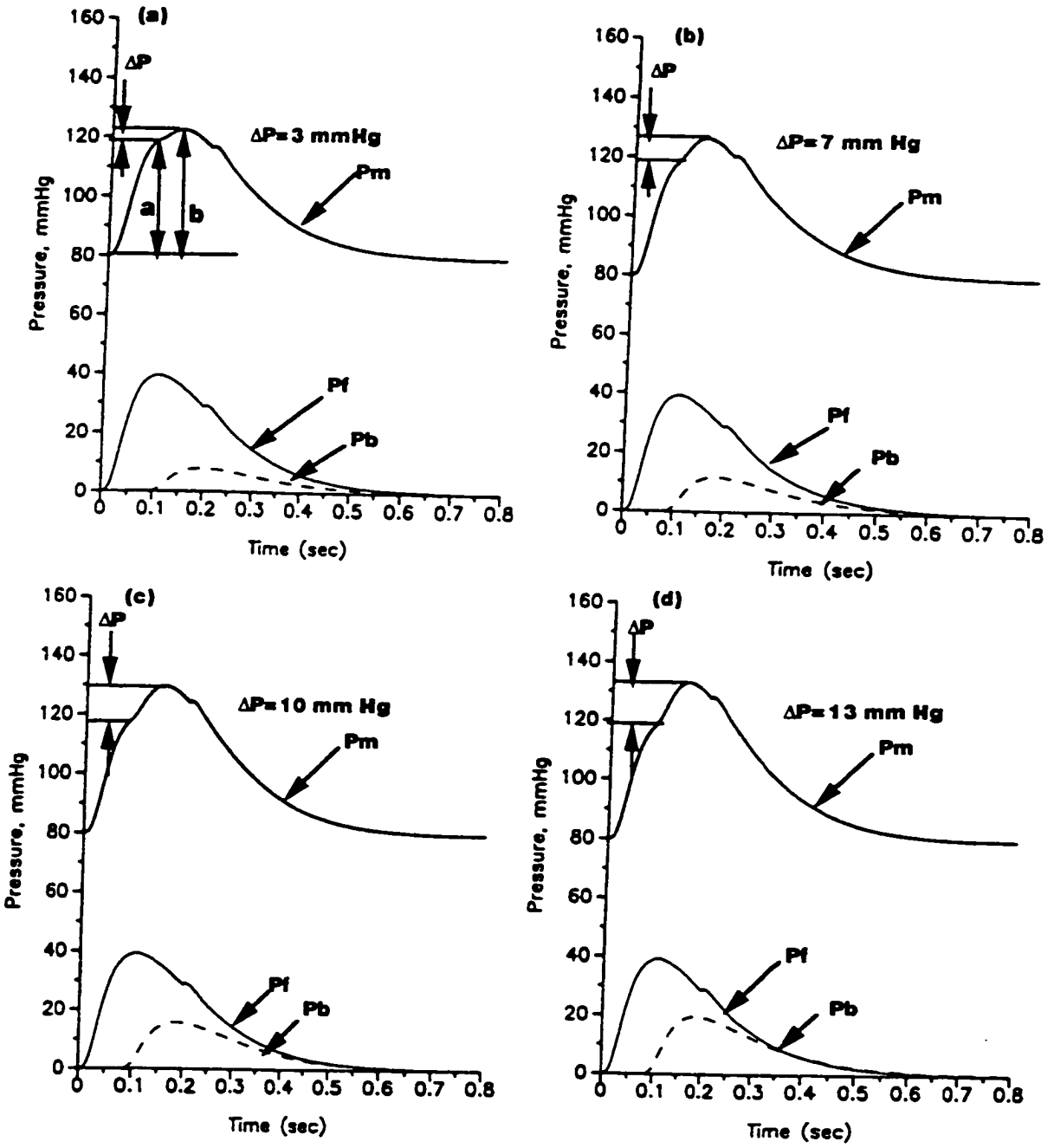


Figure 5.8 A series of simulated aortic pressure waves. The arrival time of the reflected wave in all four waves is 0.08 seconds. The amplitude of the reflected wave is (a) 20% of the forward wave; (b) 30% of the forward wave; (c) 40% of the forward wave; and (d) 50% of the forward wave. The distance between the inflection point and the peak systolic pressure represents the amplitude of the reflected wave, and is labeled as ΔP. Pm = measured pressure wave; Pf = forward pressure wave; Pb = backward pressure wave.

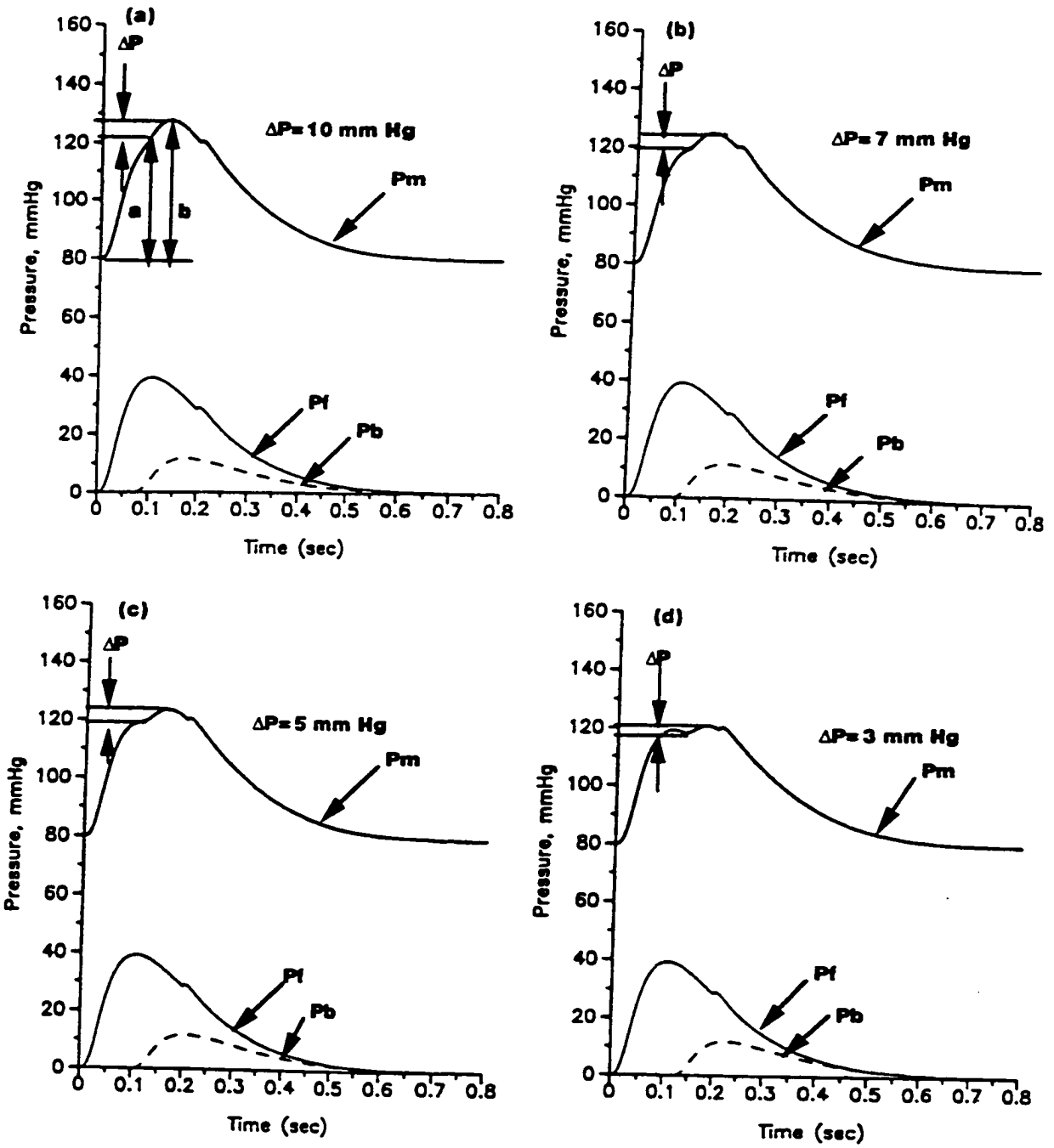
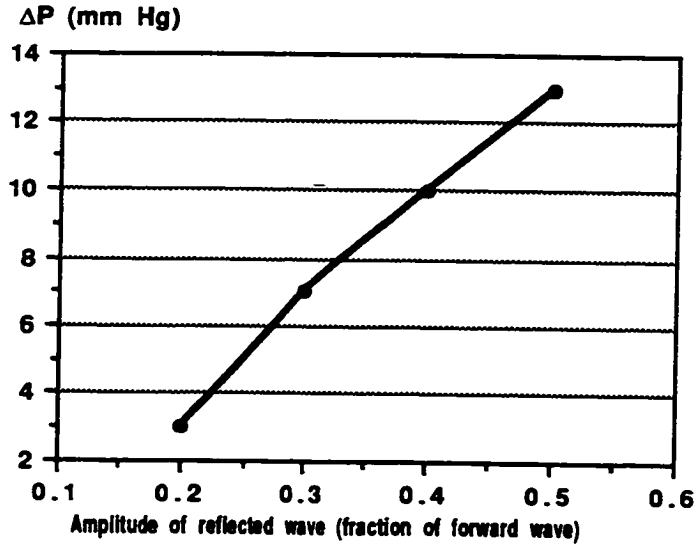


Figure 5.9 A series of simulated aortic pressure waves. The amplitude of the reflected wave is 30% of the forward wave. The arrival time of the reflected wave in all four waves is (a) 0.07 second after ventricular ejection; (b) 0.09 second after ventricular ejection; (c) 0.10 second after ventricular ejection; and (d) 0.12 second after ventricular ejection. The distance between the inflection point and the peak of systolic pressure represents the amplitude of the reflected wave, and is labeled as ΔP. Pm = measured pressure wave; Pf = forward pressure wave; Pb = backward pressure wave.

(a)



(b)

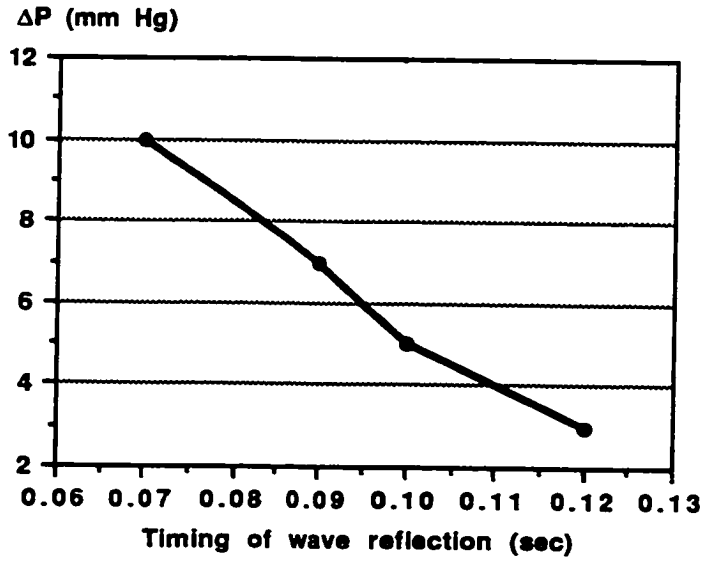


Figure 5.10 A plot of the height of systolic peak above the inflection point (ΔP) as a function of (a) the amplitude of the reflected wave (expressed as a fraction of the amplitude of the forward wave), and (b) the timing of wave reflection.

An examination of the simulated type A (Figure 5.6) and C (Figure 5.7) pressure wave clearly indicates that the ratio of the height of the systolic peak above the inflection point to the pulse pressure (ΔP/PP) affects the shape of the pressure waves differently. For type A waveform, the earlier return and the larger amplitude of the reflected wave account

for a larger value in this ratio. In the type C waveform, the peak systolic pressure is near the mid-systole, and precedes the inflection point. The ratio ΔP/PP does not influence the pulse pressure amplitude or the peak systolic pressure. This type of wave configuration corresponds to a delayed return of the wave reflection with a "boosting" effect on the protodiastolic pressure and the diastolic pressure-time index [67].

In view of the divergent effects of wave reflections on the shape of the carotid pulse, separate analyses for each type of waveform were carried out to identify factors related to the age-related differences in the pressure wave contour.

Cook's D influence statistic [145] was used to detect unusually influential observations in the data; the application of this statistic for managing outliers was described in detail in Section 2.6. Ten and eight observations of type A and type C waveforms were excluded from the analysis of the relationship between parameters defining the shape of the pressure wave and age.

The data of the amplitude of reflected waves for the type A waveform were transformed logarithmically to improve the approximation to normality. The rationale for data transformation has been discussed in Chapter 2.6. No transformation was necessary for data of the timing of wave reflections for both types of waveform.

Multiple regression models were constructed to examine 1. the effect of age on the shape of the carotid pressure wave, and 2. how the hemodynamic and anthropometric factors are related to differences in the pressure wave contour. Body height, and pulse transit time were included in the models as covariates.

The effects of arterial stiffening on the pressure wave contour are predictable. At a given location in the ascending aorta, the forward-traveling pressure generated by the left ventricular ejection, first appears; but while systole is still in progress, a backward-traveling (reflected) pressure wave arrives at this location. This reflected wave is added to the forward wave, and depending on their respective timing, "boosts" the peak of systolic

pressure. The large systolic peak in the type A waveform observed in older individuals can be attributed to a large-amplitude reflected wave.

The major effect of body size on the arterial pressure wave contour is in determining the distance between reflecting sites in the upper and lower body [208]. Comparative physiology has shown that pulse wave velocity is similar in the different mammalian species, despite different diameters of the vessels. Under normal conditions [84,86,193], body length is an important determinant of the pressure wave contour. In a study of the effect of body size on the carotid pulse contour in humans, London et al. [184] found that body height is a major determinant of the contour and amplitude of the carotid pulse.

Pressure and flow measured in the ascending aorta are the result of the interplay of the heart and the arterial system. The wave shapes of pressure and flow change in response to peripheral vasodilatation or alteration in the function of the heart [209]. O'Rourke [67] stated that the duration of ventricular ejection can alter the shape of the aortic pressure wave in the normal adults. In these adults, there is usually no diastolic wave and wave reflection is manifest as a late-systolic pressure peak. A separate diastolic wave becomes apparent when the ejection duration is abbreviated or when the wave velocity is decreased.

5.2.3 Results

In Section 5.2.3.1, I examined how the three types of wave contour were distributed in these populations. In addition, I also tested for a difference in the wave contour between the left and right pressure waves.

5.2.3.1 Descriptive statistics

Table 5.11 presents the comparisons of age, height, and the parameters defining the wave contour for type A and type C waveforms between the Yugoslavian and Japanese cohorts.

Table 5.12 Comparison of the age, height, and the parameters defining the wave contour for type A and type C waveforms between two cohorts.

Variable	Type A		Type C	
	Japan (n = 109)	Yugoslavia (n = 85)	Japan (n = 2)	Yugoslavia (n = 27)
Age (years)	60.4±5.7	54.8±6.1*	58.4±1.4	54.3±0.9**
RWINT (msec)				
Left	103.3±2.0	100.2±2.9	79.2±1.8	127.0±5.0
Right	95.4±2.5	97.3±2.2	127.9±8.9	135.3±5.4
RWAMP (mV)				
Left	451.9±35.4	555.9±54.6	79.2±18	98.2±2.9*
Right	600.0±41.1	573.1±42.3	93.0±22	98.9±2.9*
SPINT (msec)				
Left	226.8±4.0	266.5±4.6*	128.7±8.9	279.7±64.5
Right	228.6±3.2	208.7±3.7*	93.0±22	214.7±29.1
Height (cm)	160.6±0.5	167.7±0.6*	160.2±1.3	170.2±0.7*

RWINT = timing of wave reflections; RWAMP = amplitude of the reflected waves; SPINT = interval from onset of systolic upstroke to peak systolic blood pressure.
Values are given as Mean±SEM.
*$p < 0.001$.
**$p < 0.05$.
The p value is for the Student's t test for independent samples.
These measurements are raw data and do not correct for the age difference for five years between the two cohorts.

Intra-group differences in the parameters defining the pulse wave contour of type A waveform were tested by Student's t test for paired samples. No statistically significant differences were found between the left and right pulse waves in any of the parameters in the Yugoslavian cohort. In the Japanese cohort, significant differences were observed between the left and right pulse in the following parameters: timing of wave reflections (Mean±SE = 5.99±2.52, $t = 2.31$, $p = 0.02$), and amplitude of reflected waves (Mean±SE

= -148.6±42.37, t = -3.49, p = 0.0007). In type C pulse waves, no statistically significant differences were found between the left and right pulse waves in any of the parameters defining the wave contour.

Compared to Yugoslavian men, the Japanese men who showed both types of waveforms were significantly older, and shorter in stature. Their interval from onset of the systolic upstroke to peak systolic pressure of type A waveform was significantly shorter in the left pulse, but longer in the right pulse than those of the Yugoslavians.

Univariate analysis of the carotid pulse waves of the pooled sample indicated a predominance of Murgo's type A waveform [85]. This pattern of distribution is also observed in the separate cohort analysis. A significantly higher percentage of the Japanese men showed a type A waveform than that of the Yugoslavian men (χ^2<0.001). Table 5.12 summarizes the frequency distribution of the three types of carotid pressure waveforms.

Table 5.13 Frequency distribution of the three types of carotid pressure waveforms in the pooled sample, the Yugoslavian, and Japanese male cohorts.

Type	Left carotid pressure waveform	Right carotid pressure waveform
Pooled sample		
A	216 (72.8%)	242 (81.5%)
C	65 (21.8%)	50 (16.8%)
D	16 (5.4%)	5 (1.7%)
Japan		
A	113 (88.2%)	126 (96.2%)
C	13 (10.2%)	5 (3.8%)
D	2 (1.6%)	0 (0.0%)
Yugoslavia		
A	103 (65.2%)	116 (71.6%)
C	52 (32.9%)	45 (27.8%)
D	3 (1.9%)	1 (0.6%)

Values are given as frequency and percentage shown in bracket.

The paired t test was used to test for difference in the pressure wave contour between the left and right pulse of the pooled sample, as well as of the individual cohorts. The shape of the wave was defined by 1. the timing of wave reflections, 2. the amplitude of the reflected waves, and 3. the interval from systolic upstroke to the peak of systolic pressure (Figure 5.11). Table 5.13 presents the comparison of these parameters between the left and right pulse of type A waveforms in the pooled sample.

Table 5.14 Comparison of the parameters defining the pulse wave contour between the left and right pulse of type A waveforms in the pooled sample.

Parameter	Type A (n = 194) Left	Right	Type C (n = 29) Left	Right
Timing of wave reflection (msec.)	101.93±1.73	96.48±1.67	127.07±4.68	134.78±5.04
Mean difference	5.43		-7.71	
SE	1.98		6.07	
t	2.76		-1.27	
p value	0.03		0.21	
Amplitude of reflected waves (mV)	497.54±31.26	588.21±29.57	266.79±60.61	203.51±28.2
Mean difference	-90.68		63.21	
SE	36.41			
t	-0.24		-1.27	
p value	0.01		0.21	
SPINT (msec.)	217.88±3.12	219.87±2.53	98.62±2.75	203.51±28.2
Mean difference	-1.99		-2.18	
SE	3.08		3.70	
t	-0.65		-0.59	
p value	0.52		0.56	

SPINT = interval from rapid systolic upstroke to peak of systolic pressure.

Values are given as Mean±SEM.

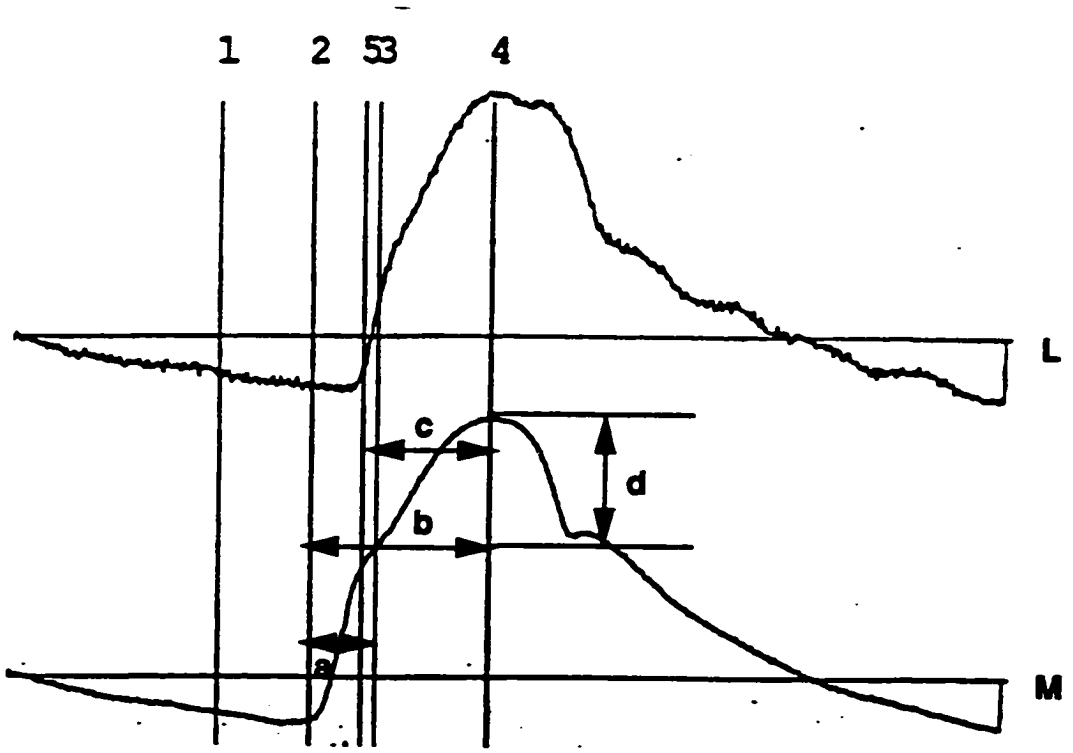


Figure 5.11 A diagram of type A waveform signal (the bottom tracing), showing the timing of wave reflection (labeled as a), interval from systolic upstroke to the peak of systolic pressure (labeled as b), and the interval from the onset of wave reflection to the peak of systolic pressure (labeled as c). The distance between inflection point and peak systolic pressure (labeled as d) represents the amplitude of the reflected wave. Vertical line 1 represents the onset of Q-wave, vertical line 2 the onset of rapid systolic upstroke, vertical line 3 inflection point, vertical line 4 peak of systolic pressure, and vertical line 5 early systolic shoulder. Signal L is the femoral pulse wave, and signal M the carotid pulse wave.

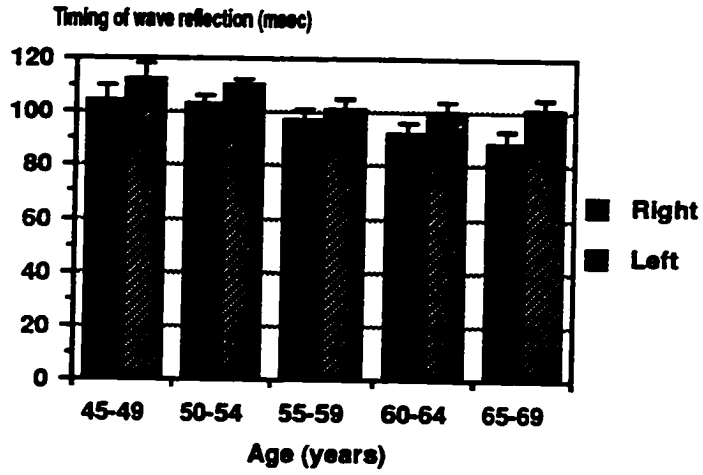
In the pooled sample, timing of wave reflections of type A left pulse was statistically significantly longer, but the amplitude of the reflected waves were significantly smaller than that of the right pulse. No statistically significant difference was found in the interval from systolic upstroke to the peak systolic pressure between the left and right pulse. In type C waveform, the contour of the left pulse was not statistically significantly different from that of the right pulse (see Table 5.13).

In the Japanese cohort, the timing of wave reflections of the left pulse was significantly longer than that of the right pulse for type A waveform (Mean±SE = 5.99±2.52 msec., $t = 2.38$, $p = 0.02$). A statistically significant difference was observed in the amplitude of the reflected waves between the left and the right pulse (Mean±SE = -148.06±42.37 msec., $t = -3.49$, $p = 0.0007$). But the interval from the systolic upstroke to the peak of systolic pressure of the left pulse was not significantly different from that of the right pulse for type A. In Yugoslavia, there was no statistically significant difference between the left and right pulses in the timing of wave reflection and the amplitude of reflected waves.

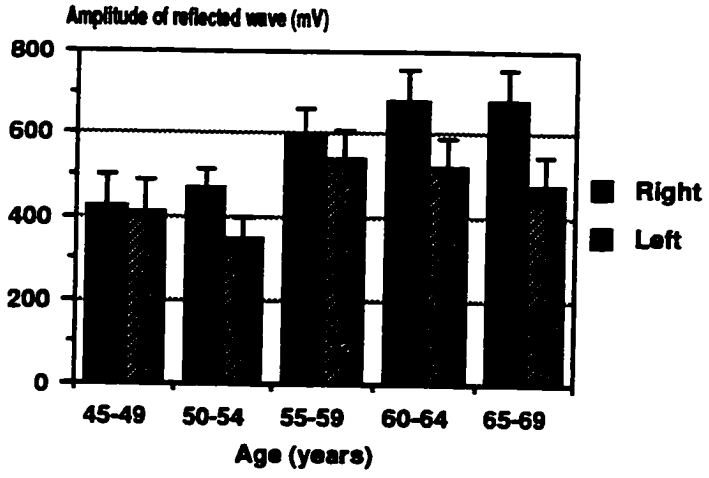
5.2.3.2 Age and parameters defining the shape of the carotid pressure wave

The literature has consistently shown that aortic pressure wave contour varies with different ages. This difference is characterized by a shortened timing of wave reflection, an augmented amplitude of the reflected wave, and a prolonged interval from systolic upstroke to late systolic peak. In this section, I present the descriptive statistics on those parameters that define the pressure wave contour. Figure 5.12 is a graphic presentation of the distribution of these parameters in the pooled sample.

(a)



(b)



(c)

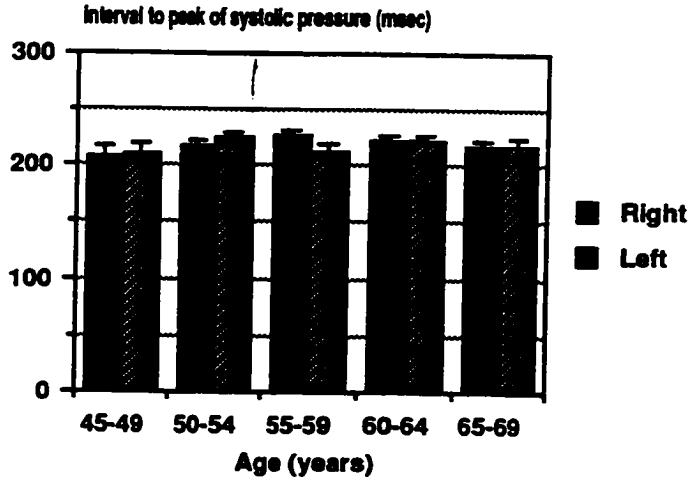


Figure 5.12 Graphs showing the age trend of (a) timing of wave reflections, (b) amplitude of reflected waves, and (c) interval from the systolic upstroke to the late systolic peak of type A waveform, by age groups in the pooled sample. The error bars represent SEM.

The bivariate analysis of type A waveforms of the pooled sample showed a general downward age trend in the timing of wave reflections, and an upward age trend in the amplitude of reflected waves. No age trend was observed in the interval from systolic upstroke to the peak systolic pressure. Age trend was not observed in any of the variables that defined the shape of type C waveform.

I analyzed type A waveforms of each cohort by age groups. Table 5.14 presents results of this analysis.

Table 5.15 Analysis of type A pressure wave contour of both male cohorts.

Age group (years)	Timing of wave reflections (msec.)		Amplitude of reflected waves (mV)		SPINT (msec.)	
	Left	Right	Left	Right	Left	Right
Japan						
50-54	110.08±2.94	110.92±3.44	315.83±50.67	403.74±45.25	231.37±9.66	230.30±6
55-59	105.44±5.00	103.15±4.59	420.13±53.78	498.35±82.89	226.83±8.04	236.60±6
60-64	100.99±4.28	91.34±3.90	563.38±92.53	722.21±104.24	229.55±9.09	229.32±8
65-69	100.99±4.25	87.96±5.15	476.14±69.79	679.43±73.42	217.99±6.27	217.14±5
Yugoslavia						
45-49	112.21±6.26	103.95±5.93	408.45±75.93	421.55±73.54	211.09±8.35	208.23±9
50-54	110.10±4.09	91.34±4.83	397.60±91.64	537.00±104.39	210.87±7.98	198.61±7
55-59	97.32±5.84	92.03±5.03	655.08±112.61	682.92±101.82	199.99±10.11	216.78±1
60-64	98.51±7.80	94.67±6.05	445.18±110.49	609.18±82.68	206.68±6.96	207.50±4

SPINT = interval from rapid systolic upstroke to the peak systolic pressure.
Values are given as Mean±SEM.

The results of a separate cohort analysis showed that older age groups had a shorter timing of wave reflections and the interval from the systolic upstroke to the peak systolic pressure than the younger ones in the Japanese cohort. But this age trend was not observed in the Yugoslavian men. Furthermore, no age trend was noted in type C pulse in either cohort.

5.2.3.3 Determinants of the carotid pressure wave contour

In this section, I examined the pulse wave contour-age relationships by using the multiple regression method. In the regression model, the parameters that defined the shape of the pressure wave were the dependent variables, and age was the independent variable. Covariates that have been shown to influence the parameters defining the wave contour were controlled for in the model. These covariates were discussed in the method section 5.2.1. The results of the multiple regression analysis of pulse wave contour and age are summarized in Table 5.15. The heterogeneity of slopes was also tested in instances where a significant difference was observed in the wave contour/age relationship in the two cohorts.

Table 5.16 Regression analysis of the parameters defining the contour of type A pressure wave on age, controlling for pulse transit time, heart rate, and height in the pooled sample.

Variable		Parameter estimate	p Value
RWINT			
Age		- 1.4348	0.0001
PTT		- 0.0182	0.8832
LGHR		- 25.950	0.0164
Height		- 0.2411	0.4200
Area	12	- 49.2723	0.1656
	13	0.0000	.
Age*area	12	0.8748	0.1558
	13	0.0000	.
Intercept		329.1671	0.0001
R^2		0.1395	
LGRWAMP			
Age		0.0209	0.1946
PTT		- 0.0014	0.8051
LGHR		- 1.4917	0.0021
Height		- 0.0161	0.2332
Area	12	1.1753	0.4510
	13	0.0000	.
Age*area	12	- 0.0011	0.6764
	13	0.0000	.
Intercept		13.2259	0.0001
R^2		0.0828	
LGSPINT			
Age		- 0.0020	0.3783
PTT		0.0007	0.3360
LGHR		- 0.3320	0.0001
Height		- 0.0007	0.7021
Area	12	- 0.0759	0.7282
	13	0.0000	.
Age*area	12	0.0012	0.7450
	13	0.0000	.
Intercept		6.9621	0.0001
R^2		0.2376	

Models tested: (a) RWINT = age + PTT + LGHR + height + area + area*age.
(b) LGRWAMP = age + PTT + LGHR + height + area + area*age.
(c) LGSPINT = age + PTT + LGHR + height + area + area*age.
RWINT = timing of reflected wave; LGRWAMP = log-transformed amplitude of reflected wave; LGSPINT = log-transformed interval from systolic upstroke to peak of systolic pressure; PTT = pulse transit time; LGHR = log-transformed concurrent heart rate; Age*area = an interaction term between age and the study area; Area 12 = Yugoslavia, Area 13 = Japan.

Testing of the regression model indicated that the timing of the wave reflection was inversely, and significantly correlated with age. There was no statistically significant association between age and the amplitude of reflected waves, or the interval from systolic upstroke to the peak of systolic pressure. The study area had no significant effect on all of the parameters that defined the shape of the pressure wave. In addition, there was no significant difference in the relationship between these parameters and age for the two cohorts. In view of these findings, data analysis from each cohort separately was not carried out. However, the heterogeneity of slopes was tested. Table 5.16 presents the regression data of parameters defining the wave contour with age, and the predicted mean and standard deviation.

Table 5.17 Parameters defining the pressure wave contour in each study cohort: observed mean, standard error, and regression on age.

(a) Timing of wave reflection

Study cohort	Observed mean (msec)	SE	Regression on age		
			Slope	SE	p Value
Japan	103.75	2.51	-0.0685	0.3743	0.0691
Yugoslavia	104.76	2.61	-1.1209	0.5324	0.0368

(b) Log-transformed amplitude of reflected wave

Study cohort	Observed mean (mV)	SE	Regression on age		
			Slope	SE	p Value
Japan	5.68	0.11	0.0096	0.0161	0.1946
Yugoslavia	6.08	0.15	0.0209	0.0225	0.6704

(c) Log-transformed interval from systolic upstroke to peak of systolic pressure

Study cohort	Observed mean (msec)	SE	Regression on age		
			Slope	SE	p Value
Japan	5.40	0.01	-0.0019	0.0022	0.3783
Yugoslavia	5.39	0.02	-0.0007	0.0032	0.8224

There was a statistically significant difference in the slope of the timing of wave reflection-age relationship between the two cohorts. The slope for this relationship was significantly steeper in the Yugoslavian cohort. However, no statistically significant difference was observed between the two cohorts in either the amplitude of reflected wave-age relationship or the interval from upstroke to systolic peak-age relationship.

The regression model shows that age had a significant effect on the timing of the reflected waves, while the concurrent heart rate was significantly correlated with timing of wave reflections as well as the interval from systolic upstroke to the peak of systolic pressure. To estimate the contribution of age and each of the covariates to the shape of the pressure wave, I performed a univariate regression analysis, to compare the R^2 value from each analysis (Table 5.17).

Table 5.18 Regression coefficients, p value, and R^2 values derived from the univariate regression analysis of parameters defining type A pressure wave contour on age, pulse transit time, heart rate, and height in the pooled sample.

Variable	Coefficient	p Value	R^2
RWINT			
Age	- 0.8799	0.0023	0.0534
LGHR	-20.1044	0.0186	0.0290
PTT	0.1350	0.2306	0.0076
Height	0.0890	0.7518	0.0006
LGRWAMP			
LGHR	- 0.6308	0.0752	0.0167
Age	0.0177	0.1335	0.0132
PTT	0.0039	0.4002	0.0038
Height	- 0.0138	0.2236	0.0087
LGSPINT			
LGHR	- 0.3401	0.0001	0.1628
Height	0.0761	0.2356	0.0025
Age	0.0010	0.6371	0.0013
PTT	0.0119	0.1381	0.0012

Models tested: (a) RWINT= age; RWINT= LGHR; RWINT= PTT; RWINT= height.
(b) LGRWAMP = age; LGRWAMP = LGHR; LGRWAMP = PTT; LGRWAMP = height.
(c) LGSPINT = age; LGSPINT = LGHR; LGSPINT = PTT; LGSPINT = height.
RWINT = timing of wave reflections; LGRWAMP = log-transformed reflected wave amplitude; LGSPINT = log-transformed interval from systolic upstroke to peak systolic pressure; PTT = pulse transit time; LGHR = log-transformed concurrent heart rate; Area 12 = Yugoslavia; Area 13 = Japan.

In the pooled sample, 5% of the variance in the timing of wave reflection can be explained by age, and 3% by the concurrent heart rate. In contrast, both age and heart rate can account for only 1% of the variance in the amplitude of reflected waves. For the interval from systolic upstroke to the peak of systolic pressure, the concurrent heart rate can explain 16% of the variance. Age contributes minimally to this interval, less than 1% of the variability in this interval can be understood in terms of the variability in age.

Freis et al. [206] and Murgo et al. [85] have shown that age has a different effect on the pressure wave contour of type C configuration. I examined the relationship of type C wave contour to age, controlling for the same covariates as in type A waveform regression analysis. Table 5.18 summarizes the findings.

Table 5.19 Regression analysis of parameters defining the contour of type C pressure wave on age, controlling for pulse transit time, heart rate, and height in the pooled sample.

Variable		Parameter estimate	p Value
RWINT			
Age		24.7824	0.2463
PTT		0.9317	0.1526
LGHR		-12.2985	0.7704
Height		1.1519	0.3891
Area	12	1133.9772	0.2996
	13	0.0000	.
Age*area	12	-21.7589	0.3009
	13	0.0000	.
Intercept		-1361.9585	0.2460
R^2		0.3043	
RWAMP			
Age		-134.8043	0.2761
PTT		- 3.6026	0.3773
LGHR		103.1935	0.7060
Height		- 15.2579	0.0832
Area	12	-7480.2123	0.2409
	13	0.0000	.
Age*area	12	142.0871	0.2465
	13	0.0000	.
Intercept		9256.6324	0.0001
R^2		0.5279	
LGSPINT			
Age		- 0.0020	0.3783
PTT		0.0007	0.3360
LGHR		- 0.3320	0.0001
Height		- 0.0007	0.7021
Area	12	- 0.0759	0.7282
	13	0.0000	.
Age*area	12	0.0012	0.7450
	13	0.0000	.
Intercept		6.9621	0.0001
R^2		0.2376	

Models tested: (a) RWINT = age + PTT + LGHR + height + area + area*age.
(b) LGRWAMP = age + PTT + LGHR + height + area + area*age.
(c)LGSPINT = age + PTT + LGHR + height + area + area*age.
RWINT = timing of reflected wave; RWAMP = amplitude of reflected wave; LGSPINT = log-transformed interval from systolic upstroke to peak of systolic pressure; PTT = pulse transit time; LGHR = log-transformed concurrent heart rate; Area 12 = Yugoslavia, Area 13 = Japan.

The regression analysis shows that age has no significant effect on any of the parameters defining the type C wave contour. The concurrent heart rate was the only significant predictor of the interval from systolic upstroke to the peak systolic pressure. This observation was also found in the type A pressure waveforms (see Table 5.15).

5.2.3.4 Effects of age on the ratio of the height of peak pressure above the inflection point to the pulse pressure (ΔP/PP)

Kelly et al. [86] reported age-related differences in the ratio of the height of peak pressure above the inflection point to the pulse pressure (ΔP/PP) in type A waveform. This ratio is a common index for detecting the contribution of wave reflection to changing carotid pulse contour. In my simulated type A pressure waves (Figure 5.10), the timing and amplitude of the reflected wave have been shown to affect the height above the inflection point (ΔP). In this section, I set out to 1. describe the distribution of the ratio ΔP/PP in different age groups; and 2. determine if by regression analysis, age significantly influenced the ratio of the height above the inflection point to pulse pressure. In the regression model, concurrent heart rate, pulse transit time, timing of wave reflection, and the interval from onset of wave reflection to peak of systolic pressure were included as covariates. Initially, I analyzed the pooled data to examine the relationship of the ratio of ΔP/PP to age. If a difference existed, then analysis of data from the individual cohorts would be carried out.

The paired t test indicated a statistically significant difference in the ratio of height above the inflection point to pulse pressure (ΔP/PP), between the left and right pressure wave of the type A contour in the pooled sample (Mean±SE = -0.03±0.01, t = - 2.02, p = 0.04), as well as in the Japanese cohort (Mean±SE = -0.006±0.02, t = - 3.32, p = 0.001). In the Yugoslavian cohort, the ratio of ΔP/PP of the left pulse was not significantly different from that of the right pulse (Mean±SE = 0.006±0.02, t = 0.27, p = 0.79).

Figures 5.13 and 5.14 present the bivariate analysis of age trend in the distribution of the ratio of ΔP/PP in the pooled sample, and in individual cohorts.

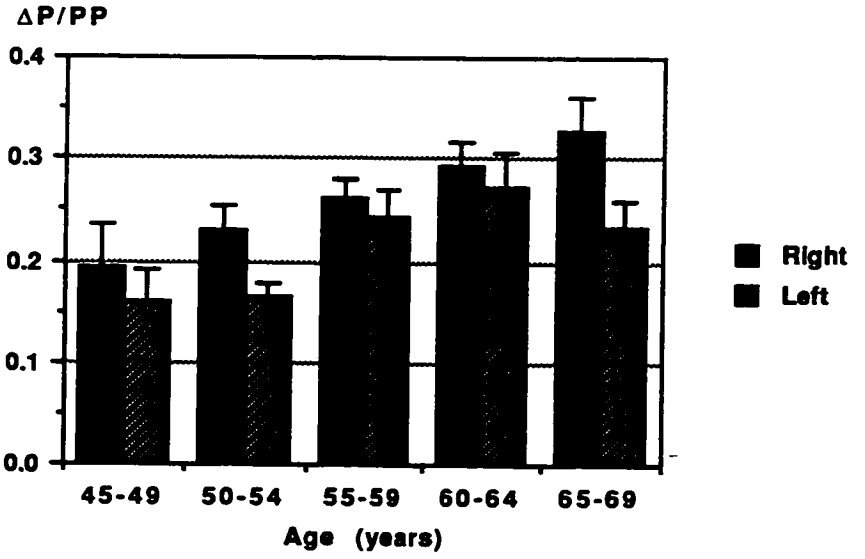
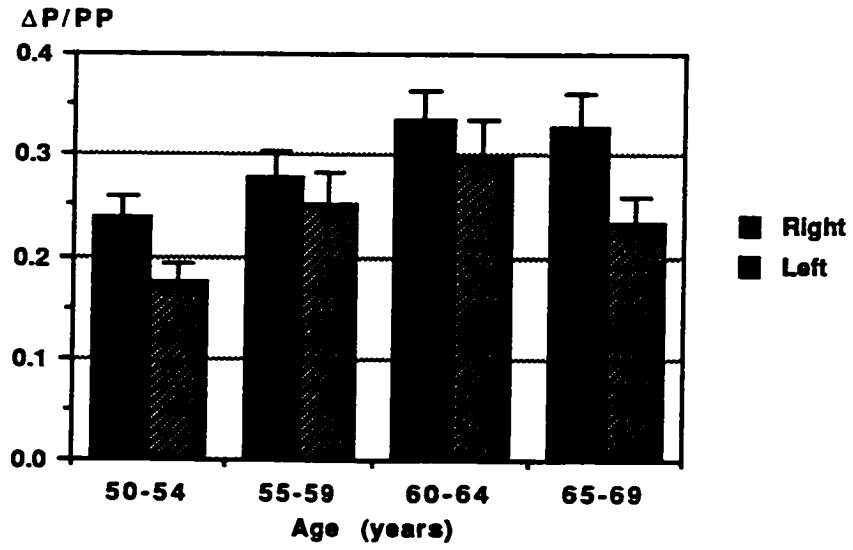


Figure 5.13 The age trend of ΔP/PP of type A waveform in the pooled sample. The error bars represents SEM.

(a)



(b)

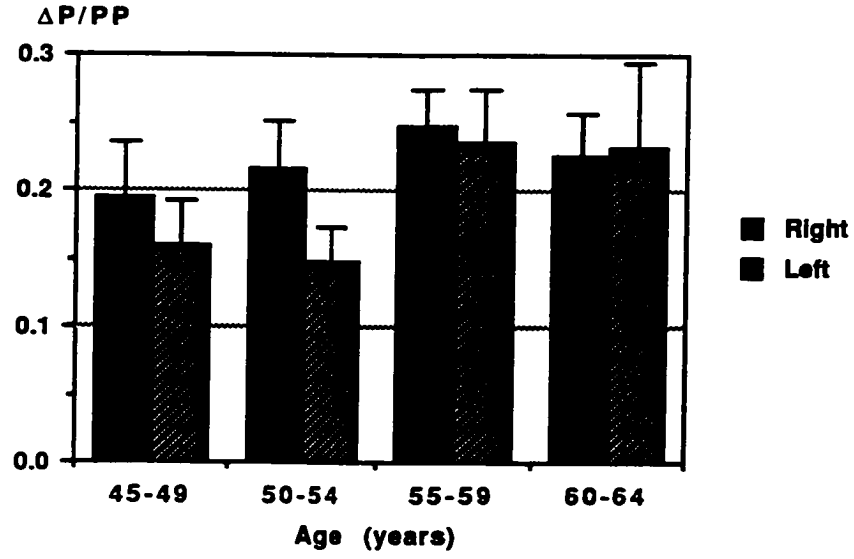


Figure 5.14 The age trend of ΔP/PP of type A waveform in the (a) Japanese, and (b) Yugoslavian male cohorts. The error bars represent SEM. These measurements are raw data, and do not correct for the age difference for 5 years between the two cohorts.

The pooled sample shows an upward age trend in the distribution of the ratio of height above the inflection point to pulse pressure of the right, but not of the left type A pressure wave. When type A pressure waves of each cohort were analyzed separately, no age trend in this ratio was observed in the either cohort.

Table 5.19 summarizes the analysis of relationship between the ratio ΔP/PP and age in the pooled sample, controlling for the heart rate and pulse transit time.

Table 5.20 Regression analysis of the ratio ΔP/PP of type A waveform on age in the pooled sample, controlling for heart rate, and pulse transit time.

Variable		Estimate of coefficient	p Value
Left			
Age		0.0061	0.0216
LGHR		- 0.1751	0.0255
PTT		0.0001	0.9035
Area	12	0.1741	0.5144
	13	0.0000	.
Age*area	12	- 0.0027	0.5562
	13	0.0000	.
Intercept		0.5832	0.1145
R^2		0.0874	
Right			
Age		0.0067	0.0032
LGHR		- 0.1369	0.0476
PTT		- 0.0003	0.6696
Area	12	0.1252	0.5787
	13	0.0000	.
Age*area	12	- 0.0247	0.5292
	13	0.0000	.
Intercept		0.4650	0.1535
R^2		0.1475	

Model tested: ΔP/PP = age + LGHR + PTT + area + age*area.
LGHR = log-transformed concurrent heart rate; PTT = pulse transit time; Age*area = an interaction term between age and area of study; Area 12 = Yugoslavia; Area 13 = Japan.

Testing of the model showed a significant correlation between the ratio of ΔP/PP and age in the right pulse, and a marginally significant relationship in the left pulse, controlling for the concurrent heart rate and pulse transit time. Heart rate contributed marginally significantly to the ΔP/PP-age relationship in both left and right pulse. Although no statistically significant difference was observed in both cohorts, a test of the

heterogeneity of slopes was performed, to determine the difference in the estimates of the regression coefficients (Table 5.20).

Table 5.21 (a) The ratio of the height above the inflection point to the pulse pressure of the type A waveform in each cohort: observed mean value , standard error, and regression on age in left pulse. (b) The same analysis, but in the right pulse.

(a) Left pulse

Study cohort	Observed mean	SE	Regression with age		
			Slope	SE	p Value
Japan	0.21	0.02	0.0061	0.0026	0.0216
Yugoslavia	0.23	0.02	0.0033	0.0039	0.3918

(b) Right pulse

Study cohort	Observed mean	SE	Regression with age		
			Slope	SE	p Value
Japan	0.27	0.02	0.0067	0.0022	0.0032
Yugoslavia	0.25	0.02	0.0042	0.0033	0.1968

The slope of this ΔP/PP-age relationship was significantly steeper in the Japanese cohort (estimated coefficient: left pulse: 0.006 for the Japanese versus 0.003 for the Yugoslavians; right pulse: 0.007 for of the Japanese versus 0.004 for the Yugoslavians). It suggests that the magnitude of the age-related increase in this ratio is greater in the Japanese than in the Yugoslavians.

Since the study area had no significant effect on the ratio of the height above inflection point to pulse pressure (ΔP/PP), no further analysis of data from each cohort separately was carried out.

5.2.3.5 Sites of wave reflections

It is well known that in the systemic arterial tree, wave reflections arise from any discontinuity of caliber or distensibility where there is a change in vascular impedance [212]. Latham et al. [210] identified two major sites of arterial wave reflection in humans: at the aortic level of the renal arterial branches and the terminal aortic bifurcation. A recent study [211] has provided evidence that the middle cerebral artery is also a site of wave relection in cerebral circulation. Wave reflections could also occur at the aortic valve, but there is no published report supporting this speculation. Furthermore, reflections from the aortic valve would be difficult to record for two reasons: 1. they would occur very early due to the short distance for the waves to travel, and 2. the recording system which was used in the Seven Countries Study is not sensitive enough to record them. In the present study, the "effective" reflection site was estimated using the relationship [213]:

$$Lp = PWV\frac{\Delta t_p}{2} \qquad (5.6)$$

where Lp is the "effective" reflection site from the pressure wave measured and Δt_p represents the travel time of the wave from the heart to the reflection site and back.

In both the pooled sample and the Yugoslavian cohort, the estimated reflection site distance of the left pulse was significantly longer than that of the right pulse. But in the Japanese cohort, the left estimated distance did not differ significantly from that of the right. A comparison between the groups indicates that the estimated "effective" reflection site distance of the right pulse of Japanese men was significantly longer than that of Yugoslavian men (Mean±SE = 6.25±1.13, t = 2.34, p = 0.02). No statistically significant difference in the estimated distance of the left pulse was observed between these two cohorts (Table 5.21).

Table 5.22 Comparisons of the estimated "effective" reflection site distance of the left and right pulse.

	Left pulse (cm)	Right pulse (cm)
Pooled sample	58.28±1.50	54.18±1.30*
Japan	59.18±1.82	57.09±1.90
Yugoslavia	57.36±2.40	51.11±1.72**

Values are given as Mean±SEM.
* $p < 0.01$.
** $p < 0.05$.
The p value is for the Student's t test for independent samples, comparing between the estimated "effective" reflection site distance of the left and right pulse.

5.3 Discussion

5.3.1 Differences in the pressure wave contour

Data analysis shows a predominance of type A pressure waveforms in the pooled sample as well as in both cohorts. A higher proportion of Japanese men had type A waveforms than in the Yugoslavian men. This difference may be explained by the following factors. 1. Comparatively, Japanese men with type A contour were significantly older, with a shorter pulse transit time, longer interval between the onset of systolic upstroke and peak systolic pressure, and a shorter stature. These characteristics were also observed in Japanese men with the type C waveform. Previous studies [85,86,214] have consistently shown that older subjects (>50 years) generally have a type A pressure wave contour, and younger subjects (<30 years) have a type C contour. 2. The pulse transit time of the Japanese men was significantly shorter than that of the Yugoslavian men, even after controlling for age and height (Observed Mean±SE for Japanese men: 55.45±1.34 msec; for Yugoslavian men: 60.44±1.41 msec, $p = 0.02$). This difference suggests that the Japanese men have much stiffer arteries than the Yugoslavian men. Reflected waves return earlier from the peripheral sites as a consequence of increased arterial stiffness; in fact the early return of wave reflections has been shown to be a mechanism responsible for the

altered pressure wave contour associated with advancing age [86]. (3) Body height has been reported to be a major determinant of carotid pulse contour in humans [184]. This is due to the early return of the reflected waves caused by the shorter body length and shorter distance to the reflecting site. The Japanese men were significantly shorter than the Yugoslavian men; their shorter stature, coupled with older age, may offer an explanation for the higher proportion of Japanese men with type A contour, when compared to their counterparts in the Yugoslavian cohort (see Table 5.12).

I observed statistically significant differences in parameters defining the shape of the carotid pressure wave, between the left and right pulse. This finding is unexpected and difficult to explain. Studies of the pulse wave velocity and wave contour have been traditionally unilateral, in that measurements are made on either the left or right carotid artery [65,71,205], assuming that no differences exist between these two sides. In fact, Van Merode et al. [215] reported no significant difference in the vessel wall properties of the left and right common carotid arteries. However, the findings of my study are at variance with their result. Two explanations for these differences are suggested. 1. In the Seven Countries Study, the pulse wave tracings were recorded with a Sanborn Apexcardiograph displacement transducer. It was placed over the external carotid artery, and affixed with a strap around the subject's neck. An identical transducer was placed over the femoral artery, affixed with a specially designed device. This measurement technique is limited by movement artifact, by the difficulty in affixing the strap around the neck and over the femoral artery, by the need for a significant "hold-down" force for optimal signal registration, and by a high damping coefficient causing attenuation of high frequency characteristics [86]. 2. The differences reflect a dissimilarity in flow patterns, as a consequence of the anatomical difference between the left and right common carotid arteries (see Figure 5.15). The left common carotid originates in the thorax from the arch of the aorta, while the right common carotid artery begins at the bifurcation of the innominate

artery. It has been documented that bifurcations are sites of accelerated atherosclerosis and consequent endothelial injury, resulting from the disturbance of laminar flow [206]. As the right common carotid artery arises from the bifurcation of the innominate artery, it is conceivable that blood flow at the flow-divider may be either slow or disturbed. Atherosclerotic plaques and wall thickenings are usually localized in regions of disturbed flow that contain the points of flow separation and stagnation [217]. Thus it is speculated that the arterial walls of the right common carotid artery may be stiffer and thicker than those of the left common carotid, resulting in a shortened return time of the reflected wave

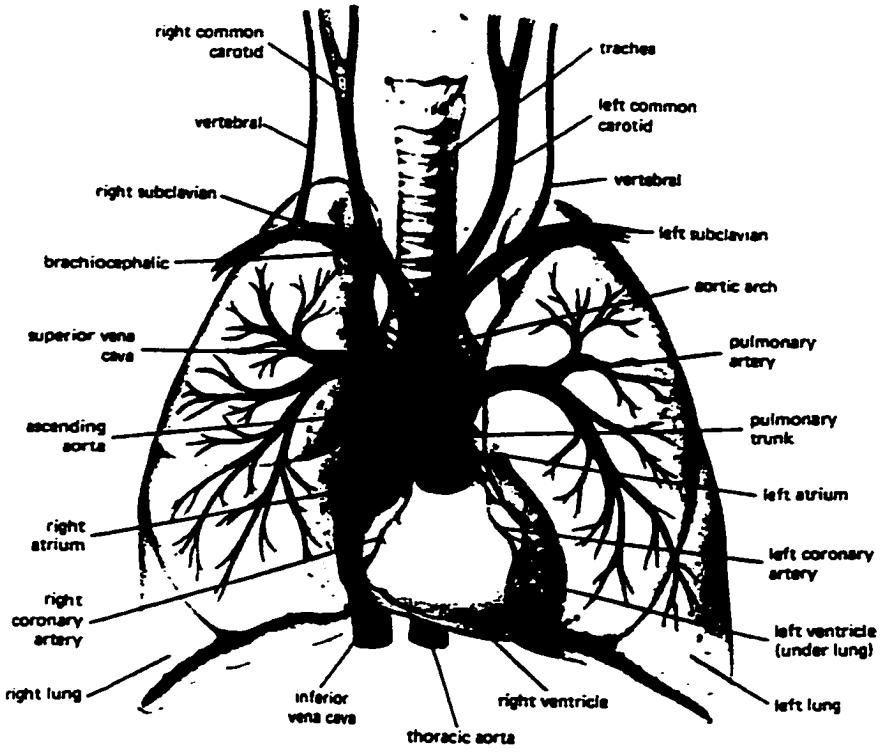


Figure 5.15 The aortic arch and its major branches. The right common carotid and the right subclaivain arteries arise from the brachiocephalic trunk. The left common carotid and the left subclavian arteries branch off directly from the aortic arch.

5.3.2 Factors influencing the shape of the carotid pressure wave

Studies of wave propagation in the human arterial system have established that wave reflection is an important factor in the magnitude and shape of the pressure wave at different locations in the arterial tree [67,210,218]. Wiggers [219] attributed modification of the peripheral pulses to the following factors: 1. damping or attenuation of wave and the viscosity of blood within; 2. dispersion of wave due to different frequency components traveling at different velocities; 3. reflected waves return from the peripheral sites which result in summation with the forward waves; and 4. the occurrence of natural vibrations in various parts of the arterial tree. O'Rourke [80] identified the form and duration of ventricular ejection, and the timing and intensity of wave reflection from the peripheral sites, as the major determinants of pressure wave contour. Propagation properties of the elastic conduit arteries determine the timing of wave reflection. The intensity of wave reflection is determined by the degree of impedance mismatch at junctions and terminations, with the main contributor being at the level of vasoconstriction of the peripheral vascular beds [207]. Advancing age affects both components.

The finding of a significant correlation between the timing of wave reflection and age, of type A waveform in the pooled sample, confirms the results reported by Fries [206], Murgo et al. [85], and Kelly et al. [86]. Two mechanisms responsible for the shortened timing of wave reflection are proposed. 1. Increased stiffness of large elastic arteries associated with age, results in an increase in both the intensity of reflected energy and the pulse wave velocity. This increase in wave velocity then results in the early return of reflected waves from peripheral sites, and is largely responsible for the appearance of the early systolic shoulder, and the amplification of the systolic peak. 2. Capillary density has been found to decrease with age [220,221], reducing the total cross-section of microcirculation, thereby contributing to increased flow resistance [88]. With elevated peripheral resistance, the reflections increase for high frequency, and the pulse wave

velocity also increases. A faster speed with which the pressure wave travels results in its earlier return.

A marginally significant correlation was found between the timing of wave reflection and the concurrent heart rate; it explains 3% of the variance in the reflected wave return time. Gow and O'Rourke [222] and O'Rourke [88] have demonstrated that the contour of ventricular ejection waves is similar in different mammals of different sizes and shapes, so that the frequency band of maximal flow harmonic amplitude is dependent on heart rate. Under ideal conditions, the minimal value of impedance corresponds to the maximal value of flow wave harmonics [223], so that maximal pulsatile flow from the heart generates minimal pressure fluctuation. However, with increasing age, this favorable relationship progressively deteriorates. There is a dissociation between the increasingly high values of the impedance modulus at low frequencies and unchanged or reduced flow harmonics. At the same time the reflected wave moves from diastole to systole and increases the late systolic peak [80], thus altering the contour of the pressure wave.

The location in the arterial system from which wave reflections arise was estimated from the carotid pulse wave velocity in both cohorts. The estimated "effective" reflection site distance for Yugoslavian and Japanese men was between 51 to 59 cm from the carotid artery. This distance is longer than a reflection site of 44-48 cm reported by Murgo et al. [85]. It is speculated that such a distance would approximate the region of the terminal abdominal aorta and bifurcation into the iliac and femoral arteries, in the adult population. An explanation for the longer reflection site distance in the two cohorts may be related to an over-estimation of the surface distance. As discussed in Section 5.1.2, this distance was estimated from a regression model, using the standing and sitting height of 67 men in two field studies. The regression model could explain only 51% and 26% of the variance in the acromial sitting height for the Caucasian and Asian men, respectively. Over-estimation of the distance between the recording sites results in an inaccurate estimation of the pulse wave velocity. As shown in equation 5.6, an "effective" reflection site is a function of the

pulse wave velocity, and one half of the travel time of the wave from the heart to the reflection site and back. Inaccurate determination of the pulse wave velocity leads to a longer "effective" reflection site distance.

Another explanation for the longer "effective" reflection site distance may be due to the inadequacy of the algorithm with which I used to identify the inflection point, and the onset of the systolic upstroke. Imprecise identification of these points can produce erroneous travel time of the reflected wave (Δt). This in turn can over or under estimate the "effective" reflection site distance.

5.4 Summary

Cohort differences were found in the pulse transit time; the pulse transit time of the Japanese men was significantly shorter than that of the Yugoslavian men (54.5±1.2 msec. versus 62.6±1.3 msec.). In the Yugoslavian cohort, age and the heart rate were the significant predictors of the pulse transit time, and can account for 14% and 9.8% of the variance in the pulse transit time in this population. In the Japanese cohort, the pulse transit time did not correlate significantly with age, but was significantly and inversely correlated with the systolic and diastolic blood pressure, controlling for the body mass index, serum cholesterol concentrations, height, and the amount of cigarettes smoked per day. The heart rate accounts for 17%, while the systolic pressure accounts for 12%, and the diastolic pressure 15% of the variability in the pulse transit time in this cohort.

A higher percentage of the Japanese men showed a type A waveform than of the Yugoslavian men (96% versus 72%). In the Japanese cohort, the timing of wave reflections of the left pulse was significantly longer than that of the right pulse type A waveform (103.3±2.0 msec. versus 97.3±2.2 msec.). In the Yugoslavian cohort, the parameters that defined the shape of the left and right pressure waveforms were not significantly different.

The study area had no significant effect on any of the parameters that defined the contour of type A and type C pressure waves. The bivariate analysis of the pooled data showed a downward age trend in the timing of wave reflections, but an upward age trend in the amplitude of the reflected waves of type A pressure waveforms. A statistically significant association was observed between timing of wave reflections and age; age can explain 5% of the variance in this parameter defining the shape of type A pressure waves. The concurrent heart rate was a significant predictor of the timing and amplitude of the reflected waves, and the interval from systolic upstroke to the peak systolic pressure. In type C wave contour, none of the parameters which defined the wave contour, was significantly correlated with age. The heart rate, however, was a significant predictor of the interval from the systolic upstroke to the peak of the systolic pressure.

The major limitation of the present study is the narrow age range (i.e. 20-year age span) of my subjects. To determine the age-related changes in the pulse transit time, and the carotid pressure wave contour, a wider age range is necessary. Furthermore, my data of the pulse transit time and the pressure wave contour are cross-sectional, and do not allow me to study how the transit time and the contour of carotid pulse change with age. Prospective investigations into the effects of aging on the wave velocity, and the shape of pressure waves would shed light on the relationship between vascular aging and wave reflections, and may have profound implications for the therapeutic strategies for hypertension in the elderly.

In this investigation, an age effect on the wave contour was found even after adjusting for the pulse transit time, which has been shown to be age-dependent. It suggests that other age-dependent factors such as increased heterogeneity of the vessel properties, may contribute to a different shape of the pressure wave in persons of different ages. It is necessary to identify these factors, and further investigate their effects on the wave contour. Only after that may a full explanation for the effects of age on the pressure wave configuration be found.

The finding of a significant correlation between the pulse transit time and the heart rate lends support to the claim that the sympathetic nervous system has an influence on the vasculature of the large arteries. Further investigations are needed to assess the possible role of the sympathetic activity in the early alterations of the arterial walls.

Chapter 6

Effects of Age on the Heart

6.1 Introduction

The effects of age on the arterial functions have been well documented [57,58,65]; alterations in the arterial system have a profound influence on ventricular function [224,225]. The arterial system has been described as an external vascular load placed on the ventricle during fiber shortening and ejection [214]. This load has two components: the pulsatile and the nonpulsatile. The pulsatile component depends on the physical properties of the large arteries and wave reflections [226]. The nonpulsatile component is determined primarily by blood viscosity and the arteriolar caliber. Age affects both components.

The vascular load increases through increases in arterial blood pressure [227], peripheral resistance [224], and arterial stiffness [228]. These alterations are associated with advancing age [203], and increasing age is known to be associated with mild left ventricular hypertrophy [180,229]. However, the exact mechanism responsible for the age-related increase in the cardiac mass remains uncertain. In the past, blood pressure has been considered as a primary determinant of left ventricular hypertrophy, but studies of the relationship between arterial blood pressure and left ventricular mass in normotensives, hypertensives, and elderly, have produced inconclusive and inconsistent results. In recent years, age-related augmentation in the stiffness of large arteries has been implicated in the development and/or maintenance of cardiac hypertrophy [72,108].

The present study set out to assess the effects of age on the heart. Specifically, it 1. evaluated the effects of blood pressure aging trend on the electrocardiographic estimate of the left ventricular mass index ($LVMI_{ECG}$), an end point of the age effect on the heart, and 2. determined the relationship between the $LVMI_{ECG}$ and pulse transit time, a proxy for arterial stiffness.

6.2 Method

The electrocardiographic (ECG) data used for the analysis were those obtained during the 5-year follow-up of the Yugoslavian cohort, and the 10-year follow-up of the Japanese cohort of the Seven Countries Study. The recording, digitization, and analysis of the Frank-lead ECG signals have been described in detail in Chapter 2.3, 2.4, and 2.5. Of 511 Yugoslavian men recruited in the Study, only 123 (24%) had undergone this investigative procedure. Similarly, 127 (25%) Japanese men had the special lead ECG recordings. The left ventricular mass index was estimated from an established multivariate regression model [134] (see Section 2.4.1).

A multiple regression analysis method was used to examine the effects of age on the electrocardiographic estimate of the left ventricular mass index ($LVMI_{ECG}$). The distribution of the $LVMI_{ECG}$ data did not approximate normality; they were transformed according to the following equations:

$$LVMI_{ECG} \text{ of the pooled sample and Japan} \rightarrow LOG(LVMI_{ECG}) \qquad (6.1)$$

$$LVMI_{ECG} \text{ of Yugoslavia} \rightarrow \frac{1}{LVMI_{ECG}} \qquad (6.2)$$

Logarithmic manipulation was used to transform the data of blood pressure and body mass index to improve the normality of their distribution. The transformed data were used in all analyses.

The systolic and diastolic pressure load was defined as the load on the myocardium that existed during the observation period, and was estimated by the integral of the time-pressure curve. The total pressure load as defined by Menotti et al. [230] was the sum of the systolic and diastolic pressure load imposed on the myocardium during the observation

period. The pressure load for the Yugoslavian and Japanese cohorts was calculated according to the following equation:

$$\text{Pressure Load} = \frac{(BP_1+BP_2)\times(T_2-T_1)}{2} \qquad (6.3)$$

Where T_2-T_1 = 5 year interval in Yugoslavia, and 10 year interval in Japan. BP_1 = the blood pressure measured at time 1 (entry to study); BP_2 = the blood pressure measured at the 5-year follow-up in Yugoslavia, and at the 10-year follow-up in Japan (see Figure 6.1).

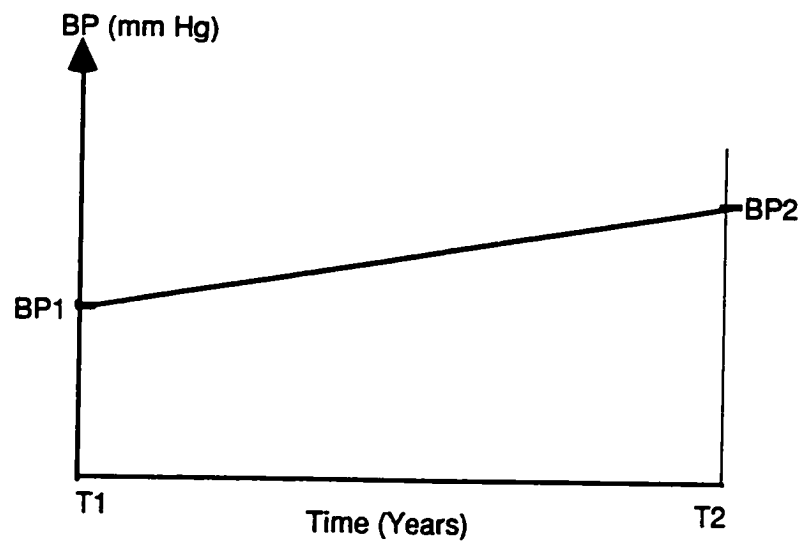


Figure 6.1. A schematic representation of the pressure load in the Yugoslavian and Japanese male cohorts. The pressure load is calculated algebraically, the entire area under the time-pressure curve.

Cook's D influence statistic was used to isolate peculiarities in the data. This statistical measure has been described in detail in Section 2.6. Outliers were removed from the linear regression models, which examined the relationship of the electrocardiographic

estimate of the left ventricular mass index to age. Four observations in Japan, and three in Yugoslavia were identified as outliers, and as such, were excluded from the data analysis.

Blood pressure, body mass index, and pulse transit time were included in the models that examined the $LVMI_{ECG}$-age relationships. These covariates were added because they have been shown to have a significant influence on the cardiac mass [108,117,231].

Influence of the arterial blood pressure on the cardiac mass has been reported in a number of studies [113,114]. The blood pressure exerts its effects on the heart by increasing the systolic pressure generated by the ventricle in order to overcome the increasing afterload. This results in an increase in the tension of the ventricular wall. An augmented wall tension induces the addition of sarcomere in parallel, leading to increased wall thickness.

Obesity has been found to associate with increased left ventricular wall thickness and left ventricular mass in both hypertensive and normotensive subjects [232,233]. The Framingham data showed a powerful influence of weight on the left ventricular mass [234]. It was reported that the age-adjusted prevalence of left ventricular hypertrophy increased 15-17-fold between the lean (body mass index < 23 kg/m^2) and the obese (body mass index > 30 kg/m^2) subjects.

Non-invasive studies of the aortic vascular properties have shown that the degree of cardiac hypertrophy is closely related to the physical properties of the arterial system [108,109]. The metabolic needs of the left ventricle are greatly influenced by the level of systolic blood pressure, which, in turn, is influenced by the systemic arterial distensibility [235]. An augmented systolic pressure results in an increase in wall stress; this in turn induces the addition of sarcomeres in parallel, leading to increased wall thickness.

6.3 Results

6.3.1 Descriptive statistics

One hundred and twenty-seven Japanese men, and 123 Yugoslavian men had the special ECG lead recordings. Table 6.1 presents the age distribution of subjects with and without the special ECG lead recordings.

Table 6.1 The age distribution of subjects with and without the $LVMI_{ECG}$ in both male cohorts.

Age group	Yugoslavia		Japan	
	With $LVMI_{ECG}$	Without $LVMI_{ECG}$	With $LVMI_{ECG}$	Without $LVMI_{ECG}$
40-44	28	111	0	0
45-49	28	56	31	61
50-54	37	99	29	91
55-59	30	122	36	103
60-64	0	0	31	108
Total	123	388	127	363

$LVMI_{ECG}$ = the electrocardiographic estimate of the left ventricular mass, with the special Frank-vector lead system.

I applied the Student's t test for independent samples to determine if subjects who had the $LVMI_{ECG}$ were significantly different from those without the $LVMI_{ECG}$ in each cohort. I compared them on the entry values of age, arterial blood pressure, body mass index, serum cholesterol levels, resting heart rate, and the number of cigarettes smoked per day. No statistically significant difference was observed between groups in any of these variables in either cohort. When the groups were compared by age groups, still no statistically significant difference was observed between the group with $LVMI_{ECG}$ and the one without $LVMI_{ECG}$ in any of these variables. In this multiple comparison analysis, a p value of 0.01 is accepted as the level of statistical significance. Table 6.2 presents the

comparisons of the $LVMI_{ECG}$ group and the non-$LVMI_{ECG}$ group by age groups in both cohorts.

Table 6.2 Comparisons of the $LVMI_{ECG}$ group and the non-$LVMI_{ECG}$ group on the entry values of some variables in the Japanese and Yugoslavian male cohorts.

(a) Japan

Variable	Age group 50-54	55-59	60-64	65-69
SBP (mm Hg)				
$LVMI_{ECG}$	110.53±15.66	121.43±19.83	122.07±10.06	120.40±21.89
Non-$LVMI_{ECG}$	110.29±17.71	115.30±20.46	120.40±20.24	134.55±27.06
DBP (mm Hg)				
$LVMI_{ECG}$	63.98±11.35	70.29±13.10	72.49±12.74	69.69±11.60
Non-$LVMI_{ECG}$	64.06±13.04	69.27±11.84	70.63±13.78	76.43±15.29
BMI (kg/m^2)				
$LVMI_{ECG}$	22.39±1.83	21.43±1.35	21.80±2.68	21.48±2.07
Non-$LVMI_{ECG}$	22.13±1.73	21.73±2.20	22.14±2.84	21.84±2.29
SC (mg/dl)				
$LVMI_{ECG}$	171.24±42.90	166.85±49.93	172.56±34.52	167.81±43.11
Non-$LVMI_{ECG}$	169.60±41.21	165.13±38.67	172.32±35.57	162.91±41.91
HR (b/min)				
$LVMI_{ECG}$	61.31±10.48	59.04±9.16	58.31±9.65	59.07±11.01
Non-$LVMI_{ECG}$	61.46±11.05	59.08±9.40	60.52±10.11	62.25±11.58
SMC (amount smoked)				
$LVMI_{ECG}$	5.22±2.78	6.20±1.59	5.54±2.25	4.83±2.99
Non-$LVMI_{ECG}$	4.71±2.75	4.88±2.91	4.77±2.85	4.92±2.79

SBP = systolic blood pressure measured; DBP = diastolic blood pressure; BMI = body mass index; SC = serum cholesterol concentrations, HR = resting heart rate; SMC = number of cigarettes smoked per day. Amount of cigarettes smoked is coded as follows: 0-1 = none or stopped smoking for at least one year; 4 = smoking 1-4 cigarettes per day,; 5 = smoking 5-9 cigarettes per day; 6 = smoking 10-19 cigarettes per day; 7 = smoking 20-29 cigarettes per day; and 8 = smoking > 30 cigarettes per day.
Values are given as Mean±SD.

(b) Yugoslavia

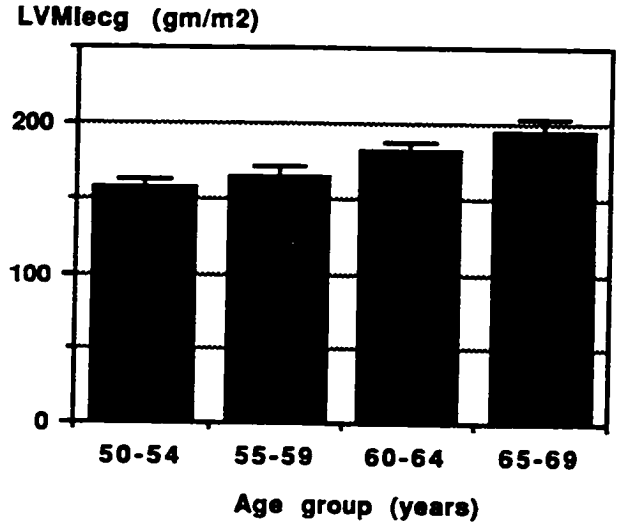
Variable	Age group 45-49	50-54	55-59	60-64
SBP (mm Hg)				
$LVMI_{ECG}$	128.07±9.45	133.44±14.11	144.20±17.62	135.73±15.72
Non-$LVMI_{ECG}$	130.23±13.79	132.19±15.44	137.31±19.60	143.46±19.27
DBP (mm Hg)				
$LVMI_{ECG}$	78.92±6.68	81.97±7.68	85.33±11.22	82.96±9.17
Non-$LVMI_{ECG}$	80.41±10.34	80.53±7.60	81.90±9.244	84.13±10.43
BMI (kg/m^2)				
$LVMI_{ECG}$	22.37±2.84	22.38±2.89	21.79±2.44	21.55±2.14
Non-$LVMI_{ECG}$	22.42±2.45	22.48±3.07	22.20±2.61	21.87±2.69
SC (mg/dl)				
$LVMI_{ECG}$	163.15±29.38	166.25±42.54	155.14±29.91	160.30±37.12
Non-$LVMI_{ECG}$	152.25±26.12	160.66±33.14	163.39±34.32	163.73±30.90
HR (b/min)				
$LVMI_{ECG}$	69.63±8.99	22.38±3.07	71.89±14.98	70.57±12.45
Non-$LVMI_{ECG}$	69.23±11.49	22.48±3.066	71.57±10.59	67.70±11.88
SMC (amount smoked)				
$LVMI_{ECG}$	3.74±3.03	2.52±2.90	2.86±3.07	3.15±2.77
Non-$LVMI_{ECG}$	3.03±3.02	3.19±3.15	3.54±2.93	2.82±2.88

SBP = systolic blood pressure measured; DBP = diastolic blood pressure; BMI = body mass index; SC = serum cholesterol concentrations, HR = resting heart rate; SMC = number of cigarettes smoked per day. Amount of cigarettes smoked is coded as follows: 0-1 = none or stopped smoking for at least one year; 4 = smoking 1-4 cigarettes per day,; 5 = smoking 5-9 cigarettes per day; 6 = smoking 10-19 cigarettes per day; 7 = smoking 20-29 cigarettes per day; and 8 = smoking > 30 cigarettes per day.
Values are given as Mean±SD.

6.3.2 Age trend in the distribution of electrocardiograpic estimate of the left ventricular mass index ($LVMI_{ECG}$)

It is well documented that the cardiac mass increases with advancing age. I used the bivariate analysis to examine if an upward age trend was present in the distribution of the left ventricular mass index in these ethnically different populations. Figure 6.2 presents this analysis graphically.

(a)



(b)

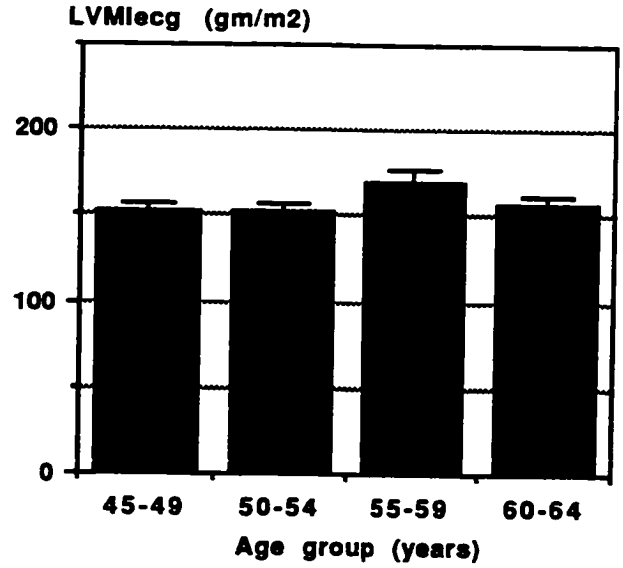


Figure 6.2 The age trend of the $LVMI_{ECG}$ distribution in the (a) Japanese and (b) Yugoslavian male cohorts. The error bars represent SEM. These measurements are raw data, and do not correct for the age difference for 5 years between the two cohorts.

A significantly higher value of the electrocardiographic estimate of the left ventricular mass index was observed among the older male cohorts of Japan, but not of Yugoslavia. In the Yugoslavian male cohort, there was no apparent age trend in the cardiac mass.

I compared the $LVMI_{ECG}$ of the two cohorts by the linear regression analysis method, controlling for age. This statistical method was chosen because of a different age distribution in these cohorts at the time when the $LVMI_{ECG}$ recordings were made (mean age for the Japanese men: 59.6±0.5 years, and for the Yugoslavian men: 54.9±0.5 years). The observed $LVMI_{ECG}$ was then compared by the Student's t test for the independent samples. Table 6.3 presents this comparison.

Table 6.3 A comparison of the $LVMI_{ECG}$ value of the Japanese and Yugoslavian men: observed mean value and standard deviation.

Study area	N	Observed mean (gm/m^2)	SEM	t	p Value
Japan	127	174.78	3.32	2.97	0.001
Yugoslavia	123	160.57	3.42		

Comparatively, the Japanese men's electrocardiographic estimate of the left ventricular mass index was statistically significantly higher than that of the Yugoslavian men, corrected for the age difference for five years between these two cohorts.

6.3.3 Relationship of the electrocardiographic estimate of the left ventricular mass index to age

The contribution of age to increased cardiac mass is apparent. Age has been shown to associate significantly with increased stiffness of the arterial walls [84,181], and shortened reflected wave travel time [83,236]. These alterations are two primary factors responsible for augmenting the systolic blood pressure. Systolic pressure is a major

determinant of oxygen requirement and therefore of myocardial blood requirement [80]. These same two factors, in turn, determine the hypertrophic process of the heart. In addition, the systolic pressure is one of the important components of the end-systolic stress [237] which is the principal stimulus of cardiac hypertrophy.

Initially, a bivariate correlation analysis was performed to determine the extent to which $LVMI_{ECG}$ is related to age, systolic pressure, and pulse transit time. This was followed by an analysis of the relationship between the $LVMI_{ECG}$ and age on the pooled sample, controlling for the covariates. Since a significant difference in the slopes was observed, analyses of data from the individual cohorts was performed. Table 6.4 presents the correlation matrix between age, systolic pressure, pulse transit time, and $LVMI_{ECG}$. Table 6.5 summarizes the regression analysis of the $LVMI_{ECG}$-age relationships in the pooled sample.

Table 6.4 A correlation matrix of age, concurrent systolic blood pressure, and $LVMI_{ECG}$ in the (a) Japanese and (b) Yugoslavian male cohorts.

(a) Japanese cohort

	Age	Log-transformed concurrent SBP	Log-transformed $LVMI_{ECG}$	PTT
Age	1.000	–		
Log-transformed Concurrent SBP	0.2931**	1.0000		
Log-transformed $LVMI_{ECG}$	0.3775**	0.4465**	1.0000	
PTT	-0.1521	-0.3529**	-0.1716**	1.0000

(b) Yugoslavian cohort

	Age	Log-transformed Concurrent SBP	Exponentially-transformed $LVMI_{ECG}$	PTT
Age	1.000			
Log-transformed Concurrent SBP	0.2574**	1.0000		
Exponentially-transformed $LVMI_{ECG}$	-0.0703	-0.0895	1.0000	
PTT	-0.3768**	-0.3216**	0.1185	1.0000

Concurrent SBP = systolic blood pressure measured at the time the electrocardiographic recordings using the special Frank-lead system; $LVMI_{ECG}$ = electrocardiographic estimate of the left ventricular mass index; PTT = pulse transit time.
The negative correlation between the $LVMI_{ECG}$ and age and the log-transformed systolic pressure in the Yugoslavian cohort is the result of the exponential transformation of the $LVMI_{ECG}$. Since the exponential transformation is the inverse of the logarithmic transformation, the correlation analysis will result in a negative coefficient.
*$p < 0.005$.
** $p < 0.001$.

Results of the Pearson r correlation analysis indicated a moderate and significant correlation among $LVMI_{ECG}$, the concurrent systolic blood pressure, and age in the Japanese cohort. The $LVMI_{ECG}$ was significantly, and inversely correlated with the pulse transit time. In the Yugoslavian cohort, the $LVMI_{ECG}$ did not correlate significantly with age, the concurrent systolic blood pressure, or the pulse transit time. But a significant correlation among age, the concurrent systolic pressure, and pulse transit time was noted in this cohort, as already described in previous chapters.

Table 6.5 (a) Regression analysis of $LVMI_{ECG}$ on age, controlling for the concurrent systolic blood pressure and various study variables in the pooled sample. (b) The same analysis, controlling for the concurrent diastolic blood pressure and other covariates.

(a)

Variable		Estimate of coefficient	p Value
Age		0.0054	0.0649
Log-transformed SBP		0.3849	0.0019
Log-transformed PP		0.0016	0.9656
PTT		0.00004	0.9669
Log-transformed BMI		0.4566	0.0001
Area	12	0.2401	0.0023
	13	0.0000	.
Age*area	12	0.0057	0.1734
	13	0.0000	.
Intercept		4.3350	0.0001
R^2		0.2492	

(b)

Variable		Estimate of coefficient	p Value
Age		0.0023	0.0507
Log-transformed DBP		0.0055	0.4099
Log-transformed PP		0.1699	0.0003
Log-transformed BMI		0.4640	0.0001
Area	12	0.2885	0.0010
	13	0.0000	.
Age*area	12	0.0066	0.0997
	13	0.0000	.
Intercept		4.7091	0.0001
R^2		0.2593	

Models tested: (a) $LVMI_{ECG}$ = age + LogSBP + LogPP + PTT + LogBMI + area + age*area.
(b) $LVMI_{ECG}$ = age + LogDBP + LogPP + PTT + LogBMI + area + age*area.
SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; BMI = body mass index; Area = study area (area 12 = Yugoslavian cohort, area 13 = Japanese cohort); Age*area = an interaction term between age and the study area .
The regression analysis was performed with the PROC GLM procedure in the SAS system for linear models. The estimate of coefficients are for the parameters (intercept and coefficient), and are not standardized coefficients as those produced with the PROC REG procedure.

Testing of the regression model showed a marginally significant relationship between $LVMI_{ECG}$ and age in the pooled sample. The concurrent systolic but not diastolic blood pressure was a significant predictor of the cardiac mass. The concurrent body mass index correlated significantly with the $LVMI_{ECG}$. The study area had an effect on the cardiac mass at any given age ($p = 0.002$ when controlling for systolic pressure, and $p = 0.001$ when controlling for diastolic pressure). In view of this significant difference, a test of heterogeneity of the slopes was carried out. The result indicated a marginally significant difference in the slope for the $LVMI_{ECG}$-age relationship between the two areas. The regression data of the $LVMI_{ECG}$ on age for each cohort, and its observed mean and standard error are summarized in Table 6.6.

Table 6.6 (a). The $LVMI_{ECG}$ in each study cohort: observed log-transformed mean value standard error, and regression on age, controlling for the concurrent systolic blood pressure and other covariates. (b) the same analysis, controlling for the concurrent diastolic blood pressure and other covariates.

(a)

Study cohort	Observed log-transformed mean (gm/m^2)	SEM	Regression on age		
			Slope	SE	p Value
Japan	5.12	0.02	0.0054	0.0029	0.0649
Yugoslavia	5.04	0.02	0.0003	0.0039	0.9344

(b)

Study cohort	Observed log-transformed mean (gm/m^2)	SE	Regression on age		
			Slope	SE	p Value
Japan	5.12	0.02	0.0055	0.1080	0.0507
Yugoslavia	5.03	0.02	0.0011	0.1698	0.7146

SBP = concurrent systolic blood pressure; DBP = concurrent diastolic blood pressure.

The marginally significant difference in the $LVMI_{ECG}$-age relationship warrants further analyses on the individual cohort. Tables 6.7 and 6.8 present results of these analyses.

Table 6.7 (a) Regression analysis of the log-transformed $LVMI_{ECG}$ on age, controlling for concurrent systolic blood pressure and other study variables in the Japanese male cohort. (b) The same analysis, controlling for diastolic blood pressure and other covariates.

(a)

Variable	Parameter Estimates	Standardized Estimates	p Value
Age	0.0067	0.2173	0.6668
Log-transformed SBP	0.2829	0.3050	0.0001
Log-transformed PP	0.0718	0.1287	0.2957
PTT	0.0004	0.0348	0.0114
Log-transformed BMI	0.3127	0.1929	0.0313
Intercept	3.9959	0.0000	0.0001
R^2	0.2885		

(b)

Variable	Parameter Estimates	Standardized Estimates	p Value
Age	0.0057	0.1867	0.0377
Log-transformed DBP	0.1967	0.1722	0.0631
Log-transformed PP	0.1900	0.3452	0.0002
PTT	0.0003	0.0234	0.7834
Log-transformed BMI	0.3564	0.2223	0.0138
Intercept	4.2510	0.0000	0.0001
R^2	0.2996		

Models tested: (a) $LVMI_{ECG}$ = age + LogSBP + LogPP + PTT + LogBMI.
(b) $LVMI_{ECG}$ = age + LogDBP + LogPP + PTT + LogBMI.
SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; BMI = body mass index; PTT = pulse transit time. The coefficients labeled Standardized Estimate are the estimates that would be obtained if all variables were standardized to a zero mean. These coefficients are independent of the scales of measurements of independent variables, and might offer a comparison of the magnitude of the effects of the variables.

Table 6.8 (a) Regression analysis of exponential-transformed $LVMI_{ECG}$ and age, controlling for concurrent systolic blood pressure and other study variables in the Yugoslavian male cohort. (b) The same analysis, controlling for diastolic blood pressure and other covariates.

(a)

Variable	Parameter Estimates	Standardized Estimates	p Value
Age	0.00001	0.0469	0.6291
Log-transformed SBP	- 0.0027	- 0.1787	0.3283
Log-transformed PP	- 0.00006	- 0.0073	0.9678
PTT	- 0.000007	- 0.0652	0.5303
Log-transformed BMI	0.0044	0.3453	0.0003
Intercept	0.01027	0.0000	0.2860
R^2	0.1285		

(b)

Variable	Parameter Estimates	Standardized Estimates	p Value
Age	0.00002	0.0724	0.4584
Log-transformed DBP	- 0.000002	- 0.0088	0.9275
Log-transformed PP	- 0.0014	- 0.1852	0.0660
PTT	- 0.00001	- 0.0954	0.3617
Log-transformed BMI	0.0041	0.3345	0.0006
Intercept	0.0034	0.0000	0.4912
R^2	0.1325		

Models tested: (a) $LVMI_{ECG}$ = age + LogSBP + LogPP + PTT + LogBMI.
(b) $LVMI_{ECG}$ = age + LogDBP + LogPP + PTT + LogBMI.
SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; BMI = body mass index; PTT = pulse transit time. The coefficients labeled Standardized Estimate are the estimates that would be obtained if all variables were standardized to a zero mean. These coefficients are independent of the scales of measurements of independent variables, and might offer a comparison of the magnitude of the effects of the variables. The negative correlation is the result of the exponential transformation of LVMIECG data to improve normality. Since the exponential transformation is the inverse of logarithmic transformation, analyses of the exponentially transformed data will produce negative results.

In the Japanese cohort, the electrocardiographic estimate of the left ventricular mass index was marginally significantly correlated with age, controlling for the concurrent systolic and diastolic pressure, the pulse pressure, the pulse transit time, and the body mass index. The concurrent systolic blood pressure has a significant, more important effect on the $LVMI_{ECG}$ (coefficient of standardized estimate = 0.3053) than age (coefficient of standardized estimate = 0.2173). The pulse pressure was also a highly significant predictor of the cardiac mass in this population when controlling for the concurrent diastolic but not systolic pressure, and had an important effect on the $LVMI_{ECG}$, (coefficient of standardized estimate = 0.3452). My model failed to detect a significant relationship between the cardiac mass and the pulse transit time. In fact, the pulse transit time exerted a minimal effect on the $LVMI_{ECG}$, as reflected in its coefficient of standardized estimate (0.0348). Its minimal effect on the cardiac mass is further confirmed by the small R^2 value in the univariate regression analysis of these variables; only 2.2% of the variance in the $LVMI_{ECG}$ can be understood in terms of the variability in the pulse transit time. The concurrent body mass index was also a significant predictor of the cardiac mass.

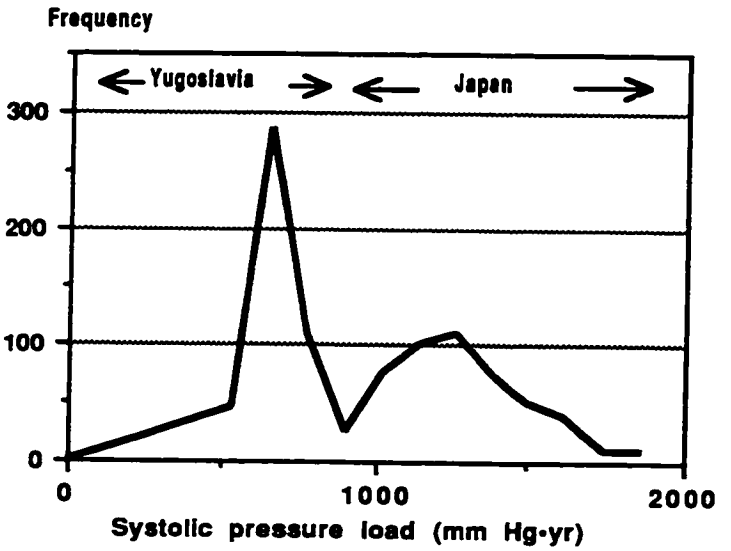
In the Yugoslavian cohort, no statistically significant association between the $LVMI_{ECG}$ and age was observed. Age had a minimal effect on the $LVMI_{ECG}$ (coefficient estimate = 0.05 when controlling for systolic pressure, and 0.07 when controlling for the diastolic blood pressure). Among the covariates, only the concurrent body mass index was a significant predictor of the $LVMI_{ECG}$, and had an important effect on it (coefficient of standardized estimate = 0.35). The pulse transit time was not a significant predictor of the $LVMI_{ECG}$ and can explain less than 1% of the variance in the cardiac mass in this cohort.

6.3.4 Effects of the pressure load and pulse transit time on the electrocardiographic estimate of the left ventricular mass index

Clinical observations indicate that the height of arterial blood pressure in itself may not be correlated totally with developed left ventricular mass. In part, this may be because the single time point pressures used to correlate with the mass do not reflect the integrated arterial pressures actually occurring throughout the 24-hour period and over a longer term, that are "seen" hemodynamically by the left ventricle [238]. Menotti et al. [230] demonstrated that the spontaneous changes of systolic blood pressure over a 10-year observation period, calculated as integrated pressure changes, were significant predictors of atherosclerotic cardiovascular and coronary heart diseases.

Thus far, my data failed to show a significant relationship between the electrocardiographic estimate of the left ventricular mass and the concurrent arterial pressures in both cohorts. In view of the fact that the height of blood pressure is not an accurate representative of the actual load imposed on the myocardium, I investigated the effects of the pressure load on the $LVMI_{ECG}$. This analysis was performed on individual cohorts as the distribution of the systolic, diastolic, and total pressure load was bimodal, with the Yugoslavian cohort occupying one distribution, and the Japanese cohort the other (see Figure 6.3). The bimodal distribution of the systolic and diastolic pressure load is related to the difference in the observation period. In the Japanese cohort, this observation period was from year 0 to year 10. In the Yugoslavian cohort, it was from year 0 to year 5.

(a)



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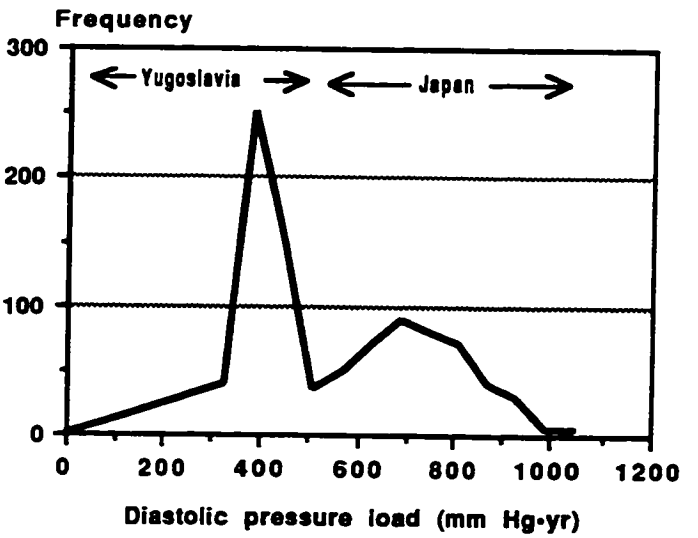
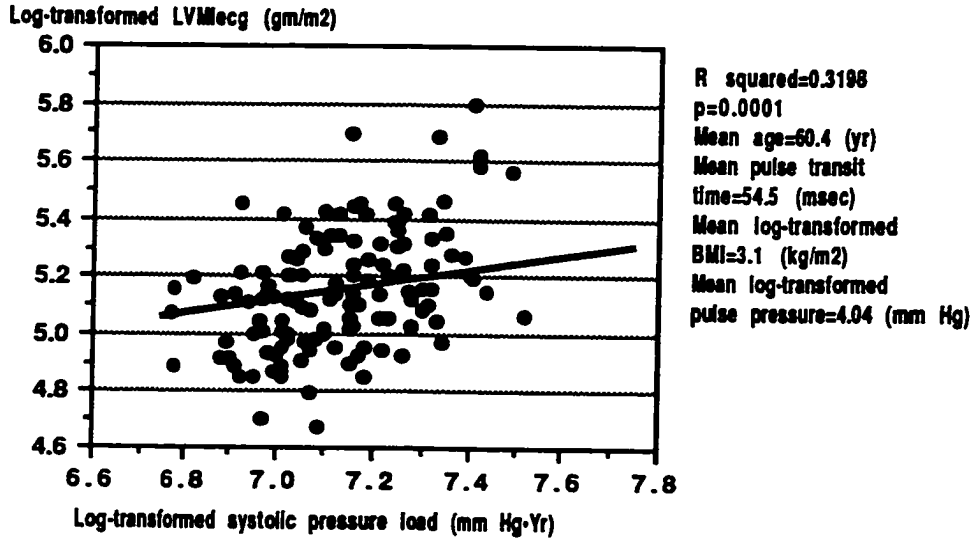


Figure 6.3 A bimodal distribution of the (a) systolic pressure load, and (b) the diastolic pressure load.

Regression models were constructed to determine the extent to which the systolic, diastolic, and the total pressure load influenced the cardiac mass. Age, concurrent pulse pressure, the pulse transit time, and the concurrent body mass index, were included in the models as covariates. Figures 6.4, 6.5, and 6.6 present the relationship between the $LVMI_{ECG}$ and the pressure load in the Japanese and Yugoslavian male cohorts.

(a)



(b)

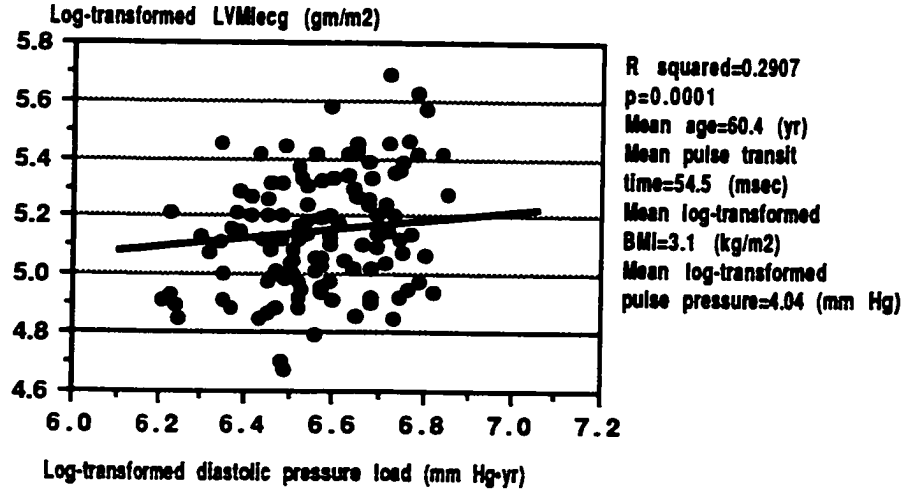
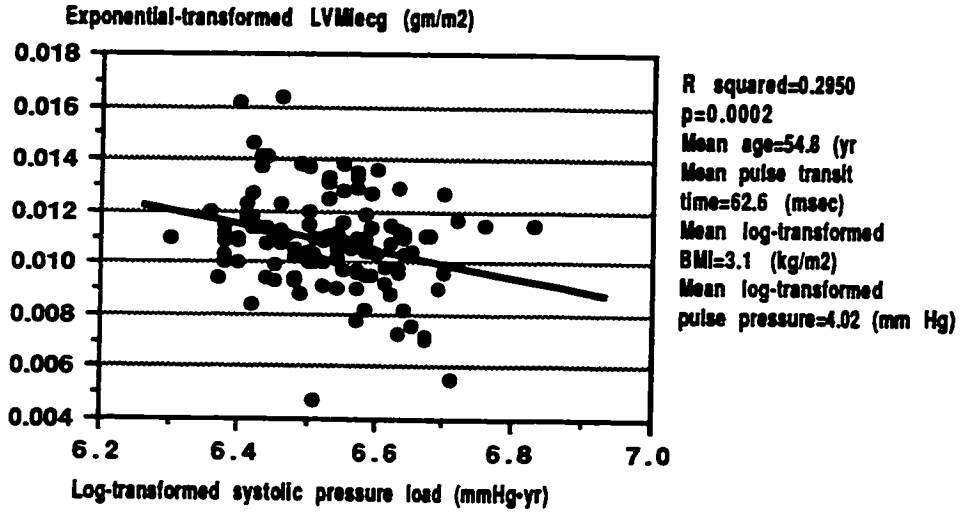


Figure 6.4 (a) A scatterplot showing the relationship between the log-transformed $LVMI_{ECG}$ and the log-transformed systolic pressure load in the Japanese male cohort. The straight line represents the predicted relationship between these two covariates, controlling for the covariates. the regression equation is: Y=3.1032+0.2492(log-transformed systolic pressure load)+0.1655(log-transformed pulse pressure)+0.0077(age)+0.0002(pulse transit time)-0.2868(log-transformed BMI). (b) A scatterplot showing the relationship between the log-transformed $LVMI_{ECG}$ and the log-transformed diastolic pressure load in the Japanese cohort. The straight line represents the predicted relationship between these two covariates, controlling for the covariates. the regression equation is: Y=3.6997+0.15124(log-transformed diastolic pressure load)+0.2119(log-transformed pulse pressure)+0.0071(age)-0.00003(pulse transit time)-0.2722(log-transformed BMI).

Testing of the regression model failed to detect a statistically significant association between the $LVMI_{ECG}$ and the systolic or diastolic pressure load in the Japanese cohort. However, age and the pulse pressure were significant predictors of the $LVMI_{ECG}$. Age can explain 14% of the variance in the cardiac mass, while the pulse pressure accounts for 19% of the variance in the $LVMI_{ECG}$.

(a)



(b)

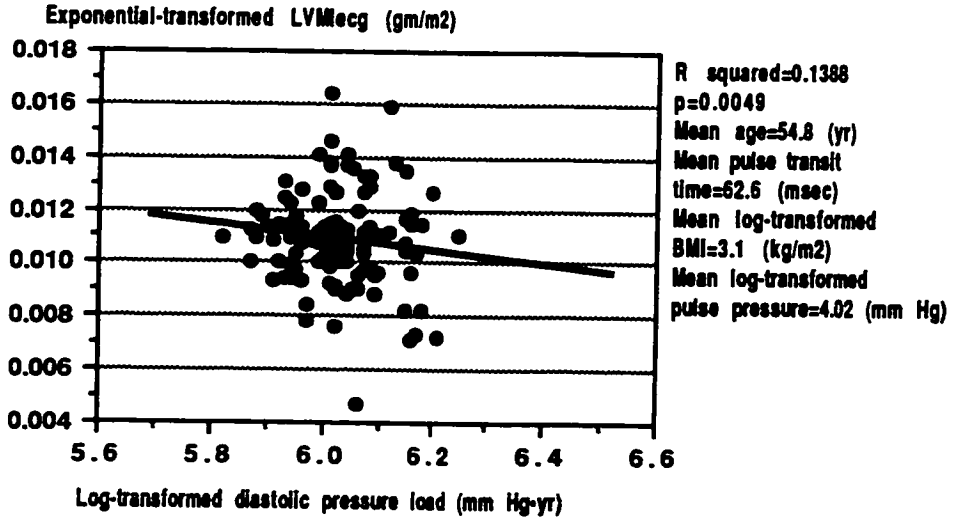
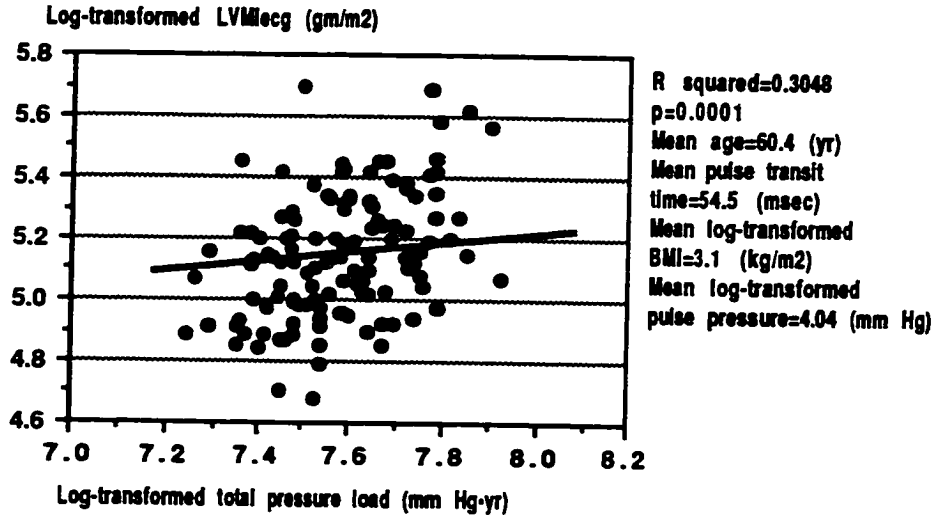


Figure 6.5 (a) A scatterplot shows the relationship between the exponential-transformed $LVMI_{ECG}$ and the log-transformed systolic pressure load in the Yugoslavian male cohort. The straight line represents the predicted relationship between these two variables, controlling for the covariates. The regression equation is Y=0.0263-0.0052(log-transformed systolic pressure load)+0.00003(log-transformed pulse pressure)+0.000026(age)-0.000002(pulse transit time)+0.0054(log-transformed concurrent BMI). (b) A scatterplot shows the relationship between the exponential-transformed $LVMI_{ECG}$ and the log-transformed diastolic pressure load in the Yugoslavian cohort. The straight line represents the predicted relationship between these two variables, controlling for the covariates. The regression equation is Y=0.0121-0.0025(log-transformed diastolic pressure load)-0.0006(log-transformed pulse pressure)+0.000016(age)+0.000003(pulse transit time)+0.0049(log-transformed BMI). The negative slope is the result of the exponential transformation of the $LVMI_{ECG}$ data to improve normality. Since the exponential transformation is the inverse of the logarithmic transformation, the plotting of the exponentially transformed data will produce a negative slope.

In the Yugoslavian cohort, a marginally significant correlation was observed between the $LVMI_{ECG}$ and the systolic pressure load ($p = 0.032$), but not with the diastolic pressure load. The systolic pressure load can account for 4% of the variance in the cardiac mass in this population. The concurrent body mass index was a significant, and important predictor of the $LVMI_{ECG}$ (coefficient of standardized estimate = 0.4212). The R^2 value of the univariate regression analysis indicates that the body mass index can explain 10% of the variability in the cardiac mass. Unlike the Japanese cohort, the pulse pressure was not significantly correlated with the $LVMI_{ECG}$ in this cohort.

Since the total pressure load is the sum of the systolic and diastolic pressure load imposed on the heart, it represents the integrated afterload which the left ventricle must overcome during ejection. I investigated the relationship of this load on the cardiac mass in the individual cohorts, controlling for age, pulse pressure, the pulse transit time, and the concurrent body mass index. Figure 6.6 presents the findings.

(a)



(b)

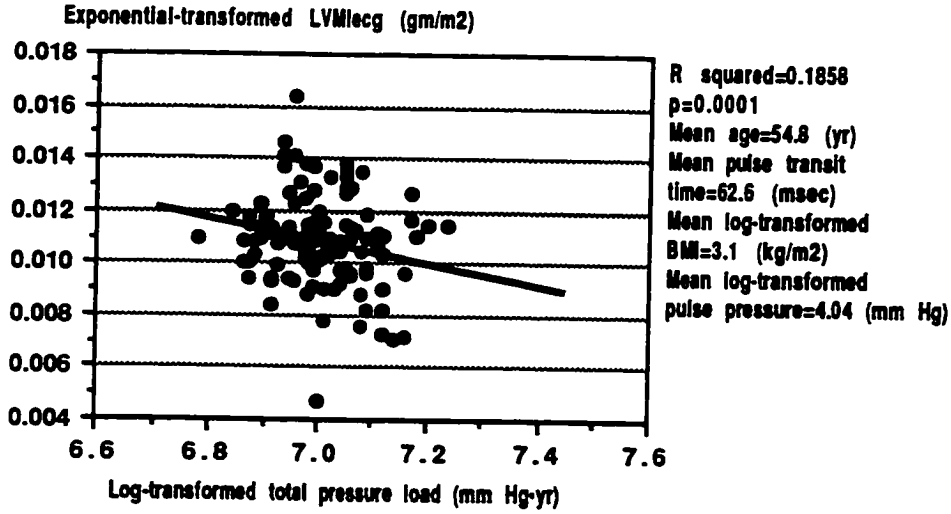


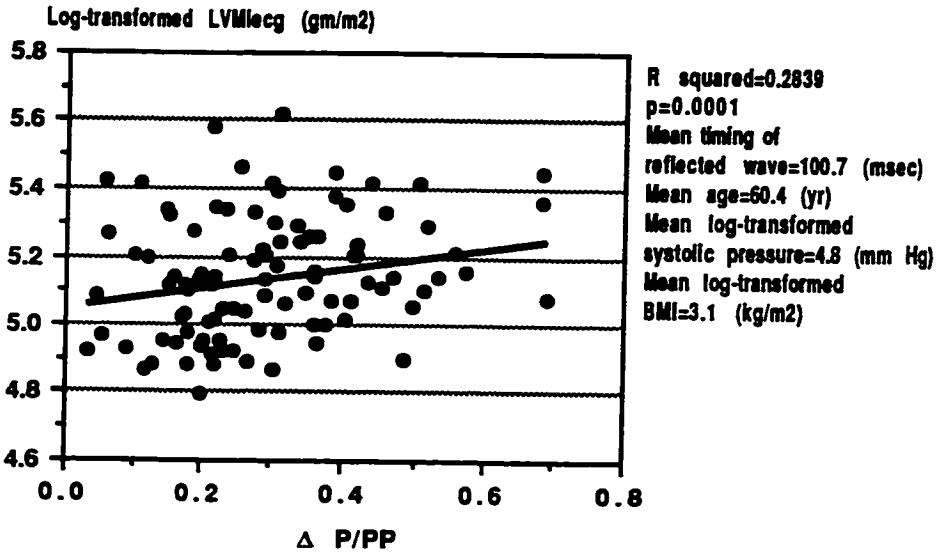
Figure 6.6 (a) A scatterplot showing the relationship between the log-transformed $LVMI_{ECG}$ and the log-transformed total pressure load in the Japanese male cohort. The straight line represents the predicted relationship between these two variables, controlling for the covariates. The regression equation is Y=3.5187+0.1554(log-transformed total pressure load)+0.20104(log-transformed pulse pressure)+ 0.0080(age)+0.00003(pulse transit time)-0.2753(log-transformed BMI). (b) A scatterplot showing the relationship between the exponential-transformed $LVMI_{ECG}$ and the log-transformed total pressure load in the Yugoslavian cohort. The straight line represents the predicted relationship between these two variables, controlling for the covariates. The regression equation is Y=0.0250-0.0042(log-transformed total pressure load)-0.0004(log-transformed pulse pressure)+0.00002(age)-0.000007(pulse transit time)+0.0053(log-transformed concurrent BMI). The negative slope is the result of the exponential transformation of the $LVMI_{ECG}$ data to improve normality. Since the exponential transformation is the inverse of the logarithmic transformation, the plotting of the exponentially transformed data will produce a negative slope.

The data analysis failed to detect a significant correlation between $LVMI_{ECG}$ and the total pressure load in either cohort, controlling for age, pulse pressure, and body mass index. In this regression model, the pulse pressure and body mass index were significant predictors of the $LVMI_{ECG}$ in both cohorts.

6.3.5 The contribution of wave reflection to the cardiac mass

It has been shown that the ideal timing of wave reflection is an important determinant of an optimal ventricular-vascular coupling [239]. The purpose of the following analysis was to investigate the contributory role of the wave reflection of type A waveform in the development and/or maintenance of the elevated left ventricular mass in each cohort. The contribution of wave reflection was quantified as the ratio of the height of systolic peak above the inflection point to the pulse pressure ($\Delta P/PP$), and the multiple regression analysis method was used to answer the research question. The timing of wave reflection, age, the concurrent systolic pressure, and the concurrent body mass index were included in the regression model. The rationale for their inclusion was discussed in detail in Section 6.2. Figure 6.7 illustrates the relationship between the electrocardiographic estimate of the left ventricular mass and the ratio of the height of systolic peak above the inflection to the pulse pressure.

(a)



(b

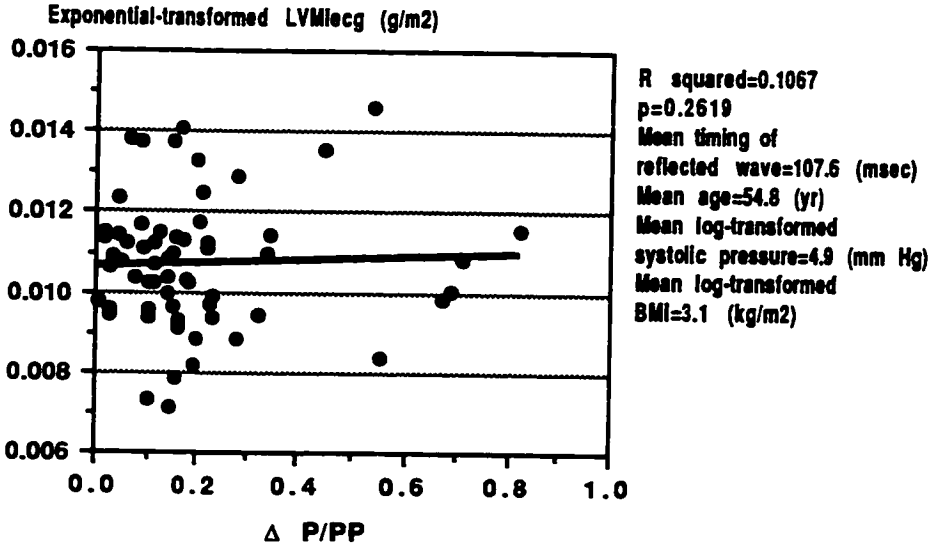


Figure 6.7 (a) A scatterplot showing the relationship between the log-transformed $LVMI_{ECG}$ and the ratio of the systolic peak above the inflection point to the pulse pressure in the Japanese male cohort. The straight line represents the predicted relationship between these two variables, controlling for the covariates. The regression equation is: Y=3.4972+0.0282(ΔP/PP)+0.0010(timing of wave refection)+0.0055(age) +0.3871(log-transformed systolic pressure)-0.2475(log-transformed BMI). (b) A scatterplot illustrating the relationship between the exponential-transformed $LVMI_{ECG}$ and the ratio of the systolic peak above the inflection point to the pulse pressure in the Yugoslavian cohort. The straight line represents the predicted relationship between these two variables, controlling for the covariates. The regression equation is: Y=0.0301+0.0004(ΔP/PP)-0.000003(timing of wave reflection)+0.00003(age)-0.0056(log-transformed systolic pressure)+0.0022(log-transformed BMI). The negative slope is the result of the exponential transformation of the LVMIECG data to improve normality. Since the exponential transformation is the inverse of the logarithmic transformation, the plotting of the exponentially transformed data will produce a negative slope.

In the Japanese cohort, there was a marginally significant correlation between the cardiac mass and the ratio of $\Delta P/PP$ ($p = 0.04$), suggesting that wave reflections of type A waveform contributed to the $LVMI_{ECG}$. In addition, the concurrent systolic pressure was a highly significant predictor of the left ventricular mass index in this cohort. In the Yugoslavian cohort, my model failed to detect such a significant relationship.

6.4 Discussion

6.4.1 Ethnic differences in the cardiac mass

Racial differences in the electrocardiographic (ECG) amplitudes are well documented. Simonson [240] documented systematic racial differences in ECG amplitudes between Japanese and American men and women. The Japanese group showed higher voltages in the precordial leads compared to the American group. Macfarlane and Lawrie [241] demonstrated significant differences in some chest lead amplitudes between younger adult Chinese men and women compared to white men and women from Glasgow. In a recent study, Rautaharju et al. [242] observed substantial racial differences in the ECG amplitudes; ECG amplitudes and amplitude combinations used in left ventricular hypertrophy criteria were larger in blacks than in whites. In my study, the electrocardiographic estimate of the left ventricular mass was estimated from an established multivariate model using six ECG measurements [134]: RV5, SV1, S111, V6, aVR, and QRS duration. Significant differences in the ECG amplitudes among various ethnic and racial groups may explain my observation that Japanese men have significantly higher $LVMI_{ECG}$ values than those of the Yugoslavian men.

The significant difference in the $LVMI_{ECG}$ between the two cohorts may suggest an inappropriateness of using the model for estimating $LVMI_{ECG}$ in other racial groups [136,243]. The ECG model was developed for Caucasian men, and may produce biased results in the Asian population.

6.4.2 Effects of age and blood pressure aging trend on the $LVMI_{ECG}$

Findings of my study suggest that different mechanisms responsible for an increase in the left ventricular mass index may be operating in the Japanese and Yugoslavian men. In the Japanese cohort, the $LVMI_{ECG}$ was significantly correlated with age, and pulse pressure was a significant predictor of the cardiac mass. In the Yugoslavian cohort, the $LVMI_{ECG}$ did not correlate significantly with age; the concurrent body mass was a significant predictor of the $LVMI_{ECG}$. Several mechanisms may be postulated to explain these observations.

The finding of a different $LVMI_{ECG}$-age relationship in the two cohorts agrees with some studies [114,117] but disagrees with others [113,116]. The significant contribution of age to augmented cardiac mass may be explained on the basis of age-related differences in the vascular properties and myocardial structures. Increased intimal thickness, elastin fragmentation, calcification, and increased collagen in tissues are common differences in the vasculature associated with advancing age. They contribute to vascular stiffness and elevated total vascular resistance. There are also differences in the myocardial structures associated with increasing age. Lakatta [244] observed a modest increase in heart weight and the size of the individual myocytes in older humans. Differences in the vascular properties and myocardial structures are claimed to present an increased afterload to the heart, resulting in an augmentation of the cardiac mass.

The effect of blood pressure aging trend on the heart is apparent. In the Yugoslavian cohort, the $LVMI_{ECG}$ was significantly correlated with the systolic pressure load. As this pressure load represents an aggregate of workload on the myocardium, it responds to the prolonged, repeated increases in afterload by a hypertrophic adaptive process [245]. This finding suggests that the systolic pressure load is a more consistent predictor of the electrocardiographic estimate of the left ventricular mass index than the systolic or diastolic blood pressure in this cohort.

The statistically significant correlation between the $LVMI_{ECG}$ and the pulse pressure in the Japanese men, supports the observation that increased systolic and pulse pressure are independent cardiovascular risk factors in men over 50 years [246]. Pulse pressure represents the cyclic systolic stress [229]. Studies on biomaterials have shown that the higher the pulsatility the faster the damage of the biomaterial [67,229]. Previous clinical investigations [141,247] have confirmed the significant influence of increased pulse pressure on the development of left ventricular hypertrophy in hypertensive patients. It is speculated that increased pulse pressure may reflect a disturbance between ventricular ejection and impedance affecting the ventricle; this may result in pulsatile energy loss and further augment the cardiac mass [74].

The finding of a statistically significant relationship between $LVMI_{ECG}$ and body mass index in both cohorts, supports the hypothesis that obesity participates in the development of cardiac hypertrophy. Several studies have found a significant correlation between increased cardiac mass and obesity in both normotensive [248], and hypertensive subjects [249,250]. MacMahon et al. [231] reported a significant association of weight reduction and decreased left ventricular mass, independently of the related falls in blood pressure, among young overweight patients with mild hypertension.

Increased subcutaneous fat in the upper body with obesity is generally considered to accentuate ECG amplitudes, in left lateral leads like V5 [242]. This is partly due to the higher impedance of fat tissue, and partly because of the larger distance from the cardiac source to the recording sites. This increase in the ECG amplitudes of some leads provides a basis for explaining the close association between the electrocardiographic estimate of the left ventricular mass index and the body mass index in both cohorts.

Compared to the Japanese men, the concurrent heart rate of the Yugoslavian men, i.e., heart rate measured simultaneously with the ECG recordings with the Frank-vector lead system, was significantly higher (Yugoslavian men: 75.84±0.99 bpm, Japanese men: 58.61±0.78 bpm, $t = 13.66$, $p = 0.0001$). A higher heart rate means heightened

sympathetic activity. It is possible that adrenergic influences may participate in the cardiac hypertrophy in the Yugoslavian cohort. There is compelling evidence relating the development of left ventricular hypertrophy to adrenergic influences. *In vitro* tissue culture studies demonstrate the development of hypertrophy and increased myocardial protein synthesis when norepinephrine or angiotensin 11 is added to tissue culture [251]. However, there is no evidence of a possible adrenergic role in my study, because the resting heart rate was poorly and non-significantly correlated with the $LVMI_{ECG}$ ($r = 0.004$). As I have no data of the plasma catecholamine concentrations measured at the time when the special ECG lead recordings were made, I could not adequately determine if adrenergic influences indeed play a role in cardiac hypertrophy in this study group.

The effects of age-related differences in the properties of the arterial system on the ventricular functions have been reported in previous studies [72,108,252]. In my study, $LVMI_{ECG}$ was not significantly correlated with pulse transit time in either cohort; the pulse transit time was used as a proxy for arterial stiffness. It is possible that arterial stiffness may not participate significantly in the development and/or maintenance of the increased cardiac mass in these two ethnically different cohorts. This finding is consistent with the observation reported by Cohen-Solal et al. [253] that coupling between the left ventricle and the arterial system expressed in term of left ventricular elastance and effective input elastance of the arterial, changes little with age. On the other hand, the pulse transit time may not be a good marker for the stiffness of the aorta, which would be mostly affecting the heart.

6.4.3 Contribution of the wave reflection to the cardiac mass

The finding of a significant association between the electrocardiographic estimate of the left ventricular mass index and the ratio of $\Delta P/PP$ in the Japanese cohort is in agreement with the results of the published studies [214,239]. In healthy, young humans, reflected waves return to the ascending aorta after ventricular ejection has stopped. This is desirable

because the rise in the ascending aortic pressure caused by the wave reflection occurs in diastole rather than in systole. As a result of this pressure rise, the early diastolic blood pressure is augmented, this in turn boosts the pressure perfusing the coronary arteries and the left ventricle without increasing the left ventricular afterload. With advancing age, the ideal relationship seen in children and young people between duration of the cardiac systole and timing of wave reflection is altered. The pulse wave velocity is augmented due to increased stiffness of the arterial walls. Wave reflection occurs earlier and earlier in the cardiac cycle, augmenting the left ventricular systolic pressure, while reducing the diastolic pressure. This has an adverse effect on the heart. An elevated ventricular systolic pressure imposes an increased afterload on the heart, leading to the subsequent adaptive left ventricular hypertrophy.

6.5 Summary

To the best of my knowledge, this is the first study that compares the effects of age on the heart, using the $LVMI_{ECG}$ as a proxy, between the Oriental and Caucasian populations. Published studies of racial differences in left ventricular hypertrophy focused mainly on the black and white populations [254,255]. Comparatively, the electrocardiographic estimate of left ventricular mass index of the Japanese men was significantly higher than that of the Yugoslavian men, even after controlling for age (174.8 ± 3.34 gm/m^2 versus 160.6 ± 3.4 gm/m^2). In the Japanese cohort, an upward age trend was noted in the distribution of the $LVMI_{ECG}$. Age was marginally significantly correlated with the cardiac mass, but the pulse pressure was a highly significant predictor of the electrocardiographic estimate of the left ventricular mass index. The wave reflections, quantified by the ratio of the height of the systolic pressure above the inflection point to the pulse pressure ($\Delta P/PP$), contributed marginally significantly to an increase in the cardiac mass in this cohort.

In the Yugoslavian cohort, no apparent age trend in the distribution of the $LVMI_{ECG}$ was observed. The concurrent body mass index and the systolic pressure load were the significant predictors of the electrocardiographic estimate of the left ventricular mass index. Neither the pulse transit time nor the wave reflections, both of which are proxies for arterial stiffness, contributed significantly to the cardiac mass.

The differential effect of age on the $LVMI_{ECG}$ in the Yugoslavian and Japanese cohorts can be due to the fact that the ECG model was developed for the Caucasian men, and may produce biased results in the Asian population. Improved models for the left ventricular mass estimation, specifically developed for the Asian men are needed so that results from the present study can be re-evaluated.

Evidence is accumulating suggesting that adrenergic activity could play an important pathogenic role in the cardiac hypertrophy of hypertension. Large-scale population studies which determine the association between the $LVMI_{ECG}$, plasma catecholamine concentration, and age can be expected to produce more definitive indications of the contributions of the neuroendocrine system to cardiac hypertrophy in the normotensives and hypertensives.

Chapter 7

The Process of Cardiovascular Aging

7.1 Introduction

I have presented and discussed data of the effects of age on the major arteries and the heart in previous chapters. The purpose of this chapter was to examine how blood pressure aging interacts simultaneously with the pulse transit time, wave reflections, and the electrocardiographic estimate of the left ventricular mass index.

Most of the published work on cardiovascular aging focus on one facet of the aging process in isolation. This approach towards studying cardiovascular aging cannot provide a comprehensive view on the events that take place during the aging process. The present investigation is new and unique in that it examined the simultaneous interactions between aging of blood pressure and the effects of age on the arterial vasculature and the heart in two ethnically different cohorts.

7.2 The interactions between blood pressure aging and the age effects on arterial vasculature and the heart

7.2.1 The vascular responses to blood pressure aging

Studies of blood pressure-age relationships demonstrate consistently that both the systolic and diastolic pressures increase with age in developed societies. But analyses of the prospective data of blood pressure in my study cohorts indicate that the blood pressure of a small group of the Japanese and Yugoslavian men actually declined with age. For the purpose of illustrating how large arteries and the heart responded to blood pressure aging, I selected two groups of people from each cohort, who showed a distinctly different pattern of systolic pressure change over the observation period. The "upward" group comprised individuals whose systolic pressure increased with age, and the "downward"

group those whose pressure decreased with age.

The pressure wave signals and the special-lead ECG signals were recorded at different times of the follow-up period for each cohort. These signals were acquired during the 10-year follow-up in the Japanese cohort, and the 5-year follow-up in the Yugoslavian cohort. The difference in the observation period in which these signals were recorded, has necessitated the use of the 10-year and the 5-year systolic pressure change for the Japanese and Yugoslavian men. Therefore, the blood pressure definitions I used for categorizing the "downward" group in the Japanese cohort were: 1. the entry systolic pressure equal to, or less than, 140 mm Hg, and 2. the decline of the systolic pressure during the observation period equal to, or greater than 10 mm Hg. The definitions for the "upward" group were 1. the entry systolic pressure equal to, or greater than, 100 mm Hg, and 2. the rise of the systolic pressure during the observation period equal to, or greater than, 10 mm Hg. Similar definitions were applied in classifying the groups in the Yugoslavian cohort, except that a $\pm$5.0 mm Hg in the 5-year systolic pressure change was used. Figure 7.1 is a sketch of the blood pressure definitions for the two groups. These limits of the systolic pressures were chosen to reduce the effect of the "regression towards the mean". Thus the downward group excluded the higher systolic blood pressure values that are more likely to show a downward trend, and the upward group excluded the lower systolic blood pressure values with their greater tendency to increase.

"Downward Group"		"Upward Group"	
Yugoslavia	Entry SBP ≤ 140 mm Hg	Yugoslavia	Entry SBP ≥ 100 mm Hg
Japan	Entry SBP ≤ 140 mm Hg	Japan	Entry SBP ≥ 100 mm Hg
Yugoslavia	Δ SBP ≤ -5.0 mm Hg	Yugoslavia	Δ SBP ≥ +5.0 mm Hg
Japan	Δ SBP ≤ -10.0 mm Hg	Japan	Δ SBP ≥ +10.0 mm Hg

Figure 7.1 A sketch of the definitions for categorizing the subjects into the "downward" and "upward" group in each cohort. Entry SBP = the systolic pressure value on entry to the study. ΔSBP = change in systolic pressure, and is calculated as the 10-year minus entry systolic pressure for the Japanese male cohort, and the 5-year minus entry systolic pressure for the Yugoslavian male cohort.

A window of 5 and 10 mm Hg increase and decrease in the systolic pressure was used as a criterion for classifying the groups. I selected this window size for defining the groups to ascertain that the values of systolic pressure change for the downward and upward groups, fall within one half of the standard deviation away from the mean of the systolic pressure change distribution.

To gain an insight into the status of the systemic arterial tree and the heart during aging of blood pressure, I compared the "downward" and the "upward" groups. I also examined how blood pressure aging interacts simultaneously with the electrocardiographic estimate of the left ventricular mass index, an end point of age effect on the heart, as well as with the pulse transit time and timing of wave reflections, both of which are proxies of arterial stiffness, in the "downward" and "upward" groups. Table 7.1 presents this comparison.

Table 7.1 A comparison of the Mean±SEM values of the proxies for arterial stiffness and for effect of age on the heart between the "downward" and the "upward" groups in each male cohort.

	ΔSBP (mm Hg)	Pulse transit time (msec)	Timing of wave reflection (msec)	LVMI$_{ECG}$ (gm/m^2)	ΔHR (b/min)
Japanese cohort					
10-year ΔSBP					
Downward	-16.54±0.98 (n=29)	51.08±3.95 (n=18)	110.32±5.88 (n=19)	153.02±7.74 (n=12)	-5.0±2.89 (n=12) Pr>|T|=0.1106
Upward	32.32±1.38 (n=196)	50.81±1.82 (n=50)	99.18±3.47 (n=50)	187.49±5.30 (n=51)	0.29±1.62 (n=48) Pr>|T|=0.8574
t value	-28.88	0.24	1.59	-2.98	-1.49
p value	0.0001	0.8432	0.1158	0.0042	0.1404
Yugoslavian cohort					
5-year ΔSBP					
Downward	-11.16±0.93 (n=50)	69.65±2.48 (n=50)	109.46±4.96 (n=48)	152.03±7.39 (n=10)	1.73±4.22 (n=11) Pr>|T|=0.69
Upward	15.66±0.69 (N=223)	60.25±1.65 (n=61)	102.26±4.64 (n=61)	154.12±4.03 (n= 62)	4.89±1.48 (n=62) Pr>|T|=0.001
t value	-23.09	2.97	0.32	-0.20	-0.80
p value	0.0001	0.0037	0.7469	0.843	0.4245

10-year ΔSBP = the 10-year change of systolic pressure, and is calculated as the difference between the 10-year follow-up systolic pressure and the entry systolic pressure; 5-year ΔSBP = the 5-year change of systolic pressure, and is calculated as the difference between the 5-year follow-up systolic pressure and the entry systolic pressure; ΔHR = the change of the resting heart rate, and is calculated as the difference between the 10-year follow-up resting heart rate and the entry resting heart rate in the Japanese cohort, and the difference between the 5-year follow-up resting heart rate and the entry resting heart rate in the Yugoslavian cohort.

Values are given as Mean±SEM.

Student's t test compares the means of the "downward" and "upward" groups.

In the Japanese cohort, the "upward" group's systolic blood pressure increased at a rate of 3.2 mm Hg/year, while the "downward" group's systolic pressure decreased at a rate of 1.7 mm Hg/year. Compared to the "downward" group, the pulse transit time and the reflected wave travel time of the "upward" group were shorter, but this difference did not reach statistical significance. The shortened pulse transit time and the reflected wave travel time suggest increased arterial stiffness, and is believed to be the consequence of arterial wall degeneration. However, findings of this study suggest that arterial stiffness in this population is most likely caused by the increased systolic and diastolic blood pressure with age, and the data presented in Chapters 3 and 5 provide some evidence supporting this speculation. In the Japanese cohort, there was a small age trend in the pulse transit time, but a large age trend in the systolic pressure. Furthermore, my analysis of the determinants of pulse transit time showed that both the systolic and diastolic pressure but not age, were significant predictors of the pulse transit time.

The effects of elevated blood pressure on the structure of the arterial walls are well documented. The elevated blood pressure causes arterial stiffness, because stress is transferred from the more extensible elastin to the less extensible collagenous fibers. The increased stress causes further structural degeneration, further dilatation, and further stiffening of the arterial walls, with greater stresses resulting. Arterial stiffness shortens the pulse transit time, thus allowing the reflected waves to return to the heart much earlier and move into the systolic part of the wave. This results in the merging of the forward and reflected waves in the systole, augmenting the systolic peak pressure.

In the Yugoslavian cohort, similar observation can be made regarding the 5-year systolic pressure change. The "upward" group's systolic blood pressure increased at a rate of 3 mm Hg/year, and the "downward" group decreased at a rate of 2.2 mm Hg/year. The pulse transit time of the "upward" group was significantly shorter than that of the "downward" group. Although statistically nonsignificant, the "upward" group demonstrated shorter reflected wave travel time than the "downward" group. Unlike the

Japanese cohort, shortened pulse transit time and wave reflection time in the Yugoslavian cohort are most likely caused by arterial stiffness secondary to the age-related degenerative changes in the structure of the arterial walls. Findings presented in Chapter 5 showed a large age trend in the pulse transit time in this cohort. Although the systolic pressure also contributed significantly to the transit time, its contribution was relatively small, compared to that of age, as reflected in the R^2 value. This speculation is supported by a small age trend in blood pressure in the Yugoslavian cohort, suggesting that pressure-induced degeneration in the arterial walls is not likely to be the stimulus for increased arterial stiffness.

With advancing age, the blood vessel dilates, and its wall thickens. Mural changes involve hyperplasia of the intima, a loss of the orderly arrangement of elastic fibers and laminae in the media. Degeneration of the elastic fibers is associated with an increase in the collagenous material and in ground substance, and often with deposition of calcium. The outcome of these alterations is progressive stiffening of the arterial walls, with the consequent shortening of the pulse transit time and early return of the reflected waves from the peripheral sites.

7.2.2 The cardiac responses to blood pressure aging

Alterations in the arterial system have a profound influence on the ventricular function [224,226]. This is because the ventricle and the vascular system into which the blood is ejected, can be viewed as a pump working against a load. The amount of energy and thus the oxygen consumed by the left ventricle to produce the cardiac output depends not only on the contractile properties of the myocardium, but also on the physical properties of the blood and arterial system [256]. In the Japanese cohort, the electrocardiographic estimate of the left ventricular mass index of the "upward" group was significantly higher than that of the "downward" group. In the Yugoslavian cohort, the "upward" group also

had a higher cardiac mass index than the "downward" group, but this difference was not statistically significant.

Different mechanisms responsible for the augmented cardiac mass in these two populations can be postulated. In the Japanese cohort, the increased cardiac mass of the "upward" group is not likely due to age per se, but is mediated by the age-related increase in systolic and pulse pressure. This speculation is supported by my finding of a large age trend in systolic pressure, a small age trend in diastolic pressure, and a large age trend in the pulse pressure. The effects of elevated blood pressure on the pulse transit time and timing of wave reflections have been addressed in previous discussions. Under normal conditions, the appropriately timed wave reflection maintains the pressure during systole. But when the arterial walls stiffen, the reflected waves return to the heart much earlier, increasing the systolic pressure even further [120]. The initial stimulus of pressure overload induces an immediate biochemical response of the myocardium to increase protein synthesis and thereby initiate the adaptive structural process of ventricular hypertrophy [256].

In the Yugoslavian cohort, no statistically significant difference in the left ventricular mass index was found between the "upward" and "downward" groups, although this index was higher in the former than in the latter. As reported in Chapter 6, the body mass index was the only significant predictor of the cardiac mass in this cohort. This finding strongly implies the participation of the non-hemodynamic factors in the development and/or maintenance of elevated left ventricular mass index in this population. Obesity and the sympathetic nervous system activity appear to play a role in the development of left ventricular hypertrophy.

In the Japanese cohort, the resting heart rate was increased in the "upward" group but declined in the "downward" group during the 10-year observation period; neither of these changes was statistically, significantly different from zero. The Student's t test that compared the means of heart rate change of these groups, also failed to detect a statistically

significant difference. A different scenario emerged in the Yugoslavian cohort. The 5-year resting heart rate of both the "upward" and "downward" groups rose, but such an increase was statistically significantly different from zero in the "upward" group only. This rise in the resting heart rate with age in the "upward" group in both cohorts can be taken as indirect evidence of an increased cardiac autonomic drive. Sympathetic stimulation increases the force with which the heart muscle contracts, therefore increasing the volume of blood pumped as well as increasing the ejection pressure. This increase augments the blood pressure of the "upward" group even further. The effects of elevated blood pressure on the structure of the arterial walls and the heart have been discussed previously.

The data shown in Table 7.1 suggest that the "downward" group in both cohorts presumably did not "age". However one can argue that increased systolic blood pressure does not qualify as an aging phenomenon, since a fraction of the subjects in the Japanese and Yugoslavian cohorts showed either no change or a decline in their systolic blood pressure over the 10-year period. But compared to the "upward" group, the "downward" group demonstrated a lower value in the pulse transit time, timing of wave reflections, and the left ventricular mass index. It is possible that this difference reflects the effects of blood pressure aging, rather than those of age on the status of the large arteries and the heart .

7.3 Summary

The cardiovascular system undergoes a variety of structural, biochemical, and functional changes with age, though the actual aging process has not been clarified. However, an examination of the simultaneous interactions among the various facets of age effects on the vascular system and the heart permits the development of two views of cardiovascular aging. The first holds that age-related structural deterioration of the arterial walls is the primary cause responsible for a progressive increase in systolic blood pressure, a widening of the pulse pressure, and shortening of pulse transit time and reflected wave travel time. The second maintains that elevated blood pressure secondary to an augmented

cardiac sympathetic drive or to alterations in arterial function occurring with advancing age, is the stimulus initiating the structural degenerative changes in the major arteries.

The interrelation of the muscle tone, size, and the mechanical properties of the arteries, dictates the functional state of the vascular system and its interaction with the heart. An understanding of the cardiovascular aging process requires a comprehensive approach to the study of this process, in order to elucidate the mechanisms that result in the observable functional decline and diseases of this system characteristic of the aging population.

Chapter 8

Summary and Conclusions

8.1 Introduction

Alterations in arterial structure and function occur with advancing age in healthy individuals. These changes include thickening of the arterial walls secondary to hyperplasia of the intima, addition of medial lamellae, and loss of the orderly arrangement of elastin in the media. In addition, there is progressive fibrosis in the media, with partial replacement of elastin with the less compliant forms of collagen. Changes in the load-bearing media contribute to increased arterial wall stiffness, with important functional implications. Hypertension and cardiac hypertrophy are two common diseases of the elderly.

The majority of published studies of age effects on arterial vasculature has been conducted principally on Caucasian populations. Thus far, there have been only two population surveys [65,71] that investigated the relationship between age and arterial wall stiffness in the Oriental populations. Similarly the influence of age on the cardiac mass has been studied mainly on white men and women, although there are several investigations of black-white differences in electrocardiographic left ventricular hypertrophy [255,257,258]. Results of these few studies on the "non-Caucasian" populations indicate different vascular and cardiac responses to aging as compared to those of the Caucasian populations. But the mechanisms responsible for these differential responses have not been adequately explained.

8.2 Major contributions of the thesis

Although the material for the thesis was obtained from the Seven Countries Study of coronary heart disease, its objectives were completely different from those of the Seven Countries Study. The major aim of the thesis was to examine biological aging as it relates to the systemic arterial tree and the heart. It investigated how blood pressure changed

during the 10-year observation period, and the effects of age on the major arteries and the heart in the Yugoslavian and Japanese cohorts.

Published cross-sectional and prospective studies have shown that arterial blood pressure increases with age in developed countries. The age effects on blood pressure are determined by either examining differences in blood pressure among various age groups, or by studying how blood pressure values change with the passage of time. The influences of other factors besides age on blood pressure are not considered in these studies. The present investigation used a different approach to the study of blood pressure aging; I separated out that part of blood pressure change that is due to aging per se. Using the blood pressure values corrected for cohort-period effect, I demonstrated blood pressure age trends that were distinctly different from the age trends analyzed with blood pressure values without correction of the this effect. To the best of my knowledge, the separation of age trends and those due to the cohort-period effect in blood pressure has never been done before. Furthermore, my analysis challenged the observation reported in the literature that the higher the initial blood pressure, the greater the increment. Compared to the Yugoslavian men, the Japanese men showed a steeper rise in blood pressure with age in spite of their lower entry blood pressure levels. Findings of the type described in this thesis have not been reported previously.

The thesis provided evidence of ethnic differences in the age effects on the arterial vascular status. In the Yugoslavian cohort, the pulse transit time shortened as a function of age. In contrast, age had a minimal effect on the pulse transit time in the Japanese cohort; instead, the heart rate was the most important determinant of the pulse transit time in this cohort. These findings suggest that different mechanisms are responsible for arterial stiffness in these ethnically different populations. There has been no report of differential age effects on the arterial walls in various racial groups.

The present study demonstrated significant differences between the left and right carotid pulses in the travel time and the amplitude of reflected waves. This finding is novel

and has not been reported in the literature. In the past, studies of pulse wave velocity and pressure wave contour have been primarily unilateral; measurements are made on either the left or right carotid artery, assuming that no differences exist between these two sides. But data in my study invalidated this assumption.

The present investigation was the first to compare the effects of age on the heart, using the electrocardiographic estimate of the left ventricular mass index ($LVMI_{ECG}$) as an end point, between the Oriental and Caucasian populations. Results confirmed the published reports that there are racial differences in the left ventricular hypertrophy. In addition, this study provided clarification to the conflicting views about the contributory role of blood pressure in the development and/or maintenance of left ventricular hypertrophy. Since the systolic pressure load represents an aggregate of workload imposed on the myocardium, it appears to be a more consistent predictor of the $LVMI_{ECG}$ than the discrete measurements of systolic or diastolic blood pressure.

8.3 Summary of the major findings

The present study provided evidence indicating that biological aging and trends due to the cohort-period effect, influenced the arterial blood pressure in both cohorts. My method of separating these two trends is supported by their association with trends in population and cohort stroke mortality. The Yugoslavian cohort demonstrated a slight upward trend ascribable to the cohort-period effect, and a modest age increase in systolic (0.81 mm Hg/year), diastolic (0.17 mm Hg/year), and pulse pressure (0.66 mm Hg/year). In contrast, the Japanese cohort showed a downward trend due to the cohort-period effect, but a steep upward age trend in systolic (1.67 mm Hg/year), diastolic (0.56 mm Hg/year), and pulse pressure (0.97 mm Hg/year). The entry blood pressure values (after correction for the cohort-period effect) of the Yugoslavian men were significantly higher than those of the Japanese men (129.84±1.11/79.90±0.81 mm Hg versus 109.92±1.79/63.47±1.28 mm Hg). Compared to the Japanese men, a higher percentage of the Yugoslavian men's blood

pressure was reduced during the 10-year follow-up (systolic: 35% in the Yugoslavian cohort versus 23% in the Japanese cohort; diastolic: 50% in the Yugoslavian cohort versus 33% in the Japanese cohort). The biological factors contributing to blood pressure elevation with age are related to the aging changes of the arterial vasculature. The cohort-period effect that may be responsible for the rise in blood pressure include cardiac output, peripheral resistance, sympathetic nervous system activity, alcohol consumption, cigarette smoking, and some nutritional deficiency or abundance.

Cohort differences were found in the pulse transit time; the pulse transit time of Japanese cohort was significantly shorter than that of the Yugoslavian cohort (54.5±1.2 versus 62.6±1.3 msec.). Age had significantly different effects on the major arteries of these cohorts. In the Yugoslavian cohort, the pulse transit time, a proxy for arterial stiffness, was significantly and inversely correlated with age, even after controlling for blood pressure, heart rate, serum cholesterol, body mass index, height, and cigarette smoking habits. Age had the most important effect on the pulse transit time ($r = 0.38$, $p = 0.0001$). In the Japanese cohort, the pulse transit time was also inversely correlated with age, but this relationship did not reach statistical significance. Heart rate ($r = 0.42$, $p = 0.0001$), systolic ($r = 0.38$, $p = 0.0001$) and diastolic pressure ($r = 0.38$, $p = 0.0001$) were significant predictors of the pulse transit time in this cohort.

Analyses of the pooled sample indicated that timing of wave reflections was inversely and significantly correlated with age, controlling for the pulse transit time, heart rate, and height ($r = 0.23$, $p < 0.01$). Age explained 5% of the variance in the timing of wave reflections. But it had no statistically significant effects on either the amplitude of the reflected waves or the interval from systolic upstroke to peak of systolic pressure, both of which were parameters defining the shape of the carotid pressure waveform. Instead, heart rate was a significant determinant of these parameters.

Comparatively, the electrocardiographic estimate of the left ventricular mass index ($LVMI_{ECG}$) of the Japanese cohort was significantly larger than that of the Yugoslavian

cohort, even after correction for the 5-year difference in the age distribution between these cohorts (174.8±3.34 gm/m^2 versus 160.6±3.4 gm/m^2, p = 0.01). Once again, an ethnic difference was noted in the $LVMI_{ECG}$-age relationship. Age was marginally significantly correlated with $LVMI_{ECG}$ in the Japanese cohort, controlling for diastolic blood pressure, pulse pressure, pulse transit time, and body mass index (p = 0.038). The pulse pressure was a significant predictor of the cardiac mass, while wave reflections expressed as the ratio of ΔP/PP contributed marginally significantly to the $LVMI_{ECG}$ in the Japanese cohort. In the Yugoslavian cohort, neither age nor pulse pressure was significant determinant of the cardiac mass, but the body mass index and the systolic pressure load were significant predictors of the left ventricular mass index in this cohort. The differential effect of age on the $LVMI_{ECG}$ in the two cohorts can be due to the bias produced by using the ECG model that was developed exclusively for the Caucasian men.

A different configuration of interactions between blood pressure age trends and the proxies for age effects on the large arteries and the heart, was observed in two groups of people from each cohort who showed a distinctly different pattern of systolic pressure change with age. Individuals whose systolic blood pressure increased with age demonstrated shorter pulse transit time, earlier return of reflected waves, a larger left ventricular mass index, and a greater change in the resting heart rate with age than those of individuals whose systolic blood pressure declined with age.

8.4 Limitations of the study

The Seven Countries Study data were obtained during the time period when antihypertensive treatment was not widely used. The analysis of these data permits me to observe the effects of age on blood pressure without the confounding influence of the treatment. However, my study has three major limitations. The first one relates to the relatively short observation period for studying the age effect on the cardiovascular system in both cohorts. I analyzed the 10-year blood pressure changes in the Japanese and

Yugoslavian cohorts. The age range was 50 to 70 years for the Japanese and 45 to 65 years for the Yugoslavians. Studies have shown that aging changes in vasculature do not simply develop in the elderly, but begin in childhood. These changes are progressive throughout life, and are well developed by early adulthood. The process of vascular and cardiac aging cannot be understood fully without tracking the subjects from childhood to old age, to examine how blood pressure, pulse transit time, pressure wave contour, and the cardiac mass change with age.

The second limitation relates to the measurement issues. The pulse wave signals from both cohorts were recorded at least 25 years ago, with a displacement transducer and apex cardiograph technique. This method of signal acquisition is subject to a number of limitations. The pressure wave signals, particularly those of the Yugoslavian men, contained a high level of noises. I suspected that this was the primary reason for the failure of my computer algorithm to identify accurately the different points on some of the pressure waves. The acquisition of pressure wave signals with a more sensitive and accurate noninvasive method, will improve the performance of my algorithm.

The third limitation of the present work is that the study population was composed exclusively of males. Therefore the results cannot be generalized to the female population because of gender-related differences in anthropometric and biological measurements, and perhaps the cardiovascular responses to aging. Similar studies including women should be conducted to explore the effects of age on the arterial system and the heart.

8.5 Future directions for the study of cardiovascular aging

It is clear that further investigation in many areas of this field is desirable. Given the results and limitations of my study, I may suggest on the future works to be carried out in the field of cardiovascular aging.

The available literature regarding human aging and sympathetic nervous system activity suggests a hyperadrenergic state, and a reduced vascular responsiveness to β-

adrenergic stimulation in old age. In the past, it was believed that structural changes in the arterial walls were the exclusive explanation for increased aortic rigidity in the elderly. More recently, age-related differences in the sympathetic nervous system activity have been proposed as another mechanism responsible for reduced aortic distensibility in the elderly, in particular those with sustained systolic hypertension [259,260,261]. In a pilot study investigating the relationship of plasma norepinephrine levels to aortic distensibility in elderly with isolated systolic hypertension, Handa et al. [262] observed that the aortic diameters were significantly increased while the diastolic blood pressure and heart rate were decreased, in response to combined α- and β-receptor blockade (labetalol). A trend towards a positive correlation between aortic distensibility and the plasma norepinephrine levels was observed, and the correlation almost reached statistical significance. Increased aortic diastolic diameters with decreased diastolic blood pressure implied that the aortic distensibility may be increased. The suggestion of increased aortic distensibility was confirmed by the observation that the pulsatile diameter (aortic diameter at systole minus aortic diameter at diastole) was significantly increased, but the pulse pressure did not change significantly after 2-week oral labetalol. Results of this pilot study provided evidence that sympathetic nervous system activity affects, at least in part, the distensibility of the aorta even in the elderly hypertensives. Furthermore, this pilot study also showed that plasma norepinephrine is not a useful marker for aortic distensibility in that elderly population (see Appendix B). Much research remains to be done to determine the influence of age-related alterations in the function of the sympathetic nervous system on the elastic properties of the aorta.

In the present investigation, I used the pulse wave velocity as an index of arterial stiffness. Although this method of estimating arterial stiffness is viewed by some as simple and easy, it does not describe the whole arterial system, is not based directly on the arterial pressure-volume relationships, and does not account for reflections or the viscoelastic properties of the vessel walls. The recent development of a high-resolution echo-Doppler

technique permits the noninvasive clinical determination of pulsatile pressure and diameter changes *in vivo*. This allows for the direct measurement of the various indices of arterial stiffness, thereby permitting *in vivo* recognition of the great heterogeneity of the arterial tree.

In a steady-flow system, the peripheral flow resistance represents the total load placed on the ventricle by the system. However, in a pulsatile-flow system such as the cardiovascular, the total hydraulic load placed on the myocardium during ejection must include the viscoelastic properties of the aorta and other major arteries, and wave reflections within the system, in addition to the total peripheral resistance. Since few data were available, of necessity, the hydraulic load in my study was represented by the pulse wave velocity, one of the several components of the load "seen" by the ventricle during its ejection. Future prospective investigations assessing the influence of aortic input impedance, the best available index of the hydraulic load, on the performance of the left ventricle, will provide a better understanding of the effect of aging on the heart.

I estimated the left ventricular mass index from an established multivariate model, using electrocardiographic measurements. The accuracy of this estimation of the cardiac mass can be improved with the echocardiographic measurement of the cardiac chamber dimensions.

Findings of the present investigation show a difference in the effects of the age on the status of the large arteries and the heart, associated with blood pressure aging in the two ethnically different cohorts. Although the effects of genetic and environmental factors on the function and structure of the arterial walls have not been clearly established, there is some preliminary, indirect evidence indicating aortic morphological differences between occidental and oriental populations [159]. Further studies of the structural and functional parameters of the aorta and large arteries in various racial and ethnic groups, would shed light on the role of genetic factors in influencing the aging changes in the structure and function of the systemic arterial tree.

8.6 Concluding remarks

Knowledge about the aging process of the systemic arterial vasculature, provides a basis for a better understanding of the pathophysiology of systolic hypertension in the elderly population. This information has important clinical implications regarding the choice of appropriate antihypertensive therapy, and provides a rational framework for evaluating the effectiveness of therapy in elderly patients.

Appendix A

Smoking Questionnaire*

1. (a) Do you smoke cigarettes now?
   Yes, regularly _____
   No _____
   If "NO", go to question 2(a).
   Occasionally (usually less than one cigarette per day) _____

   (b) Do you inhale?
   Yes _____
   NO _____

   (c) What kind of cigarettes do you smoke?
   Manufactured, with filters _____
   Manufactured, without filters _____
   Hand-rolled _____

   (d) How many manufactured cigarettes do you usually smoke per day? _____

   (e) About how many ounces (or grams) of tobacco do you use per week for rolling your own cigarettes _____

   (f) What is the maximum of cigarettes that you have smoked per day for as long as a year?
   Record total number of manufactured and hand-rolled cigarettes, counting 1 oz of tobacco as 25 cigarettes and 1 g as 1 cigarette _____

   (g) How many cigarettes did you smoke per day a year ago? _____

   (h) How old were you when you began to smoke cigarettes? _____
   After asking this question, go to question 3 (a).

2. (a) Did you ever smoke cigarettes?
   Yes, regularly _____
   No, never _____
   Occasionally (usually less than one cigarette per day) _____

   (b) What is the maximum of cigarettes that you have smoked per day for as long as a year?
   Record total number of manufactured and hand-rolled cigarettes, counting 1 oz of tobacco as 25 cigarettes and 1 g as 1 cigarette _____

   (c) Do you inhale?
   Yes _____
   NO _____

   (d) How old were you when you began to smoke cigarettes? _____

(e) When did you stop smoking cigarettes? Give year. _____

(f) Why did you stop? _____

3. (a) Have you ever smoked cigars?
NO _____
If "NO", go to question 4 (a)
Used to, but not now _____
If "Not now", go to question 4 (a).
Now smoke occasionally (less than one per day) _____
Now smoke regularly _____

(b) About how many do you smoke per week? _____

(c) Do you inhale?
Yes _____
No _____

4. (a) Have you ever smoked a pipe?
No _____
Used to, but not now _____
Now smoke a pipe occasionally (less than once a day) _____

(b) About how many ounces (or grams) of tobacco do you smoke per week? _____

(c) Do you inhale?
Yes _____
No _____

* From Rose G.A. and H. Blackburn [14].

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The Relationship of Plasma Norepinephrine Levels and Aortic Distensibility in Elderly With Isolated Systolic Hypertension

S. Paul Handa, Julia Wong, and Hermann K. Wolf

The present study evaluated the relationship of plasma norepinephrine (NE) and aortic distensibility (AOD) in a group of elderly patients with isolated systolic hypertension. Aortic distensibility was calculated as AOD = 2 × (Δ aortic diameter)/ diastolic aortic diameter) × (Δ aortic pressure). Results indicate that the aortic diameters were significantly increased while the diastolic blood pressure and heart rate were decreased, in response to combined α- and β-receptor blockade (labetalol). However, we found that AOD was not significantly related to plasma NE. We therefore conclude that AOD can be increased in elderly hypertensives by α-β-blockade, as has been found in younger patients, but plasma NE is not a useful marker for AOD in this population. Am J Hypertens 1995;8: 422–425

KEY WORDS: **Plasma norepinephrine, aortic distensibility, labetalol.**

Overactivity of the sympathetic nervous system has been implicated in the pathogenesis of essential hypertension because of its role in blood pressure regulation.[1,2] Plasma catecholamines provide a useful index of the sympathetic neurogenic activity. A number of investigations have reported increased levels of norepinephrine (NE) in response to emotional stress,[3] physical stress,[4] and with age.[5] Aortic compliance is considered to be one of the most important mechanisms which can induce and sustain systolic hypertension. Factors affecting aortic compliance and therefore systolic pressure have been identified. Alicandri et al.[6] have shown that aortic rigidity, the reciprocal of aortic compliance, correlated significantly with plasma NE in older patients (age range 46 to 66 years) with essential hypertension. Aortic rigidity was reduced significantly after the administration of α-adrenoreceptor blockade alone and combined α- and β-adrenoreceptor blockade. The aim of the present study was to 1) estimate the impact of temporary α-β-adrenoreceptor blockade on the heart and large arteries of a group of elderly patients with isolated systolic hypertension, and 2) evaluate the relationship of plasma NE levels and aortic distensibility in these patients.

MATERIALS AND METHODS

Twenty-seven patients (12 males and 15 females; age range 60 to 81 years) with isolated systolic hypertension were included in the study. The blood pressure criteria used for defining isolated systolic hypertension were clinic cuff systolic blood pressure (SBP) ≥ 160 mm Hg, and cuff diastolic blood pressure (DBP) ≤ 95 mm Hg. Exclusion criteria were insulin-dependent diabetes mellitus, manifest abnormality of mental function, Canadian Cardiovascular Society (CCS) grade 3 or 4 angina pectoris, currently taking

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From the Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada.

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Address correspondence and reprint requests to Julia Wong, Department of Physiology and Biophysics, Dalhousie University, 3A Tupper Building, College Street, Halifax, Nova Scotia, Canada B3H 4H7.

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antihypertensive agents or diuretics, metastatic malignancies, history of physical disability, secondary hypertension, and serum creatinine > 200 μmol. Supine cuff blood pressure (BP) was recorded by a nurse in the clinic, using a mercury sphygmomanometer following 5-min resting. Korotkoff sounds phase 1 and 5 were taken as the systolic and diastolic pressure, respectively. The mean of three readings taken this way at a 10-min interval was considered as the casual cuff BP.

Immediately after cuff BP determination, all patients underwent 24-h BP monitoring using a noninvasive automatic recorder (Spacelabs Model 90207 Ambulatory Blood Pressure Monitor, Redmond, WA). The monitor used the oscillometric method to measure BP and was preset at 20-min intervals during daytime (0601 to 1800 h) and at 30-min intervals during nighttime (1801 to 0600 h). Following the completion of 24-h BP monitoring, venous plasma samples were taken after 30-min recumbence for basal NE level estimation. Patients were instructed to refrain from cigarette smoking, alcohol, tea or coffee consumption on the morning when plasma NE levels were determined. Blood samples were drawn acutely by venipuncture 60 sec after the needle was inserted into the vein, to allow for clearance of venous blood stasis resulting from the application of tourniquet. No difficulty was encountered in drawing blood in any of the subjects. This was followed by a 2-D and M-mode echocardiogram (echo) and 12-lead electrocardiogram (ECG). Patients were placed on an oral α- and β-adrenoreceptor blockade (labetalol) for a period of 2 weeks, to allow for the maximum vasodilatory effect to take place. The echocardiogram, electrocardiogram, and supine cuff BP were repeated in the third week. Of 27 patients, six were not placed on 2-week oral labetalol for medical reasons; only 16 patients had post-labetalol supine cuff BP measurements.

The aortic diameter was recorded by M-mode echocardiogram 3 cm above the aortic valve. Measurement of aortic diameters was obtained according to the methods recommended by the American Society of Echocardiography. Aortic diastolic diameter was measured at the onset of QRS complex, from the leading edge of anterior wall echo to the leading edge of posterior wall echo. Aortic systolic diameter was measured as the maximum dimension from the leading edge of anterior wall echo to the leading edge of posterior wall echo. Distensibility of the aorta was calculated using the formula described by Stefandis et al.[7]

$$\text{AOD} = 2 \times (\Delta \text{ aortic diameter})/(\text{diastolic aortic diameter}) \times (\Delta \text{ aortic pressure}),$$

where Δ aortic diameter = systolic − diastolic aortic diameter, and Δ aortic pressure = cuff SBP − cuff DBP (brachial artery pressure by sphygmomanometer). Calculation of the aortic distensibility was based on the assumption that the resting stroke volume at the time of casual cuff BP measurements remained unchanged when echocardiogram measurements were made.

Patients were classified as dippers and nondippers according to their BP circadian variation. Circadian variation was defined by O'Brien et al[8] as a difference in the mean daytime and mean nighttime SBP ≥ 10 mm Hg, or a difference in the mean daytime and mean nighttime DBP ≥ 5 mm Hg.

Data of aortic distensibility and plasma NE were logarithmically transformed to approximate normal distribution. Cook's D influence statistic[9] was employed to isolate peculiarities in the data. Observations which exerted an unusually strong influence were removed from the linear regression model which evaluated the relationship between aortic distensibility and plasma NE levels. $P < .05$ was considered significant.

RESULTS

Univariate analysis shows no significant difference between male and female patients in age, plasma NE, aortic distensibility, cuff BP, and mean daytime BP. The mean plasma NE of our isolated systolic hypertensive elderly was comparatively higher (3.39 ± 0.43 nmol/L) than that reported by Lake et al.[10] (2.31 ± 1.9 nmol/L), Sever et al.[11] (2.47 ± 1.8 nmol/L), and Gavras et al.[12] (1.6 ± 0.29 nmol/L) on hypertensive patients younger than 60 years of age.

In our study 22 patients (81%) could be classified as dippers and five patients (19%) as nondippers. These two groups were not significantly different in age, height, plasma NE, aortic distensibility, cuff SBP, and mean daytime BP.

The impact of α-β-adrenoreceptor blockade on the heart and large arteries was evaluated by examining the changes observed in the aortic diameters, cuff BP, and heart rate. DBP and heart rate decreased significantly while the aortic diastolic diameter and the pulsatile diameter, ie, the difference between aortic diameter at systole and diastole, increased significantly. Table 1 summarizes these findings.

A statistically nonsignificant correlation was found between aortic distensibility and plasma NE ($r = 0.35$, $P = .1295$). Age did not contribute significantly to this correlation. Figure 1 shows this relationship.

DISCUSSION

The decrease in DBP in response to labetalol is due to vasodilatation, suggesting that the peripheral resistance may be reduced. Increased aortic diastolic diameter coupled with decreased DBP, implies that the aortic distensibility was augmented. The finding that

TABLE 1. CHANGES INDUCED BY 2-WEEK ORAL LABETALOL (N = 16)

	DBP (mm Hg)	ADd (cm)	PP (mm Hg)	ADs-d (cm)	HR (b/min)
Prelabetalol					
Mean	90.67	2.58	84.21	0.33	68.92
SE	1.59	0.09	2.96	0.04	4.20
Postlabetalol					
Mean	84.96	2.82	83.44	0.46	60.52
SE	2.11	0.08	4.85	0.06	2.40
t values	3.25	−3.21	0.19	−2.43	2.82
P values	<.01	<.01	NS	<.05	<.05

DBP, diastolic blood pressure; ADd, aortic diameter at diastole; PP, pulse pressure; ADs-d, pulsatile aortic diameter; HR, heart rate; NS, not significant.

the pulse pressure did not change, but the pulsatile aortic diameter was significantly increased after 2-week oral labetalol, provides further evidence of increased aortic distensibility. The significant reduction in the heart rate is possibly associated with a compensatory increase in stroke volume, to maintain cardiac output, which is reflected in the increased pulsatile aortic diameter. The ejection phase is related to heart rate. Since heart rate was decreased, the ejection velocity probably was also decreased, making it unlikely that the ejection velocity accounts for the increase in postlabetalol pulsatile aortic diameters.

We recognize that labetalol may not be the most appropriate agent for evaluating the effects of diminished adrenergic stimulation to the aortic vasculature, since its action on β-adrenergic receptors is four times stronger than on the α-receptors. A pure α-adrenergic antagonist such as phentolamine would more effectively block the effects of NE on the vasculature.

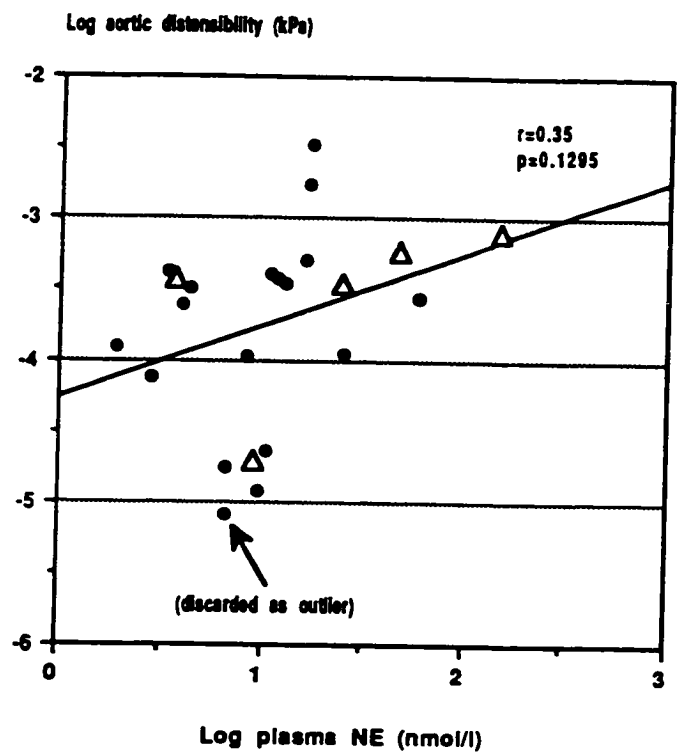

FIGURE 1. *The relationship between aortic distensibility and plasma NE levels in dippers (·) and non-dippers (Δ).*

Contrary to findings reported by Alicandri et al[6,13] and Agabiti-Rosei et al,[14] our study indicates a trend toward positive correlation between aortic distensibility and plasma NE levels, and the correlation almost reaches statistical significance. A possible explanation for this observation is that 1) the inverse relationship between these two variables reported in the literature was observed in hypertensive patients who were younger (<60 years) than our subjects (>60 years), and in older people, clearance of plasma NE may not be as effective as it is in younger people[15]; and 2) β-adrenergic receptor concentration has been found to decrease with age.[16] This may account for the elevated plasma NE levels in the elderly. Therefore, NE levels may not have the same relationship to sympathetic nervous system activity in the old as in the young.

Consistent with results of previous studies, our study also shows a group of elderly hypertensive patients whose 24-h BP differed from most hypertensives and normotensives in not having a nocturnal dip. It has been suggested that failure to present a fall in nocturnal BP may signify dysregulation of the autonomic nervous system.[17] Kobrin et al[18] reported a loss of diurnal variation in the elderly, who have a higher incidence of cardiovascular disease.

In conclusion, we have provided evidence suggesting that adrenergic neuro activity influences aortic distensibility of these elderly patients in a similar fashion as in younger people. The aortic distensibility in isolated systolic hypertensive elderly may not be as related to plasma NE as it is in young people. A major limitation of our study is the small sample size, which seriously limits the statistical power to detect significant relationship of aortic distensibility and plasma NE levels.

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Appendix C

Macfarlane, P.W., and P. Rautaharju. Electrocardiology '93. Proceedings of the XXth International Congress on Electrocardiology. Kananaskis, Alberta, 26 -30 July, 1993.

Influence of Arterial Blood Pressure on Cardiac Mass: The Yugoslavian and Japanese Cohorts of the Seven Countries Study

Julia Wong
Department of Physiology and Biophysics, Dalhousie University
Halifax, Nova Scotia, Canada B3H 4H7

A. Menotti
Ist. Sup. di Sanita, Rome, Italy

S. Nedeljkovic
Dept Medicine, University of Belgrade, Yugoslavia

H. Toshima
3rd Dept Medicine, Kurume University, Japan

Hermann K. Wolf
Department of Physiology and Biophysics, Dalhousie University
Halifax, Nova Scotia, Canada

ABSTRACT

The contributory role of arterial blood pressure to increased cardiac mass remains controversial. We analyzed the prospective blood pressure data in a Yugoslavian and Japanese cohort of the Seven Countries Study to determine the effects of blood pressure and the pressure load on electrocardiographic estimate of left ventricular mass index (ECG-LVMI). Results indicate that pressure load is a more consistent predictor of the ECG-LVMI than either the systolic or diastolic blood pressure.

Introduction

Left ventricular hypertrophy (LVH) represents a wide range of mild to severe increase of left ventricular mass (LVM), and its development is believed to be a response to the demands imposed on the heart through increasing hemodynamic workload.[1] Although LVH is often thought of as an adaptive process to augmented pressure or volume load,[2,3] it is difficult to define a value of LVM at which compensatory hypertrophy ends and pathologic hypertrophy begins.[4]

Studies on the relationships between arterial blood pressure and LVM in normotensives and hypertensives have produced inconclusive and inconsistent results. The contributory role of blood pressure to augmented cardiac mass remains controversial. In our study, we analyzed the prospective blood pressure data in a Yugoslavian and Japanese cohort of the Seven Countries Study,[5] to (1) describe the distribution of the ECG-LVMI of the normotensives and hypertensives in these two cohorts, (2) evaluate the effects of arterial blood pressure on ECG-LVMI, and (3) determine the relationships between pressure load and ECG-LVMI.

Method

The study population consisted of 511 Yugoslavian men and 508 Japanese men, aged 40-59 years at the time of entry into the study. The ECG data used for the analyses were those obtained after a 5- and 10-year observation period in Yugoslavian and Japanese cohort, respectively. The LVMI was estimated from an established multivariate model using a variety of ECG measurements.[6] The regression equation used for estimating LVMI, according to the Penn convention was:

$$\begin{aligned} LVMI(g/m^2) = &-36.4 + 0.10 \times R(V5) + 0.020 \times S(VI) + 0.028 * (III) \\ &+ 0.182 Tneg(V6) - 0.148 pos(aVR) + 1.049 \times QRSdur \end{aligned} \qquad \text{Eq.(1)}$$

Where * = S or Q or QRS amplitude, whichever is larger.

Systolic and diastolic pressure load was defined as the load on the myocardium that existed during the observation period, and was estimated by the integral of the time-pressure curve. Total pressure load (TPL) as defined by Menotti et al,[7] was the sum of the systolic and diastolic pressure load imposed on the myocardium during the observation period. Figure 1 is a schematic representation of the systolic pressure load (SPL) in the two cohorts.

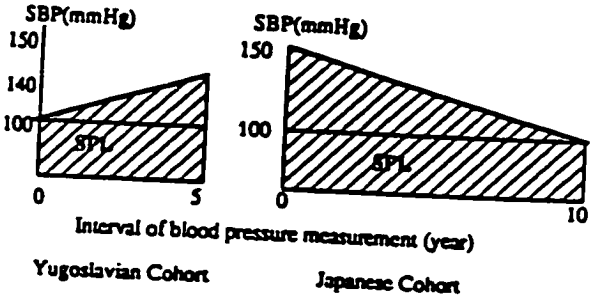

Figure 1 A schematic representation of SPL in two cohorts.

Results

The mean of the entry blood pressure was 131.4±18.4/81.9±10.2 mmHg for Yugoslavian men and 133.1±24.4/73.7±14.1 mmHg for Japanese men. Univariate analysis of the cross-sectional data indicates significantly higher ECG-LVMI values in older cohorts of Japan, but not of Yugoslavia. The ECG-LVMI of Yugoslavian men with systolic hypertension (SBP≥140 mmHg) was higher than that of the normotensive (SBP<140 mmHg, and DBP<90 mmHg) and diastolic hypertensive men (DBP≥90 mmHg), but this difference did not reach statistical significance. In contrast, in the Japanese cohort, ECG-LVMI of systolic hypertensive men was significantly higher than that of the normotensive and diastolic hypertensive men. Table 1 summarizes these findings.

Table 1 Comparison of mean ECG-LVMI (kg/m^2) by blood pressure levels in two cohorts

	Yugoslavian Cohort	Japanese Cohort
Normotension	153.9± 33.2	163.7± 30.2
Systolic hypertension	170.1± 35.5	202.2± 46.5
Diastolic hypertension	165.6± 56.2	195.1± 41.7

The effects of arterial blood pressure on ECG-LVMI were analyzed by linear regression analysis. In the Yugoslavian cohort, ECG-LVMI significantly correlated with the entry, but not the concurrent systolic blood pressure, adjusting for age and body mass index (BMI). On the other hand, ECG-LVMI significantly correlated with both the entry and the concurrent systolic blood pressure in the Japanese cohort. Similarly, a significant relationship was observed between ECG-LVMI and entry diastolic pressure in the Yugoslavian cohort, and with the concurrent diastolic blood pressure in the Japanese cohort, after statistically controlling for age and BMI. The systolic, diastolic and total pressure load were all significantly related to ECG-LVMI in both cohorts, adjusting for age and BMI. Figure 2 shows the predicted relationship of TPL to ECG-LVMI in both cohorts.

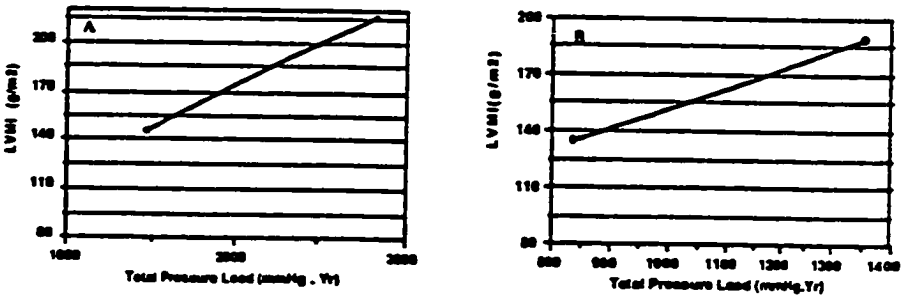

Figure 2 The predicted relationship between TPL and ECG-LVMI in (A) Japanese cohort and (B) Yugoslavian cohort.

Discussion

Our finding of a disparate relationship between age and cardiac mass in the two cohorts agrees with some studies,[8,9] but disagrees with others.[10,11] Several mechanisms have been proposed to elucidate the significant contribution of age to augmented cardiac mass; among them are age-related alterations in vascular properties and changes in the myocardial structures during aging. These structural changes present an increased afterload to the heart, requiring it to accelerate blood against the inertial force in the aorta when systole begins, and from this standpoint increases impedance. With advancing age, prolonged augmented impedance would be expected to result in an age-associated increase in wall thickness with unchanged cavity dimensions.

The significant relationship between systolic pressure and the ECG-LVMI in both cohorts indicates that increased systolic stress may play an important role in the

mechanism of augmented cardiac mass. The positive, significant correlation between diastolic blood pressure and ECG-LVMI suggests a contributory role of arterioles in the development of left ventricular hypertrophy.

The systolic, diastolic and total pressure load independently and significantly influenced the ECG-LVMI in the Yugoslavian as well as the Japanese cohort. As this pressure load represents an aggregate of workload on the myocardium, it responds to the prolonged, repeated increases in afterload by a hypertrophic adaptive process.

In conclusion, results of our study show that pressure load is a more consistent predictor of ECG-LVMI than either the systolic or diastolic blood pressure. The significant association between pressure load and LVMI suggests that cumulative workload on the myocardium may be the inciting stimulus of cardiac hypertrophy. The significant difference in the ECG-LVMI between normotensives and hypertensives of the Japanese cohort, but not of the Yugoslavian cohort can be due to the fact that the ECG model was developed primarily for Caucasian men, and may produce biased results in the Asian population. Improved models for LV mass estimation specifically developed for Asian men are needed so that results from the present study can be re-evaluated.

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IMAGE EVALUATION
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