VALINE-CATABOLITE, 3-HYDROXYISOBUTERATE ALTERS MYOTUBE METABOLISM AND REDUCES INSULIN SIGNALING

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ABSTRACT

Recently, circulating branched-chain amino acids (BCAA) have been consistently correlated with severity of insulin resistance. The valine catabolite 3-hydroxyisobuterate (3HIB), was shown to enhance lipid uptake contributing to insulin resistance in skeletal muscle. PURPOSE: This study investigated the effect of 3HIB on skeletal muscle insulin signaling, metabolism, and related gene expression in vitro. METHODS: C2C12 myotubes were treated with 3HIB for up to 48 hours with various concentrations. Metabolic gene expression was measured via qRTPCR, cell metabolism was measured via O2 consumption (mitochondrial) and extracellular acidification rate (glycolysis), insulin sensitivity was measured using western blot, and lipid content was assessed using lipid-specific staining (each of which were analyzed using either t-test, one-way ANOVA, or MANOVA with correction for pair-wise comparison). RESULTS: 3HIB did not alter expressional indicators of mitochondrial biogenesis, glycolysis, BCAA catabolism, or lipogenesis. Chronic physiological 3HIB treatment significantly increased peak oxygen consumption ($p<0.05$), while supraphysiological 3HIB treatment suppressed basal and peak mitochondrial and glycolytic metabolism ($p<0.05$ for each). Both physiological and supraphysiological 3HIB reduced pAkt expression during insulin stimulation ($p<0.05$). CONCLUSION: 3HIB may reduce insulin sensitivity in vitro, supporting a potential role of 3HIB in the development of insulin resistance.

METHODS

Cell Culture

- Mouse myotubes (C2C12) were treated with 3HIB at varied concentrations for up to 48 hours.
- Acute insulin stimulation was accomplished using 100nM for 30 min.

Experimental Protocol

- mRNA expression was quantified via qRT-PCR
- Protein expression was quantified via western blot
- Mitochondrial metabolism was measured using O_2 consumption and glycolytic metabolism was measured via extracellular acidification rate.
- Lipid content was measured using ORO extraction and quantification, and confirmed with Nile Red staining.

RESULTS

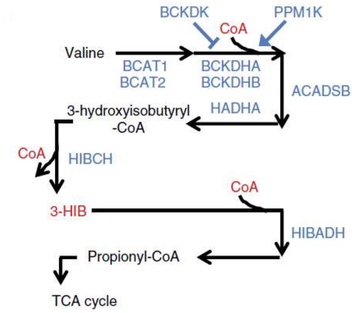


Figure 1. Catabolic pathway of valine.[2]

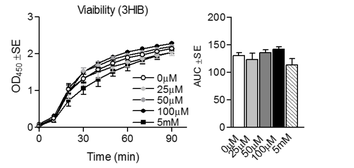


Figure 2. Effect of 3-hydroxylisobutyrate on myotube viability.

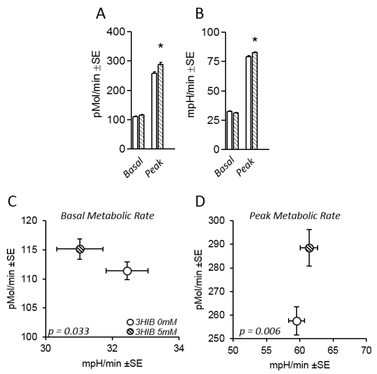


Figure 4. Effect of acute supraphysiological 3HIB concentrations on myotube metabolism. (A and B) Effect of 3HIB treatment on (A) myotube oxygen consumption (O2 pMol/min) and (B) glycolytic metabolism (mpH/min) under basal and peak metabolic conditions. (C) Basal and (D) peak metabolic capacity of cells treated with and without acute 3HIB at 5mM.

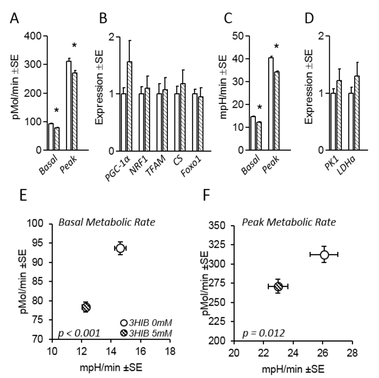
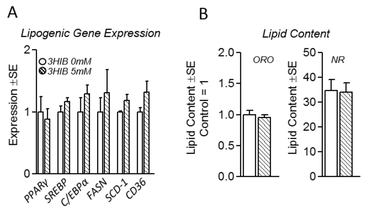


Figure 5. Effect of supraphysiological 3HIB treatment for 48-hours on (a) mitochondrial and (b) related gene expression, as well as (c) glycolytic metabolism and (d) related gene expression. (E) Corresponding basal metabolic rate and (F) peak metabolic capacity.



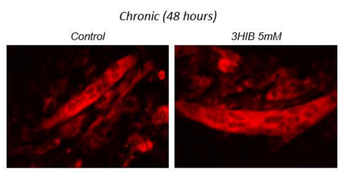


Figure 8. Effect of 3HIB on myotube lipogenic signaling and lipid content. (A) Effect of 3HIB at 5mM for 48 hours on regulators of lipogenesis and lipid uptake. (B) Lipid content following treatment with and without 3HIB for 48 hours indicated by Oil Red O extraction (left) and Nile Red fluorescent microscopy (right) with representative images.

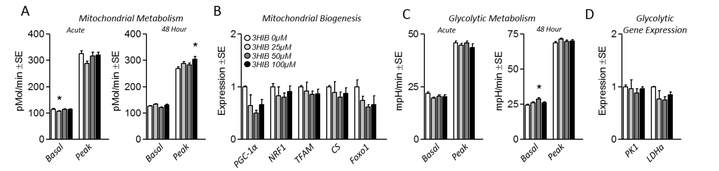


Figure 3. Effect of 3HIB on mitochondrial biogenesis and metabolism. (A) Effect of 3HIB on oxygen consumption (O_2 pMol/min). (B) Effect of 3HIB treatment on mitochondrial biogenesis. (C) Effect of 3HIB on glycolytic metabolism (mpH/min). (D) Effect of 3HIB on expression of PK1 and LDHa.

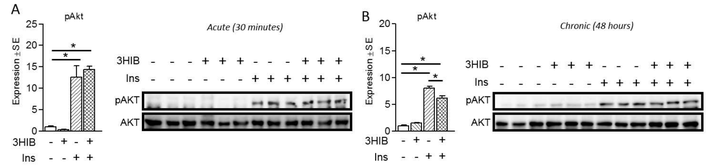


Figure 6. Effect of acute and 48-hour physiological 3HIB treatment on myotube insulin signaling. (A and B) Effect of (A) acute and (B) chronic physiological 3HIB-treatment (100μM) with and without 100nM insulin stimulation for 30 minutes on myotube insulin signaling (indicated by pAkt:Akt).

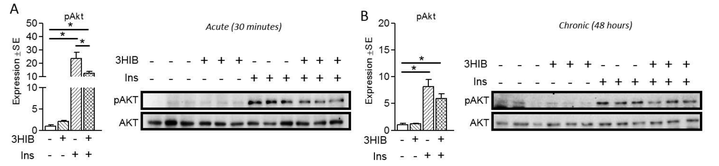


Figure 7. Effect of acute and 48-hour supraphysiological 3HIB treatment on myotube insulin signaling. (A and B) Effect of (A) acute and (B) chronic supraphysiological 3HIB-treatment (5mM) with and without 100nM insulin stimulation for 30 minutes on myotube insulin signaling (indicated by pAkt:Akt).

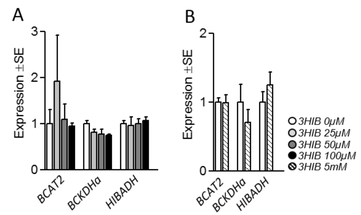


Figure 9. Effect of 3HIB on myotube BCAA-catabolic enzymes. (A) Effect of physiological 3HIB treatment for 48 hours on mRNA expression of BCAT2, BCKDH, and HIBADH. (B) Effect of supraphysiological 3HIB (5mM) for 48 hours on mRNA expression of BCAT2, BCKDHa, and HIBADH.

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