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Research Paper

Assessment of mechanical properties of porcine aortas under physiological loading conditions using vascular elastography

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ABSTRACT

Non-invasive assessment of the elastic properties of the arterial wall is often performed with ultrasound (US) imaging. The purpose of this study is to estimate mechanical properties of the vascular wall using *in vitro* inflation testing on biological tissue and two-dimensional (2-D) US elastography, and investigate the performance of the proposed methodology for physiological conditions.

An inflation experiment was performed on 12 porcine aortas for (a) a large pressure range (0–140 mmHg); and (b) physiological pressures (70–130 mmHg) to mimic *in vivo* hemodynamic conditions. Two-dimensional radiofrequency (RF) data were acquired for one longitudinal and two transverse cross-sections for both experiments, and were analyzed to obtain the geometry and diameter–time behavior. The shear modulus (G) was estimated from these data for each pressure range applied. In addition, an incremental study based on the static data was performed to (1) investigate the changes in G for increasing mean arterial pressure (MAP) for a certain pressure difference (30, 40, 50 and 60 mmHg); (2) compare the results with those from the dynamic experiment, for the same pressure range.

The resulting stress–strain curves and shear moduli G (94 ± 16 kPa) for the static experimentare in agreement with literature and previous work. A linear dependency on MAP was found for G, yet the effect of the pulse pressure difference was negligible. The dynamic data revealed a G of 250 ± 20 kPa, whereas the incremental shear modulus (G_{inc}) was 240 ± 39 kPa. For all experiments, no significant differences in the values of G were found between different image planes. This study shows that 2-D US elastography of aortas during inflation testing is feasible and reproducible under controlled and physiological circumstances. In future studies, the *in vivo*, dynamic experiment should be repeated for a range of MAPs, and pathological vessels should be examined.

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1. Introduction

Current clinical decision making of aortic aneurysms is based on geometrical data, i.e., diameter measurements, to decide on surgical intervention (Brewster et al., 2003; Zankl et al., 2007). Patients with a diameter that exceeds 5.5 cm (or an increase of 0.6–0.8 cm in the last year) are elected for surgery (UK Small Aneurysm Trial, 1998). However, diameter assessment does not provide insight or quantitative data on the mechanical state of the wall, which is desirable to monitor or predict wall deterioration and possible rupture, since the diameter measurements have proven to be inadequate in individual cases (Choksy et al., 1999; Conway et al., 2001). Imaging modalities can be used to assess the mechanical properties of the aneurysmal tissue (Satriano et al., 2015; Tierney et al., 2012; Van 't Veer et al., (2008). However, ultrasound (US) is the modality of choice for non-invasive assessment of mechanical properties of vessels due to the high resolution, lack of radiation hazard, and its bed side applicability.

The assessment of mechanical properties of thoracic and abdominal aortic aneurysms using ultrasound has been reported in literature. *In vivo* studies include transesophageal vector velocity imaging (Kim et al., 2013; Petrini et al., 2014), pulse wave imaging (Li et al., 2013), and strain imaging (Bihari et al., 2013; Brekken et al., 2006; Fromageau et al., 2008; Taniguchi et al., 2014), whereas *in vitro* and *in silico* studies have been performed using acoustic radiation force impulse imaging (Tierney et al., 2010), inflation testing (Lopata et al., 2014), and strain imaging (Brekken et al., 2012). Studies on the assessment of intrinsic material properties are sparse and limited to distensibility measurements (Long et al., 2005; Oyamada et al., 2012).

Studies on the ultrasonic estimation of mechanical properties such as the elastic modulus are more abundant in other vessels, including work on coronary arteries (Baldewsing et al., 2006; Mikola et al., 2015), carotids (Beaussier et al., 2008; Gamble et al., 1994; Hoeks et al., 1990; Karimi et al., 2008; Schmitt et al., 2007) and brachial arteries (Kim et al., 2004).

The problem of *in vivo* studies lies in the verification, or validation, of the measurements. One does not simply obtain the ground truth. For that purpose, many studies have been conducted using simulations (Brekken et al., 2012; Larsson et al., 2009), experimental studies on phantoms (De Korte et al., 1997; Maurice et al., 2004), *in vitro* inflation testing on biological samples (Boekhoven et al., 2014; Lopata et al., 2014), and comparison of *in vivo* results obtained with different modalities (Naim et al., 2013).

There is a long history of determining the mechanical properties of human and animal aortic tissue *in vitro*. Numerous studies have performed uni- and bi-axial tensile testing on normal and aneurysmal tissue (Di Martino et al., 2006; Forsell et al., 2014; Kamenskiy et al., 2014; Okamoto et al., 2002; Raghavan et al., 1996; Thubrikar et al., 2001; Vande Geest et al., 2006). Studies on porcine tissue, used in this study, include reports from the early eighties on stress–strain behavior and stiffness of thoracic pig aortas using tensile testing (Purslow, 1983). More recently, Peña et al. (2015) performed uniaxial and biaxial tensile tests on thoracic porcine aortas. Alternatives to tensile testing are compression (Han and Fung, 1995) and inflation testing using pressurization (Kim and Baek, 2011; Lopata et al., 2014) or an uniaxial tensile tester (Lillie et al., 2010).

Inflation testing on biological tissue is preferred over phantoms since the non-linear material behavior will be closest to that of *in vivo* tissue, and over tensile testing, since the tissue structure is left intact and a more physiological load is applied. Recently, inflation testing was used to assess the mechanical behavior of healthy aortas using ultrasound and was validated using tensile testing (Lopata et al., 2014). In this particular study, hydrostatic pressurization was performed by injecting fluid and measuring the pressure using a water column, similar to the work presented in other studies on phantoms and carotids (Ribbers et al., 2007; Ryan and Foster, 1997). This method is relatively labor intensive, which makes reproducible measurements time consuming, whereas the type of loading is not realistic for the *in vivo* situation. A solution lies in pulsatile pressurization (Boekhoven et al., 2014; Li et al., 2013; Van den Broek et al. (2011). A motorized setup can be used to apply a pressure pulse that is realistic in shape and magnitude, thereby mimicking the *in vivo* loading of the tissue in an experimental setup (Boekhoven et al., 2014).

In this study, an experimental framework is introduced to perform ultrasonic measurements of diameter changes and mechanical properties under physiological loading conditions by closely mimicking the *in vivo* hemodynamics. Moreover, full inflation testing will be performed on porcine aortas as shown in the aforementioned previous study, but now in a reproducible and controlled fashion using an automated setup. The feasibility of assessing material properties in different imaging planes and for different pressure ranges is assessed and the relation between elastic modulus and mean arterial pressure is examined.

2. Materials and methods

2.1. *Sample preparation*

Porcine tissue was obtained from a local slaughterhouse and comprised a large part of the aorta (11–18 cm) with the heart still attached ($n = 12$). The pigs were between 5 and 7 months of age and had a body weight varying between 100 kg and 120 kg. Right after the excision at the slaughterhouse, the porcine tissue was stored in phosphate buffered saline solution (PBS-solution) and transported to the laboratory. All aortas were dissected from the heart and excess connective and fat tissue was removed from the aortas. Each aorta was stored in PBS-solution in a freezer at $-20\,^{\circ}\mathrm{C}$. This method of preservation did not lead to any significant changes in the mechanical properties of the tissue (Stemper et al., 2007), even for longer periods of time (O'Leary et al., 2014).

Prior to the experiment, the aorta was thawed slowly and all remaining connective and fat tissue was removed from the outer layer of the aorta. From time to time, the aorta was rinsed with fresh PBS-solution to keep it moist. The side branches were closed using suture thread and tissue glue (superglue). Finally, the aorta was cannulated at both ends, after which a leakage test was performed to check whether the side branches were correctly closed and to detect other possible leakage sites. If so, additional superglue was added to completely close the side