EFFECT OF VALINE ON MYOTUBE METABOLISM AND INSULIN SENSITIVITY

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ABSTRACT

BACKGROUND: Branched chain amino acids (BCAA) have become increasingly popular because of consistent correlations between circulating BCAA levels and insulin resistance. Most recently valine catabolite, 3-hydroxyisobutyrate (3HIB), has emerged as a potential cause of BCAA-mediated insulin resistance through increased endothelial lipid uptake. However, it is unclear if valine independently causes insulin resistance and related metabolic perturbations. Therefore, this study investigates the effect of valine on muscle metabolism *in vitro*. METHODS: C2C12 myotubes were treated with varying concentrations (0.5mM – 2mM) of valine for up to 48 hours. qRT-PCR was used to measure metabolic gene expression. Mitochondrial and glycolytic metabolism were measured via oxygen consumption and extracellular acidification rate, respectively. BCKDH protein expression was evaluated using western blotting, as was insulin sensitivity following insulin stimulation (indicated by pAkt). RESULTS: Valine did not affect genes which regulate mitochondrial biogenesis, mitochondrial metabolism or glycolytic metabolism, however valine significantly reduced basal and peak cell metabolism. And although valine altered BCKDHa gene expression following both 24- and 48-hour treatment, BCKDH protein content remained unaltered in valine-treated cells versus control. Additionally, valine treatment had no discernable effect on pAkt expression following both acute and 48-hour treatment. CONCLUSION: Despite consistent population data demonstrating an inverse relationship between circulating BCAAs (and related metabolites) valine does not appear to independently alter insulin sensitivity, however, valine may reduce skeletal muscle metabolism *in vitro*.

METHODS

- C2C12 myotubes were treated with valine from 0-2mM for up to 48 hours
- Gene expression was measured via qRT-PCR
- Protein expression was measured via Western Blot
- Cell viability was assessed using WST-1 assay
- Metabolism was measured via O_2 consumption or cellular acidification
- Data were analyzed via 1-way ANOVA, 2-way ANOVA, or 1-way MANOVA.
  - Dunnett's or Bonferroni's correction was used for ANOVAs
  - * indicates $p<0.05$ versus control
  - Dissimilar letters indicate $p<0.05$ between groups

RESULTS

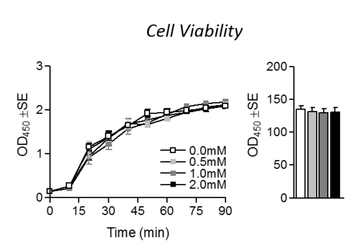

Figure 1. Effect of valine on myotube viability. *Viability of cells treated with valine at 0.0mM, 0.5mM, 1.0mM, or 2.0mM for 48 hours.*

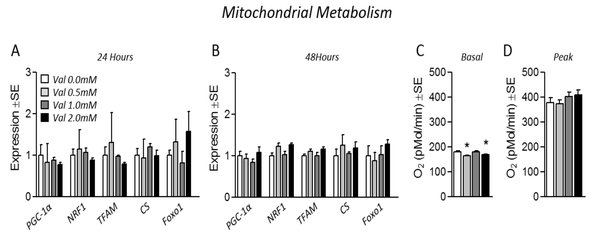

Figure 2. Effect of valine treatment on mitochondrial biogenic gene expression and metabolism. *(A and B) Effect of valine (0.0mM, 0.5mM, 1.0mM, or 2.0mM) for (A) 24 or (B) 48 hours on myotube mRNA expression of peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α), nuclear respiratory factor 1 (NRF1), mitochondrial transcription factor A (TFAM), citrate synthase (CS), and Forkhead box protein O1 (Foxo1). (C and D) Effect of valine treatment for 48 hours on oxygen consumption (O_2 pMol/min) under (C) basal and (D) peak metabolic conditions.*

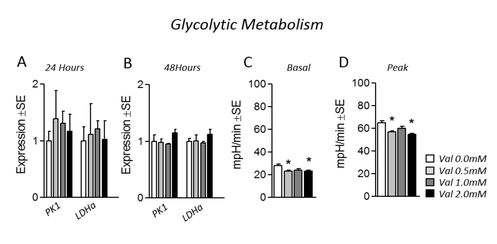

Figure 3. Effect of valine treatment on glycolytic gene expression and metabolism. *(A and B) Effect of valine (0.0mM, 0.5mM, 1.0mM, or 2.0mM) for (A) 24 or (B) 48 hours on myotube mRNA expression of pyruvate kinase (PK) and lactate dehydrogenase a (LDHa). (C and D) Glycolytic metabolism (mpH/min) under (C) basal and (D) peak conditions.*

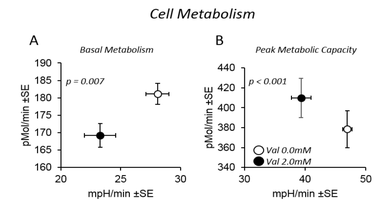

Figure 4. Effect of valine treatment on cell metabolism. *(A and B) Effect of valine at 2.0mM for 48 hours on myotube (A) basal metabolic rate (indicated by oxygen consumption (O_2 pMol/min) and glycolytic metabolism (mpH/min)) and (B) peak metabolic capacity.*

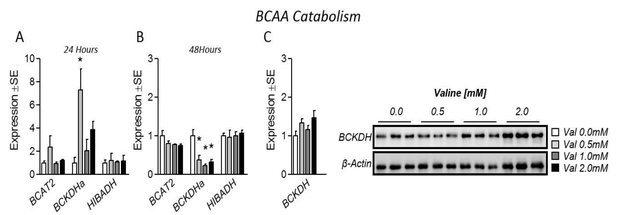

Figure 5. Effect of valine on myotube expression of BCAA-catabolic enzymes. *(A) Effect of valine at 0.0mM, 0.5mM, 1.0mM, or 2.0mM for (A) 24 and (B) 48 hours on mRNA expression of branched-chain aminotransferase (BCAT2), branched-chain-alpha-keto acid dehydrogenase (BCKDH), and 3-hydroxyisobutyrate dehydrogenase (HIBADH). (C) Effect of 48 hour valine treatment on BCKDHa protein expression.*

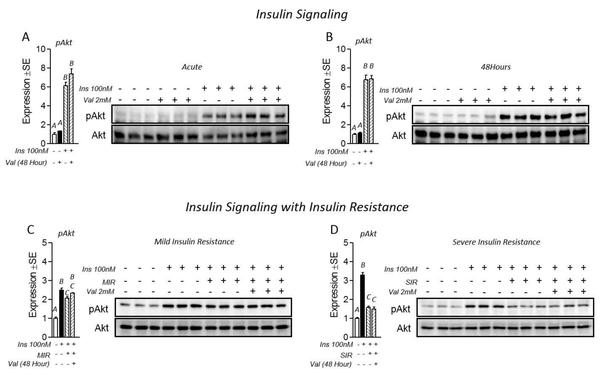

Figure 6. Effect of acute and chronic valine on myotube insulin signaling. *(A) Effect of 30-minute valine treatment at 2.0mM both with and without concurrent insulin stimulation (100nM) on insulin sensitivity (indicated by pAkt:Akt expression). (B) Effect of 48-hour valine treatment at 2.0mM on insulin sensitivity. (C and D) Effect of 2.0mM valine for 48 hours on insulin signaling during mild (C) or severe (D) insulin resistance. Notes: Insulin resistance was achieved by differentiating myotubes in the presence of 2nM (mild insulin resistance) or 100nM (severe insulin resistance) insulin for the final 3 days of differentiation plus the 48-hour treatment period.*

CONCLUSIONS

- Valine may alter myotube metabolism in a dose-dependent fashion without altering related gene expression.
- Valine temporally altered BCKDHa mRNA expression without altering BCKDHa protein content.
- Valine did not alter insulin signaling under normal cell conditions.
- Valine partially rescued insulin signaling during mild, but not severe, insulin resistance.

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