SKELETAL MUSCLE RESEARCH ACROSS STRUCTURAL LEVELS

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INTRODUCTION
Skeletal muscles have a natural structure, and the different structural elements can be exploited to address problems in muscle mechanics. In human biomechanics, muscles are often studied as entire groups spanning a joint. The next lower level of investigation is the isolated muscle typically activated by direct electrical stimulation. Much of the 20th century research aimed at elucidating the mechanisms of contraction was performed on isolated fascicles or single fibres, either in the intact (fibres with intact membranes that can be stimulated electrically) or skinned preparation (membranes are removed chemically or mechanically and activation occurs by controlled fluid exchange). For the past 30 years, systematic mechanical experiments have also been performed on single myofibrils (a sub-cellular organelle that has a number of sarcomeres arranged in series), and from that preparation, our group pioneered dynamic measurements on mechanically isolated, single sarcomeres [1]. Finally, Finer et al. [2] were the first to publish work on single protein-protein interactions using laser trapping techniques, and used this technique to measure the forces and step sizes of single cross-bridges attaching to an isolated actin molecule. Laser trapping, atomic force microscopy and other micro- and nano-scale techniques have also been used to explore the properties of isolated contractile (actin, myosin) or structural muscle proteins, such as titin, nebulin, and desmin.

METHODS
Our group is working on all structural levels identified above. For the purpose of this presentation, I will focus on approaches and questions we have asked on the sub-cellular level which includes single myofibrils, single sarcomeres, single proteins and actin-myosin interactions.

Single myofibrils can be isolated easily from any muscle. We mostly use the rabbit psoas, but for specific questions have also isolated myofibrils from hearts with cardiac myopathy, spastic muscles from children with cerebral palsy, and mdx knockout mice. Myofibrils are attached to a nano-lever or an atomic force lever for force measurements and a glass needle attached to a motor for mechanical perturbation. Single sarcomeres are set up in an identical manner as myofibrils, but then a sarcomere is identified and mechanically isolated by gripping it at its z-bands using micro-manipulator technology. Actin-myosin interactions (or more precisely, interactions of a single cross-bridge with an isolated actin protein) are performed using a laser trap approach [2] in which a self assembled actin is decorated at either end with a micron-sized silica bead that is then captured and held in place through the forces exerted by a focussed laser beam. The actin can then be manipulated and brought into contact with a single cross-bridge head that we place on top of another silicon bead attached to a cover slip. The cross-bridge can then interact with the actin while experimental perturbations (cross-bridge stretching or shortening in the attached state) can take place and the dynamics of single cross-bridge interactions can be measured. Single protein properties, such as we have determined with recombinant fragments of the molecular spring “titin”, can be performed using standard atomic force microscopy.

RESULTS
Among the many results we have obtained using sub-cellular micro- or nano-based approaches to study the mechanics of skeletal muscle, I would like to highlight four that have direct implications for whole muscle function.

Laser trapping: Measuring the attachment dynamics of single cross-bridges during isometric, concentric, and eccentric contractions, we found that dwell times were unaffected by the mode of contraction, dispelling the commonly held view at the time that the enhanced muscle forces following active muscle stretching were caused by an increase in the proportion of attached cross-bridges [3].

Atomic force microscopy: Measuring the molecular unfolding of forces of fragments of titin (the so-called Ig domains), we found that Ig-domains can bind calcium upon muscle activation, and that calcium binding made the titin spring stiffer, thereby supporting the idea that passive forces in an actively stretched muscle are much higher than the equivalent passive forces in a passively stretched muscle.

Single sarcomere: Measuring the forces in a single sarcomere for purely isometric contractions and for isometric contractions following active stretching, we found force enhancement in excess of 100%, dispelling the long-held belief that force enhancement was caused by sarcomere length non-uniformities [1].

Single Myofibrils: When producing stretch-induced damage in single myofibrils, we observed that sarcomere overextensions (“popping”) were consistently associated with reduced force loss compared to when such overextensions did not occur, suggesting that sarcomere popping is a protective mechanism in an actively stretched muscle, rather than the cause of force loss and damage, as previously assumed [4].

DISCUSSION & CONCLUSIONS
Great insight into mechanistic questions of skeletal muscle contraction can be gained by judiciously designed experiments on sub-cellular structures and isolated proteins.

REFERENCES

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