L-type Amino Acid Transporter expression and function during *in vitro* myogenesis

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Introduction

- Amino acid supplementation with the essential amino acid leucine has been shown to have anabolic effects on skeletal muscle.
- Leucine is selectively transported into myofibers by the L-type amino acid transporter (LAT1), which directly activates mTORC1, an intermediate in protein synthesis that stimulates cell growth and proliferation pathways.
- Myogenesis is the process of forming new myofibers from precursor satellite cells. The four progressive phases of myogenesis include quiescence, activation, proliferation and differentiation.
- Although the mechanisms by which LAT1 senses and transports leucine is known, little is known about the direct effect of LAT1 on myoblasts, or of the presence and expression of LAT1 throughout myogenesis.

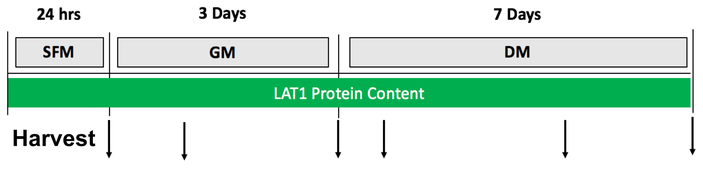
Purpose: To examine the expression and function of LAT1 on myoblasts during different stages of myogenesis *in vitro*.

Hypothesis: LAT1 will be expressed on myoblasts throughout all stages of myogenesis, the inhibition of LAT1 will impair myogeneis, and LAT1 will be responsive to leucine supplementation on myoblasts.

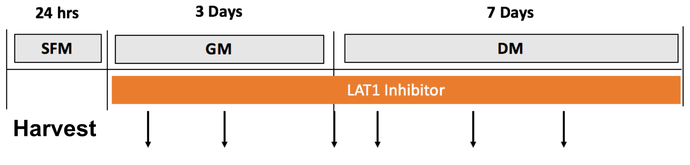
Methods

C2C12 myoblasts were made quiescent in serum free media (SFM) for 24 hours, then re-activated by incubation in growth media (GM) for 3 days. Myoblasts were then stimulated to differentiate in differentiation media (DM) for 7 days.

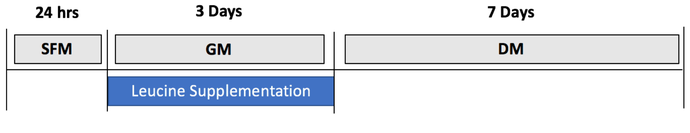
Objective 1: LAT1 protein content was evaluated across the time course in C2C12 myoblasts.



Objective 2: LAT1 specific inhibitor 2-Aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) was used to assess the effect of LAT1 inhibition on myoblast proliferation and differentiation.



Objective 3: LAT1 protein content was evaluated across a time course following incubation with leucine.



Results

LAT1 protein content increased during early differentiation

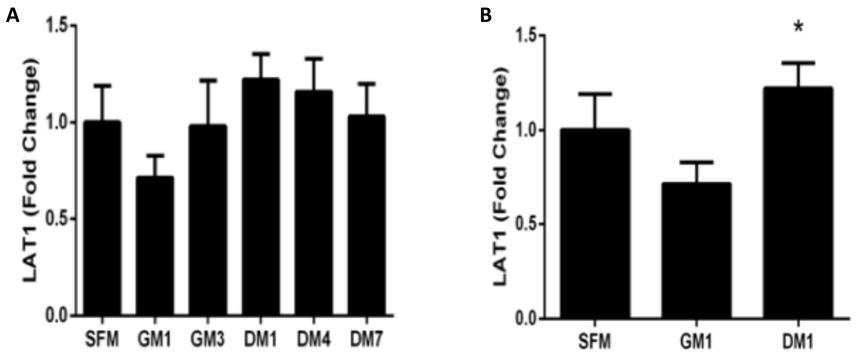


Figure 1: C2C12 myoblasts were harvested under SFM conditions, after 1 and 3 days in GM, and after 1, 4, and 7 days in DM. LAT1 protein content was examined by western blot. Quantifications are shown in (A) and (B). Data are mean ± SEM of n=5-8/group. *p<0.05 vs. GM1.

Inhibition of LAT1 results in decreased proliferation and impaired differentiation

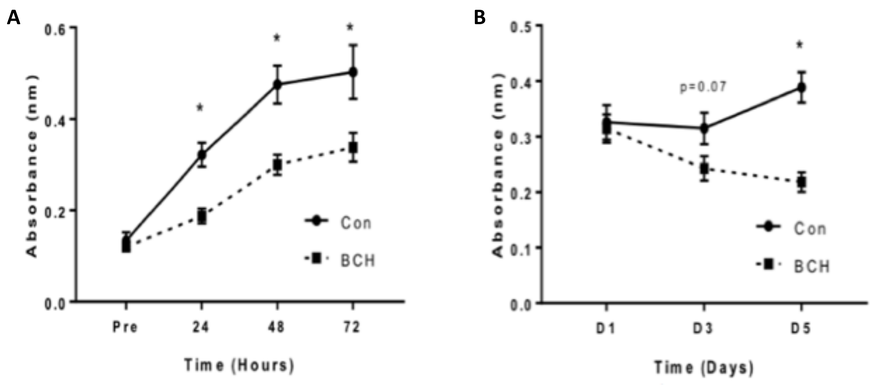


Figure 2: Myoblast protein content was assessed by MTT assay after 24, 48, and 72 hours in GM (A) and after 1, 3, and 5 days in DM (B), with or without 25mM of BCH. Data are mean ± SEM of n=8/group for (A), and n=5-6/group for (B). *p<0.05 vs. control.

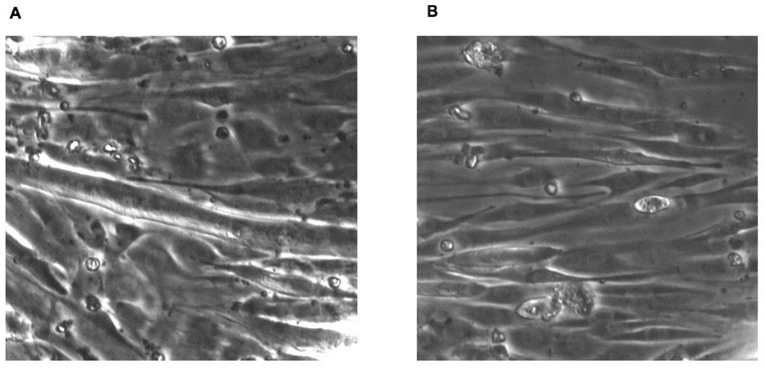


Figure 3: Effect of LAT1 inhibition on C2C12 myoblasts after 5 days in DM, where (A) represents cells without treatment, and (B) represents cells treated with 25mM of BCH.

LAT1 is not responsive to leucine supplementation

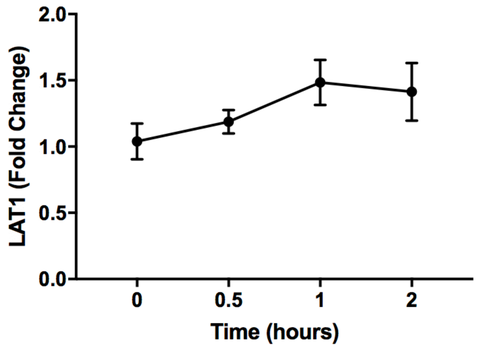


Figure 4: C2C12 myoblasts were harvested under SFM conditions, changes in LAT1 protein content was assessed by western blot and examined over a time course following incubation with 5mM leucine for 0.5h, 1h, and 2h. Data are mean ± SEM of n=9/group.

Conclusions

LAT1 is expressed on C2C12 myoblasts throughout all stages of myogenesis, where expression peaks during early differentiation relative to proliferation. Additionally, LAT1 inhibition impairs *in vitro* myogenesis which suggests an important role for the transporter in muscle development. However, LAT1 is not responsive to leucine supplementation.

These data suggest that myoblasts may be directly regulated by amino acids and could be used to prevent muscle atrophy, improve conditions that require muscle repair, and provide therapeutic options for individuals suffering from muscle wasting conditions.

In order to evaluate the effect of amino acid supplementation *in vivo*, future evaluation is necessary to examine the impact of leucine supplementation on LAT1 in a dose-dependent manner.

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