

# **Disruption of Branched-chain Amino Acid Catabolism Improves Anabolic Signaling in Rat Myotube**

Glory Madu and Olasunkanmi A. J. Adegoke

School of Kinesiology & Health Science, Muscle Health Research Centre,  
York University, Toronto, Canada

Branched-chain amino acids (BCAAs) are essential amino acids that are crucial for skeletal muscle protein anabolism. Thus, alterations in their levels are associated with several muscle atrophic diseases such as cancer, chronic inflammatory and neurological disorders. In addition to their anabolic effect, BCAAs are catalyzed in the mitochondria through a multi-step process to generate acetyl CoA, its derivatives and other metabolites. Studies have shown that decreased BCAA catabolism leads to the buildup of BCAAs and their metabolites as seen in maple syrup urine disease and insulin resistance disorders (obesity and type 2 diabetes mellitus (T2DM)). BCAA catabolism starts with the reversible transamination by the enzyme, branched-chain aminotransferase 2 (BCAT2) followed by the irreversible carboxylation catalyzed by branched-chain ketoacid dehydrogenase (BCKD) complex. Data from our lab have shown that BCAT2 and BCKD are essential for the differentiation of skeletal myoblasts into myotubes. This current study investigates the effect of BCAT2 and BCKD depletion in differentiated skeletal muscle cells. On day 4 of differentiation, myotubes were transfected with scrambled siRNA (SCR siRNA, control), BCKD siRNA or BCAT2 siRNA oligonucleotides. Forty-eight hours after transfection, we observed improved myotube structure in the BCKD-depleted cells compared to those treated with scrambled or BCAT2 siRNA. Additionally, BCKD depletion augmented myofibrillar protein levels in comparison to control: myosin heavy chain (MHC, 2-fold,  $p < 0.05$ ,  $n = 3$ ), tropomyosin (4-fold,  $p < 0.05$ ,  $n = 3$ ), troponin (14%,  $p = 0.85$ ,  $n = 3$ ). When compared to BCAT2-depleted myotubes, BCKD-depleted myotubes exhibited a 4-fold increase in MHC ( $n = 3$ ,  $p < 0.05$ ), and increases in troponin and tropomyosin. To further analyze the increase in myofibrillar protein content, we examined signaling through mTORC1 (mammalian/mechanistic target of rapamycin complex 1), a vital complex necessary for skeletal muscle anabolism. BCKD depletion increased the phosphorylation of mTORC1's upstream activator, AKT (52%,  $p < 0.05$ ,  $n = 3$ ), and of mTORC1 downstream substrates by 25%-86%, consistent with the observed increase in myofibrillar proteins. Results from this study suggest that the depletion of BCKD enhanced myofibrillar protein content and anabolic signaling in myotubes. Future research will examine protein breakdown to better understand mechanisms involved in myotube protein metabolism. The development of dietary based interventions that target BCKD abundance may promote muscle protein anabolism in individuals with muscle wasting conditions.