Mechanical stimulation of C2C12 cells increases m-calpain expression, focal adhesion plaque protein degradation and cell differentiation
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Conclusions. We have shown that mechanical signals transmitted through the C2C12 cells interaction with laminin cause an increase in cellular differentiation. This signaling results in not only an increase in the expression of the proteolytic enzyme m-calpain, but a relocation of the enzyme to the cell surface and subsequent breakdown of focal adhesion proteins.

Background: The past 10 years has seen a dramatic increase in the understanding of how proteolytic enzymes such as calpains can affect the growth of muscle. In vivo studies have shown that m-calpain is necessary for myoblast fusion leading to the formation of muscle fibres and that inhibition of this enzyme restricts myotube formation. Whether there is a link between stretch- or load induced signaling and m-calpain expression and activation is not known.

Objectives: The goal of this work was to determine whether a mechanically stimulated cell population showed differences in expression and localization of m-calpain, an enzyme required for myotube formation in vitro.

![Figure 1](image1.png)
Figure 1. The effect of mechanical stimulation on CPK activity in C2C12 cells. C2C12 cell cultures stimulated with laminin coated microspheres showed a higher CPK activity than controls (a). CPK activity was unaffected by either the presence of, or stimulation by fibronectin coated microspheres (b).

![Figure 2](image2.png)
Figure 2. The effect of mechanical stimulation via laminin receptors on m-calpain expression in C2C12 cells. Mechanically stimulated (a,c) and un-stimulated (b,d) myoblasts stained for either m-calpain (a,b) or µ-calpain (c,d). Mechanical stimulation increases m-calpain but not µ-calpain expression.

![Figure 3](image3.png)
Figure 3. Localization of m-calpain after mechanical stimulation with laminin coated spheres. A. Prior to stimulation. B. 30 min of stimulation. C. 4 h of stimulation. Arrows - focal adhesion complexes. Expression of m-calpain increases shortly after mechanical stimulation begins, and the enzyme relocates to the cell surface. After a longer stimulation time, the staining at the cell surface disappears.

![Figure 4](image4.png)
Figure 4. Breakdown of focal adhesions after mechanical stimulation of myoblasts. Stimulated (Bottom) and un-stimulated (Top) myoblasts were stained for the focal adhesion plaque protein paxillin. Paxillin staining can be seen in punctate focal adhesions in un-stimulated cells but not in stimulated cells.

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