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Research Paper

Assessing the microstructural response to applied deformation in porcine passive skeletal muscle

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ABSTRACT

The passive micro-structural mechanical response of muscle tissue is important for numerous medical applications. However, the recently observed tension/compression asymmetry in porcine muscle remains poorly explained. In particular there remains a lack of understanding of how external tension or compression applied in the fibre or cross-fibre direction translates internally to deformation of muscle fibres and the extra-cellular matrix. Accordingly, fresh porcine skeletal muscle tissue was harvested, deformed by 30% in uniaxial tension or compression in both the fibre and cross-fibre directions and prepared for optical microscope, polarised light microscope and SEM analysis. The average deformed specimen results were compared to the average control results in each case. For compressive or tensile stretch applied in the muscle fibre direction the average measured muscle fibre cross-sectional area changes are in close correspondence with predictions based on global Poisson's ratio measurements and these deformation modes did not cause shape changes in the muscle fibre cross-sections. However, muscle tissue reacted to the applied cross-fibre direction deformations as follows: compression flattened muscle fibre cross-sections, aligning them perpendicular to the direction of the applied deformation while tensile deformations stretched the cross-sections of muscle fibres, aligning them parallel to the direction of applied deformation. No evidence of structural reorganisation of endomysium collagen fibres in response to applied stretch was observed. The observed micro-structural responses do not appear to be influenced by the surrounding endomysium, but appear to be significantly influenced by proximity to the perimysium network. It is hypothesised that the perimysium and its interaction with the surrounding muscle fibres is therefore likely to be predominantly responsible for the tension/compression asymmetry observed in macroscopic tests of passive skeletal muscle stress strain behaviour.

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1. Introduction

Skeletal muscle fibres are embedded in extracellular matrix (ECM) which is composed of proteoglycans, elastin and collagen fibres. The role of the ECM is to provide two important functions: (1) a structure that organizes muscle fibres into aligned hierarchal groups and (2) to act as a retaining mechanism during muscle deformation (Rowe, 1974, 1981; Huijing, 1999, 2009). Collagen is the main fibrous protein of the extracellular matrix, accounting for approximately 1–9% of the mass of a fat free skeletal muscle. It is also reported that the mechanical properties of passive skeletal muscle depend on the collagen intermolecular cross-linking as well as the size, orientation and organisational arrangement of the collagen fibres (Nishimura et al., 1996; Purslow, 2010; Rowe, 1974).

The arrangement of the ECM is functionally divided into three hierarchical levels: the endomysium network surrounds individual muscle fibres, the perimysium network encloses a collection of skeletal muscle fibres called fascicles, and the epimysium encapsulates the entire muscle. Collagen is assumed to be the main load bearing component of passive skeletal muscle (Boerboom et al., 2007). In the meat industry the collagen content has been directly linked with the toughness of meat (Bailey and Light, 1989; Fang et al., 1999; Light et al., 1985).

The tensile response of passive skeletal muscle is anisotropic (Takaza et al., 2013; Morrow et al., 2010; Hernandez et al., 2011) and some authors describe this as specifically being transversely isotropic (Blemker and Delp, 2005; Morrow et al., 2010), nonlinear (Grieve and Armstrong, 1988; Morrow et al., 2010; Takaza et al., 2013) and viscoelastic (Martins et al., 1998; Myers et al., 1991; Yamada, 1970; Hernandez et al., 2011). Similarly, for compressive deformations, the following characteristics have been observed: anisotropic (Van Loocke et al., 2006, 2008, 2009) or specifically transversely isotropic (Song et al., 2007), nonlinear (Grieve and Armstrong, 1988; Song et al., 2007; Van Loocke et al., 2006) and viscoelastic (Grieve and Armstrong, 1988; Van Sligtenhorst et al., 2006; Song et al., 2007; Van Loocke et al., 2008, 2009).

However, the tensile stress/strain response is approximately two orders of magnitude higher than the compressive response (see Fig. 1). In compression, the stress–strain plot of all muscle fibre testing angles shows that the 45 degree loading direction is the least stiff (Van Loocke et al., 2006), see Fig. 1A. In tension the stress–strain plot of all muscle fibre testing angles shows a more a sinusoidal shape response, with the muscle fibre direction being the most compliant (Takaza et al., 2013), see Fig. 1B.

This asymmetrical tension/compression response of passive skeletal muscle when subjected to external deformations is not fully understood. A partial explanation has been offered by Gindre et al. (2013), where a simplified structural model was developed to assess the hypothesis that the mechanical response of skeletal muscle is dominated by the interaction between the soft but incompressible muscle fibres and stiff but initially soft collagen and the connective tissue. However, although Purslow performed seminal work (Purslow, 1989, 1999, 2010; Purslow and Trotter, 1994; Trotter, 1995; Trotter and Purslow, 1992) in studying the microstructure of skeletal muscle tissue and relating this to the stiffness, the recent observation of the tension/compression asymmetry in muscle remains poorly explained. In particular there remains a lack of understanding how external tension or compression applied in the muscle fibre or cross-fibre direction translates internally to deformation of muscle fibres and the connective tissue.

The aim of this paper is therefore to evaluate the relationship between the macroscopic response to applied deformation and the microscopic response in passive skeletal muscle and to apply the results to study how the microstructure of the tissue gives rise to the tension/compression asymmetry recently observed. It is anticipated that this approach is necessary as a precursor to developing appropriate microstructurally based constitutive models of passive skeletal muscle, which can capture both the tension and compression characteristics observed.

2. Methods

Fresh *Longissimus dorsi* skeletal muscle tissue was harvested from two 3 month old female pigs. The experimental protocols

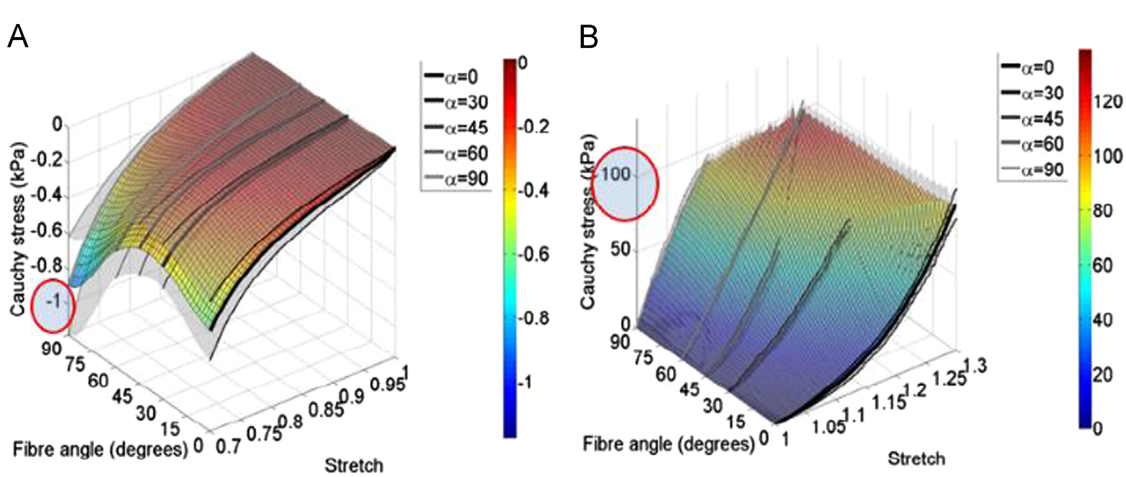

Fig. 1 – The (A) compressive and (B) tensile passive quasi-static stress–strain response of porcine skeletal muscle under uniaxial loading at various angles to the muscle fibre direction.