Review of doctoral thesis by Tereza Voňavková with title *Mechanical Properties of Perivascular Adipose Tissue and its Effect on Biomechanics of Abdominal Aorta*

The submitted doctoral thesis deals with a field, which is mostly missed out in computational models of aorta and in related considerations, and may be worth of deeper investigation. The presented experiments with perivascular tissue try to fill a gap in our knowledge on mechanical properties of a specific type of soft tissue ─ perivascular adipose tissue. Unfortunately, the amount of author's work done in this field is rather small and suffers from many factual and methodological drawbacks. Also the presented analyses succumb many limitations which are not at all mentioned or discussed in the thesis; even the main paper where the results were published miss a Limitation section completely. Already the formulation of the aim of the thesis sounds strange (p. 37): "**This work aims to confirm the significant influence of boundary conditions on the mechanical condition of the abdominal aorta.**" The hypothesis on significant impact of PVAT is rather original and new, it contradicts most of generally accepted approaches, thus it is relevant to investigate it but strictly without any presumptions on the result. Such a biased approach is not acceptable in science and no wonder that it brings confirmation of the formulated hypothesis, even if this confirmation is not well substantiated.
I start the review with addressing the required issues:

Objective of the doctoral thesis
The above objective was accomplished, but in my opinion only partially, and substantiation of conclusions is poor and doubtful. In fact, no comparison with the aorta without PVAT is presented. The sub-goal No. 2 "application of axial prestretch on ends of the abdominal aorta" is in fact not fulfilled but replaced by a reduced axial force which results in substantial changes in the prestretch and length of the aorta during pressurization (axial strains change by up 5%). This approach limits the boundary conditions investigated in the thesis to the thickness of PVAT only, while some others, e.g. the way how the axial prestretch is introduced, are not addressed although they may be very relevant. Consequently, axial displacements of more than 10 mm would occur and cause extreme shear strains of the PVAT especially in the narrow gap between the aorta and the spine. The reason for this simplification is not explained.

The level of analysis of the state of art
The state of art is sufficiently described with exception of the constitutive models, representing a bridge between experimental and computational parts of the work. The chapter on constitutive models (Chapter 3.5) is very brief, does not include any of the models used later in the computational part of the thesis and any analysis of suitability of individual models is missing. Although I do not impugn the suitability of Yeoh model, its choice is not substantiated and advantages in comparison with Demiray model used in another author's publication with the same geometry and boundary conditions are not shown.

Theoretical contribution of the thesis
Theoretical contribution of the thesis is not relevant and I do not expect if in such a study. The analytical model of bilayer cylindrical vessel used for the aorta in many studies, e.g. Holzapfel et al. (2000), was transformed to represent the whole aorta as the inner layer and the PVAT as the outer one, and different constitutive models were used. However, high mathematical knowledge is needed for realization of the computational model applied in the thesis.

Practical contribution of the thesis
I cannot confirm a significant practical contribution of the thesis because in my opinion the results suffer from many limitations or even errors. This is given by absence of analysis of limitations in the thesis, as well as absence of a validation experiment (inflation-extension test of the aorta with and without PVAT).

Suitability of the applied methods
Also suitability of the applied methods is disputable for me. Uniaxial testing under room temperature suffers from limitations related to drying-out of the tissue, high temperature dependence of mechanical behaviour of soft tissues and limited relevance of uniaxial tests for simulation of multiaxial behaviour of the tissue. As the lab disposes of biaxial testing machine capable of testing in heated solution, it should be at least discussed why uniaxial testing of dry specimen under room temperature was preferred. Moreover, predictive capability of the applied constitutive models is not at all mentioned although it is decisive in simulations of multiaxial stress states on the basis of uniaxial tests only. Fortunately, one of the best phenomenological models for the PVAT was chosen, although without any substantiation. Also application of a cylindrical model does not represent the highest actual level; the model should be compared with a corresponding finite element model which would enable the author to analyse also some non-symmetric shapes. It is evident (e.g. from Figure 1b in Voňavková and Horný 2020) that the PVAT is not (and cannot be) axisymmetric.

The way of application of the chosen method
Application of the chosen methods in experiments and analytical calculations is correct. Only the transmission of constitutive models into the computation suffers from significant extrapolation of the results, which makes the results hardly acceptable. If most uniaxial tension tests terminated with failure under some 8-10% elongation and the simulations cover the range of 25 % circumferential and 20% longitudinal strains at the same time, then it is evident that the results are behind the loadbearing capacity of the PVAT.

Knowledge of the author in the field
I have also some doubt on knowledge of the author. For instance, she presents the original Mooney two-parametric model as Mooney-Rivlin model (p. 35), confuses viscoelasticity with some other non-elastic effects (p. 59), and has no idea on impact of individual constitutive parameters. Otherwise she could not declare that the three-parameter Yeoh model published by Omidi (2014) is "somewhat lower" than the same model identified by herself although their constants differ by orders. Consequently, the former shows no strain stiffening and the latter is comparable with the arterial wall and even exceeds its stiffness under certain conditions. Moreover, the resulting stresses contradict not only the well-known hypotheses on uniform stress or strain throughout the wall (they show high stress gradients with maximum value being on the outer surface of the aortic wall) but sometimes also basic knowledge on elasticity (negative stresses on the inner surface of the aorta under inner pressure ─ see Figure 28).

Formal level of thesis
Formal level of the thesis is very good, it is written in very good American English, with few grammatical or syntax errors, which only exceptionally complicate understanding.

Publication activity of the candidate (related directly to the doctoral thesis) appears sufficient (1 IF journal, 2 conference papers indexed in databases, 1 paper in local peer reviewed journal, all as the first author). However, in the list of references in the fundamental paper in

Computer methods in Biomechanics and Biomedical Engineering (Voňavková, Horný, 2020) the earlier three publications are not referenced although they publish substantial parts of the presented final results (experiments, constitutive model, computational model).

Some other **major drawbacks** of the thesis are summarized below:

1. The constitutive models of PVAT are extrapolated behind the experiment (see Figure 24, extrapolation is much higher than in the published paper). This is not only in the figure but even more in the simulations where they are used for a several times larger range of strains than measured in experiments and also behind failure strain. Although the failure strain of most samples is about 10% and only one specimens shows a value higher than 15% (in uniaxial tension), the model is extrapolated for biaxial state of strain with up to some 25% circumferential and 20% longitudinal strain in the simulations.
2. The constitutive model used for PVAT contradicts any other knowledge on perivascular tissue. Under 25% strain calculated in the analyses the stresses are by 3 orders higher than in the model by Omidi (which shows no strain stiffening) and by 2 orders higher than those in Sommer (2014) while another reference (Calvo-Gallego et al., 2018) is missing in the list and the reviewer could not identify it. The maximum tangential moduli are between 1500 and 2000 kPa for all the models and may overestimate the stresses in the PVAT for strains above 5-10 %. There is no discussion on these discrepancies in the thesis, except for the mentioned decellularization of the tissue in Omidi (2014) which cannot be convincing.
3. Residual stresses in the aortic wall were included by means of the opening angle, taken from literature together with dimensions of the specific aorta. As their impact depends significantly on the applied constitutive model, they seem to be overestimated in this combination because the stress distribution contradicts the hypotheses on uniform stress or strain throughout the wall thickness.
4. Discussion often repeats information given already in Results Section. A relevant discussion (e.g. quantitative comparison with other studies and explanation of differences) is almost missing and limitations of the study (representing its unavoidable part) are missing totally.
5. The maximum thickness of PVAT (40 mm) is not substantiated and it results in negative stresses (both circumferential and axial) on the inner surface of the aorta. For loading by inner pressure and high axial prestrain, these results contradict any expertize in mechanics.
6. Circumferential as well as axial strains exceed 20% with which is beyond the realized experiments and thus probably above the failing strain of PVAT; such extrapolation of experiments is unacceptable.
7. The conclusions drawn from figure 25 (p. 49) are often trivial and sometimes disputable:
   - Repeatedly stated that the inner radius increases during pressurization – this is really trivial, nothing else can be expected.
   - Deformability decreases with PVAT thickness on two innermost radiuses – again highly intuitive, quantitative comparison is not present.
   - In contrast, the statement „The outer radius of the PVAT is almost constant for all 3 PVAT thicknesses“ is misleading. The graphs in figure 25 show that for the PVAT thickness of 0,2 mm, the radial displacement is more than 1 mm, comparable with that of the inner and interface radiuses, for the thickness of 1.22 mm it is on the same order and only for thickness of 40 mm it is smaller by order (0,14 mm). But here this

conclusion does not require any continuum mechanics, it can be calculated directly from incompressibility condition.

Minor or formal errors:

- Abstract: "Axially prestrained aortas exhibited higher distensiblity than nonprestrained aortas." This statement is not true generally, it does not hold for PVAT thickness of 40 mm (see figure 27).
- It is not explained how the average response of PVAT was obtained. Different ways of averaging may give very different constitutive models and it might contribute to the extremely high strain stiffening of the models.
- p. 56, Table 4: It is not always true that increasing thickness of PVAT decreases the distensibility of aorta.
- p. 59: The statement "with pre-cycling to eliminate the viscoelastic effects" is not true. Pre-cycling does not eliminate viscoelastic effects.
- Initial modulus is comparable with the other studies on the same tissue but the high strain stiffening is contradictory, not well substantiated and impugnes the credibility of the results.
- „It was also showed that perivascular tissue carries some of the pressure load and reduces mechanical stresses inside the wall of aorta by approximately twice (Figure 28)" I cannot see any evidence for this statement being in contradiction with the evident influence of PVAT thickness. There is even no comparison with the aorta without PVAT.
- „ decreased arterial distensibilty is compensated by the axial prestretch"; this statement is disputable, the thickness of PVAT has no relation to axial prestretch (which decreases with age) and cannot anyway compensate its influence.
- In linear elasticity theory, gradient of radial stress decreases with increasing radius. As hyperelasticity generally enhances the stress gradients, the results are contradictive.
- In Nomenclature the specification of units is often incorrect.

Final recommendation

The candidate has presented a doctoral thesis which suffers from many drawbacks. Most important, she has not shown capability of a critical analysis of the results which is fundamental for scientific work. I **can recommend the thesis for defence only under condition** that she will be capable to present a deeper analysis of the results and a relevant list of limitations of the model which is missing in the thesis.

Questions to be answered:

1. Strain stiffening of your model is very high. Can you compare uniaxial and equibiaxial responses of your average model with those of Omidi (2014) (and possibly others) and with that of aortic wall. Explain in detail the way how the average uniaxial responses of the PVAT were obtained?
2. Can you support your conclusions on almost constant outer radius of PVAT with numerical results of the analysis?
3. Can you explain the statement at p. 51 "…we conclude that axial prestretch causing enlargement of the aortic circumference in case of a thin PVAT layer." This statement contradicts Figure 27. And why the circumferential stress is lower than 1 for no axial prestretch and no pressure?
4. Can you explain the basic mechanical principles resulting in negligible or even negative stresses on the inner surface of aorta under systolic blood pressure as presented in figure

28? The axial stresses are negative even for 20% longitudinal strain and the strangest results occur for PVAT thickness of 40 mm. How this value was chosen?

5. Can you summarize limitations of your computational model applied in the thesis?

Brno, 31st August 2020 Prof. Ing. Jiří Burša, PhD.