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● *Original Contribution*

INFLATION AND BI-AXIAL TENSILE TESTING OF HEALTHY PORCINE CAROTID ARTERIES

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Abstract—Knowledge of the intrinsic material properties of healthy and diseased arterial tissue components is of great importance in diagnostics. This study describes an *in vitro* comparison of 13 porcine carotid arteries using inflation testing combined with functional ultrasound and bi-axial tensile testing. The measured tissue behavior was described using both a linear, but geometrically non-linear, one-parameter (neo-Hookean) model and a two-parameter non-linear (Demiray) model. The shear modulus estimated using the linear model resulted in good agreement between the ultrasound and tensile testing methods, $G_{US} = 25 \pm 5.7$ kPa and $G_{TT} = 23 \pm 5.4$ kPa. No significant correspondence was observed for the non-linear model $a_{US} = 1.0 \pm 2.7$ kPa vs. $a_{TT} = 17 \pm 8.8$ kPa, $p \sim 0$); however, the exponential parameters were in correspondence ($b_{US} = 12 \pm 4.2$ vs. $b_{TT} = 10 \pm 1.7$, $p > 0.05$). Estimation of more complex models *in vivo* is cumbersome considering the sensitivity of the model parameters to small changes in measurement data and the absence of intraluminal pressure data, endorsing the use of a simple, linear model *in vivo*. (E-mail: r.lopata@tue.nl) © 2016 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Ultrasound elastography, Carotid artery, Inflation testing, Bi-axial tensile testing.**

INTRODUCTION

Methods of non-invasive diagnosis of arterial dysfunction are of great clinical interest (Ammirati et al. 2013; Czernuszewicz et al. 2015; Hansen et al. 2013; Magnoni et al. 2015). The current methods used to diagnose carotid atherosclerosis are based on geometric information, which does not provide any functional information on the vessel at risk and results in overtreatment of around 80% of the patient population (Medical Research Council 1998; Rothwell and Warlow 1999). Vascular strain imaging and elastography are non-invasive techniques, allowing the assessment of strains and mechanical properties of the arterial wall from ultrasound, respectively (Baldewsing et al. 2004; de Korte et al. 1998; Fromageau et al. 2008; Schmitt et al. 2007). These techniques, however, have been validated experimentally on simple phantoms with known properties (Cinthio et al. 2010; Larsson et al. 2015; Ribbers et al. 2007; Ryan and Foster 1997; Widman et al. 2015) and on biological vascular tissue (Keyes et al. 2013; Lopata et al. 2014; van den Broek et al. 2011). Studies that compare functional ultrasound measurements with measurements obtained with another experimental method are scarce.

Several experimental methods used to acquire material properties of different vascular tissue components have been described in the literature. Tensile testing, either uni-axial or bi-axial, is most commonly applied. Both tests apply a stretch to the tissue, and the resulting force is measured. For vascular tissue, bi-axial tensile testing is preferred over uni-axial testing, because of the tissue's anisotropy and the loading of the tissue in all directions *in vivo*. Several studies have reported on uni- and bi-axial tensile testing of carotid arteries, either with or without atherosclerotic plaque (Choserot et al. 2005; Kural et al. 2012; Lawlor et al. 2011; Maher et al. 2009). Similar measurements were reported for coronary arteries (Lally et al. 2004) and healthy or aneurysmal aortic tissue (Duprey et al. 2010; Holzapfel and Ogden 2006; Nicosia et al. 2002; Okamoto et al. 2002). Inflation testing characterizes the arterial tissue in more physiologic circumstances *in vitro*, that is, less destructive circumstances, and was previously employed on several types of arteries (Agianniotis

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et al. 2012; Beattie et al. 1998; Boekhoven et al. 2014; Lopata et al. 2014; Schulze-Bauer et al. 2003; Vychytil et al. 2010).

For mechanical characterization of tissue, the change in both luminal diameter and wall thickness needs to be assessed. Moreover, the local radial displacements and strains in the wall can be of help in understanding the mechanical behavior of the tissue. Both can be measured with ultrasound (strain) imaging techniques, either *in vivo* or using the *in vitro* inflation method (Boekhoven et al. 2014; Hansen et al. 2013; van den Broek et al. 2011). From the radiofrequency (RF) data, local deformation fields are estimated and displayed as color overlays on the regular B-mode images, that is, strain imaging (Talhami et al. 1994). The combination of inflation testing and ultrasound strain imaging has been used in healthy and atherosclerotic tissue (Boekhoven et al. 2013; Ribbers et al. 2007) and in other types of arteries, mostly in a quasi-static fashion (Brusseau et al. 2001; de Korte et al. 1998; Lopata et al. 2014; Schaar et al. 2003).

One step beyond strain imaging is so-called elastography or elastometry. The aim in vascular elastography is to identify the local mechanical properties of vessels *in vivo*, using invasive or non-invasive ultrasound imaging (Baldewsing et al. 2004; Maurice et al. 2008). Elastometry is defined as the estimation of a global parameter set from ultrasound data (Bensamoun et al. 2008; Saito et al. 2004). Validation of vascular strain imaging- and vascular elastography-related techniques is based mainly on simulation and phantom studies. The main advantage of simulation data is the known ground truth of the motion and deformation; however, the contrast of the images and speckle patterns are unrealistic. The same disadvantages are valid in the phantom studies, which have the advantage of known material properties and geometry. For the validation of results from vascular elastometry, bi-axial tensile testing has proven to be a good candidate. The direction and magnitude of the forces acting on the vessel sample can be matched for both methods. Lopata et al. (2014) reported good agreement between the two methods when modeling the porcine aortic arterial wall as a neo-Hookean solid.

By choosing an appropriate material model, the intrinsic material properties of the tissue can be estimated, which requires information not only on deformation of the tissue, but also on the force causing it. Numerous material models are described in the literature. The arterial wall is mostly described as a linear or non-linear hyperelastic solid material, implying that the stress–strain relation is derived from a strain energy density function. The constitutive material models advance from one-parameter models (*e.g.*, neo-Hookean model), to two-parameter models (*e.g.*, two-term Ogden, Fung, Demiray and Mooney-Rivlin models, Delfino et al. (1997), Fung et al. (1979), to non-linear, fiber-reinforced models such as those described by Driessen et al. (2005), Gasser et al. (2006) and Holzapfel and Gasser (2000). For translational purposes simple models are required, because the number of parameters to be obtained from clinical data is small. Moreover, the stress–strain relation of vascular tissue in the physiologic range is quite linear; hence the use of more simple models could be valid.

The goal of this study was to compare ultrasound elastographic measurements in carotid arteries obtained with inflation testing to those obtained with bi-axial tensile testing. In a previous study by our group, such a comparison was performed in relatively large vessels (aortas) using a simple, quasi-static approach (Lopata et al. 2014). In this study, 2-D RF-based displacement estimation was investigated for the much smaller carotid artery, which limits the performance because of the limitations in resolution. Inflation experiments were performed in a dedicated mock-circulation loop under more realistic, physiologic conditions with respect to pressurization, osmolarity and temperature. The complete mechanical behavior of 13 porcine carotids was assessed by pressurizing the wall for a full range of 0–120 mm Hg in a controllable and reproducible fashion. Moreover, dynamic pressurization was performed, which allowed testing for the more limited, physiologic pressure range, typically 80–120 mm Hg. Finally, mechanical behavior was described using a constitutive linear, one-parameter, material model (neo-Hookean) and a non-linear, two-parameter model (Demiray), for both the inflation and tensile testing data. The models were chosen to have a minimal number of parameters to allow accurate and robust estimation of the material parameters from this type of experimental data.

METHODS

Inflation testing

Sample preparation and data acquisition. Porcine carotid arteries were obtained from a local slaughterhouse (n = 13). The pigs were between 5 and 6 months of age and had a body weight in the range of 100–120 kg. The arterial segments were excised from the aortic arch to the bifurcation (common carotid artery), at the left side. The arteries were frozen at −20°C within 2 h of excision. For inflation testing, the carotid arteries were thawed, and excess material (mainly connective and adipose tissue) was removed from the total length of the segments and tested for leaks. Samples were discarded when leaks were found. The extracted samples had a stress-free length of about 30 mm and were selected to have a similar diameter (3.4 ± 0.5 mm). Cannulas were