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Research Paper

# Effect of glucose on the biomechanical function of arterial elastin

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ABSTRACT

Elastin is essential to provide elastic support for blood vessels. As a remarkably long-lived protein, elastin can suffer from cumulative effects of exposure to biochemical damages, which can greatly compromise its biomechanical properties. Non-enzymatic glycation is one of the main mechanisms of aging and its effect is magnified in diabetic patients. The purpose of this study is to investigate the effects of glucose on mechanical properties of isolated porcine aortic elastin. Elastin samples were incubated in 2 M glucose solution and were allowed to equilibrate for 4, 7, 14, 21 or 28 days at 37 °C. Equibiaxial tensile tests were performed to study the changes of elastic properties of elastin due to glycation. Significant decreases in tissue dimension were observed after 7 days glucose incubation. Elastin samples treated for 14, 21 or 28 days demonstrate a significant increase in hysteresis in the stress–stretch curves, indicating a greater energy loss due to glucose treatment. Both the longitudinal and the circumferential directions show significant increases in tangent modulus with glucose treatment, however only significant increases are observed after 7 days for the circumferential direction. An eight-chain statistical mechanics based micro-structural model was used to study the hyperelastic and orthotropic behavior of the glucose-treated elastin and the material parameters were estimated using a nonlinear least squares method. Material parameters in the model were related to elastin density and fiber orientation, and, hence, the possible microstructural changes in glucose-treated elastin. Estimated material parameters show a general increasing trend in elastin density per unit volume with glucose incubation. The simulation results also indicate that more elastic fibers are aligned in the longitudinal and circumferential directions, rather than in the radial direction.



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## 1. Introduction

Elastic fibers, one of the major structural components of the extracellular matrix (ECM), are essential to provide elasticity to the aortic wall and to accommodate intermittent deformations encountered during cardiac cycles. These resilient networks are made primarily of a protein called elastin. Elastic fibers consist of an inner crosslinked elastin core surrounded by a mantle of fibrillin-rich microfibrils (Mecham, 2008). In elastic arteries, such as the aorta, elastic fibers form thick concentric fenestrated layers of elastic laminae, with inter-laminar connecting fibers distributed radially through the vessel wall (O'Connell et al., 2008). Each elastic lamina alternates with a layer of smooth muscle cells (SMCs), and together, organize into a laminar unit which is considered as the functional unit of the vessel wall (Brooke et al., 2003; Wolinsky and Glagov, 1967; Davis, 1993).

Pathogenesis of vascular diseases, including hypertension, atherosclerosis, stenosis and aneurysms has been associated with elastin disorders in structure and function and its altered interaction with other arterial constituents (Campa et al., 1987; Menashi et al., 1987; Kobs et al., 2005; Arribas et al., 2006; Krettek et al., 2003). From a biomechanical viewpoint, abnormalities in elastin can alter mechanical homeostasis and promote SMC proliferation, migration and synthesis of ECM and eventually contribute to the development of disease (Ito et al., 1997; Ito et al., 1998; Li et al., 1998; Karnik et al., 2003; Ailawadi et al., 2009; Yamamoto et al., 1993). Therefore, it is important to have a better understanding of the mechanical properties of elastin and its alteration in disease, as well as the mechanisms by which elastin is degraded/damaged, influencing the arterial remodeling. To understand the mechanical function of elastin in the arterial wall, purified elastin was obtained and mechanically tested using both uniaxial stretching (Lillie and Gosline 2002; Watton et al., 2009; Stephen et al., 2013) and biaxial tensile testing methods (Gundiah et al., 2007; Zou and Zhang, 2009, 2012). It has been shown that the elastin network in the thoracic aorta possesses anisotropic mechanical behavior, and it becomes increasingly anisotropic with distance from the heart (Zou and Zhang, 2009; Watton et al., 2009).

Elastin is a long-lived ECM protein and it can suffer from cumulative effects of biochemical damages. Non-enzymatic glycation of elastin has been shown to increase with aging (Konova et al., 2004). Stiffening of the arterial wall among diabetic patients has been attributed to glycation crosslinks of elastin and collagen (Sims et al., 1996). In vitro studies have also shown that glucose treatments stiffens arterial elastin (Winlove et al., 1996, Zou and Zhang, 2012), increases the storage and loss modulus (Lillie and Gosline, 1996), and also changes the viscoelastic stress relaxation behavior of elastin (Zou and Zhang, 2012). We have previously studied the mechanical behavior of elastin using a biaxial tensile testing method and demonstrated that short-term glucose treatment (48 h) induces changes in both the elastic and viscoelastic properties of elastin (Zou and Zhang, 2012). The present study aims to fully understand the mechanical properties of elastin with longer period of glucose treatment. To this end, the changes in the mechanical properties of elastin were studied with 4, 7, 14, 21 or 28 days of glucose treatment. Histology studies and Transmission electron microscopy (TEM) were performed to examine the elastin samples and to identify possible ultrastructure changes due to glucose treatment. A statistical mechanics based constitutive model was implemented to describe the orthotropic hyperelastic behavior of elastin with glucose effects.

## 2. Materials and methods

### 2.1. Sample preparation

Porcine thoracic aortas were harvested from a local abattoir and transported to laboratory on ice. Aortas were cleaned of adherent tissues and fat and rinsed in distilled water. Squared samples of about 20 mm × 20 mm were cut from the cleaned aortas. All samples were taken from the similar longitudinal region of aortas to minimize the changes of mechanical properties with longitudinal position (Zou and Zhang, 2009). Purified elastin was obtained using cyanogen bromide (CNBr) treatment while cells, collagen and other ECM components were removed (Zou and Zhang, 2009). Briefly, aortic samples were treated with 50 mg/ml CNBr in 70% formic acid solution for 19 h at room temperature with gentle stirring. Tissue samples were then gently stirred for 1 h at 60 °C and followed by 5 min of boiling to inactivate CNBr. Elastin tissue samples were kept in 1 × phosphate buffered saline (PBS) solution before further experiment.

### 2.2. Glucose treatment

Before glucose treatment, the side lengths and thickness of each elastin sample were carefully measured using a digital caliper. Thickness was determined by averaging the measurements taken at 8 positions over the sample. Tissue samples ($n=7$) were then incubated in 2 M glucose solution and were allowed to equilibrate at 37 °C for 4, 7, 14, 21 or 28 days. The dimensions of the tissue samples were re-measured after glucose treatment.

### 2.3. Histology studies

Aorta and elastin samples were fixed in 10% formaldehyde (Fisher Scientific) for histological studies. A cross-section of about 6 μm in thickness was cut along the longitudinal direction and stained with Movat's pentachrome, which stains collagen fibers yellow, nuclei/elastic fibers purple to black, SMCs red and ground substance blue (Taylor et al., 1999; Arbustini, 2002; Chow et al., 2013).

### 2.4. Transmission electron microscopy (TEM)

Aorta and purified elastin samples were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). The samples were then washed in buffer, and sequentially treated with 1% osmium tetroxide in buffer, 2% tannic acid in buffer and 2% uranyl acetate in water as previously described (Davis, 1993). The samples were dehydrated through a graded series of methanol to propylene oxide, inflitrated with propylene oxide: Epon mixtures and embedded in pure Epon. The blocks were polymerized at 60 °C for 3 days. Thin sections (60 nm) were counterstained with methanolic uranyl acetate and lead citrate