EFFECT OF SATURATED FATTY ACID ON MARKERS OF BCAA-MEDIATED MITOCHONDRIAL BIOGENESIS AND BCAA CATABOLISM IN VITRO

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ABSTRACT

PURPOSE: Branched chain amino acids (BCAA) such as leucine, stimulate favorable metabolic processes involved in lean tissue preservation and skeletal muscle metabolism. However, higher levels of circulating BCAAs correlate with severity of metabolic disease (including diabetes/insulin resistance), and may result from deregulated BCAA catabolism. This study investigated the relationship(s) between BCAA concentration and expression of regulators of metabolism and BCAA catabolism in cultured skeletal muscle cells. METHODS: C2C12 myotubes were treated with or without varying concentrations of either leucine or valine for 24 hours, both with and without concurrent palmitate. Data were analyzed via Spearman correlation. RESULTS: Increasing leucine treatment significantly correlated with elevated mRNA expression of metabolic targets including peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α) and mitochondrial transcription factor A (mtTFA). Furthermore, increasing leucine treatment only correlated with PGC-1α expression. Interestingly, leucine-induced metabolic gene expression was abolished by concurrent palmitate. Additionally, branched-chain amino transferase 2 expression positively correlated with increasing leucine treatment, which was not observed for branched-chain α-ketoacid dehydrogenase BCAA catalytic enzyme expression. CONCLUSION: These data suggest leucine possesses unique metabolic effects compared with other BCAAs. Moreover, the presence of palmitate diminished the metabolic effects of leucine, suggesting lipids may suppress leucine-mediated cell adaptations.

METHODS

- C2C12 myotubes were treated with leucine from 0-2mM for 24 hours, both with and without concurrent palmitate at 0.5mM.
- Gene expression was measured via qRT PCR
- Protein expression was measured via Western Blot
- Lipid content was measured using Oil Red O extraction
- Cell viability was assessed using WST-1 assay
- Data were analyzed via Spearman correlation, 1-way ANOVA, 2-way ANOVA, or student’s t test.

- * or dissimilar letters indicates p<0.05
- Bonferroni’s correction was used for ANOVAs

RESULTS

Figure 1. Leucine-induced mitochondrial biogenesis is abolished by concurrent palmitate. Effect of varying leucine concentration with and without palmitate at 0.5mM on mRNA expression of (A) PGC-1α, (B) mtTFA, (C) Sirtuin 3, (D) FFH3, and (E) Sirtuin 1 of myotubes (n=3).

Figure 2. Cell viability of cells treated with and without leucine at 2mM both with and without palmitate at 0.5mM.

Figure 3. Palmitate increases lipid content without altering indicators of mitochondrial biogenesis. (A) Effect of palmitate on mRNA expression of peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α) and myotubes, nuclear common factor 1 (NRF1), mitochondrial transcription factor A (mtTFA), sirtuatn 3 (Sirt3), forkhead box protein O1 (Foxo1) and citrate synthase (CS). (B) Lipid content (indicated by oil red O extraction) of cells treated with and without leucine at 2mM both with and without palmitate at 0.5mM for 24 hours.

REFERENCES


WORKING HYPOTHESIS

Under conditions of energy homeostasis, BCAAs may promote improved metabolic phenotypes including improved glucose uptake/insulin sensitivity. Under conditions of chronic excess energy, cells (especially adipose) appear to lose the ability to degrade BCAAs causing an accumulation of BCAAs and related metabolites in circulation. As with many metabolites during metabolic disease (such as lipids and glucose), BCAA accumulation appears to correlate and may have predictive value of metabolic disease. It is hypothesized that obesity-suppression of molecular targets that regulate BCAA metabolism precedes deregulated BCAA catabolism and accumulation.