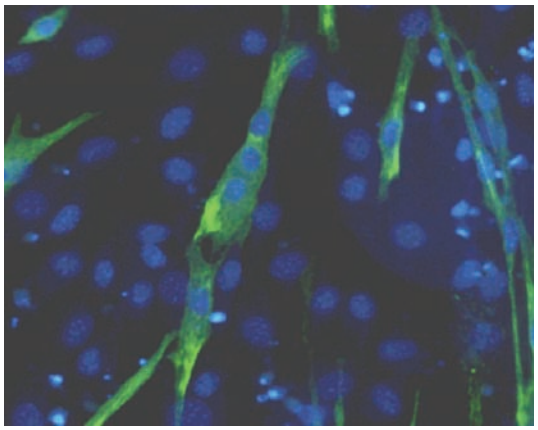
THE EFFECTS OF ARACHIDONIC ACID SUPPLEMENTATION ON MYOTUBE DEVELOPMENT



Depicts C2C12 stained with DAPI, MHC antibody, and phalloidin at 200X

Corporate Report

David Bisterfeldt, Howard Hughes Medical Institute Student Researcher

Allison Wilson , Ph.D. - Department Chair - Biological Sciences

Craig Broeder, Ph.D. FACSM, FNAASO
Director - Activities of Daily Living Performance Enhancement Research Center
Director of the Masters of Clinical Exercise Physiology Program

BENEDICTINE UNIVERSITY
DEPARTMENT OF BIOLOGICAL SCIENCES
LISLE, IL 60532
cbroeder@ben.edu

Study Summary

The current cell-culture studies we are conducting are designed to determine what effects arachidonic acid (AA) may have on the development and molecular constitutions involved in the differentiated myoblast to skeletal muscle myotube cells in vitro. We are targeting three specific areas of muscle growth including actin-myosin cell development-cell fusion, androgen receptor expression, and how protein cell-signaling activities are affected by various AA concentrations. To date, we have focused on the myotube hypertrophy and cell fusion effects of AA using immunohistochemical staining of myosin and actin. We are also in the final stages of setting up the optimum in-vitro model for identifying androgen receptor expression with and without AA added to our cell-culture medium.

Cell differentiation, myosin hypertrophy and fusion studies using C2C12 myoblasts (Precursor muscle cells) cells were conducted initially. These C2C12 cells were replenished with either fresh 10% fetal bovine serum (FBS) or the appropriate 2% horse serum (HS) media (containing no AA or 12.5 µM, 25 µM, or 50 µM AA) and then incubated for 96, 120, or 144 hours. These results indicated that the best differentiation was noted for at 144 hour incubation time in 2% HS and when comparing 25 µM or 50 µM AA to control cultures. Thus, a 144 hour incubation times were used as the experimental condition for all measurements.

For the myosin hypertrophy and fusion studies, the same experimental conditions described above were used. Thus far, we have observed clear phenotypic changes in C2C12 cells to myotubes whose medium contains arachidonic acid. These changes occurred most notable in 25 µM and 50 µM concentrations. The myotubes have an increased lateral diameter, enhanced levels of myosin deposition, and a greater concentration of nuclei than controls (Figures 1 and 2). These results suggest that arachidonic acid may play a role in enhancing protein synthesis and myotube development at the cellular level. Moreover, we observed increased levels of nuclear fusion suggesting arachidonic acid may augment a muscle's hypertrophic response through enhanced cellular myotube growth in our mouse myoblast cell-culture model.

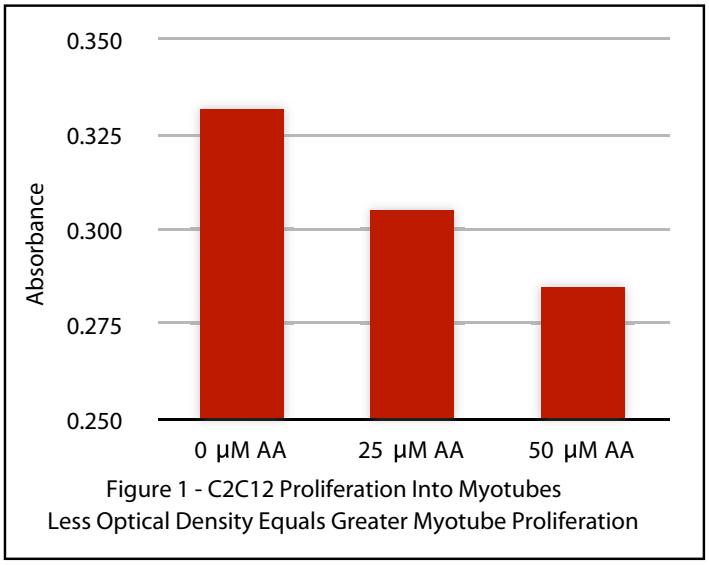
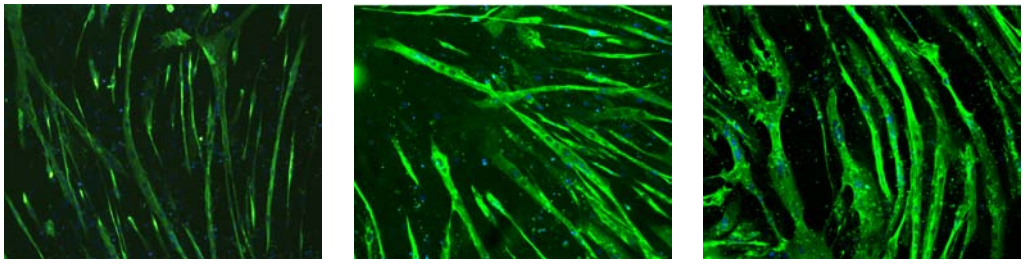


Figure 1 - C2C12 Proliferation Into Myotubes
Less Optical Density Equals Greater Myotube Proliferation



Controls No AA | 25 µM AA | 50 µM AA

Figure 2 - Differentiated C2C12 cells into myotubes with or without arachidonic acid.