CZECH TECHNICAL UNIVERSITY IN PRAGUE
Faculty of Mechanical Engineering
Department of Mechanics, Biomechanics and Mechatronics

HOW AGEING IS REFLECTED BY LONGITUDINAL PRETENSION IN ABDOMINAL AORTA

DOCTORAL THESIS
By

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2012 Prague

Type of publication	Ph.D. thesis
Title	How ageing is reflected by longitudinal pretension in abdominal aorta
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Address	Technická 4, 16607, Prague, Czech Republic
Study branch	Biomechanics
Number of pages	89
Number of figures	19
Number of tables	10
Number of supplements	1 CD-ROM

Annotation

Studies on the influence of ageing on the longitudinal mechanical response of elastic arteries are rare, though longitudinal behaviour may have a significant effect on pressure pulse wave transmission. Longitudinal prestrain in physiological conditions ensures almost constant length of the artery during the cardiac cycle. This may not, however, be true in aged arteries. Our study was designed to elucidate how ageing is reflected in the longitudinal prestress, prestretch and pretension force. The study involved ten human samples (six female, four male) of the abdominal aorta with longitudinal prestretch determined in autopsy (age 47/12; prestretch 1.13/0.1; mean/SD). Cylindrical samples underwent a longitudinal elongation test in order to estimate the force necessary to attain the in situ length and to determine the corresponding axial stress (prestress). The elastic modulus was estimated employing a limiting chain extensibility model to describe the stress–strain relationship. It was found that pretension force, longitudinal prestress and prestretch are negatively correlated with age. The axial prestress decreases with age within one order of magnitude (from tens of kPa to units of kPa). The decreased longitudinal force necessary to obtain the in situ length suggested that the decrease in the prestress occurs not only due to the age-related increase in the cross-section area. Since elastin is the main constituent responsible bearing the prestretch, this suggests that the observed decrease in the longitudinal prestress and prestretch reflects ageing-induced damage to the elastin. Finally, constitutive modelling showed that limiting chain extensibility is a concept that is suitable for describing the ageing effect.

Keywords: ageing; aorta; arteriosclerosis; constitutive modelling; elastin; prestress; prestretch.

Anotace

Ačkoliv mohou axiální mechanické vlastnosti elastických tepen významně ovlivnit přenos pulsní vlny, byla vědecká pozornost vždy více zaměřena na obvodovou roztažnost tepen. Podélné předpětí za fyziologických podmínek zajišťuje téměř konstantní délku aorty během srdečního cyklu. Ve stáří tyto podmínky ovšem nemusí být zachovány. Tato studie byla zaměřena na mapování podélného mechanického chování břišní aorty, zejména na její předpětí a sílu, která ho zajišťuje. Při elongačních testech cylindrických vzorků infrarenální aorty (10 dárců – 6 žen a 4 muži ve věku 47/12 let; průměr/SD), bylo zjištěno, že předpětí v dospělosti s věkem prudce klesá. Ačkoliv celková mechanická odezva byla charakterizována výrazným zvýšením podélné tuhosti, hodnoty modulu pružnosti odpovídajícího předepjatému stavu spíše klesaly. Vzhledem k tomu, že klesala nejenom hodnota předpětí (měřeno deformací i napětím), ale i síla potřebná k předepnutí, je vysvětlení pomocí remodelace geometrie tepny nedostatečné, neboť tato síla je vtištěna vazbami. Hypotézy vysvětlující tento jev zní: (1) předpětí s věkem klesá dík degradaci elastických membrán ve stěně tepny (což je jev známý, s tímto fenoménem ovšem dosud nespojovaný); (2) degradují vazby podélně upínající tepnu (což je fenomén zcela neprozkoumaný).

Klíčová slova: aorta, arterioskleróza, elastin, konstitutivní modelování, předpětí.

Declaration

Herewith I declare that the thesis comprises only my original work except where indicated and due acknowledgement has been made in the text to all other material used.

Lukáš Horný

in Prague 2nd of February 2012

Acknowledgments

I am inexpressibly thankful to Vanda for her prodigious love, patience and generosity.

I would like to give thanks to my mentor and supervisor, Prof. Ing. Rudolf Žitný, CSc., for his invaluable encouragement and support during my entire study.

I thank to MUDr. et MVDr. Tomáš Adámek for the access to autopsy measurements which was indispensable to accomplish this study.

I would also like to give thanks to the Head of the Laboratory of Human Biomechanics, Prof. Ing. Svatava Konvičková, CSc.

I am thankful to my colleagues, Ing. Hynek Chlup, Ing. Hana Macková, Ing. Martin Hulan, Ing. Jakub Kronek, Ing. Jan Veselý, Ing. Eva Gultová and Ing. David Hromádka, for their assistance during laboratory experiments and for inspiration.

This study would not be accomplished without the financial support provided by the Czech Ministry of Education, Czech Science Foundation, Technology Agency, and Czech Technical University in Prague. The research has been partially supported form these projects:

- MSM6840770012 - Transdisciplinary Research in the Field of Biomedical Engineering (Czech Ministry of Education, Youth and Sport)
- GA106/08/0557 - Material properties of veins and their remodelling (Czech Science Foundation)
- GAP108/10/1296 - Development and Characterization of Active Hybrid Textiles with Integrated Nanograin NiTi Micro Wires (Czech Science Foundation)
- TA01010185 - New materials and coatings for joint replacement bionical design (Technology Agency of the Czech Republic)
- SGS10/247/OHK2/3T/12 – Student research grant (Czech Technical University in Prague)

Preface

This study "How Ageing is Reflected by Longitudinal Pretension in Abdominal Aorta" results from a cooperation between the Faculty of Mechanical Engineering of the Czech Technical University in Prague (FME CTU) and the 3rd Faculty of Medicine of the Charles University in Prague (3FM CU) realised by Ing. Lukáš Horný and MUDr. et MVDr. Tomáš Adámek. It comprises autopsy measurements of the longitudinal prestretch of the human abdominal aorta which were conducted at the Department of Forensic Medicine (3FM CU). During the past two years, more then 250 cadavers have been investigated. Complementary to autopsy examinations, laboratory measurements of the mechanical response of aortic tissue have been conducted at the Laboratory of Human Biomehcanics (FME CTU) by the author of the thesis. All autopsy measurements were performed by MUDr. Tomáš Adámek. He provided the author of the thesis with raw data. Subsequently, the author processed all the data (statistical analysis, regression analysis, constitutive modelling). The author also formulated the hypotheses presented in the study and written in all resulting papers which have recently appeared in several peer-reviewed scientific journals.

The organization of the thesis was predetermined by the above mentioned journal articles. The main body of the thesis is based on "***How Ageing is Reflected by Longitudinal Pretension in Abdominal Aorta***" submitted to the *Biomechanics and Modeling in Mechanobiology*. This article combines previous forensic-anthropological investigation with cardiovascular biomechanics and presents their natural complementarity necessary to understand ageing-induced changes in conduit arteries. It especially deals with the consequences of the ageing for biomechanics and mechanobiology (which together should be referred to as physiology) of the artery wall. The longitudinal pretension force, prestress and prestretch are here correlated on the basis of continuum mechanics and consequences for artery wall elastin are discussed.

This main body text is followed by three appendices. The appendices represent manuscripts of papers written by the author of the thesis. The appendices are presented in a consecutive manner with respect to the date of the submission to the journal (from the oldest, A, to the newest, C). They are edited to conform to a uniform style. However, one difference still remains. The author has not changed the reference style of articles (it holds the format corresponding with specific journal rules). The author hopes readers will not feel it too uncomfortable.

Appendices are as follows:

Appendix A – full bibliographic citation

Horny L, Adamek T, Vesely J, Chlup H, Zitny R, Konvickova S (2012) **Age-related distribution of longitudinal pre-strain in abdominal aorta with emphasis on forensic application**. *Forensic Sci Int* 214(1–3):18-22. doi: 10.1016/j.forsciint.2011.07.007
Submission date December 15th 2010 IF(JCR,2010) 1.821

In this text, the longitudinal prestretch of human abdominal aorta was proposed as simple measure of human age at time of the death suitable for forensic investigation purposes. The correlation with other anthropological parameters (heart weight, thickness of left ventricle, etc.) was also investigated.

Appendix B – full bibliographic citation

Horny L, Adamek T, Gultova E, Zitny R, Vesely J, Chlup H, Konvickova S. (2011) **Correlations between age, prestrain, diameter and atherosclerosis in the male abdominal aorta**. *J Mech Behav Biomed Mater* 4(8):2128-2132. doi: 10.1016/j.jmbbm.2011.07.011
Submission date May 5th 2011 IF(JCR,2010) 3.297

In this article, the correlation between longitudinal prestretch, atherosclerosis and aortic diameter was investigated. A detailed comparison with observations conducted by predecessors was included.

Appendix C – full bibliographic citation

Horny L, Adamek T, Chlup H, Zitny R. (2011) **Age estimation based on a combined arteriosclerotic index**. *Int J Leg Med*, in press. doi: 10.1007/s00414-011-0653-7
Submission date August 18th 2011 IF(JCR,2010) 2.939

In this article, the new quantity, combined arteriosclerotic index, was proposed. This quantity combines both longitudinal prestretch and diameter of an artery and is suitable for forensic age estimation. It was found that combined arteriosclerotic index is linearly proportional to the age which has a favourable property of simple use and almost constant confidence of the prediction.

All parts of the thesis (body text and appendices) bring original and new results. They significantly extend our knowledge of the physiology and biomechanics of human abdominal aorta and altogether give a possibility to interpret the functioning of aorta within ageing in the new context which has not appeared in scientific literature before the study.

List of symbols and abbreviations

a, b	regression parameters [arbitrary unit]
AAA	abdominal aortic aneurysm
ATH	degree of atherosclerosis [-]
$\mathbf{b}$	left Cauchy-Green strain tensor
c_i	model parameters [arbitrary unit]
c_{ijkl}	component of $\mathbb{c}$ [kPa]
C	reference circumference [mm]
C_{IJKL}	component of $\mathbb{C}$ [kPa]
$\mathbf{C}$	right Cuachy-Green strain tensor
$\mathbb{c}$	elasticity tensor in spatial description
$\mathbb{C}$	elasticity tensor in material description
F	axial force [N]
F_{iK}	component of deformation gradient [-]
F_{20}	force normalization constant [N]
$\mathbf{F}$	deformation gradient
I_i	i^{th} invariant of the right Cuachy-Green strain tensor [-]
$\mathbf{I}$	second order unit tensor
J	volume ratio [-]
J_m	limiting extensibility parameter [-]
l	in situ length [mm]
L	ex situ length [mm]
p	Lagrangean multiplier [kPa]
p_k	p–value related to k^{th} parameter [-]
P	transmural pressure [kPa]
PMI	post mortem time interval [hour]
r_i, r_o	inner and outer deformed radius of a tube [mm]
R	linear correlation coefficient [-]
S	reference cross-section area [mm^2]
S_{IJ}	component of $\mathbf{S}$ [kPa]
$\mathbf{S}$	second Piola-Kirchhoff stress tensor
T	reference thickness [mm]
W	strain energy density function [kPa]
x	arbitrary independent variable (usually calendar time, [year])
y	arbitrary dependent variable [arbitrary unit]
y_{20}	arbitrary normalization constant [arbitrary unit]

α significance level [-]

ε engineering strain [-]

ε_{20} strain normalization constant [-]

λ stretch ratio [-]

$\lambda_{AUTOPSY}$ autopsy prestretch [-]

λ_{zZ} longitudinal stretch ratio [-]

μ infinitesimal shear modulus [kPa]

σ_{20} stress normalization constant [kPa]

σ_{ij} component of $\boldsymbol{\sigma}$ [kPa]

σ_{zzP} longitudinal Cauchy stress induced by pressure acting in closed vessel [kPa]

σ_{zzF} longitudinal Cauchy stress induced by prestretching (due to force F) [kPa]

$\boldsymbol{\sigma}$ Cauchy stress tensor

Table of content

...
What are the roots that clutch, what branches grow
Out of this stony rubbish? Son of man,
You cannot say, or guess, for you know only
A heap of broken images, where the sun beats,
And the dead tree gives no shelter, the cricket no relief,
And the dry stone no sound of water. Only
There is shadow under this red rock,
(Come in under the shadow of this red rock),
And I will show you something different from either
Your shadow at morning striding behind you
Or your shadow at evening rising to meet you;
I will show you fear in a handful of dust.
...

excerptum from Burial of the Dead, T.S. Elliot *The Waste Land*

How Ageing is Reflected by Longitudinal Pretension in Abdominal Aorta?

1. Introduction

1.1 State of the art

In this paper it is continued in a study of the longitudinal mechanical behavior of human abdominal aorta, started with a detailed description of age-related changes in longitudinal prestretch and its correlation with the diameter and with atherosclerosis (Horny et al., 2011, 2012; see Appendix A, B, and C). Previous autopsy measurements of prestretch have shown a nonlinear age-related decrease in the prestretch magnitude accompanied by a nonlinear increase in the aortic diameter, and both were correlated with the occurrence of atherosclerosis. Reported diameter enlargement, in conjunction with age-related stiffening of elastic arteries, is referred to as arteriosclerosis (Greenwald 2007; McEniery at al. 2007; O'Rourke and Hashimoto 2007). Previous studies, however, have not revealed the magnitudes of the corresponding longitudinal pretension force and prestress. To extend our knowledge further, the results of laboratory measurements of these quantities will be herein presented.

It is probably the Windkessel function of the aorta (quantified with easily obtainable circumferential distensibility) that has caused the circumferential behaviour of elastic arteries to receive more scientific attention than the longitudinal behaviour. During the past decade, however, it has been found that longitudinal stress and displacement are closely interrelated with arterial development, remodelling and adaptation (Cardamone et al. 2009; Davis et al. 2005; Han et al. 2003; Humphrey et al. 2009; Jackson et al. 2002, 2005; Lawrence et al. 2009; Valentín and Humphrey 2009; Wagenseil 2011). Technical progress in imaging methods has also brought evidence of the axial motion of elastic arteries during the cardiac cycle (Cinthio et al. 2006; Tozzi et al. 2001). These findings have clearly shown the fundamental role of the axial behaviour of elastic arteries in understanding their biomechanics and mechanobiology.

When dealing with the longitudinal mechanical properties of an artery, the key phenomenon is the prestress that the artery sustains in situ. This is observed as artery retraction in the excision from a body. It was studied in detail by P.B. Dobrin, D.H. Bergel, B.M. Learoyd and M.G. Taylor, among others, in the third quarter of the twentieth century. They found that the greater the distance of the aortic segment from the heart, the greater the prestrain (Bergel 1961; Learoyd and Taylor 1966). The observed prestrain was between 0.15 and 0.4 (quantified as the relative retraction), and canine samples showed higher prestrain than human samples. Learoyd and Taylor (1966) also found age dependency of the prestrain. Dobrin and Doyle (1970) studied the retractive force and the longitudinal elastic modulus in dog carotid arteries. They found that the pretension force was approximately constant when normalized to the subject's

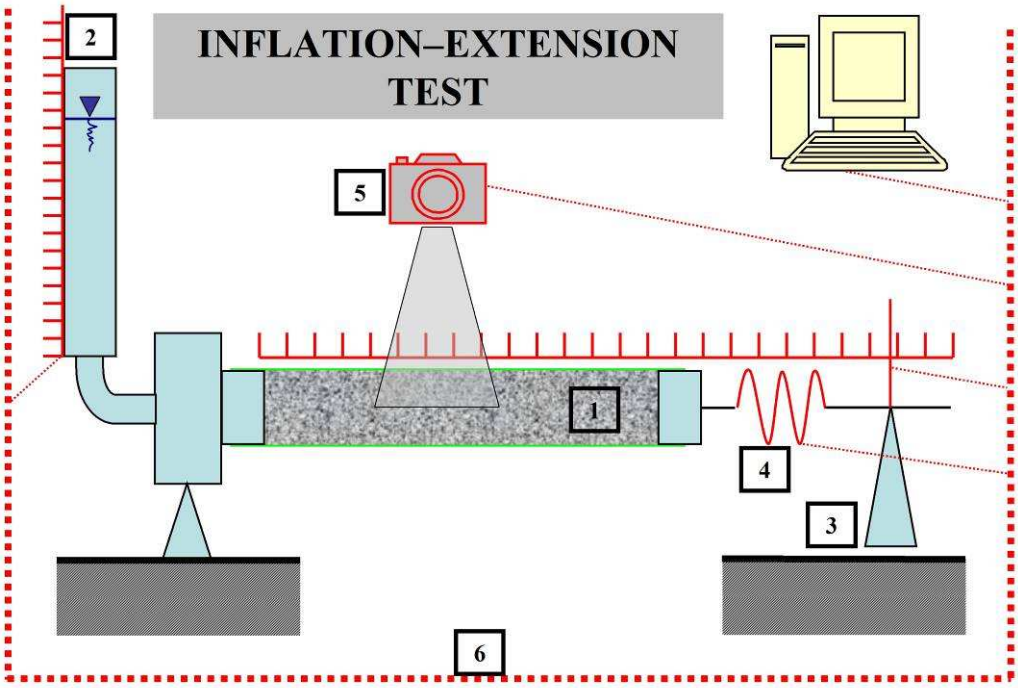

Figure 1 Scheme of the inflation-extension test. Sample of an artery – 1; arbitrary pressure generator (e.g. water column with scale; syringe with transducer etc.) – 2; adjustable longitudinal positioning (with arbitrary measurement method depicted as the rule) – 3; longitudinal force transducer – 4; radial displacement measurement method (e.g. optical sensor) – 5; data channels (bus) to PC – 6. Colour highlights: measurement and data transfer – red; sample – green; ground – black.

body weight (≈0.01 N/kg), and the longitudinal elastic modulus was almost independent from the smooth muscle activation (≈410 kPa at in situ length and zero pressure).

In the years that followed, Langewouters et al. (1984) studied the dimensions of human thoracic and abdominal aortas, and published raw data suitable for modelling (Wuyts et al. 1995; Zulliger and Stergiopulos 2007). Han and Fung (1995) investigated prestress and prestrain in canine and porcine aortas. They mapped the position dependence in detail, and found a correlation between the prestrain and the cross-sectional area. They also estimated the longitudinal stress and tension force acting in the wall due to the prestrain (the stress increased distally from 10 to 50 kPa, and the pretension force was approximately independent of the location, ≈1N).

Axial prestretch is advantageous from a biomechanical viewpoint. Inflation-extension experiments showed that, at a certain axial strain, arteries can be pressurized with no change in their length (Schulze-Bauer et al. 2003; Sommer et al. 2010). Under these conditions, a pressure pulse wave can be transmitted along an artery without a significant change in the axial force (Van Loon et al. 1977). Such conditions would be physiologically optimal. However, it seems to be rather unrealistic to expect these conditions in aged persons.

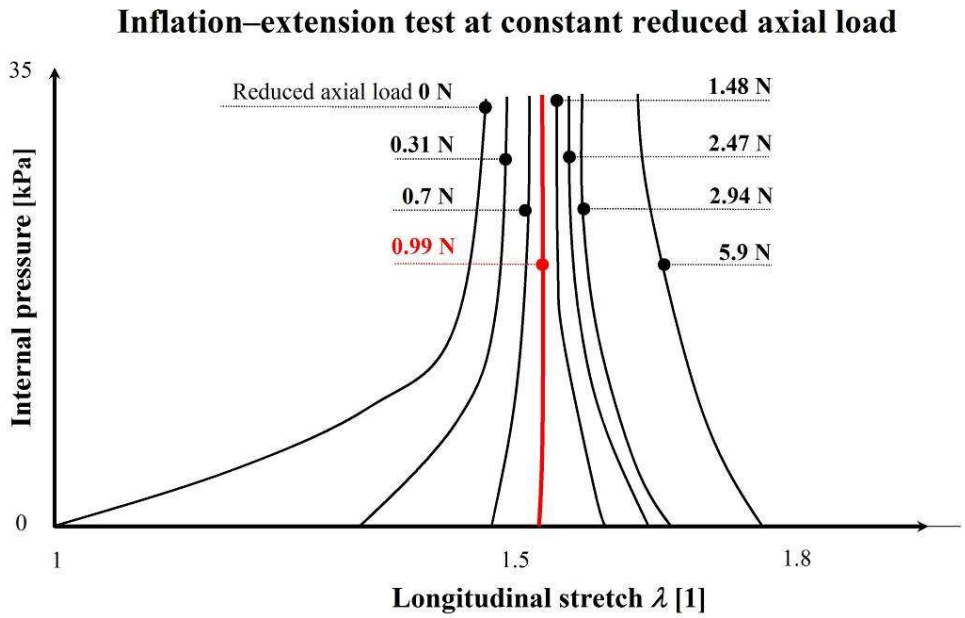

Figure 2 Sketch of the longitudinal mechanical response of the artery during pressure inflation (with constant reduced axial load = constant pretension force). Sketch adapted form Ogden and Schulze-Bauer 2000. Specific values correspond to the experiment with human iliac artery. At longitudinal prestrain highlighted with red colour, the pressurization induces almost no longitudinal displacement.

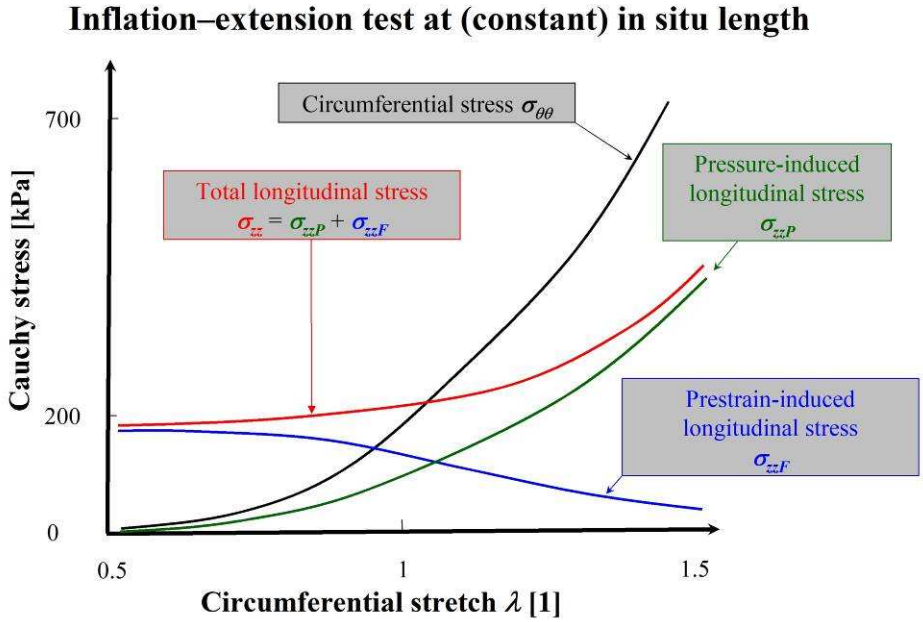

Figure 3 Longitudinal wall stress during pressurization. Adapted from Dobrin and Doyle 1970. Total longitudinal stress is induced by blood pressure acting at closed end of the sample σ_{zzP}, and from pretension force σ_{zzF}. Note that the artery is longitudinally prestretched (radius decreased), thus circumferential stretch does not begin at $\lambda = 1$. Compare the gradient of red curve (total axial stress) with the gradient of green curve (pressure-induced stress). The difference results from blue curve (effect of longitudinal prestress).

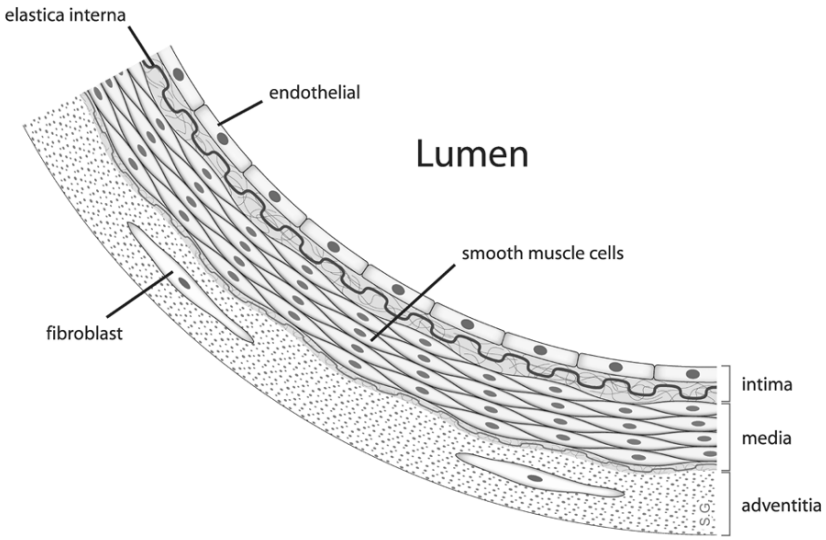

Figure 4 Artery architecture in the cross-section. Prestrained elastine membranes (fenestrated lamellae) pass along smooth muscle cells (oriented circumferentially) in out-of-plane direction (longitudinally). Adopted from http://upload.wikimedia.org/wikipedia/commons/0/05/Anatomy_artery.png.

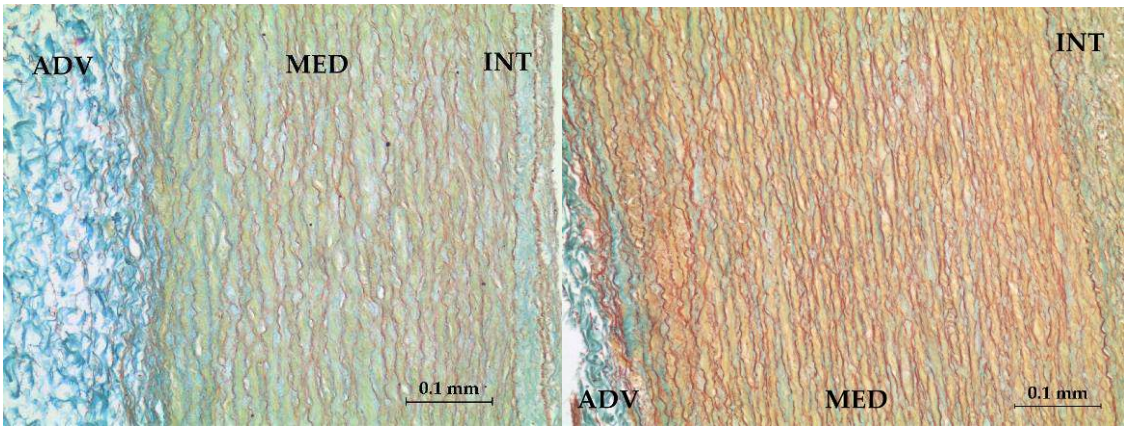

Figure 5 Histological sections of the wall of abdominal aorta. Stained with orcein – collagen in blue/green, elastin in brown, smooth muscle cells in grey/green (left) and orange/brown (right). In elastic arteries, three main wall layers are distinguished: predominantly collagenous tunica adventitia (ADV), middle musculo-elastic tunica media (MED), and inner endothelial tunica intima (INT).

The magnitude of the species-dependent prestretch seems to be explainable by means of the intramural collagen-to-elastin ratio (the higher the ratio, the lower the prestretch; Humphrey et al. 2009). Distally increasing the magnitude of the axial prestretch along the aorta conforms to the idea that the resulting axial force carried by elastin membranes is approximately constant in the aorta (the smaller the number of lamellae, the higher the prestretch). This is consistent with the crucial role of elastin in the pretension-bearing capacity revealed by enzyme digestion (Dobrin et al. 1990). Animal models with an elastin insufficiency also show the essential role of elastin (elastin insufficiency led to a loss of pretension; Wagenseil et al. 2009).

1.2 Aim of the study

The primary aim of this study is **to investigate age-related changes in longitudinal prestretch, pretension force and prestress in human abdominal aorta**. **As has been shown above, this lacks in the scientific literature. The model describing a loss of pretension will be proposed. Ageing induced changes in the constitutive behaviour (stress-strain relatinship) will be elucidated. The consequences for the stiffness, related to prestrained artery wall, will be shown.**

The study is organized as follows. The ex vivo elongation test with tubular samples of infrarenal aorta and autopsy measurement are described first. Constitutive modelling follows. The results are discussed especially with respect to the age of the donors. It is generally accepted that elastin is the main artery wall component responsible for the hypo-physiological response. The consequences for elastin mechanics are therefore also discussed.

2. Materials and methods

In contrast to the circumferential mechanical behavior of elastic arteries, little is known about their longitudinal mechanical response. An experiment aimed at determining the effect of ageing on the force that preloads the aorta in the axial direction (the force needed to attain in situ length) and the corresponding axial prestress is herein presented. The experimental data is used in the constitutive modeling to elucidate age-dependent changes in stiffness related to longitudinal prestretch. Since autopsy measurement (excision from a body) is the only direct method available for detecting non-prestretched length, a simple elongation test of tubular samples was used. To mimic the conditions of autopsy measurement of in situ and ex situ length, the aortas were not pressurized in these tests.

2.1 Experiment – Autopsy measurement of the prestretch

Longitudinal prestretch of the aorta was quantified by ratio (1). Here l denotes the length of the artery segment in situ and L denotes the length after excision from the body.

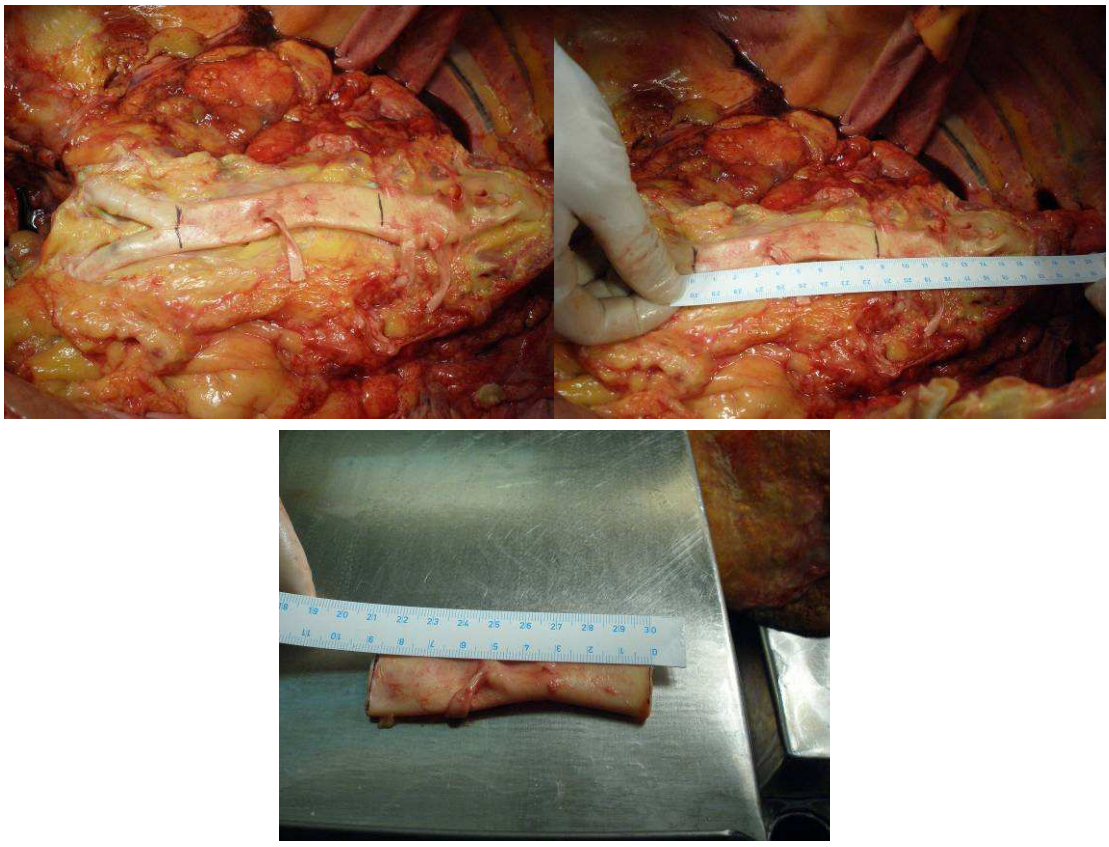

Figure 6 Autopsy measurement of the longitudinal prestretch in infrarenal aorta.

Index *AUTOPSY* is used to emphasize the fact that we are dealing with a specific value, not with a variable in some mechanical test. The measurement method for the determination of the longitudinal prestretch is also described in Horny et al. (2011, 2012 – Appendix A, B). The abdominal aorta was thoroughly removed and the distance between two markers in situ and after the excision was measured with a ruler. Markers were made just below the renal arteries and above the aortoiliac bifurcation. The method is depicted in Fig. 6.

$$\lambda = \frac{l}{L} \tag{1}$$

2.2 Experiment – Elongation test

The experiments were performed with samples of human infrarenal aorta obtained from regular autopsies in the Department of Forensic Medicine of the Na Královských Vinohradech University Hospital and the Third Faculty of Medicine of Charles University in Prague. The relevant ethical committee approved the use of human tissue in this research. After being transported to the laboratory, the samples were equilibrated to the laboratory temperature (22°C). Then they were cannulated at both ends, marked with a liquid eyeliner (which has been shown to be better than permanent ink and other alternatives) and suspended on a stand. Each cylindrical sample was consecutively elongated by a longitudinal load up to at least a force of 1.6 N. The specimen was photographed in each loading step, and the longitudinal stretch λ_{zZ} was determined by analyzing the distances between the marks in the photograph. The method is depicted in Fig. 7 (scheme) and dimension evaluation performed in NIS-Elements (NIKON software, USA) is illustrated in Fig. 8.

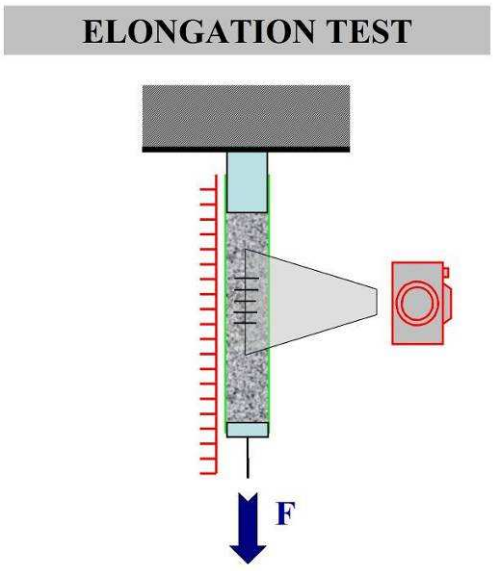

Figure 7 Sketch of the elongation test.

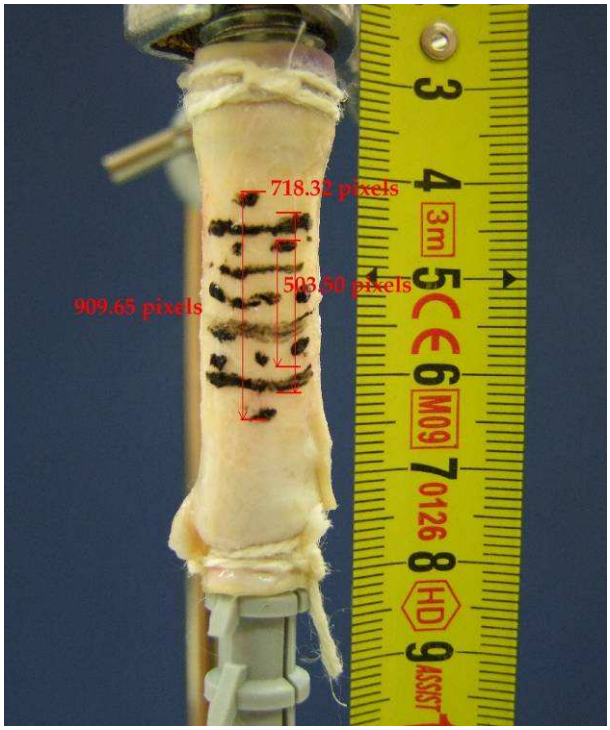

Figure 8 Representative of the tracking of marks in the photographs. At least five measurements (for different marks) were conducted in each photograph in each loading step.

The samples were preconditioned with several manual elongations before the measurement cycle. Each experiment consisted in at least fifteen loading steps, which spanned approximately three minutes. To avoid a time-dependent material response (creep), the specimen was unloaded between each pair of longitudinal weights. The average loading time in each step was approximately 5s.

The pretension force (the force necessary to extend the distance of the marks to the prestretch measured in the autopsy) was determined by means of linear interpolation between two neighbouring loading steps, if it did not exactly match any applied load.

The experimental stress was determined by (2), where F is the acting axial force and S is the reference cross-sectional area given as the product of the circumference and the thickness of the sample. Incompressibility was assumed.

$$\sigma_{zz}^{EXP} = \lambda_{zZ} \frac{F}{S} \tag{2}$$

2.3 Model – Stress-strain relationship

The hyperelastic limiting chain extensibility model was employed to determine the longitudinal stiffness of the prestretched aorta. This model was originally proposed by Gent (1996), and its application in the field of artery biomechanics was proposed by Horgan and Saccomandi (2003) and by Ogden and Saccomandi (2007). It is a two-parametric model belonging to the class of so-called generalized neo-Hookean models, which is able to describe large strain stiffening. The particular form of the model is given by equation (3).

$$W = -\frac{\mu J_m}{2}\ln\left(1-\frac{I_1-3}{J_m}\right) \tag{3}$$

W denotes the strain energy density function; μ is the infinitesimal shear modulus (a stress-like parameter); and J_m is a (dimensionless) limiting extensibility parameter which governs the rate of stiffening. I_1 is either the first invariant of the right Cauchy–Green strain tensor $\mathbf{C}$ or the first invariant of the left Cauchy–Green strain tensor $\mathbf{b}$ ($I_1 = tr(\mathbf{C}) = tr(\mathbf{b})$). They are computed from the deformation gradient $\mathbf{F}$ as follows: $\mathbf{C} = \mathbf{F}^T\mathbf{F}$, $\mathbf{b} = \mathbf{F}\mathbf{F}^T$.

The assumption of incompressibility of the artery wall was also adopted. It implies $I_3 = J^2 = det^2(\mathbf{F}) = det(\mathbf{C}) = det(\mathbf{b}) = 1$. J denotes volume ration. Employing a material description, the stress–strain relationship for a hyperelastic material is obtained via differentiation of W with respect to a strain tensor (4).

$$\mathbf{S} = 2\frac{\partial W(\mathbf{C})}{\partial \mathbf{C}} - p\,\mathbf{C}^{-1} \tag{4}$$

Here p accounts for the reaction to the incompressibility constraint, and will be determined from a boundary condition. The Cauchy stress $\boldsymbol{\sigma}$ is obtained after transformation into the spatial configuration $\boldsymbol{\sigma} = J^{-1}\mathbf{F}\mathbf{S}\mathbf{F}^T$. The final expression (in a spatial description) is written in equation (5). $\mathbf{I}$ denotes a unit second order tensor.

$$\boldsymbol{\sigma} = \mu\frac{J_m}{J_m-(I_1-3)}\mathbf{b} - p\,\mathbf{I} \tag{5}$$

This study is focused on determining the mechanical state of the aorta corresponding to the prestretch found in an autopsy. We note that before and during an autopsy excision of the aorta no blood pressure is acting in the body. Thus an experiment to find the force necessary to extend an arterial segment to in situ dimensions, to the stress induced by this preload, and to the related stiffness cannot include pressurization. It was assumed that the longitudinal force deforms a cylindrical segment of aorta to an elongated cylinder with decreased radius. The reference cylindrical coordinates (R, Θ, Z) are mapped on to the deformed coordinates (r, θ, z) by means of the equations $r = r(R)$, $\theta = \Theta$, $z = \lambda Z$ (here $\lambda_{zZ} = \lambda$). The longitudinal stretch λ is

considered to be uniform along the length of the sample. Since no transmural pressure is acting, the contribution of the collagen fibres (the main source of arterial anisotropy) to the load-carrying process is considered to be negligible. In this situation, the mechanical response is dominated by elastin and is modelled as isotropic (Holzapfel et al. 2000; Ogden and Saccomandi 2007; Watton et al. 2009). Now, incorporating the thin-walled approximation, the deformation gradient **F** is obtained in the form of (6).

$$\mathbf{F}=\begin{pmatrix}\lambda_{rR} & 0 & 0\\ 0 & \lambda_{\theta\Theta} & 0\\ 0 & 0 & \lambda_{zZ}\end{pmatrix}=\begin{pmatrix}\lambda^{-\frac{1}{2}} & 0 & 0\\ 0 & \lambda^{-\frac{1}{2}} & 0\\ 0 & 0 & \lambda\end{pmatrix} \tag{6}$$

The boundary condition necessary to complete the stress–strain relationship by p is adopted as $\sigma_{rr} = 0$. The model then results in equation (7) which describes the longitudinal stress acting in the artery. In (7), I_1 takes the form $I_1 = \lambda^2 - 2/\lambda$.

$$\sigma_{zz}^{MOD}=\mu\frac{J_m}{J_m-(I_1-3)}\left(\lambda^2-\frac{1}{\lambda}\right) \tag{7}$$

For the sake of completeness, the referential counterpart of (7) is the second Piola–Kirchhoff stress, given by $S_{ZZ} = \mu J_m(1 - \lambda^{-3})/(J_m - (I_1 - 3))$. The stress–strain relationships were obtained by fitting the model parameters to the observed data, employing a least–square algorithm (equations (2) and (7) were used). This was performed using the optimization package in Maple 15.

Generally, the elastic modulus is the slope of the tangent to a stress–strain curve. Fitted models (3) were employed to this end rather than experimental stress–strain data. In the present case, the general approach via the elasticity tensor was used. It is obtained by differentiating equations (4) or (5) with respect to the appropriate strain tensor. The result is a fourth-order tensor, where the components are functions of the specific strain state and the material parameters.

$$\mathbb{C}=2\frac{\partial\mathbf{S}(\mathbf{C})}{\partial\mathbf{C}} \tag{8}$$

Equation (8) expresses the elasticity tensor $\mathbb{C}$ generally in the material description. Substituting S_{ZZ} into (8), we will approach towards (9). A numerical value for the elastic modulus of the material is then attained after substituting specific $\lambda_{AUTOPSY}$ into (9).

$$C_{ZZZZ}=\mu\frac{J_m}{J_m-(I_1-3)}\frac{1}{\lambda^5}\left(\frac{2\left(1-\lambda^3\right)^2}{\lambda\left(J_m-(I_1-3)\right)}+3\right) \tag{9}$$

The spatial counterpart of (9) is denoted c_{zzzz}. It is obtained after transformation to the spatial configuration respecting the fourth order of $\mathbb{C}$. Since the component notation is clearer in the case of higher-order tensors, the transformation rule is written as $c_{ijkl} = F_{iA}F_{jB}F_{kC}F_{lD}C_{ABCD}$ (details can be found in Holzapfel (2000) chapter 6.6). The resulting equation is $c_{zzzz} = \lambda^4 C_{ZZZZ}$. This simple relation is the consequence of the diagonal nature of all second order tensors used in the model. Although the explication given in both the material description and the spatial description could be considered redundant, it will be shown later that the description that is used can affect the correlation between age and stiffness.

2.4 Age-related changes of the longitudinal prestretch, presrtess and pretension force

Correlation with age. All studied quantities (prestretch, pretension force, prestress, elastic modulus) were involved in the correlation analysis. The correlation of the model parameters was also investigated. The linear correlation coefficient R is supplemented by hypothesis testing. Two tests were employed. The first was a t-test with the null hypothesis H_0: $R = 0$; the corresponding p–value is denoted p_R. The second was a t-test related to the efficiency of the linear regression model with the null hypothesis H_0: $a = 0$ (in the regression equation $y = ax + b$); p–value denoted p_a. The power law was used to supplement the linear model (this was proved to be suitable in previous studies, Horny et al. 2011, 2012a – Appendix A and B). In this case, the null hypothesis states H_0: $b = 0$ (considering equation $y = ax^b$ and its logarithmic transformation). The results are considered to be significant at $\alpha = 0.05$.

Sample size effect, and loss of pretension. Previous studies, conducted by the author and coworkers, reporting statistics for the longitudinal prestretch in human abdominal aorta included 250 samples; 60 female and 190 male aortas (Horny et al. 2012b – Appendix C). The current study of longitudinal prestress involved only 10 samples, which could lead to biased results. Current data was compared with previsous observations to exclude potential outliers. Moreover, incorporation of the previous data, with supplementary presumptions, provides a more reliable basis for estimating prestretch–age, pretension force–age, and prestress–age. In what follows, it will be distinguished between the small data sample (10 specimens, Table 1) and the large data sample (containing only prestretches – Horny et al. 2012b, Appendix C).

All the data was normalized with respect to the maximum value, which was presumed to appear at the age of 20 years. A decreasing trend in prestretch with increasing age was observed in previous analyses, and it is reasonable to expect this trend also in the relationships between

pretension force–age and prestress–age. In addition to the simultaneous occurrence of the maximum value, it is also postulated that the minimum value will be reached at the same time. The prestretch was substituted by the engineering prestrain ε (= λ - 1) and the minimum value for studied quantities was prescribed to zero.

A zero value is justified by the following considerations. First, zero–prestretched arteries were observed (they do not retract upon excision). Second, it is well known that human abdominal aorta may form an aneurysm (AAA), which can be considered as a manifestation of the loss of longitudinal prestretch. Better to say, the loss of prestretch can be accompanied by the formation of AAA, because loss of pretension also exists in another form. The other form is so-called tortuosity (loss of straightness of the vessel axis). It means that the aorta does not expand radially (as in AAA), but longitudinally. However, due to fixation at the ends of the infrarenal segment, the aorta buckles laterally. This phenomenon is also frequently observed in carotid arteries.

The zero-prestress time was prescribed at 85 years of age. This value was estimated from statistics reporting the prevalence of aneurysms in the population. The prevalence (number of expected diagnoses per sample of a population) of AAA is usually reported between 1.5 – 5%, depending on the considered age and population. Svensjö et al. (2011) reported 2.2% in 65-year-old Swedish men. A comparative study of United Kingdom, Denmark and Australian populations gave an extrapolation to 3.5% and 4.5% at the same age (Comparative study, 2011). The age of 85 years was estimated with an extrapolation to 3.5% on the Czech population aged between 65 and 100 years. A 3.5% portion in each age group was computed from demographic statistics on length of life (an empirical probability density function of the age of old persons in the population) obtained from the annual statistical report of the Czech Statistical Office (2010). The sum of these portions was doubled to account for tortuosity and asymptomatic cases. The resulting number was considered as an estimate of the number of individuals with zero prestretch in the population. Strictly speaking, it is not known how these cases are distributed in the population. However, a conservative estimate of the zero-prestress age can be obtained when all non-prestretched cases are inserted into the empirical density distribution of age, decreasing from 100 years of age. A conservative estimate means that it does not overestimate ageing-induced changes (the loss of pretension). On the other hand it means that the loss of pretension may occur earlier, but in average it should not be later. These cases covered the interval between the eighty-fifth year of age and the last age in the statistics (100 years). Thus zero-prestress (-prestretch, -force) was prescribed at the age of 85 years.

The same form of the regression model (10) was adopted for prestress, prestrain and the pretension force–age relationship. Equation (10) contains three parameters, c_0, c_1 and c_2, but the number of free parameters is restricted by the conditions $y(20) = 1$ and $y(85) = 0$.

$$y = c_0 \left(1 - c_1 e^{-c_2 x}\right) \tag{10}$$

Note that the regression was performed with normalized data. Taking into account previous observations (Horny et al. 2011, 2012 – Appendix A, B, C), $\lambda_{AUTOPSY} = 1.4$ was prescribed as the expected value at the age of 20 years, which implies $\varepsilon_{20} = 0.4$ as the normalization constant. In case of prestress and pretension force, the normalization constants were considered to be unknown a priori. They were therefore optimized in the regression analysis.

3. Results

The study involved 10 human donors (6 female, 4 male) of infrarenal aorta. The elongation experiments were conducted in the Laboratory of Biomechanics of the Faculty of Mechanical Engineering of the Czech Technical University in Prague. Descriptive statistics of the samples are listed in Table 1. Sample ID identifies the gender (M, F) and the age of the donor.

3.1 Pretension force, prestress and stress–strain relationships

The experimental records of the force–stretch and stress–stretch relationships obtained in the elongation of the cylindrical samples are depicted in Fig. 1 and 2. The prestretch measured in the excision of the sample from a body is indicated by a vertical line. The prestress and the pretension force, listed in Table 1, were determined as the position of the intersection in these graphs (mean/SSD – autopsy prestretch 1.13/0.1 pretension force 0.54/0.44 N; and prestress 11.6/13.5 kPa; SSD denotes sample standard deviation). Irrespective of measurement errors, it is clearly visible that younger donors manifest a higher prestretch and pretension force (the vertical lines shorten from right to left). The mechanical responses also confirmed a presumption that older donors will yield stiffer aortas (they yield a higher force and stress for the same deformation in comparison with younger individuals).

The stress–strain responses were fitted to the limiting chain extensibility model (3). The models correspond well with the observed behaviour, see Fig. 3. The estimated parameters are listed in Table 2. The results show that age–dependency is primarily governed by J_m. This is in accordance with the physical interpretation of J_m, since this parameter is responsible for the rate of strain stiffening.

3.2 Elastic modulus.

To avoid increasing the effect of a measurement error, the elastic modulus was estimated by the model parameters of the stress–strain curves. The results are listed in Table 2. The mean values 167/210 kPa and 85/61 kPa were acquired using the spatial description and the reference description, respectively (mean/SSD).

Table 1 Data summary. The table summarizes measured quantities in the form mean/estimation of uncertainty (if available). The code indicates gender and age [years]. The circumference in the reference configuration, C, was obtained as the length of a ring cut from the specimen (determined via image processing). The thickness in the reference configuration, T, was measured by a calliper (five times and then averaged). The reference cross-section area, S, was obtained as $S = C \cdot T$. PMI denotes the time interval between death and the experiment. ATH quantifies the degree of atherosclerosis examined by the pathologist; 0 – intact artery and fatty streaks; 1 – fibro-fatty plaques; 2 – advanced plaques; 3 – calcified plaques; 4 – ruptured plaques (Kumar et al., 2010). Measurement uncertainties were estimated by means of the sample standard deviation (SSD) for C, T, S; in the case of prestress, it is the mean of SSD for the neighbouring stresses determined in the elongation test under the strain corresponding to the prestrain; and in case of the prestrain, it is the resolution unit/L. R denotes linear correlation coefficient. It is supplemented with p–value p_R and p_a (p_R – the null hypothesis $R = 0$ will be rejected if the significance level α is higher than p_R; p_a – the null hypothesis $a = 0$ (related to the regression equation $y = ax + b$) will be rejected if the significance level α is higher than p_a. We note that in the "logarithmic data" row all quantities are computed after logarithmic transformation, and p–value p_b is related to the hypothesis $b = 0$ in $\ln(y) = \ln(a) + b\ln(x)$ ($y = ax^b$). The row denoted Mean contains averaged mean values and SSD of the mean values listed in the column above. ATH was not considered in the regression analysis due to its rather ordinal-variable nature.

Code	C [mm]	T [mm]	S [mm^2]	PMI [hour]	ATH [-]	Prestrain [1]	Prestress [kPa]	Pretension force [N]
M26	34.9/1.7	1.41/0.09	49.1/5.6	32	0	1.31/0.01	25.5/3.5	0.96
M52	43.0/1.0	1.60/0.15	68.8/8.1	26	1	1.09/0.01	5.6/0.8	0.35
M58	48.5/1.7	1.91/0.36	92.4/20.6	80	2	1.10/0.01	6.0/1.5	0.52
M61	43/–	1.46/0.22	62.7/9.4	80	1	1.03/0.01	2.3/0.4	0.14
F29	35.1/0.9	1.38/0.26	48.4/10.4	99	0	1.32/0.02	45.0/10.6	1.63
F38	32.7/0.6	1.35/0.09	44.2/3.9	67	0	1.16/0.01	10.5/1.2	0.40
F47	36/–	1.95/0.23	70.1/8.3	50	2	1.07/0.01	4.5/0.6	0.30
F48	36.5/0.5	1.62/0.17	59.3/6.9	25	1	1.09/0.02	6.0/0.7	0.33
F53	40.5/1.5	1.56/0.23	63.3/11.9	16	2	1.08/0.01	7.5/1.5	0.44
F58	48.5/0.5	2.29/0.37	110/17.7	29	4	1.09/0.01	3.3/0.7	0.33
Mean/SSD (Median)	39.9/5.7	1.65/0.30	63.8/25.2	50/29	(1)	1.13/0.10	11.6/13.5	0.54/0.44
Correlation with age (row data) R	0.802	0.567	0.768	–	0.711	-0.920	-0.829	-0.767
	p_R<0.003 p_a=0.36	p_R=0.044 p_a=0.016	p_R<0.005 p_a=0.47	–	p_R<0.011 –	p_R<0.001 p_a<0.001	p_R<0.002 p_a=0.43	p_R<0.005 p_a<0.002
a b $y = ax + b$	0.3748 22.25	0.0141 0.9889	1.580 -10.45	–	–	-0.0075 1.484	-0.9134 54.55	-0.0276 1.834
Correlation with age (logarithmic data) R	0.765	0.591	0.764	–	–	-0.946	-0.919	-0.800
	p_R<0.005 p_b<0.001	p_R<0.036 p_b<0.001	p_R<0.006 p_b<0.004	–	–	p_R<0.001 p_b<0.001	p_R<0.002 p_b<0.001	p_R<0.005 p_b<0.008
a b $y = ax^b$	8.4810 0.4041	0.4021 0.3693	0.5714 1.222	–	–	3.180 -0.2713	$1.227 \cdot 10^5$ -2.496	510.9 -1.836

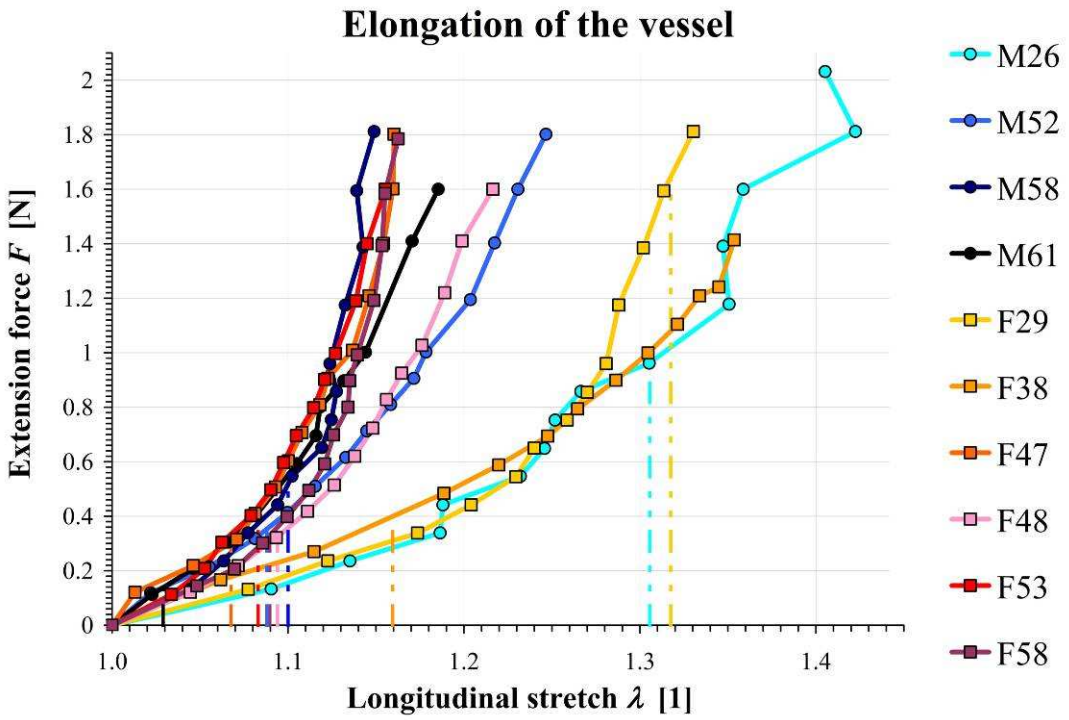

Figure 1 Longitudinal force–stretch diagram obtained during simple elongation of the cylindrical segments of infrarenal aorta; experimental records. Vertical lines highlight the prestrain determined in the autopsy. The intersection between the force record and a vertical line determines the presumed axial force acting in the vessel before it is excised. The colours indicate calendar age rising from bright to dark colour. Cold and warm colours indicate the sex of the donor. The difference between younger individuals (M26, F29 and F38) and older individuals (M52, M58, M61, F47, F48, F53 and F58) is apparent.

3.3 Correlation with age

All studied quantities showed a correlation with calendar age. This is clearly shown in Figures 1 – 5; in the force/stress–stretch relationships it is shown as a shift to the left with advancing age. Fig. 4 displays the results of the regression analysis aimed at the prestretch–age relationship, comparing the current (small) sample with previously observed data. To this end, prediction intervals (PI) for 95% confidence of prediction based on previous data (the large sample) were added. Their construction was described in detail in previous studies (Horny et al. 2011, 2012a – Appendix A, B). It turns out that the current observations are not exceptional, and may be considered as apposite representatives. The model parameters are cited in the figure legend. In the small data sample, the linear correlation coefficient R was (considering logarithmic

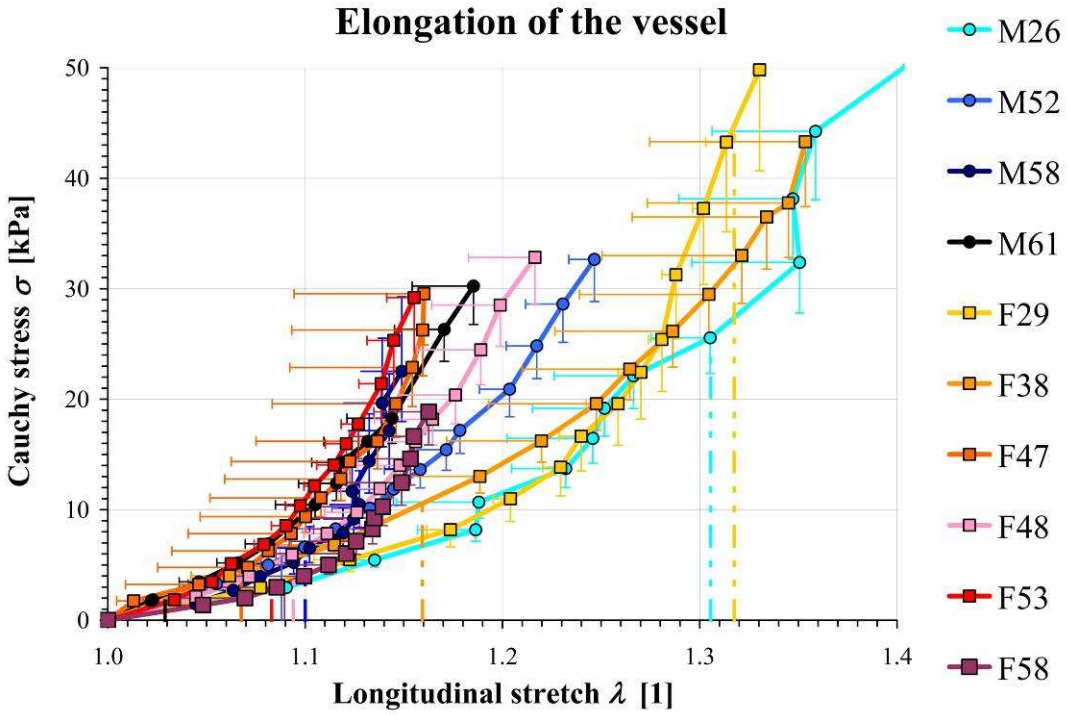

Figure 2 Experimental stress–strain records obtained in the elongation test. The error bars indicate the sample standard deviation. The vertical lines and the colour have the same meaning as in Fig. 1. This graph and the following graphs are restricted to 50 kPa in order to display the prestress–prestrain relationship effectively.

transformation of the power law) -0.919 for prestress–age dependence, -0.946 for prestretch–age dependence, and –0.800 for pretension force–age dependence. These values are higher than the correlation coefficients (see Table 1) based on raw data (implicitly presuming a linear relationship between the variables). The *p*–values (Table 1) confirm this result. They particularly suggest that a linear equation is not an efficient description for the prestress–age relationship (p_a = 0.43 vs. p_b < 0.001). Generally, the power law was more successful in describing the age–dependency of primary quantities.

However, this is not the case for the material parameters μ and J_m (deduced quantity). The statistical analysis results suggest that we should reject the hypothesis that μ is age dependent (using both linear and power law relationships, see Table 2). In the case of J_m, which correlates significantly with age, a linear description is more successful than the power law. Bearing this in mind, we went on to investigate whether J_m alone could explain the age-dependent variability in the stress-strain data. μ was fixed to its mean value (20.24 kPa), and each curve was fitted again. The results showed (Fig. 6) that this approach is capable of expressing the character of the ageing effect.

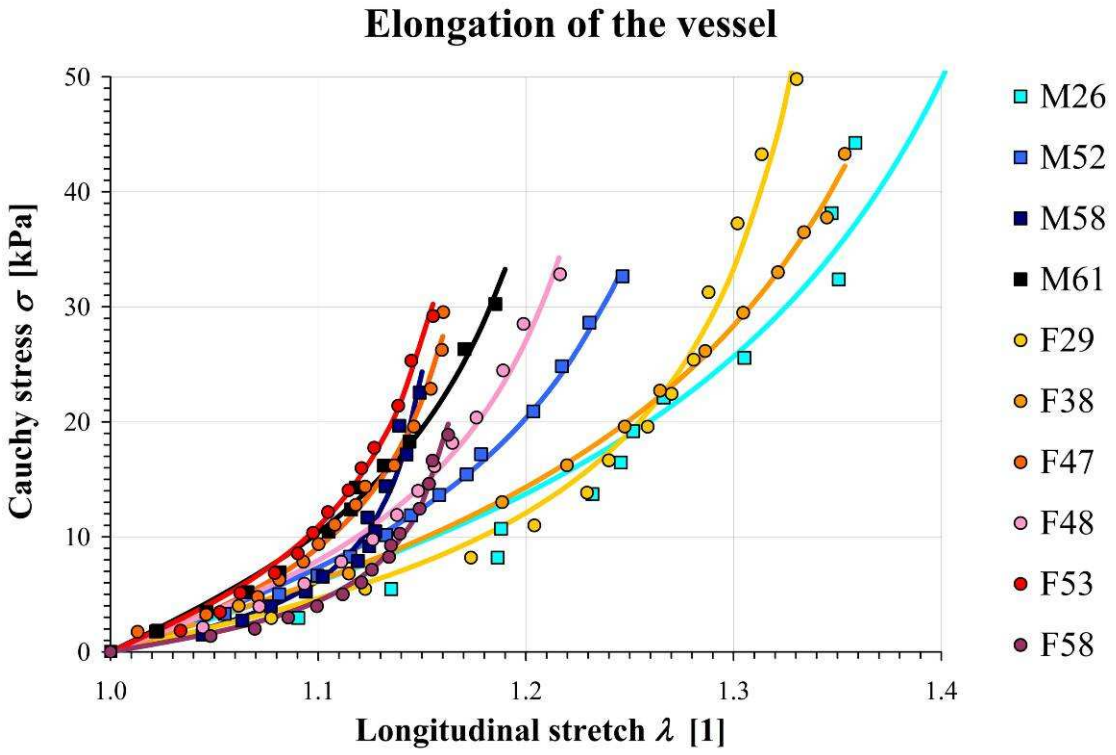

Figure 3 Experiment and model predictions. The points correspond to the experiment, and the thick curves refer to the model. The parameters of the model are listed in Table 2.

However, the data for younger donors is somewhat overestimated at the initial deformations. As might be expected, this approach also strengthened the correlation between J_m and age (R = -0.861, $p_R < 0.001$ for linear dependence).

Elastic moduli C_{zzzz} and c_{zzzz} (related to autopsy prestretch) did not have as high a correlation with age as prestretch, force and prestress. The data shows that different conclusions may be obtained using either a material description or a spatial description. The results in Table 2 suggest that the hypothesis of an age–C_{zzzz} correlation should be rejected, unlike an age–c_{zzzz} correlation. In the case of the age–c_{zzzz} correlation, the power law is more successful than a simple linear trend (measured by R), though neither pb nor pa reaches a significant level. A re-evaluation of the regression (with fixed μ) led to increased R for the spatial description (significance attained) and decreased R for the material description (not significant).

Table 2 Model parameters (μ, J_m) and elastic moduli. Elastic moduli and limiting extensibility were estimated twice, with free μ and with fixed μ, to highlight the ability of Jm to capture the effect of ageing. R, p_R, p_a and p_b have the same meaning as in Table 1.

Code	M26	M52	M58	M61	F29	F38	F47	F48	F53	F58	Mean SSD	R with age (raw data)		R with age (logarithmic data)	
μ [kPa]	19.49	22.18	13.64	29.57	14.30	19.60	22.88	23.03	27.30	10.43	20.24 6.07	0.200	p_R=0.29 p_a=0.37	0.120	p_R=0.37 p_b=0.37
J_m [1]	0.7592	0.3160	0.0826	0.1982	0.3788	0.6287	0.1196	0.2208	0.1143	0.0970	0.2910 0.2349	-0.818	p_R<0.002 p_a<0.017	-0.793	p_R=0.003 p_b=0.017
c_{zzzz} [kPa]	161.0	77.10	125.4	89.61	758.9	75.12	93.31	92.43	134.6	64.19	167.2 210.1	-0.566	p_R=0.044 p_a=0.10	-0.632	p_R=0.025 p_b=0.10
C_{ZZZZ} [kPa]	55.33	55.02	85.65	79.93	252.3	41.63	71.72	64.53	97.86	45.54	84.95 61.45	-0.383	p_R=0.138 p_a=0.31	-0.256	p_R=0.24 p_b=0.31
μ [kPa]	20.24										–	–	–	–	–
J_m [1]	0.7882	0.2851	0.1100	0.1408	0.4883	0.6561	0.1073	0.1976	0.0933	0.2008	0.3068 0.2499	-0.861	p_R<0.001 p_a<0.008	-0.823	p_R<0.002 p_b<0.008
c_{zzzz} [kPa]	159.3	72.14	134.4	62.32	435.6	76.32	85.39	84.99	114.3	81.48	130.6 111.4	-0.611	p_R=0.03 p_a=0.04	-0.671	p_R=0.017 p_b=0.039
C_{ZZZZ} [kPa]	54.75	51.49	91.80	55.59	144.8	42.30	65.63	59.34	83.07	57.80	70.66 29.94	-0.272	p_R=0.22 p_a=0.31	-0.185	p_R=0.3 p_b=0.31

3.4 Regression analysis of normalized models

Normalized models. Equation (10) was used to model age–related changes of the prestretch, prestress and pretension force considering not only decreasing trend but also the state of zero longitudinal pretension. The correlation coefficient (after transformation to the linear problem) had values of -0.910 for prestrain in the small sample, -0.863 for prestrain in the large sample, -0.914 for prestress, and -0.792 for pretension force. The parameters of the model are listed in the legend of Fig. 5. In Fig. 4, a simple power law model (based on the small data sample; red curve) is compared with (10) for the large data sample (thick black curve). It is clearly shown that at the intersection of the domains of observation the two models give similar predictions. Computation of the p–values for the regression given by (10) has therefore been omitted.

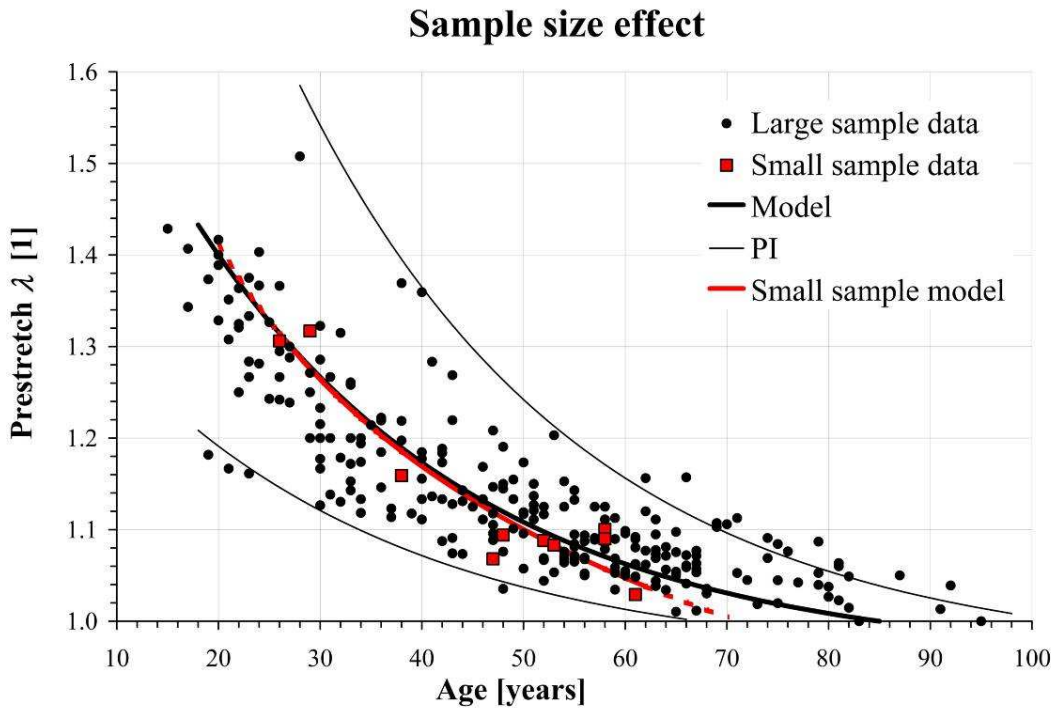

Figure 4 Comparison of data samples. Previously reported data (Horny et al. 2012b, Appendix C) is depicted with black points, current data sample with red boxes. The models are depicted with solid curves; the large sample model is based on equation $y = c_0\left(1 - c_1 e^{-c_2 x}\right)$ i.e. (10), which gives with parameters ε_{20} =0.4, c_0 = -0.1082, c_1 = 20.95, and c_2 = 0.0358 (x denotes age in years). The small sample model is based on the power law $y = ax^b$ where a = 3.1804, b = -0.2713. Extrapolation from the observation domain in the small sample model is depicted with a dotted curve. The black curve approaches $\lambda = 1$ (zero prestrain) at the age of 85 years (prescribed value). The small sample model approaches $\lambda = 1$ at the age of 71 years.

Note that although the simple power law gives similar predictions, it does not hold outside this domain. They differ significantly in their asymptotic behaviour, because the power law ($y = ax^b$) cannot predict the loss of longitudinal prestress and pretension force since it approaches zero at infinity. Contrastingly, equation (10) is able to describe this property and was employed for this purpose.

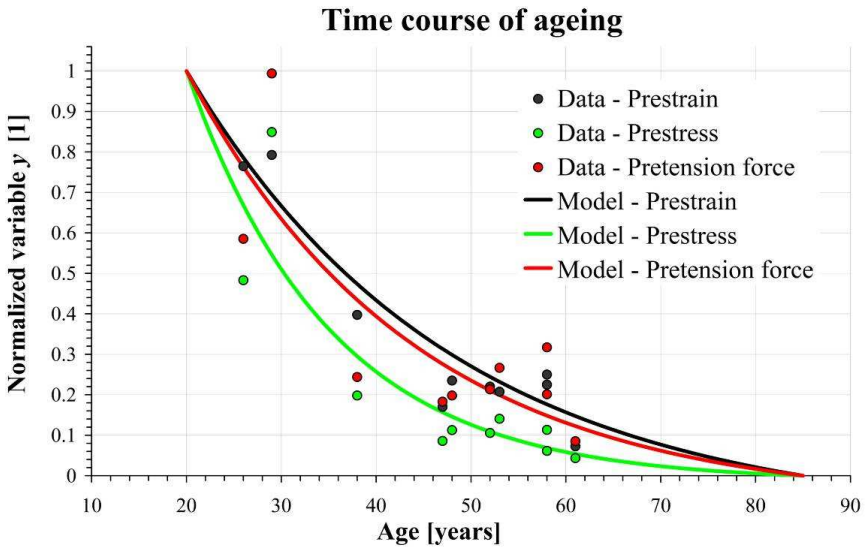

Figure 5 Normalized regression models for the effect of ageing on prestrain, prestress and pretension force. The models are based on the equation $y = c_0\left(1 - c_1 e^{-c_2 x}\right)$. The parameters were estimated as follows: c_0 = -0.1082, c_1 = 20.95, and c_2 = 0.0358 for normalized prestrain–age, c_0 = -0.0139, c_1 = 273.3 and c_2 = 0.0660 for normalized prestress–age, c_0 = -0.0711, c_1 = 34.68, and c_2 = -0.0417 for the normalized pretension force–age relationship. In order to obtain predictions in physical dimensions (non-normalized data), proportionality constants have to be included (they correspond to the values predicted for the age of 20 years); ε_{20} = 0.4, σ_{20} = 53 kPa, and F_{20} = 1.64 N.

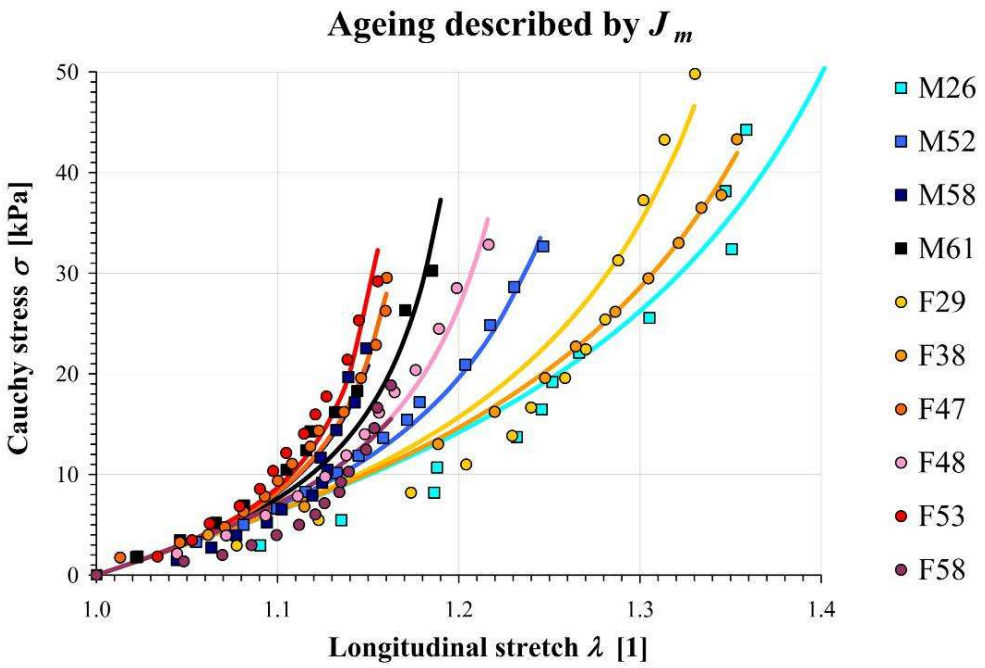

Figure 6 Limiting extensibility reflects the ageing effect. Infinitesimal shear modulus μ was fixed, and all ageing variability was left to be explained only by J_m. The model confirmed its ability to describe ageing, though the initial stresses for young donors seem to be rather overestimated (M26, F29, F38).

4. Discussion

Arteriosclerosis of elastic arteries is one of the most apparent manifestations of advanced age in the cardiovascular system. Elastic arteries enlarge their diameter and stiffen (Greenwald 2007; McEniery et al. 2007; O'Rourke 2007). This process can be attributed to several mechanobiological events at the molecular and cellular length scale. Among others, fragmentation and thinning of elastin lamellae (Arribas et al. 2006; Avolio et al. 1998; Bode-Jänisch et al. 2011; Fonck et al. 2009), which is coincident with increased matrix proteinase activity (Arribas et al. 2006; Jacob 2003), occurs in ageing. This affects the arterial elasticity, since elastin is the dominant load-bearing component under physiological loadings (less than 10% of collagen fibres are engaged in a normal physiological situation; Wagenseil and Mecham 2009; Greenwald et al. 1997). Increased calcium deposition is also usually observed (calcium damages elastic fibres – medial elasto-calcinosis; Atkinson 2008; Elliott and McGrath 1994). It can be accompanied by a change in cellular phenotype (transdifferentation of vascular smooth muscle cells to bone-like cells occurs; Persy and D'Haese, 2009). Non-enzymatic cross-linking caused by advanced glycation end-products also contributes to stiffening of the artery wall with cross-linking collagens and also elastin fibrils (Ansari and Rasheed 2009; Brüel and Oxlund 1994; Konova et al. 2004; Sherratt 2009). These changes may be viewed as the source of the mechanobiological perturbations which initialize remodelling and adaptation processes and result hand-in-hand in our empirical evidence of an enlarged diameter, a stiffened mechanical response, and loss of pretension.

In previous studies (Appendix A, B and C – Horny et al. 2011, 2012 a,b), age-related distribution of the axial prestrain has been shown in details. However, these studies did not address the question of prestress. More than 250 donors participated. Such a large number of samples was feasible mainly due to the simplicity of the measurement method. The autopsy protocol contained a regular measurement of the infrarenal circumference before the start of the study. However, it is logistically impossible to repeat such a large number of samples in laboratory prestress measurements. Only ten samples of abdominal aorta were selected for the purposes of studying the effect of ageing on prestress.

4.1 Prestretch, prestress, pretension force

The study confirmed that longitudinal prestretch decreases with advanced age, and the same finding was newly recorded for prestress and pretension force (after logarithmic transformation R = -0.946 for prestretch, -0.919 for prestress, and -0.800, for pretension force, with p_R<0.005 for all; the small data sample was taken into account). Prestress and pretension force were estimated ex vivo by an elongation test of a cylindrical sample of the infrarenal aorta. The highest force (1.63 N) was obtained for a 29-year-old female, and the lowest force (0.14 N) was for a man aged 61. The same samples also showed the highest and lowest prestretch and prestress (prestress/prestretch = 45kPa/1.32 and 2.3kPa/1.03).

The observed mean values for prestress and pretension force (11.6 kPa and 0.54 N) are somewhat smaller than the values reported in the literature. 50 kPa and 1 N were estimated by Han and Fung (1995) for porcine and canine aortas, while the estimate, obtained by the Dobrin-Doyle rule (see Dobrin and Doyle 1970) which is that "0.01 N/kg", gives 0.75N (taking 75 kg as the human body weight). The difference may be attributed to inter-species differences. In our specific case, however, lower values for the observed prestress and pretension force are affected by the age distribution in our sample (47/12; mean/SSD). However, it is more interesting to study an aged population because the results of P.B. Dobrin, J.M. Doyle, H.C. Han and Y.C. Fung can serve as estimates for physiological (non-aged) conditions.

4.2 Elastic modulus

The hyperelastic limiting chain extensibility model (3) was adopted to elucidate whether decreasing pretension is accompanied by decreased or increased stiffness (corresponding to the prestrained state of an artery), and the elastic modulus was calculated as a component of the elasticity tensor. It was found that the human abdominal aorta does not stiffen in its prestress–prestrain state (elongated under zero internal pressure). The correlations with age differed according to the description used for the elasticity tensor (material vs. spatial). Higher correlation coefficients were obtained for a spatial description (R = -0.566, p_R = 0.04 for raw data; and R = -0.632, p_R =0.03 after logarithmic transformation). Furthermore, the material description did not give significant results: R = -0.383 (p_R = 0.14) for the raw data; and R = -0.256 (p_R =0.24) for their logarithms. The difference between a material description and a spatial description originates in the transformation law, which has the form $c_{zzzz} = \lambda^4 C_{ZZZZ}$. Since ambiguous significance was obtained for the hypothesis of negative correlation with age, it was concluded

that the stiffness corresponding to a longitudinally prestretched aorta does not increase with age. This is a somewhat weaker formulation.

It is widely accepted that elastin is the most important load-bearing component in the vascular wall in initial and physiological loading (Wagenseil and Mecham 2009; Greenwald et al. 1997). Moreover, elongations of cylindrical samples of abdominal aorta (at zero transmural pressure) are accompanied by decreasing radius of the tubes (compressive circumferential strains). Hence, we can assume that collagen fibres (the main load-bearing component at supraphysiological loading), which are oriented predominantly in the circumferential direction (Haskett et al. 2010), were not operative within extensions up to $\lambda_{AUTOPSY}$ (this conclusion is only approximate because it neglects possible forms of load-carrying capacity other than tensile stiffness; e.g. bending stiffness of fibres and fibre-to-matrix interaction). In this case, the results may be attributed to the elastin component of the wall. Non-increasing stiffness of the elastin is in agreement with the interpretation of experiments given in Wuyts et al. (1995) (experiments performed by Langewouters et al. 1984). They presented an even stronger conclusion that the Young's modulus of elastin–smooth muscle complex decreases with age.

Our conclusion about non-increasing stiffness is valid, however, only for strain–state corresponding to the longitudinal prestretch (at zero pressure). Figs. 1–3 and 6 show clearly that a loading exceeding $\lambda_{AUTOPSY}$ generates steeply increasing stress–strain curves, where ageing is manifested by greater steepness of the curves. Thus non-decreased $\lambda_{AUTOPSY}$ would result in a highly stiffened response (consider equation (9) and a decreasing trend in J_m–age dependency).

4.3 Constitutive parameters

The character of the ageing effect is similar to the behaviour known from studies dealing with the circumferential response of arteries (Bader 1967; Nakashima and Tanikawa 1971). The stress–strain curves shift to the left and become steeper with advancing age. Parameter μ, which is responsible for the stiffness at infinitesimal strains, showed no demonstrable correlation with age (Table 2). This suggests that almost all age-related variability may be attributed to J_m, which is consistent with its interpretation as a measure of the limiting extensibility (Horgan and Saccomandi 2003, Ogden and Saccomandi 2007). Measured by J_m, the extensibility decreases with age; correlation R = -0.818 (p_R<0.002) was attained for the raw data and R = -0.793 (p_R<0.003) was attained for the logarithmic data. Note that that admissible deformations are given by $I_1 - 3 < J_m$ with $I_1 = \lambda^2 - 2/\lambda$ in our specific kinematics.

The observed progressive stiffening at deformations exceeding $\lambda_{AUTOPSY}$ cannot be related only to the elastin, because the extensions are supraphysiological. It is known that the adventitia (the outermost layer of the artery wall) contains collagen fibres helically wound

along the vessel axis (this is only the approximation because these fibres manifest rather high directional dispersion; Holzapfel et al. 2000, 2002; Gasser et al. 2006). We interpret the stiffening (when $\lambda > \lambda_{AUTOPSY}$) as their non-negligible engagement to the load–carrying process. It is obvious that at these deformations ageing is manifested by stiffened behaviour, which reflects the higher degree of collagen cross-linking and other ageing-induced changes in the collagens.

It should be noted that the application of equation (9), when $\lambda > \lambda_{AUTOPSY}$, gives a stiffness value which cannot be considered as direction-independent (isotropic), although isotropic strain energy function is used. Due to the engagement of collagen fibres, a loss of isotropy should be expected. In this case, our model will adequately predict the elastic modulus C_{ZZZZ} for the elongation test (because it corresponds to the longitudinal stress-strain curves fitted during modelling). However, the circumferential modulus may be different. For this reason we retain indices *ZZZZ* throughout the study, although this is not necessary for an isotropic model.

4.4 Normalized models of ageing-induced changes

To the best of our knowledge this study is the first to attempt to estimate the age corresponding to the total loss of longitudinal pretension. Apart from our data (two individuals with $\lambda_{AUTOPSY} = 1$ aged 83 and 95 years in the large sample), total loss of the pretension was experimentally confirmed by Schulze-Bauer et al. (2003) in aged human iliac arteries (one individual aged 81) and by Langewouters et al. 1984 (two individuals aged 85 years and 88 years) for human thoracic aorta. Both these publications even reported $\lambda_{AUTOPSY} < 1$ (longitudinal precompression). Bearing this in mind, and considering relatively frequent diseases such as aneurysms and tortuosity, we prescribed 85 years of age as the time when total loss of pretension may be expected. Figure 4 shows that the model incorporating this presumption gives some outliers (points exceeding a 95%-prediction interval). Although one could suggest shifting the loss of prestretch to a higher age (PI would be enlarged in this way), we note that our statistics is affected by the "sampling method". Our studies were primarily aimed at a study of individuals with well-defined prestretch, and aneurysmatic and tortuous aortas were excluded. Since known data gives 83 and 95 (our sample; these individuals had straight but non-prestretched aortas) and 85, 88 and 81 (from the literature), we hold the prescribed age (85 years) for loss of pretension as the first approximation, in spite of the outliers. Future studies will probably refine our result.

In fact, it is not known whether loss of prestress (prestretch and pretension force) is an inevitable consequence of ageing or a "rare" pathological condition. The power law regression models for prestress–age and pretension force–age are therefore also presented in this study (Table 1). These models reflect the situation (in contrast to the normalized models described by

equation (10)) when the hypothesis of inevitable loss of prestress is not accepted. These models decrease progressively with age, similarly to models based on (10), but they only asymptotically approach zero. Nevertheless, they were also helpful due to their simple linearization within hypothesis testing. It is worth noting that very little is known about longitudinal prestretch in aneurysmatic aortas (see a recent review by Humphrey and Holzapfel, 2012). We presumed that the development of an aneurysm is accompanied by total loss of the pretension (due to loss of elastin). Future studies, however could show that there is some threshold in the longitudinal force (likely very small but non-zero) sustained by an artery below which aneurysm formation can initiate. In this situation, an asymptotical approach to zero would be more appropriate.

Equation (10) for a normalized description of ageing was chosen with respect to a phenomenological analogy with a discharging capacitor and Newton's law of cooling. This means that the governing equation can be written in the form $dy/dt = c_2(c_0 - y)$. We note that negative values of c_0 (see the legend for Fig. 5) are the consequence of the prescribed zero-prestretch age.

A similar idea, a simulation employing a time rate of the longitudinal prestretch, was recently presented in Tsamis et al. (2011), who studied the evolution of age-related changes in conduit arteries. They hypothesized that ageing-induced damage to elastin fibers (fragmentation and thinning) initializes a remodelling of the arterial geometry. Ageing-induced damage can result from an elastolysis, as mentioned above, with a concurrent contribution from material fatigue due to the remarkable number of pressure pulse cycles that elastic arteries have to sustain throughout the entire lifespan (Greenwald 2007; Hodis and Zamir 2009; Lillie and Gosline 2007; O'Rourke 2007). The longitudinal prestrain is mainly carried by elsatin (in animal models, elastin-insufficient individuals do not develop prestrained elastic arteries, the arteries usually become tortuous; Wagenseil et al. 2009, 2010). It is therefore hypothesized that herein presented observation of decreasing longitudinal prestretch is also a consequence of ageing-induced damage to the elastin. Considering that longitudinal prestress (prestrain) is developed simultaneously with the elastin as a reaction to rising blood pressure to optimize the longitudinal motion of an artery (to be almost negligible within the cardiac cycle under physiological conditions), some longitudinal force (pretension force) is originated in the system. After post mortal removal of the blood pressure, this force still acts in the body and autopsy retraction of excised arteries is a consequence of it.

The decrease in longitudinal prestress can be explained by an age-related increase in the cross-sectional area (observed in our study and in many others, Table 1). Increased cross-section area gives lower prestress (at the same force, because the number of elastin lamellae does not increase when the growth period is finished). The lower prestress would certainly induce a decrease in the prestrain. But what is the source of the decrease in the pretension force observed

in the experiments? The paradigm states that an artery remodels to reduce the stress, not the force.

This force can, however, be released in permanent deformation of the elastin membranes (disruption, fragmentation), which is then macroscopically observed as a lengthened ex situ geometry (reported in details in Horny et al. 2012b – Appendix C). This leads to the hypothesis that decreased longitudinal prestretch originates in ageing-induced damage to elastin. However, at current state of knowledge it is not possible to say that this is the sole cause of the observed phenomena. Further studies are needed to distinguish between the potential roles of remodelling and elastin degradation. The role of the perivascular tissue is also unclear (we suppose that during the developmental period the pretension force is imprinted on external bonds which hold the aorta in its position, because all tissues and organs develop simultaneously). Perivascular–vascular interaction, sometimes called tethering, is frequently neglected in the literature. Thus we have no evidence on how ageing affects this bond. Theoretically, it is possible that there are some age-related changes to this interaction which may contribute to a decrease of the longitudinal preload.

4.5 Consequences for the in vivo state

In experimental (ex vivo but in situ) inflation of a canine carotid artery, Dobrin and Doyle (1970) found that the total longitudinal stress σ_{zz} increased only slightly up to circumferential (engineering) strain 0.15. This was due to a simultaneous decrease in the pretension force (they held a constant artery length during inflation, and measured the force necessary to do this) and an increase in the force induced by internal pressure P acting at the end of a closed vessel. The equation $\sigma_{zz} = \sigma_{zzP} + \sigma_{zzF} = Pr_i^2/(r_o^2 - r_i^2) + F/(\pi(r_o^2 - r_i^2))$ describes this situation; here r_o and r_i denote the outer and inner deformed radius. Inflation–extension experiments were not conducted in present study, and thus specific (numerical) values of the total longitudinal stress cannot be described. The equation, however, enables the situation to be discussed qualitatively. $\sigma_{zz} = \sigma_{zzP} + \sigma_{zzF}$ operates with the force F which we measured in its initial value (before pressurization). This initial value corresponds just to our pretension force. Considering that this force was proved to be decreasing during ageing, it implies that the term $\sigma_{zzF} = F/(\pi(r_o^2 - r_i^2))$ can approach 0 at the initial conditions (no prestrain = no prestress = no F). Thus the favourable property: $\sigma_{zzF} = F/(\pi(r_o^2 - r_i^2)) \rightarrow 0$ during pressurization, which ensures almost constant axial stress within the cardiac cycle, may be lost. This suggests that the pattern of the longitudinal stress will be governed by $\sigma_{zz} = \sigma_{zzP} = Pr_i^2/(r_o^2 - r_i^2)$.

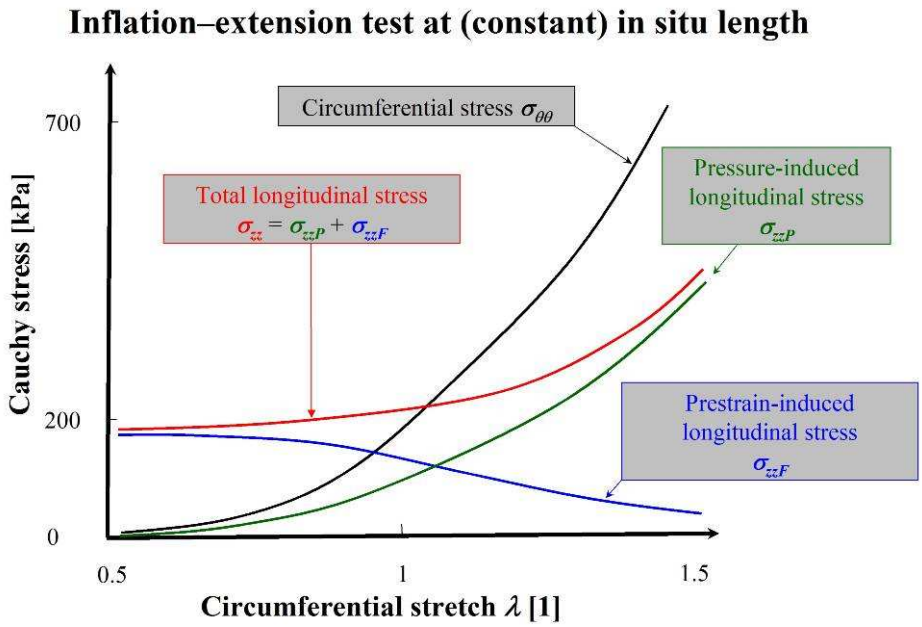

Figure 3 For convenience the figure is here reproduced. Longitudinal wall stress during pressurization. Adapted from Dobrin and Doyle 1970. Total longitudinal stress is induced by blood pressure acting at closed end of the sample σ_{zzP}, and from pretension force σ_{zzF}. Note that the artery is longitudinally prestretched (radius decreased), thus circumferential stretch does not begin at $\lambda = 1$. Compare the gradient of red curve (total axial stress) with the gradient of green curve (pressure-induced stress). The difference results from blue curve (effect of longitudinal prestress).

Thus the pattern of the longitudinal stress will mimic the variations in the blood pressure during the cardiac cycle. This variation of the longitudinal stress may be the reason for the longitudinal oscillations observed in vivo by Cinthio et al. (2006) and Tozzi et al. (2001).

4.6 Sources of errors

Simple elongation of cylindrical samples was used to estimate the pretension force and prestress. For estimating the longitudinal prestretch only the results of the autopsy measurement can be used (due to the excision of a sample, which would be unconceivable in living persons). The experiments were therefore performed without pressurization of the artery. However, this results in a deformation process that is susceptible to warping or local non-uniformity. This is the main source of measurement errors. Samples displaying obvious violation of the assumption of cylindricality during the deformation were excluded. However, with no acting pressure inside the vessel the effect of the local geometrical non-uniformities (branching points of small arteries) is stronger than in conventional inflation–extension tests.

In the modelling, some assumptions were made which idealized complex reality. To avoid misinterpretation of the results, we note the most important of them: thin-walled approximation; incompressibility; negligible engagement of collagen fibres during deformation up to $\lambda_{AUTOPSY}$; homogenization of the cross-section area (the presented mechanical response of the arteries was ascribed predominantly to the elastin, since elastin is mainly responsible for pretension, but the calculations were preformed for the total cross-section area). In particular, the numerical values of the material parameters estimated during presented experiments may not be appropriate to the kinematics resulting in non-negligible engagement of collagen fibres (e.g. an inflation–extension test). The author plans to study this generalization in future work.

The circumference of the artery was identified as the length of the ring. By cutting the ring, a circumferential residual stress is released, and this can result in a discrepancy between the circumference and the length of the ring. Bearing this in mind, we have to state that only approximations of the stresses are presented in this study.

5. Conclusion

It is concluded that the longitudinal pretension force and the prestress, as well as the longitudinal prestretch, decrease significantly during ageing. The longitudinal stiffness related to the prestretch of a human abdominal aorta observed in autopsies did not increase with advancing age. However, the overall mechanical response to longitudinal tension was characterized by significant stiffening, which was qualitatively similar to the behaviour known for the circumferential direction. Constitutive modelling suggested that limiting chain extensibility is a concept suitable for describing the ageing effect.

All these results are original and extend our knowledge of the physiology of conduit arteries in ageing. The author considers that objectives of the thesis have been met.

The present study has also revealed that further research is needed to elucidate to what extent the age-related decrease in longitudinal prestress, prestrain and related force is induced by remodelling, by damage to the elastin and by changes in the perivascular–vascular tissue interaction. Computational simulations employing age-related evolution of constitutive models and artery remodelling can be helpful for this purpose (Alford et al. 2008; Cardamone et al. 2009; Tsamis et al. 2011; Valentín and Humphrey 2009; Valentín et al. 2011). They can incorporate herein presented results as an estimate of the lower limit for the function of artery wall elastin (the functioning of elastin should not be worse than the observed normalized evolution of the prestrain; it can, however, be better if other factors contribute to a decrease in the prestrain). The data can also be incorporated in an in vivo determination of constitutive parameters. This highly promising approach (aiming directly to living persons and theoretically capable to offer patient-specific constitutive model) has to presume some reference and intermediate geometry and related boundary conditions (prestrain and prestress). Prestrain and prestress cannot, however, be accessed in living persons. Prestress and prestrain therefore have to be estimated in ex vivo studies such as this (Åstrand et al. 2011; Masson et al. 2011; Stålhand and Klarbring 2005; Stålhand 2009).

The author will be glad if readers go on with following appendices (A, B, C) which deal with detailed description of the forensic-anthropological applications of longitudinal prestretch in artereis.

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Appendix – A

Age-related Distribution of Longitudinal Pre-strain in Abdominal Aorta with Emphasis on Forensic Application

has been appeared in *Forensic Science International* 214(1-3):18-22.
doi: 10.1016/j.forsciint.2011.07.007

Abstract – It is a well-known fact that the length of an artery in situ and the length of an excised artery differs. Retraction of blood vessels is usually observed. This pre-tension plays crucial role in arterial biomechanics. It augments an artery wall load-bearing capacity. This paper presents the longitudinal pre-strain of the human aorta as an index of human age. The length of abdominal aortas was measured during autopsies before and after segment resection. The longitudinal pre-strain was calculated in 130 donors; 100 male and 30 female bodies. The pre-strain was defined as the ratio between in situ length and the length after the excision. The mean pre-strain was found to be 1.18±0.10 for male and 1.14±0.10 for female sample (mean ± standard deviation). The age in the male group was 41.6±15.9 years; and 47.7±17.7 years in the female group. Statistical analysis revealed the correlation coefficient between age and pre-strain R =-0.821 and R =-0.839 in male and female group, respectively. The analysis also confirmed close correlation between aortic circumference and age; and between circumference and pre-strain. Linear and power law regression equations were employed and prediction intervals were computed. The power law estimates the age more accurately than linear one model. Nevertheless, especially for small values of the pre-strain (aged individuals) the linear model can be advantageous.

Introduction

The estimation of the age of cadavers of unknown identity is one of the first steps in forensic identification. There are several recommended methods. They can be divided into radiological examination of dental or skeletal development, morphological examination of teeth and skeleton and biochemical analysis based on aspartic acid racemisation rate [1]. The accuracy of the above mentioned methods varies in children and adults and is the most important criterion in forensic practise. Radiological methods are accurate in children and can be used not only for cadavers but also for archeological cases and the living [2]. In adults, aspartic acid racemisation rate in dentine is the gold standard for age estimation, if teeth are available [3,4]. In case teeth are not available, accurate and reproducible results can be obtained by analysis of purified long-living proteins from other tissues [5,6,7].

Age estimation morphological methods are currently used especially for burnt bodies because the racemisation process of aspartic acid is highly temperature dependent [8]. There are also macro-morphological and histo-morphometrical methods, e.g. evaluation of pubic symphyseal and rib ageing patterns and evaluation of bone histology [9,10]. Most of these methods require experienced scientists and/or adequate laboratory equipment.

To the best of author's knowledge nobody yet considered changes in biomechanical properties of tissues in forensic age estimation. The main goal of the present study is to illustrate that the longitudinal pre-strain of the abdominal aorta is suitable and easily obtainable quantity for this purpose. This phenomenon now will be briefly reviewed.

The non-pressurized artery is not in a stress-free state. This can be confirmed within excision of a tubular segment of an artery from a body; retraction of the sample is usually observed [11-14]. Bergel [11] reported mean shrinkage ranged between 32% - 42% (percentage of original length) for canine samples depending on the position in the arterial tree. Han and Fung [12] confirmed this result when reported monotonically increasing longitudinal pre-strain for canine and porcine aortas from 1.2 to 1.5 with increasing distance from the heart. Learoyd and Taylor [13] performed measurement with 59 samples of arteries obtained from 12 human donors. Their results proved position dependence of the pre-strain of human arteries and also suggested a negative correlation between the age and the retraction. Some studies performed with human subjects gave significantly smaller values of the pre-strain; Schulze-Bauer et al. [14] reported longitudinal pre-strain in aged iliac arteries 1.07±0.09 (mean ± standard deviation).

Different authors call the same phenomenon different names – retraction, pre-stretch, pre-strain. It depends on the used reference configuration of an artery. In what follows we avoid the term "retraction" and only "pre-strain" will be used. This is defined as the ratio between the length in situ of the sample and the length of the sample measured after it was removed from a body.

The longitudinal pre-strain generates the longitudinal pre-stress remaining in an artery after a deflation of the blood pressure. This way generated pre-tension prevents an artery from a tortuosity [15,16]. Moreover, longitudinal pre-strain gives the possibility to carry the pressure pulse wave along an artery without significant change in axial force in the wall [14,17,18,19]. This is advantageous from a mechanical point of view.

The longitudinal pre-strain originates in the biological structure of an artery wall where different cells (smooth muscles, fibroblasts) and matrix proteins (collagen, elastin, and proteoglycans) interact together. They are subjected to growth, remodeling and ageing processes [15,16,20-26]. This results in age-dependent pre-tension of an artery. Our study maps age-dependency of the longitudinal pre-strain in the abdominal aorta and shows that it can be used in forensic age estimation. It also elucidates correlations between other characteristics of cardiovascular system such as aortic circumference, thickness of left ventricle, heart weight, and the degree of atherosclerosis.

Materials and methods

Measurements of the pre-strain in segments of the abdominal aorta were performed in the Department of Forensic Medicine of the Third Faculty of Medicine, Charles University and University Hospital Na Královských Vinohradech in Prague. Postmortem use of human tissue was approved by the Ethic committee of the University Hospital Na Královských Vinohradech in Prague.

Data sample was collected from 130 donors; 100 Caucasian male and 30 Caucasian female individuals. No putrefied bodies were involved. Age [years], longitudinal pre-strain [dimensionless], postmortem interval (PMI) [hour], circumference [cm], stature [cm], heart weight [gram], the thickness of left ventricle [cm], and degree of atherosclerosis [dimensionless] were documented. The thickness of the left ventricle was measured just above the anterior papillary muscle. The degree of atherosclerosis was quantified in the scale from 0 up to 4 according to the morphologic features: 0 – normal artery + fatty streaks; 1 – fibrofatty plaques; 2 – advanced plaques; 3 – calcified plaques; 4 – ruptured plaques [27].

Longitudinal pre-strain. The abdominal aorta was thoroughly preparated during autopsy and two markers were made with permanent ink just below the origin of renal arteries and just above the bifurcation into the iliac arteries. The measurement of the size has been performed two times in situ and then immediately after the excision, using a ruler. Longitudinal pre-strain, λ, was defined by (1A).

$$\lambda = \frac{l}{L} \tag{1A}$$

Here l denotes in situ length and L is the length after removal from the body. This definition of the pre-strain is in accordance with [10,12]. The relative retraction mentioned in [9] is obtained as $(\lambda - 1)/\lambda$.

Subsequently a ring was cut off from the aortic segment. This ring was then cut to the strip and the circumference of aortic segment was determined as the length of this strip. The measurements of the length were performed two times.

Correlation. Statistical dependence of all documented quantities was evaluated via correlation analysis. It was based on the simple correlation coefficient R defined in (2A).

$$R = \frac{\sum_{i=1}^{n} \left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)}{\left(n-1\right) s_x s_y} \tag{2A}$$

Here n is the number of observations, s_x and s_y denote sample standard deviations of quantities x and y. Mean values are written with bands.

Regression analysis. Linear regression equation (3A) was suggested in order to employ λ as the age estimator.

$$y = A_j \lambda + B_j \tag{3A}$$

Here the estimated age [years] is denoted y. Regression parameters A_j, B_j, were determined using least square algorithm. Male and female data are distinguished with index j ($j = M$, and F).

Linear regression model was chosen to keep simplicity. After preliminary computations it was, however, decided to employ also power law model for age–pre-strain equation. Particular form of the model is expressed in (4A). C_j and D_j (j=M, F) are the model parameters.

$$y = C_j \lambda^{D_j} \tag{4A}$$

The power law model was transformed by logarithmic transformation into the linear problem and then optimized with least squares algorithm.

Comparison of the models. Two ways were approached in the models' comparison. First comparison was obtained making use of the correlation coefficient. In case of the linear model it is the same as in (2A). The correlation coefficient for the power law model was obtained from (2A) after the logarithmic transformation. A correlation coefficient, however, can only indicate the character of statistical relationship nay predictive capability of the model.

Predictive capability evaluation was based on so-called prediction intervals (confidence intervals for model prediction). It means the range in which future observation of the

dependent variable will fall with probability equal to α (α = confidence level). Complete regression model, with implemented prediction intervals, can be written in the form (5A).

$$y = y_R \pm t_{\frac{\alpha}{2}}(m) S_e \sqrt{1 + \frac{1}{n} + \frac{(x - \bar{x})^2}{S_{xx}}} \tag{5A}$$

Here y is predicted variable and x is independent variable. y_R denotes regression equation. $t_{\alpha/2}(m)$ is the quantile of Student's t-distribution with m degrees of freedom (here $m = n - 2$, where n is the number of observations). S_{xx} and S_e are defined with equations (6A) and (7A).

$$S_{xx} = \sum_{i=1}^{n} (x_i - \bar{x})^2 \tag{6A}$$

$$S_e = \sqrt{\frac{1}{n-2} \sum_{i=1}^{n} (y_i - y_{Ri})^2} \tag{7A}$$

In (8) y_{Ri} denotes model prediction for i-observation. The logarithmic transformation was again used to linearize power law model. Confidence intervals for model parameters were also computed. $\alpha = 0.95$ was considered through entire study.

Results

Data summary for male and female group is presented in Table 3. Female autopsy data were available with complete documentation. Male data, however, were incomplete in five cases. Therefore samples in male population range from 95 to 100.

Correlation. Results of correlation analysis are presented in Table 4. Eight investigated variables generate 28 untrivial correlation coefficients. Some of them, however, do not seem to be worthwhile; e.g. correlation between height and post mortem interval. Thus only ten highest correlation coefficients in each group are reported.

Both populations (male and female) give the highest correlation between age and circumference of abdominal aorta; $R_M = 0.898$ and $R_F = 0.893$ (M and F denote the population). This is followed by age and pre-strain correlation; $R_M = -0.821$ and $R_F = -0.839$. While the circumference increases with the age, longitudinal pre-strain decreases. This is common to both populations.

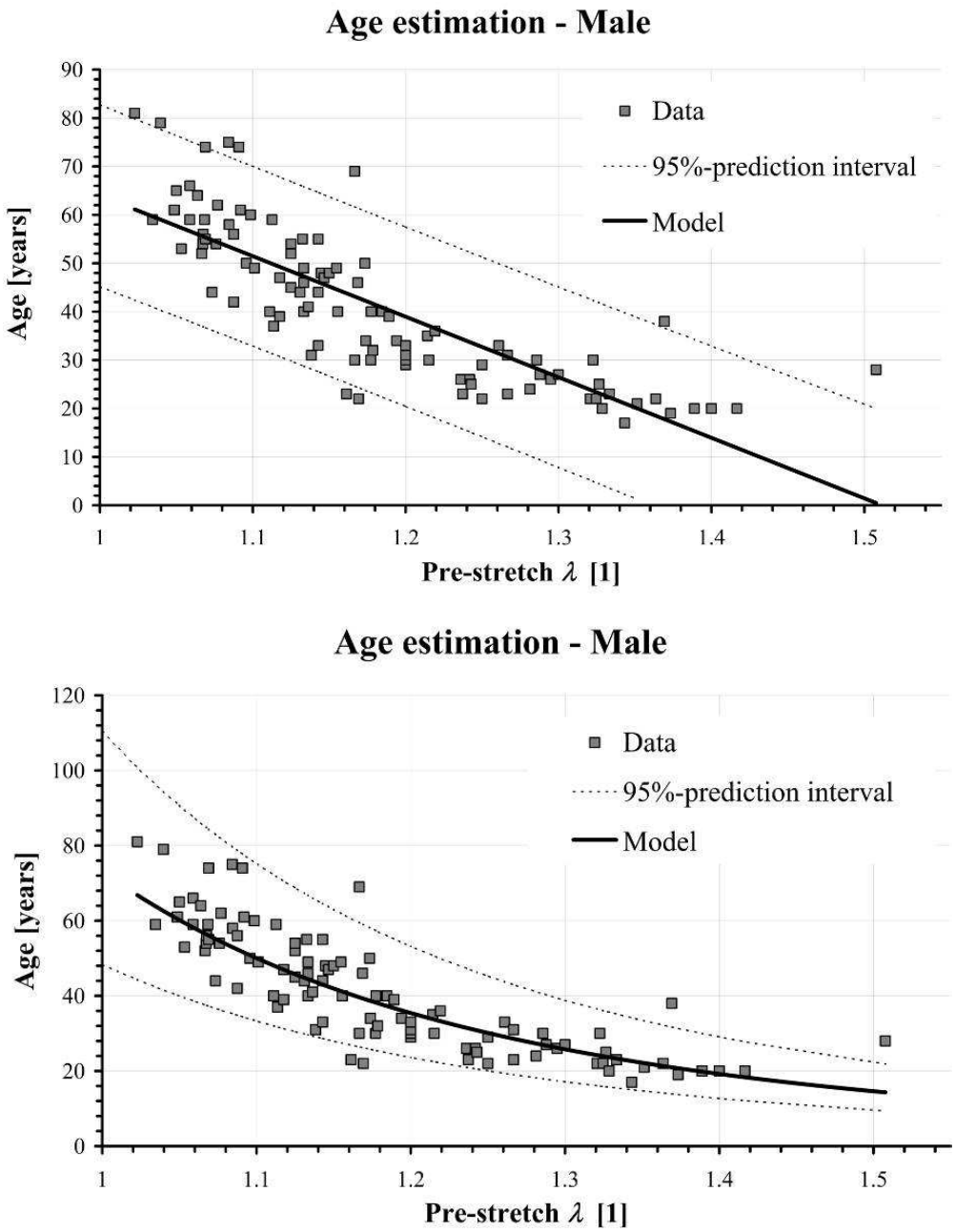

Fig. 15 The age estimated with the linear regression model (upper) and with power law (bottom) for male population. Following symbols are used: solid-box as observation points; solid line as the regression model; and dashed curves as limits of prediction intervals ($\alpha = 0.95$). The presence of outliers (especially for small pre-strains) in the linear model suggests that power law is more suitable. The correlation coefficient is R = -0.821 and -0.860 for linear and power law, respectively.

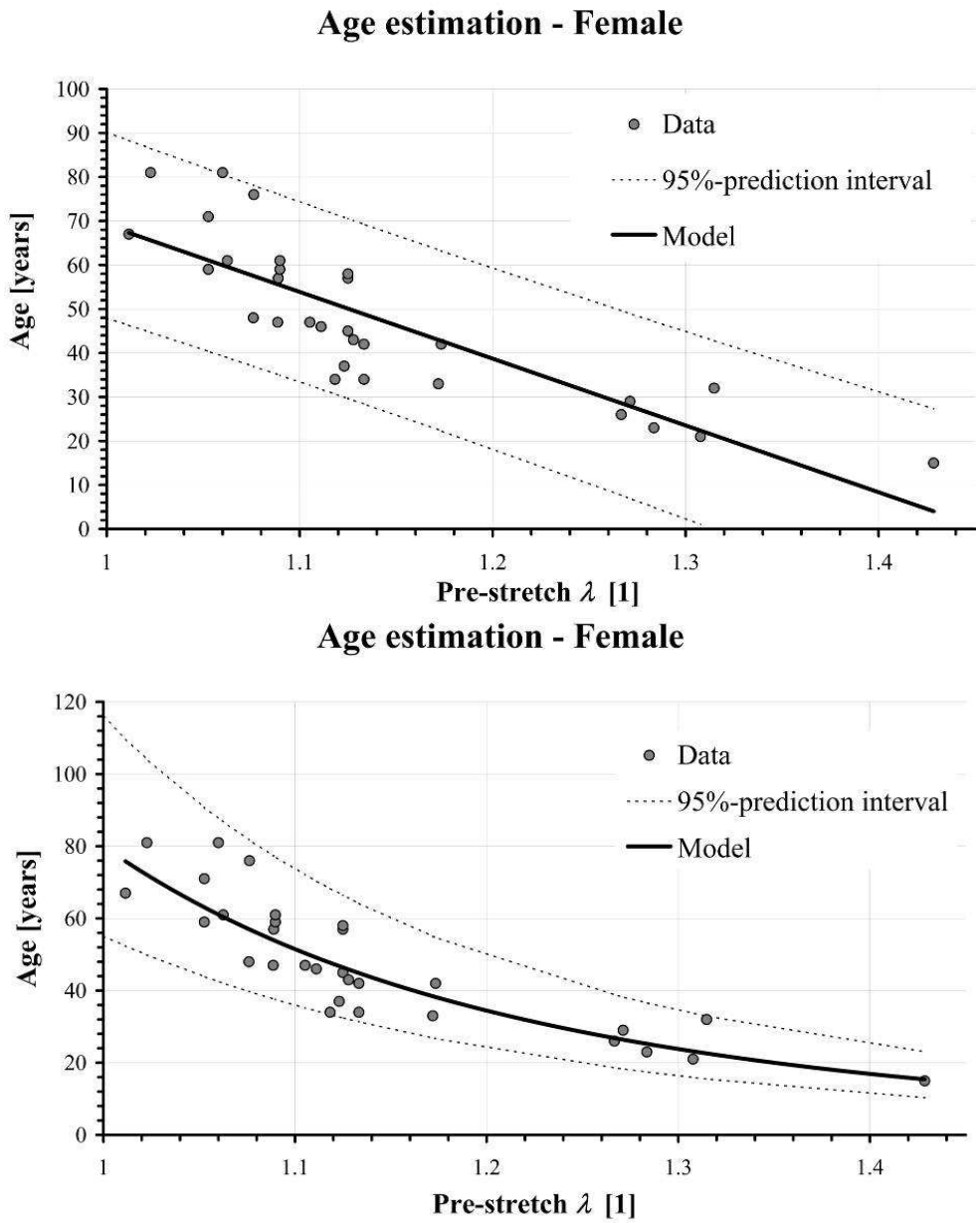

Fig. 16 The age estimated with linear and power law model for female population. Following symbols are used: solid-box as observation points; solid line as the regression model; and dashed curves as limits of prediction intervals ($\alpha = 0.95$). The correlation coefficient is R = -0.839 and R = -0.913 for linear and power law, respectively.

In what follows male and female data give different order of correlations. Male data indicate tight correlation between age and atherosclerosis (R_M = 0.768), circumference and arthrosclerosis (R_M = 0.765), and circumference and longitudinal pre-strain (R_M = -0.750). Also the correlation between pre-strain and atherosclerosis should be mentioned (R_M = -0.693), however, statistical dependence weakens rapidly.

Contrary to male population, female data reveal tighter dependence between longitudinal pre-strain and circumference (R_F = -0.802). Also age and degree of arthrosclerosis, and circumference and arthrosclerosis give noticeable correlations (R_F = 0.763 and R_F = 0.668, respectively). It is worth noting that the correlation between longitudinal pre-strain and degree of atherosclerosis falls in female population to R_F = -0.522, which is in contrast to R_F = -0.693 in male population.

Regression analysis. Obtained regression models are presented in Fig. 15, 16. Decreasing trend of longitudinal pre-strain within ageing is clear. Power law models, which were computed after the logarithmic transformation, gave additional correlation coefficients; R_M = -0.860 and R_F = -0.913. They are both higher then obtained in simple linear computations. It strongly suggests that nonlinear model is more suitable than linear one.

The prediction intervals computed with confidence level 0.95 are also presented (Fig. 15, 16). Their widths vary with change in independent variable. It is evident that the power law model gives narrower range of predicted value. Thus, such a way the age can be predicted more reliably. For example mean longitudinal pre-strain gives prediction intervals in the linear model 23.1÷60.1 years; and 27.9÷64.9 years for male and female sample, respectively. Nevertheless, the power law model gives 25.3÷57.0 years; and 30.5÷62.3 years in male and female sample, respectively.

Table 3 Summary of documented data; mean ± standard deviation. Following abbreviations were used: AGE – age [years]; PRESTR – longitudinal pre-strain [-]; CIRC – abdominal aortic circumference [cm]; HGHT – height [cm]; HWGHT – heart weight [cm]; TLVENT – thickness of left ventricle [cm]; DEGATHR – degree of atherosclerosis [-]; PMI – post mortem interval [hour]; n – number of observations; M – male; and F – female. The degree of atherosclerosis is characterized rather with the mode (the most frequent observation) than with the arithmetic mean; arithmetic mean gives 1.54 and 1.37 in male and female population, respectively.

	n	M	n	F
AGE	100	41.6±15.9	30	47.7±17.7
PRESTR	100	1.18±0.10		1.14±0.10
CIRC	99	4.0±0.7		3.7±0.6
HGHT	100	179±7		168±5
HWGHT	95	417±119		359±97
TLVENT	96	1.4±0.3		1.3±0.3
DEGATHR	97	0#		0#
PMI	100	42±25		40±20

Table 4 Ten highest correlations ordered decreasingly. Data reveal significant correlation between age, pre-strain and abdominal aortic circumference. The abbreviations are described in the caption of Table 3.

Correlation coefficient			
Male		Female	
AGE CIRC	0.898	AGE CIRC	0.893
AGE PRESTR	-0.821	AGE PRESTR	-0.839
AGE DEGATHR	0.768	PRESTR CIRC	-0.802
CIRC DEGATHR	0.765	AGE DEGATHR	0.763
PRESTR CIRC	-0.750	CIRC DEGATHR	0.668
PRESTR DEGATHR	-0.693	HWGHT TLVENT	0.591
CIRC HWGHT	0.501	AGE HWGHT	0.564
AGE HWGHT	0.471	CIRC HWGHT	0.558
HWGHT DEGATHR	0.444	HWGHT DEGATHR	0.544
HWGHT TLVENT	0.335	PRESTR DEGATHR	-0.522

Table 5 Estimated model parameters.

A_M	B_M	A_F	B_F
-125.0	189.0	-151.5	220.6
-143÷-107	168÷210	-190÷-114	177÷264
C_M	D_M	C_F	D_F
73.05	-3.968	79.94	-4.614
67.0÷79.6	-4.44÷-3.50	70.9÷90.2	-5.41÷-3.82

Discussion

The main aim of our study was to investigate the possibility of the estimation of the age based on the longitudinal pre-strain of the abdominal aorta. To this end measurements of the longitudinal pre-strain with 100 male and 30 female subjects were carried out within autopsies. Results have shown that tight correlation between the age and pre-strain exists (R_M = -0.821 and R_F = -0.839). The longitudinal pre-strain decreases with increasing age. The results of the regression analysis and the width of prediction intervals suggest that the power law model is more suitable to fit observed relationship than linear one.

Correlation. Our data confirmed previous observations of Learoyd and Taylor [13] that negative correlation between the pre-strain and the age exists. Langewouters et al. [28] published the list of the retractions found in 20 human abdominal and 45 thoracic aortas. To compare the results with [28] we computed correlation between age and longitudinal pre-strain based on their list. R = -0.790 in abdominal and R = -0.786 in thoracic aorta were obtained. It confirms our observation although our sample gives higher correlation coefficient.

Even higher correlation coefficients were obtained for circumference–age dependence; R_M = 0.898 and R_F = 0.893. This fact is in accordance with Stefan and Josifko [29] who found the aortic circumference to be suitable indicator of the human age. Because both the age-related increase in the circumference and the decrease of longitudinal pre-strain can be considered as the results of ageing process it is not surprising that the study revealed their tight inter-correlation; R_M = -0.750 and R_F = -0.802. Also the correlation between the age and the degree of atherosclerosis should be presumed; R_M = 0.768 and R_F = 0.763. Rather surprising fact is low (with respect to previous values) correlation obtained for dependences between the age, heart weight, and thickness of the left ventricle. It suggests the heart weight and thickness of the left ventricle are more dependent on pathologic changes or physical training then ageing.

Regression models. The correlation coefficient computed after the logarithmic transformation of the data (R_M = -0.860 and R_F = -0.913) indicates that the power law model is more suitable. It is also supported by the width of prediction intervals (see Fig. 15, 16). The power law model, naturally, gives the widths of prediction intervals variable with measured pre-strain. Nevertheless, they are significantly shorter than in the linear model for pre-strains higher approximately than 1.1. It implies, however, that for elderly subjects the linear model can be advantageous because of gradual increase of the length of prediction interval caused by nonlinearity.

To the authors' knowledge nobody yet considered longitudinal pre-strain as the age estimator. Thus the possibility of comparison with literature is limited. The most precise methods for age estimation in adults based on amino acid racemization can determine the age at death of an individual to ±1.5-4 years with correlation coefficients 0,97-0,99 [1]. Evaluation of skeletal morphology is less accurate and standard errors are ±2-12 years with correlation coefficients 0,60-0,90 [1].

Stefan and Josifko [29] proposed the regression model that predicts the age by means of aortic circumference. Our model can be simply extended with this idea. To keep brevity and also to track only one key idea it is omitted here. Nevertheless, it should be noted that the inter-correlation between age and longitudinal pre-strain was found. This implies that such a model would probably operate with mutually dependent variables.

Biomechanical context. The change of the longitudinal pre-strain is linked to remodeling and adaptation processes (e.g. to hypertension) or other phenomena associated with ageing (e.g. glycation). If the hypothesis of approximately constant longitudinal load in an artery through adulthood is accepted then decreased value of longitudinal pre-strain seems to be explainable with overall stiffening of arteries in increased age. This stiffening was reported widely [30,31]. Alternatively, the wall thickening and increasing of arterial diameter restore the wall stress within a remodeling. Under unchanged material properties it may also lead to a decrease in arterial pre-strain.

However, the biomechanics of the blood vessel wall is determined by its constituents. Histological and biomechanical investigations carried out by Dobrin et al. [32] and Greenwald et al. [33] proved that the amount of longitudinal pre-strain and residual strain correlate closely with the presence of elastin within the artery wall. The study performed with an animal model (haploinsufficiency in elastin) also revealed decreased value of the longitudinal pre-strain in abdominal aorta [34]. It implies the decreasing of longitudinal pre-strain could be attributed to age-related degradation and fragmentation of elastin. The decision between the mentioned hypotheses is still in question and requires detailed studies which will reflect the phenomenon of time-varying longitudinal pre-strain in living subjects.

Conclusion

We conclude that longitudinal pre-strain–age relationship is suitable to be used as a quick and easy preliminary step in estimating the age in forensic practice. The power law estimates the age more accurately than linear one. Nevertheless, especially for small values of the pre-strain (aged individuals) the linear model can be advantageous.

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Appendix – B

Correlations between Age, Prestrain, Diameter and Atherosclerosis in the Male Abdominal Aorta

has been appeared in *Journal of the Mechanical Behavior of Biomedical Materials* 4(8):2028-2132
doi: 10.1016/j.jmbbm.2011.07.011

Abstract – The longitudinal prestrain of arteries facilitates their physiological function. Remodeling, adaptation and aging result in an age-dependent magnitude of the pretension. Although the phenomenon is known, detailed statistics, especially for human arteries, are lacking. This study was designed to propose the regression model capable of estimating the prestrain of the human abdominal aorta. The length of the abdominal aorta before, l, and after excision from the body, L, the diameter, heart weight, thickness of left ventricle and degree of atherosclerosis were collected in autopsies of 156 male cadavers of known age. Longitudinal prestrain was quantified by means of the stretch ratio $\lambda = l/L$. Statistical analysis revealed significant dependence between age, prestrain, diameter and atherosclerosis, which were best fitted to the power law equation. Longitudinal prestretch reduced with age significantly; λ_{mean}=1.30±0.07 for age <30 (n=29), whereas λ_{mean}=1.06±0.03 for age >59 (n=31) with p-value <0.0001. Raw data gave linear correlation coefficients as follows: λ–age (R=−0.842); l–age (R=0.023); L–age (R=0.476); (l–L)–age (R=−0.811). It was concluded that longitudinal prestrain decreases nonlinearly with age and both age and diameter are suitable predictors of the prestrain. Data suggests that unloaded length elongates with age in contrast to the elastic retraction.

Introduction

It has been known for a long time that arteries in vivo undergo significant longitudinal prestrain; they retract upon excision 0–50% (Bergel, 1961; Learoyd and Taylor, 1966; Schulze-Bauer et al., 2003; Sommer et al., 2010; Han and Fung, 1995; Langewouters et al., 1984). Axial prestrain is advantageous from a biomechanical viewpoint. Inflation-extension experiments showed that under a certain value of axial strain arteries can be pressurized with no change of their length (Schulze-Bauer et al., 2003; Sommer et al., 2010). Under such conditions the pressure pulse wave can be transmitted along an artery without significant change in axial force (Van Loon et al., 1977).

The magnitude of the in situ prestrain was proved to be species and location dependent. It increases with increasing distance from the heart (Han and Fung, 1995). Results suggest that this dependency could be explained by means of the intramural collagen-to-elastin ratio (Humphrey et al., 2009). The crucial role of elastin was proved by enzyme digestion and in animal models with an elastin insufficiency (Dobrin et al., 1990; Wagenseil et al., 2009).

Axial prestrain plays important role in the remodelling and adaptation of arteries (Humphrey et al., 2009; Cardamone et al., 2009; Lawrence et al., 2009; Hayashi and Naiki, 2009). Significant lengthening during increased blood flow was observed (Lehman et al., 1990). Elevated axial load also led to lengthening via increased cell proliferation and matrix synthesis (Jackson et al., 2002; Han et al., 2003).

Thus magnitude of the prestrain changes during the lifespan (Learoyd and Taylor, 1966; Langewouters et al., 1984). However, detailed description of the age-dependency, especially in humans, is lacking in the literature (Zulliger and Stergiopulos, 2007).

Direct measurement of the axial prestrain in living individuals is problematic due to the requirement of a sample resection. Another method which could determine the prestrain is therefore desirable. The aim of this study is to find a regression model for longitudinal prestrain estimating.

Methods

Data describing the in situ and excised lengths of the male abdominal aorta, as well as the diameter (D), age, degree of atherosclerosis (ATH), thickness of the left ventricle, heart weight (HW), height and post-mortem interval (PMI, time span between the death and autopsy measurement) were collected during regular autopsies of Caucasian cadavers of known age. Post-mortem usage of human tissue was approved by the Ethics Committee of the University Hospital Královské Vinohrady in Prague. No putrefied bodies were involved. The degree of atherosclerosis was examined by the pathologist and quantified in a scale from 0 up to 4

according to morphological features: 0 – intact artery and fatty streaks; 1 – fibro-fatty plaques; 2 – advanced plaques; 3 – calcified plaques; 4 – ruptured plaques (Kumar et al., 2010). This study includes only male donors because female autopsies are carried out approximately three times less than male. Comparison between male and female data will be the subject of separate study.

Longitudinal prestrain and diameter. The abdominal aorta was thoroughly removed and the distance between two markers in situ and after the excision was measured with a ruler. Markers were made just below the renal arteries and above the aortoiliac bifurcation. Longitudinal prestrain was quantified by means of the stretch ratio, λ, defined in (1B).

$$\lambda = \frac{l}{L} \tag{1B}$$

Here l denotes in situ length and L is the length after removal from the body. Subsequently a ring was cut off from the aortic segment (approx. 2 cm above the bifurcation) and then cut into a strip. The circumference of the aortic segment was determined as the length of this strip and then divided by π to obtain the diameter. The measurement of excised artery was performed approximately 2 minutes after the removal from a body.

Correlation and regression analysis. Linear correlation coefficients were computed according to the equation (2B), where x and y denote appropriate variables and overbar indicates the mean value. S_x a S_y denote sample standard deviations.

$$R = \frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)}{\left(n-1\right)s_x s_y} \tag{2B}$$

Preliminary computations revealed a successful fit with the power law equation (3). It was used to express prestrain–age, diameter–age and diameter–prestrain relationships. Model parameters were estimated with least squares optimisation in Maple 13 (Maplesoft, Waterloo, Canada).

$$y = ax^b \tag{3B}$$

The predictive capability of the models was evaluated by means of prediction intervals (confidence interval in which future observations will fall with a probability equal to confidence level α). The probability $\alpha = 0.95$ was considered to be significant. Prediction intervals (PI) were computed after the logarithmic transformation into a linear problem. In such a situation they can be implemented by means of (4B) .

$$y = a + bx \pm t_{\frac{\alpha}{2}}\left(m\right)S_e\sqrt{1 + \frac{1}{n} + \frac{\left(x - \bar{x}\right)^2}{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}} \tag{4B}$$

where a and b are model parameters estimated using (3). S_e denotes residual standard deviation and $t_{\alpha/2}$ is the quantile of the Student's t-distribution with $m = n - 2$ degrees of freedom.

Results

A one-year collection of data resulted in 156 donors. Descriptive statistics are listed in Table 6. Table 8 summarizes longitudinal dimensions of arteries since the stretch ratio does not provide this notion.

Correlation. Computed correlation coefficients (Table 7) proved a strong mutual dependence between the longitudinal prestrain, age, diameter and the degree of atherosclerosis. Higher values were attained after the logarithmic transformation. This is in accordance with chosen form of the regression model (3B).

Besides the tight correlations given in Table 2, the data indicate rather high dependence for HW–D (R = 0.579); HW–ATH (R = 0.511); HW–age (R = 0.464); and HW–prestretch (R = -0.454); computed for raw data. The correlation coefficients between PMI and other quantities did not exceed $|R|$=0.22. It suggests that results were not significantly affected by post-mortem changes.

Regression analysis. Fig. 17 shows results of the regression analysis. Symbols representing observation points highlight the degree of atherosclerosis. Since the atherosclerosis is quantified in rather a qualitative manner it was not considered for the regression modelling. Nevertheless, graphs show that points with the same ATH are mostly grouped into regions with overlapping boundaries, which confirms the significant correlations presented in Table 2. Results also show that while longitudinal prestrain decreases with age, the aortic diameter increases. As correlation coefficients suggest, the power law is suitable to fit age–prestretch, age–diameter, and diameter–prestretch nonlinear relationships.

The model predictions are supplemented by prediction intervals which emphasize variances in observations and give the limits for hypothetical future observation. Confidence level of 0.95 covers almost all observations. It implies that PI must be wide enough. The nonlinearity of the problem results in variable lengths of PI. Concerning PI two facts should be mentioned: since the lower limit of the prestretch–age relationship approaches 1 at about age 60, the model suggests that non-prestrained aortas may occur. It implies that the model predicts a high risk of the pretension being lost which can be manifested as an aneurysm or tortuosity. A similar situation occurs in the prestretch–diameter relationship where the aortic diameter exceeds 1.5 cm. Secondly the outliers presented, especially in the

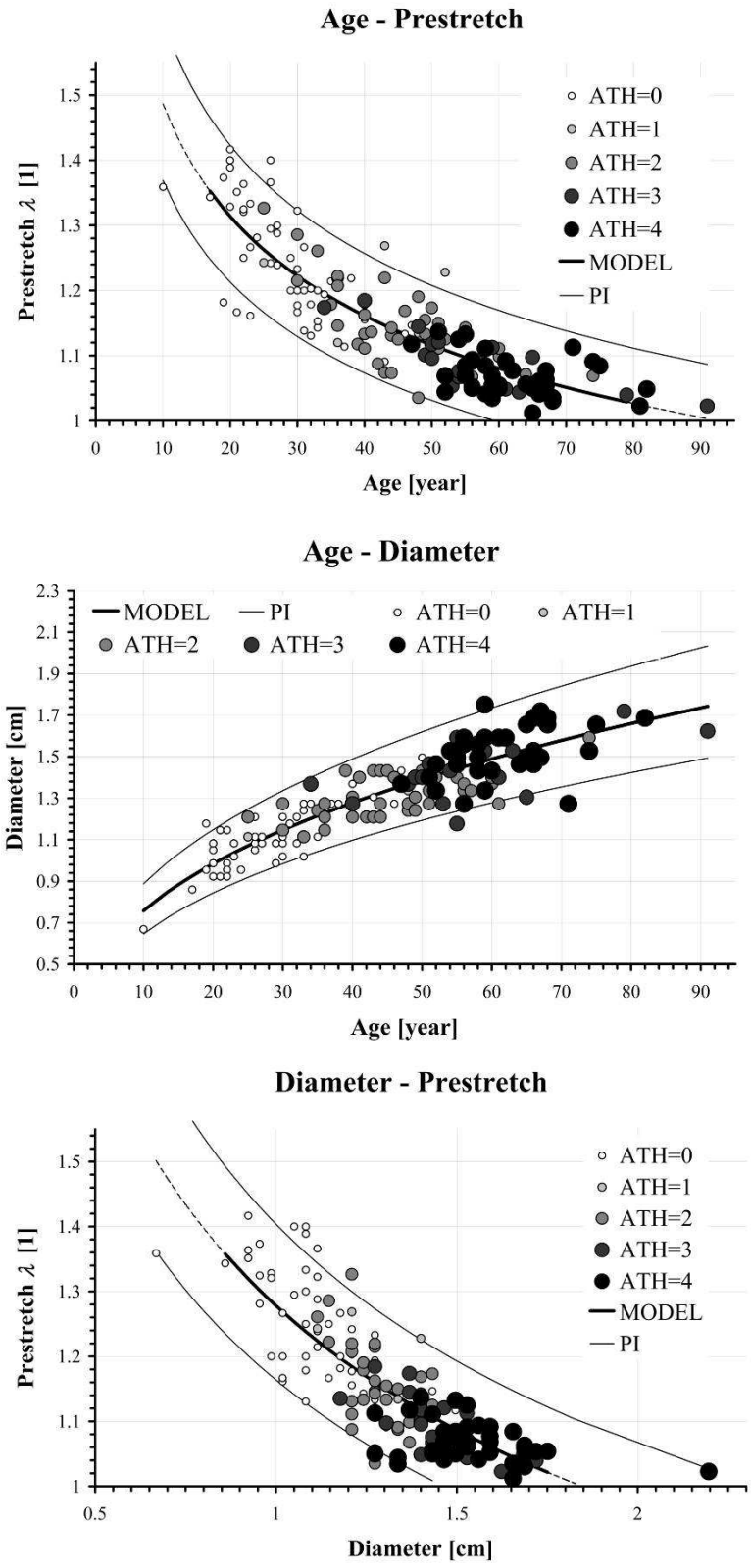

Figure 17 Models and observations. A – age predicts prestretch; B – diameter predicts prestretch; and C – age predicts diameter. The predictions are based on (1) with parameters as follows: age–prestretch a=2.2397, b=-0.1780; diameter–prestretch a=1.2781, b=-0.3999; and age–diameter a=0.3179, b=0.3771. The dashed curves are based on model equations and highlight areas where the models should be used carefully. The symbols used for data-points indicate the degree of atherosclerosis (ATH).

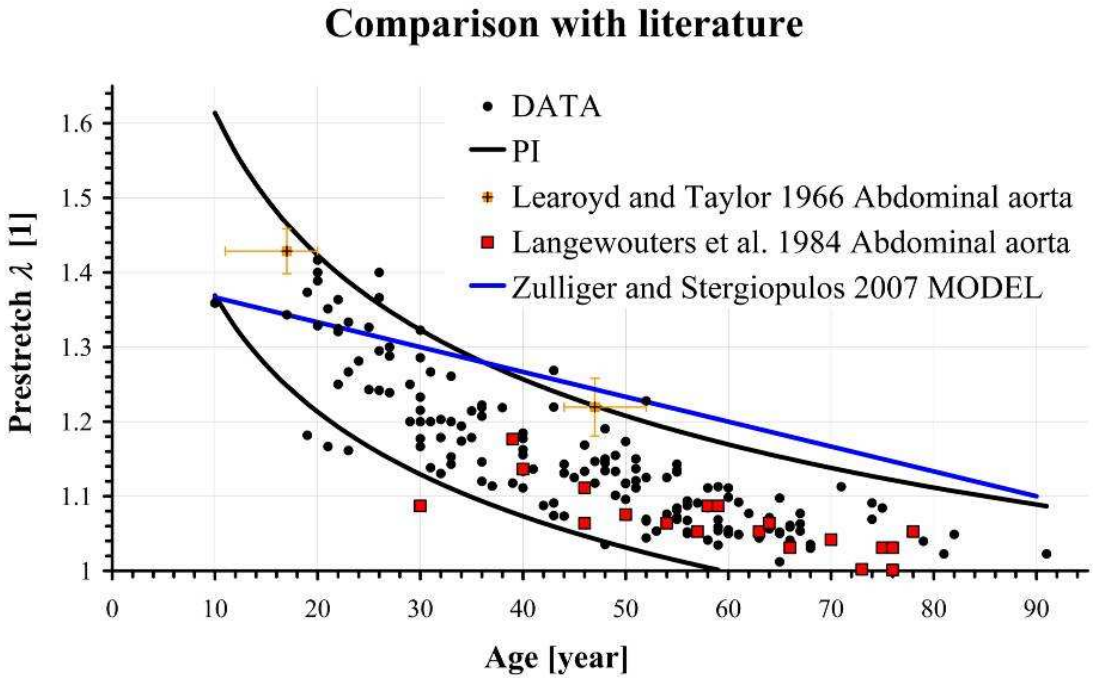

Figure 18 Comparison with literature. There are only few papers reporting longitudinal prestrain in human abdominal aorta. Learoyd and Taylor (1966) involved 12 human donors and published only averaged results (SD is indicated). Langewouters et al. (1984) involved 20 human donors, nevertheless, their sample did not cover the age < 30 years. Their results are in accordance with our data. Zulliger and Stergiopulos (2007) proposed the linear model λ =1.4-Age/300 for human thoracic aorta. The literature however suggests that λ increases with the distance from the heart (Han and Fung, 1995; Learoyd and Taylor, 1966). Thus it seems that λ = 1.4-Age/300 somewhat overestimates the prestrain when predicts higher values in thoracic aorta than in abdominal aorta.

Table 6 Data summary.

	Mean±SD	Min/Max	Median/Mode
Prestretch [-]	1.153±0.097	1.012/1.417	1.133/1.111
Age [year]	45.5±16.2	10/91	47.5/30
Diameter [cm]	1.32±0.22	0.67/2.20	1.31/1.27
Heart weight [g]	430±121	160/840	410/350
Thickness LV [cm]	1.5±0.3	0.6/2.5	1.5/1.5
Atherosclerosis [-]	1.9±1.6	0/4	2/0
PMI [h]	46±31	2/170	37/24

prestretch–diameter relationship, may raise questions about the suitability of the power law model. A better fit could likely be obtained with the curve decreasing more steeply; however, calculations proved that power law is superior to exponential, logarithmic and linear alternatives.

Table 7 Correlation coefficients. Upper triangular table (shaded) shows coefficients obtained after the logarithmic transformation; the lower one represents correlations computed with raw data. ATH were not transformed.

	λ or **ln**(λ)	Age or **ln**(Age)	D or **ln**(D)	ATH
λ or **ln**(λ)	—	-0.873	-0.819	-0.736
Age or **ln**(Age)	-0.842	—	0.890	0.798
D or **ln**(D)	-0.798	0.871	—	0.762
ATH	-0.726	0.811	0.766	—

Table 8 Descriptive statistics of measured longitudinal dimensions.

[cm]	Mean	Standard Deviation	Minimum	Maximum
Excised length *L*	7.9	1.17	3.9	11.5
In situ length *l*	9.1	1.15	5.3	13.0
Elastic retraction *l*-*L*	1.2	0.65	0.1	3.2

Discussion

Experimentally validated prestrain should be used in computer simulations (e.g. stress or pulse wave velocity analyses) and experimental techniques (bioreactor, tissue engineering) of artery biomechanics to obtain reliable results. Pretension is, however, experimentally inaccessible in living subjects due to the requirement of the excision of a sample. Hence statistical models can be helpful in estimating prestrain. It is a surprising fact that these models are lacking in the literature (Zulliger and Stergiopulos, 2007).

This study found that longitudinal prestrain of the human abdominal aorta measured post-mortem is well correlated with the age and diameter of the artery, and both can be utilized for estimating prestrain. Predictions based on age are more successful than those based on the diameter. The nonlinear decreasing trend of the prestrain was well described with the power law.

Prestrain presented here, however, can differ from in vivo pretension. In vivo pretension accounts not only for the tension developed by an artery wall itself, it also includes tension of the surrounding tissue which had to be removed during the sample excision. The purpose of the surrounding tissue, however, is especially to support artery position and to reduce the transmural pressure load. In vivo longitudinal tension is also raised with the blood pressure, although the literature suggests that this effect might be negligible (Liu et al., 2007). Presented observations should be considered rather as the lower limit of in vivo prestrain.

Low correlations with PMI support the hypothesis that the measured data were not significantly affected by post-mortem changes. It is in accordance with common opinion that the passive elasticity of arteries does not change substantially up to 7 days after death when kept in cold storage (Medynsky et al., 1998).

The presented models are purely empirical and their usability out of the range of observations is questionable. Specifically, they do not respect the obvious fact that longitudinal prestrain cannot be decreasing throughout the entire life. This fact was indicated in the dotted curves in Fig. 17. The pretension must be developed. Unfortunately, we have almost no data covering the childhood because such autopsies are not carried out in our hospital (searching the literature also did not reveal suitable sources). Likewise significantly aged (or those with a large aortic diameter) subjects could be modelled unrealistically.

The artery diameter, determined via the length of the arterial strip, can differ from the diameter of the closed ring due to residual strains acting in circumferential direction (Rachev and Greenwald, 2003). The residual strain, however, correlates with aging (Valenta et al., 2002). It suggests that presented diameters can be transformed to in vivo dimensions after applying the appropriate model.

We also tested if the predictive capability of the model can be increased considering multivariate problem. Unfortunately, the inter-correlation between variables resulted in only slight improvement. It was concluded that in terms of a phenomenological approach, the age is the most efficient predictor for both prestretch and diameter.

Interesting question is what the source of the age–prestrain correlation is. Data analysis revealed that l does not correlate with age (R = 0.023), and L correlates slightly (R = 0.476). The highest age-dependence was found for elastic retraction l-L, R = -0.811. Note also that non-prestrained length L elongates with age (possible remodelling of the unloaded state) in contrast to the elastic retraction (l-L decreases likely due to the stiffening of an artery).

Further studies are needed to obtain more reliable descriptions of age-, remodelling-, and pathology-dependent phenomena of artery pretension. Constituent-based models are especially desirable. They can account for the rate of extracellular matrix synthesis/inhibition, calcium deposition and high-cycle fatigue. Reported data can be utilized in their development.

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Appendix – C

Age Estimation Based on a Combined Arteriosclerotic Index

is going to appear in *International Journal of Legal Medicine*, accepted for publication.
doi: 10.1007/s00414-011-0653-7

Abstract – This study introduces a new quantity, the combined arteriosclerotic index (CAI), which is defined as the ratio between the diameter and the longitudinal prestrain of an artery. The longitudinal prestrain has been adopted as the ratio between the in situ length and the excised length of the abdominal aorta, and is a measure of arterial elasticity. During ageing, arteriosclerosis is manifested by the loss of pretension, and by enlargement of the diameter of the artery. CAI combines these two effects. A sample of 61 female and 194 male autopsy measurements of human abdominal aortas shows that CAI correlates significantly with chronological age (R = 0.916/0.921; female/male). The sample had the following parameters: age 53±19/48±16 years; diameter of the abdominal aorta 12.4±2.2/13.4±2.1 mm; and longitudinal prestrain 1.13±0.10/1.15±0.10 (mean ± sample standard deviation; female/male). The resulting CAI was 11.2±2.7/11.9±2.6 mm. The classical linear regression model was employed for age estimation by CAI. The model gave a residual standard deviation of 7.6/6.3 years, and a 95% prediction interval range of ±15.4/12.5 years (female/male). A two-sample t-test confirmed that there are significant differences between the female and male population during ageing, reflected by CAI, unlike longitudinal prestrain. It was concluded that CAI is a suitable predictor of age at time of death, and is easily obtainable in the autopsy room.

Introduction

Estimating the age of cadavers of unknown identity is a standard step in forensic practice. Numerous methods, differing in principle, accuracy and precision, are frequently employed [1-5]. The approach that is used also depends on the age and the character of the remains.

Radiological methods for analyzing dental and skeletal development are accurate in children, and can be used not only for cadavers, but also for archeological cases and in the living [6-9]. The aspartic acid racemization rate (AAR) in dentine is recommended as a standard for age estimation in adults [10-12]. Methods from molecular biology for investigating telomere shortening and damage accumulation in mitochondrial DNA likewise provide a basis for age estimation [3].

Age-related changes in soft tissues can also be utilized. AAR can especially employ durable proteins like elastin. Elastic arteries and yellow ligaments have been proven to be suitable sources for this purpose [13,14]. A simple analysis can be based on the color of tissues such as the intervertebral disc, the Achilles tendon or rib cartilage [15].

It has been known for centuries that elastic arteries become stiffer with ageing [16-19]. The process which macroscopically appears as stiffening and enlargement of the artery diameter is referred to as arteriosclerosis, and has significant consequences for heart function and pressure pulse transmission. The stiffening is caused, among others, by calcium deposition, increased cross-linking (especially by non-enzymatic advanced glycation end-products) and abrupt engagement of stiff collagen fibrils within a deformation [19-21]. These processes, together with fragmentation of elastin lamellae, result in age-dependent loss of longitudinal pretension in the aorta.

It was shown in our previous studies that the magnitude of the longitudinal prestrain (the ratio between the in situ length and the ex situ length of the aortic segment) and the diameter of the abdominal aorta correlate significantly with chronological age [22,23 – Appendix A, B]. However, these relationships have been found to be best fitted with the power law model. The nonlinearity results in variable variance, which is undesirable in age estimation.

This study introduces a new quantity, the combined arteriosclerotic index (CAI), which will be shown to depend linearly on chronological age. This quantity is based on the simply measurable consequences of ageing; the longitudinal prestrain and the diameter of the abdominal aorta. It will be shown that CAI is a suitable predictor of age at time of death.

Methods

This study extends previous observations reported in [22,23 – Appendix A, B]. Therefore, only basic information necessary to avoid misunderstandings will be repeated here. Detailed descriptions can be found in the references. In addition, details of the statistical approach seem to be unnecessary, and can be found in appropriate textbooks; e.g. [24].

Data describing the in situ and excised lengths of the male and female abdominal aorta, as well as the diameter (D), age, degree of atherosclerosis (ATH), and the post-mortem interval (PMI), were collected during regular autopsies of Caucasian cadavers of known age. Post-mortem usage of human tissue was approved by the Ethics Committee of the Královské Vinohrady University Hospital in Prague. No putrefied bodies were involved. The degree of atherosclerosis was examined by a pathologist and quantified on a scale from 0 to 4 [25].

Combined arteriosclerotic index – CAI. The abdominal aorta was thoroughly removed, and measurements were made of the distance between two marks in situ, and after excision. Marks were made just below the renal arteries and above the aortoiliac bifurcation. The longitudinal prestrain was quantified by means of the stretch ratio λ (1C).

$$\lambda = \frac{l}{L} \tag{1C}$$

Here l and L denote the in situ and ex situ lengths, respectively. Subsequently a ring was cut from the aortic segment (approx. 2 cm above the bifurcation) and it was then cut into a band, and its length was considered equal to the circumference by means of which the aortic diameter was calculated.

CAI was suggested as the ratio between the diameter and the prestrain (2). It is defined in such a way that CAI increases as D increases; and CAI increases as λ decreases. Since λ is a measure of loss of elasticity, this implies that the greater the loss of elasticity, the higher CAI will be (also for the diameter). The name of the parameter (combined arteriosclerotic index) was chosen since CAI includes the changes in both elasticity and diameter that are symptomatic for arteriosclerosis.

$$\mathrm{CAI} = \frac{\mathrm{D}}{\lambda} \tag{2C}$$

Statistical analysis. The statistical analysis consisted of correlation, regression analysis and descriptive statistics. The correlation analysis was based on the simple linear correlation coefficient R. The regression analysis employed a classical linear model to describe the age–CAI relationship. The predictive capability of the model was evaluated via the residual standard deviation (RSD) and the prediction interval (PI). The prediction interval gives the limits in

which future observation is expected with probability α (confidence level). α = 0.95 was considered to be significant within this study. A two-sample *t*-test was used to reveal whether there were any differences between the male population and the female population.

Results

The data samples were higher than in our previous studied, and were increased to 194 male and 61 female individuals (the previous data is included).

Statistical data. The descriptive statistics for the total number of samples were as follows [mean ± sample standard deviation; female/male]: age 53±19/48±16 years; CAI 11.2±2.7/11.9±2.6 mm; ex situ length 76.3±12.6/78.8±11.7 mm; in situ length 85.3±10.5/89.9±11.9 mm; D 12.4±2.3/13.4±2.1; ATH 2/2 (instead of the mean, the median is used); and PMI 45±25/46±30 hours.

The statistical analysis confirmed previous findings that the retraction (the difference between the in situ length and the ex situ length), the ex situ length, the longitudinal prestrain, the diameter and the degree of atherosclerosis correlate with age. The correlation coefficients are presented in Table 10. It was found that CAI provides an even higher correlation with age than the diameter and the prestrain. The in situ length of the abdominal segment of the aorta does not show significant dependence on age (R = 0.192/-0.069 female/male).

The results of the *t*-test (Table 9; significance indicated by *) show that the quantities based solely on the lengths of the aortic segments (in situ and ex situ length, retraction and λ) do not differ significantly with respect to gender. The quantities based on aortic diameter (CAI), however, do differ significantly.

Regression model. The results of the regression analysis are depicted in Fig. 19. The regression parameters are given in the caption to the figure. The analysis revealed RSD = 7.6/6.3 years; and PI = 15.4/12.5 years in the female/male group. The regression lines intersect the experimental data almost uniformly, which suggests that an appropriate model was used. The data was also tested to find whether l, L,λ, D and CAI follow a Gaussian distribution. They were adjusted for age, and the Shapiro-Wilk test was performed (confidence level 0.05). The results suggested that the hypothesis of normality should not be rejected.

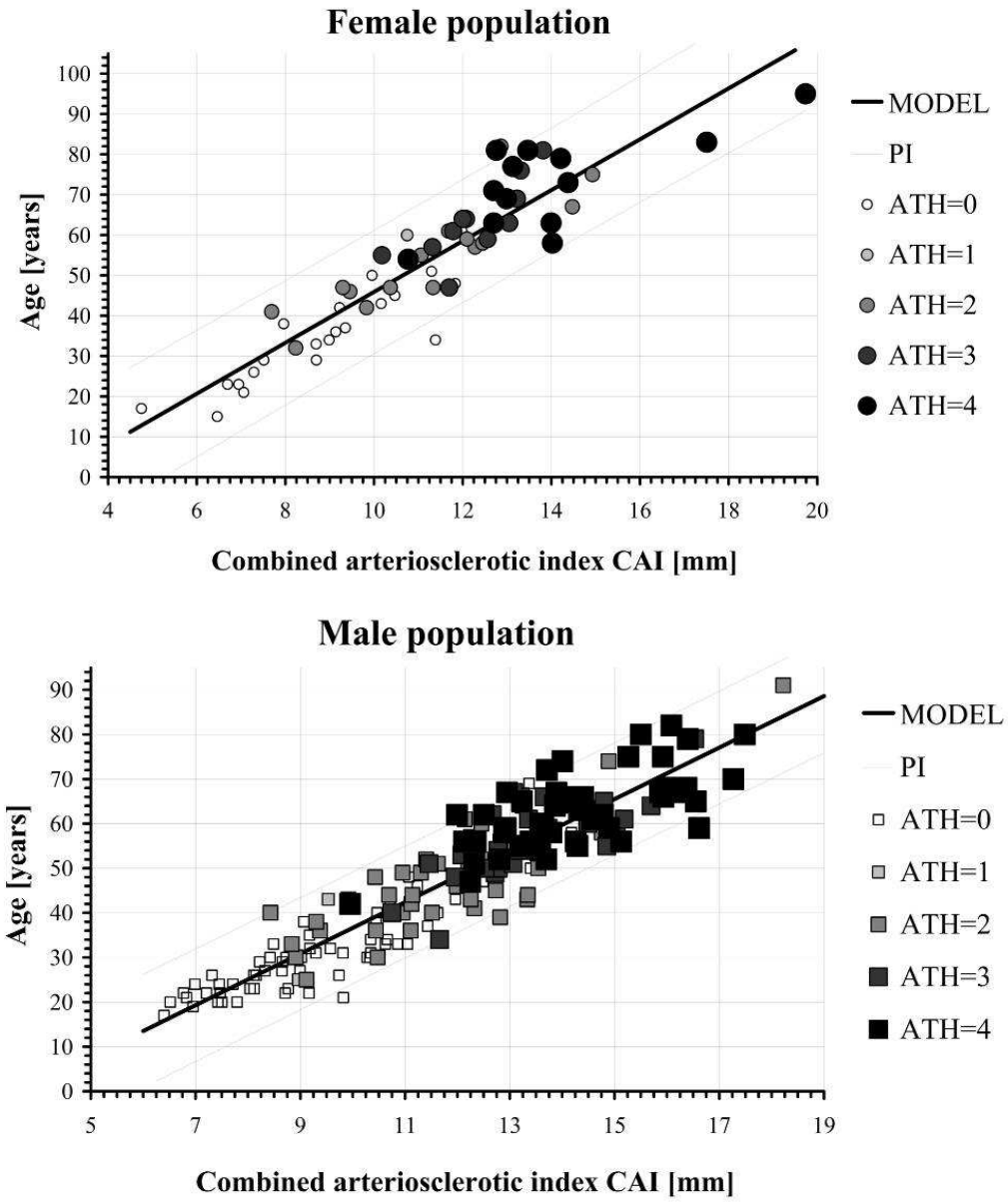

Figure 19. Regression model. The classic linear model was employed in the regression analysis; Age = *a**CAI + *b*. The parameters were computed as follows: *a* = 6.307/5.776; *b* = -17.17/-21.13 (female/male). The observation points are presented with respect to the degree of atherosclerosis. The prediction intervals are depicted with dashed lines. They show the intervals in which future observations will fall with probability equal to 0.95. The result is ±15.4 and ±12.5 years around the regression model in the female and male population, respectively.

Table 9. Measured data (mean ± sample standard deviation) sorted with respect to decades of age. Abbreviations and corresponding units: age [years]; number of observations – n [-]; combined arteriosclerotic index – CAI [mm]; ex situ length – L [mm]; in situ length – l [mm]; difference between in situ and ex situ length (i.e. retraction) – ΔL [mm]; longitudinal prestrain – λ [-]; diameter – D [mm]; degree of atherosclerosis – ATH [-]; and post mortem interval – PMI [hours]. Indices F and M indicate sex. The † symbol indicates that the median was used. A two-sample t-test was used to evaluate the differences between the mean in male and female populations in age groups older than 19 years. Differences significant with a probability 0.95 or higher are indicated by *.

Age	<20	20-29	30-39	40-49	50-59	60-69	69<
n_F	2	6	7	11	11	12	12
n_M	2	30	32	32	47	39	12
CAI_F	5.6 ±1.2	7.4* ±0.7	9.1 ±1.1	10.1* ±1.2	11.6* ±1.2	12.6* ±1.1	14.4* ±2.1
CAI_M	6.7 ±0.4	8.1* ±0.8	10.1 ±1.0	11.3* ±1.1	13.2* ±1.1	14.2* ±1.2	15.9* ±1.4
L_F	61.0 ±2.8	61.7* ±4.1	69.3 ±12.2	77.2 ±8.8	78.2 ±9.4	79.3 ±8.6	84.8 ±16.4
L_M	71.0 ±5.7	68.7* ±8.6	75.5 ±9.1	80.6 ±10.0	81.7 ±10.8	83.4 ±13.0	83.3 ±12.9
l_F	86.5 ±4.9	80.3* ±4.9	80.7 ±11.3	86.6 ±8.3	86.1 ±9.4	86.0 ±7.6	87.8 ±16.7
l_M	96.5 ±9.2	88.9* ±12.5	90.4 ±9.8	92.8 ±9.8	89.5 ±12.5	88.9 ±13.2	87.9 ±13.5
ΔL_F	25.5 ±2.1	18.7 ±2.2	11.4* ±2.8	9.5* ±3.4	7.9 ±2.1	6.7 ±2.5	3.1 ±2.4
ΔL_M	25.5 ±3.5	20.2 ±5.7	14.8* ±3.8	12.2* ±4.2	7.8 ±3.6	5.5 ±2.6	4.6 ±2.6
λ_F	1.42 ±0.02	1.30 ±0.04	1.17 ±0.07	1.13 ±0.06	1.10 ±0.03	1.09 ±0.04	1.04 ±0.03
λ_M	1.36 ±0.02	1.29 ±0.07	1.19 ±0.06	1.16 ±0.06	1.10 ±0.04	1.07 ±0.04	1.06 ±0.03
D_F	7.9 ±1.8	9.6 ±1.0	10.6* ±1.1	11.3* ±1.0	12.8* ±1.2	13.6* ±1.0	14.9* ±2.0
D_M	9.1 ±0.7	10.5 ±0.8	12.0* ±1.0	13* ±1.0	14.4* ±1.0	15.1* ±1.1	16.8* ±1.4
ATH_F	0†	0†	0†	2†	2†	3†	4†
ATH_M	0†	0†	0†	2†	3†	4†	4†
PMI_F	29 ±10	47 ±27	32 ±18	42 ±21	55 ±24	57 ±35	52 ±33
PMI_M	32 ±18	34 ±16	44 ±27	56 ±34	48 ±31	43 ±33	59 ±47

Table 10 Correlation coefficients R. The first row indicates pairs of quantities used in the calculation.

Sex	ΔL–age	L–age	λ–age	D–age	ATH–age	CAI–age	PMI–CAI
F	-0.863	0.557	-0.840	0.878	0.783	0.916	0.148
M	-0.804	0.386	-0.820	0.888	0.792	0.921	0.024

Discussion

The combined arteriosclerotic index, defined as the ratio between the diameter of the excised artery and its longitudinal prestrain (2), was proposed as a measure of ageing. It was shown that the linear model is suitable to fit the data that had been collected. The correlation between age and CAI (R = 0.916/0.921 female/male) was even higher than in the case of the diameter–age and prestrain–age dependencies. The data presented here suggests that the age-dependent distribution of the in situ length, the ex situ length, the retraction and the prestrain in the abdominal aorta are not gender-specific. As was to be expected the diameter depends on sex. This dependence is replicated in CAI.

The close correlation between CAI and chronological age makes CAI suitable for use in simple and instant age estimation. The main advantage of the method is the possibility to obtain an estimation straightaway in the autopsy room.

Comparison with existing methods. The reviews [2,26] state that age estimation methods giving a sample standard error of estimate higher than 7 years cannot provide an accurate basis for routine forensic application. From this point of view, the CAI method gives accuracy just at the limit (RSD = 7.6/6.3 years; F/M).

The CAI method provides better or comparable accuracy in comparison with methods recommended for age estimation in adults (an evaluation of dental morphology, including histological features); the standard errors published in [2] range from 4-10 years. An evaluation of bone histology (standard error of estimate 5-12 years) also gives comparable results. However, AAR determines age with a standard error of 1.5-4 years [2] which is superior to the CAI method.

In comparison with our previous studies, which employed nonlinear regression models for the prestrain–age and diameter–age relationships, the proposed combined arteriosclerotic index depends linearly on age. This fact gives the advantage of almost constant variance. Moreover, CAI yields more accurate results (the 95% prediction intervals in the power law models employed in [22 – Appendix A] yielded ranges of 30.5-62.3 and 25.3-57.0 years for the mean prestrain in the female population and in the male population, respectively).

Limits of the CAI method. Putrefied bodies were excluded from this study, since putrefaction can affect the biomechanical properties of an artery wall. In such cases, the method cannot be used. Previous studies have shown that PMI does not correlate significantly with prestrain [22,23 – Appendix A, B]. This result is replicated in the CAI method (correlation coefficient

R = 0.148/0.024 female/male). Nevertheless, we may expect that a longer PMI than was used in our study may lead to a different observation.

The method introduced here was derived from observations operating with adults. This fact limits our findings and the possible application of regression parameters. Cadavers with tortuous or aneurysmatic abdominal aorta were also not included, since the definition of prestrain is questionable in such cases.

The principal limit of CAI is its non-repeatability. Once the aortic segment has been excised, measurement of the in situ length cannot be repeated. This drawback can be overcome by including another elastic artery as a control sample. The carotid, iliac and femoral arteries have been proven to be longitudinally prestrained and are easily obtainable for measurements [27-29]. Unfortunately, suitable studies for comparison have not yet been carried out.

Biomechanics. Although the aim of our study is to introduce a new method for estimating age at time of death we should, at least briefly, clarify what CAI measures from the biomechanical viewpoint. It seems to be obvious that it is something like age-dependent deformation or elasticity. However, the true deformation must be dimensionless, and the elasticity has to be in Pascals (force per unit area, which is the unit of the Young modulus in Hooke's law). Nevertheless, CAI has a dimension of length.

Consider a cylindrical surface (a prestrained artery) of length l and diameter d. The longitudinal constraint is released upon artery excision, and the artery shrinks to length L, and expands to diameter D. The true deformation of the surface area can be defined as $\pi \cdot d \cdot l/(\pi \cdot D \cdot L)$. It is clear that CAI is only a fragment of this biomechanical information (area ratio = d/CAI). It is highly probable that the area ratio will depend on age, due to overall stiffening during ageing. Unfortunately, we are not able to measure the diameter in situ with desirable accuracy. It is also not clear whether the area ratio depends linearly on age since the prestrain, which is a dimensionless deformation measure, depends non-linearly on age [22,23 – Appendix A, B]. Finally, it should be noted that this was a somewhat simplified interpretation, since the cylindrical geometry of an artery is also prestrained in the circumferential direction (this strain is usually called "residual", and is manifested when a radial cut of an arterial ring is made as an abrupt opening to a sector [30,31]). We therefore prefer to use CAI, although its biomechanical interpretation is not as straightforward as in case of the area ratio.

Conclusion

The study has confirmed that the retraction of the abdominal aorta, the ex situ length and the longitudinal prestrain correlate with chronological age, and do not differ significantly with respect to gender. The combined arteriosclerotic index was found to depend linearly on age. This study has suggested that CAI is a suitable candidate for simple and instantaneous age estimation.

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Epilogue

Ageing, ageing, ageing concerns everyone. The ageing is one of the most challenging problems in developed countries. Due to its socio-economical significance, the discussion of this phenomenon seems to extend from purely medical circles to more general discussion in sociological community; however, public policy now also reflects it. Nonetheless, "ageing cannot be resolved." It can only be postponed and everyone sees that we have reached unprecedented success.

This success, however, gives us new evidences and information, related to human physiology, which will not likely make us happier. Empirical observations, conducted in this study, have shown that ageing is accompanied with significant changes in the physiological conditions determining the functioning of elastic arteries. Longitudinal prestress, prestrain and pretension force decrease significantly during ageing. Optimal biomechanical conditions, endowing aorta with almost no longitudinal movement within cardiac cycle, are lost in the advanced age. Ney, *the Thunder said*: ...there is no refuge, but Fig. 4 and 17 show what the truth is.

M. F. O'Rourke* and J. Hashimoto (2007)† have recently reviewed our knowledge of elasticity and stiffness of conduit arteries from the pressure pulse wave transmission point of view. They have suggested aortic elastin to be the target of future therapy. At present, the main target is the relaxation of smooth muscle cells in small distributing arteries (although not successful in reduction of peripheral resistance, this therapy reduces wave reflections). Up to date no clinically applicable (better to say industrializable) results have been achieved. Nevertheless, since the above mentioned therapy may be presumed to be long-term, one may expect that pharmaceutical industry will approach to applied research in this field, because long-term therapy is always more interesting from their point of view (naturally, this point of view is established by potential profit; this visible at present in the stagnation of developing new antibiotics because antibiotics, usually used for a short-term therapy, have worse potential to attain high commercial profit). So far the ineffectual development of elastin targeted therapy should not, to the author's opinion, be interpreted as impossibility to do it. Rather it seems that more sophisticated methods (molecular biology) will be able to affect each stage of extracellular matrix synthesis (from gen expression to final extracellular cross-linking) have to be employed. Thus, the author finally concludes that it is highly probable that followers, who will investigate

* to the author's knowledge, M.F. O'Rourke was the first stating "wear-and-tear" hypothesis (in the language of mechanical engineers is better to say "fatigue failure") of arterial elastin. He is also known to be the editor of famous McDonald's Blood Flow in Arteries (with W.W. Nichols).

† O'Rourke MF, Hashimoto J (2007) Mechanical factors in arterial aging. J Am Coll Cardiol 50:1-13.

longitudinal prestress and prestrain in fifty years, will report different observations as the result of our progress in molecular-biological therapy.

...
After the torchlight red on sweaty faces
After the frosty silence in the gardens
After the agony in stony places
The shouting and the crying
Prison and palace and reverberation
Of thunder of spring over distant mountains
He who was living is now dead
We who were living are now dying
With a little patience

Here is no water but only rock
Rock and no water and the sandy road
The road winding above among the mountains
Which are mountains of rock without water
If there were water we should stop and drink
Amongst the rock one cannot stop or think
Sweat is dry and feet are in the sand
If there were only water amongst the rock
Dead mountain mouth of carious teeth that cannot spit
Here one can neither stand not lie nor sit
There is not even silence in the mountains
But dry sterile thunder without rain
There is not even solitude in the mountains
But red sullen faces sneer and snarl
From doors of mudcracked houses
If there were water
...

excerptum from What the Thunder Said, T.S. Elliot *The Waste Land*

List of selected publications of the author

o Peer-reviewed journals indexed in Journal Citation Reports

Supplemented with impact factor based on JCR 2010 and journal ranking (rank/number of journals in a category)

- Horny L, Adamek T, Chlup H, Zitny R (2012) Age estimation based on a combined arteriosclerotic index. *Int J Leg Med*, accepted for publication. DOI: 10.1007/s00414-011-0653-7
  IF 2.939 1/13
- Horny L, Adamek T, Vesely J, Chlup H, Zitny R, Konvickova S (2012) Age-related distribution of longitudinal pre-strain in abdominal aorta with emphasis on forensic application. *Forensic Sci Int* 214(1-3):18-22. DOI: 10.1016/j.forsciint.2011.07.007
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- Horny L, Chlup H, Zitny R, Adamek T (2010) Constitutive modeling of coronary artery bypass graft with incorporated torsion. Metallurgy 49(2):273-277.
  IF 0.348 48/76

At present time submitted

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- Horny L, Chlup H, Zitny R, Vonavkova T, Vesely J, Lanzer P Ex vivo coronary stent implantation evaluated with digital image correlation. *Exp Mechanics*

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- Gultova E, Horny L, Chlup H, Zitny R (2011) A comparison between exponential and limiting fiber extensibility pseudo-elastic model for the Mullins effect in arterial tissue. *J Theor Appl Mech* 49(4):1203-1216.
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o Conference papers

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