EFFECT OF VALINE AND VALINE-CATABOLITE, 3-HYDROXYISOBUTERATE ON CELL METABOLISM, MITOCHONDRIAL BIOGENESIS AND BCAA CATABOLISM

Emily S. Lyon[1], Michele A. Johnson[1], and Roger A. Vaughan, PhD[1*]

[1]*Department of Exercise Science, High Point University, High Point NC, 27268*

Author Contact: elyon@highpoint.edu or rvaughan@highpoint.edu

HIGH POINT UNIVERSITY
CONGDON SCHOOL OF HEALTH SCIENCES
www.highpoint.edu/healthsciences

HIGH POINT UNIVERSITY
CONGDON SCHOOL OF HEALTH SCIENCES
www.highpoint.edu/healthsciences

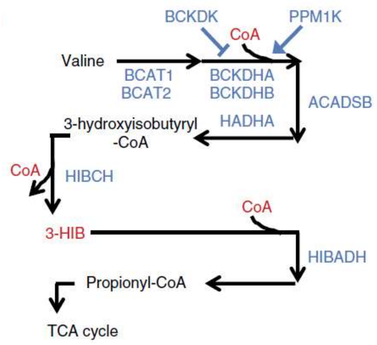
ABSTRACT

BACKGROUND: Valine and other branched chain amino acids (BCAAs) are known to have favorable effects including increased muscle mass and possibly enhanced oxidative capacity (via increased mitochondrial content) which may provide benefit for athletes. Interestingly, circulating BCAAs correlate with insulin resistance, although a causal relationship between BCAA consumption and disease development/progression has not been established. It is likely the relationship between elevated circulating BCAAs and insulin resistance is a byproduct of reduced BCAA catabolism, likely resulting from excess fat consumption. Recently, valine catabolite 3-hydroxyisobuterate (3HIB) was shown to enhance cellular lipid uptake contributing to insulin resistance. To further investigate the roles of valine and 3HIB in metabolic perturbations, this work examined the effect of both metabolites on skeletal muscle metabolism and related gene expression. METHODS: C2C12 myotubes were treated with varying concentrations of valine or 3HIB for 48 hours. Metabolic gene expression was measured via qRT-PCR, mitochondrial metabolism was measured via O2 consumption, and glycolytic metabolism was quantified using extracellular acidification rate. RESULTS: Neither valine nor 3HIB altered expressional indicators of mitochondrial biogenesis. 3HIB at 100uM significantly increased peak oxygen consumption compared to control. Conversely, supraphysiological 3HIB treatment (5mM) suppressed basal mitochondrial and glycolytic metabolism without altering metabolic gene expression. Valine treatment significantly decreased glycolytic metabolism (both basal and peak) as well as BCAA catabolic gene expression, which was not observed in 3HIB-treated cells. CONCLUSION: 3HIB may dose-dependently alter metabolism independent of mitochondrial biogenesis, while valine may uniquely suppress glycolytic metabolism and BCAA catabolic enzyme expression.

METHODS

- C2C12 myotubes were treated with valine from 0-2mM or 3-hydroxyisobutyerate (3HIB) from 0-100µM (physiological) or 5mM (supraphysiological) for 48 hours.
- Gene expression was measured via qRT PCR
- Mitochondrial metabolism was measured using O_2 consumption rate (OCR)
- Glycolytic metabolism was measured using extracellular acidification rate (ECAR)
- Cell viability was assessed using WST-1 assay
- Gene expression and metabolic data were analyzed via one-way ANOVA with Bonferonni's correction or t-test were used to determine differences between groups. Cell viability was analyzed using two-way ANOVA with Bonferonni's correction. * indicates $p < 0.05$ versus control

RESULTS



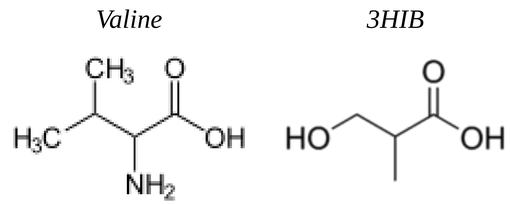


Figure 1. Depiction of valine catabolism and 3HIB biosynthesis from *Jang et al. 2015* (left) with valine and 3HIB structures (above).

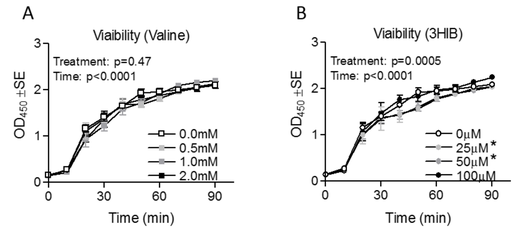


Figure 2. Effect of physiological levels of valine or 3-hydroxylisobutyrate on myotube viability.

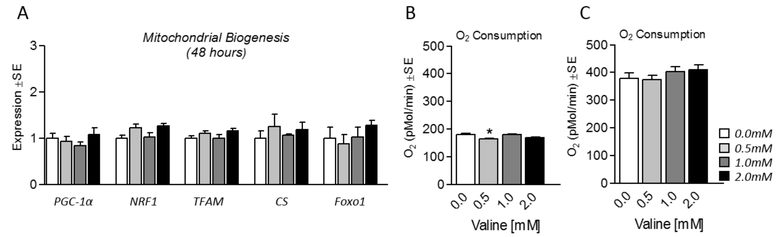


Figure 3. Effect of valine on mitochondrial biogenesis and metabolism. (A) Effect of valine treatment on myotube mRNA expression of PGC-1α, NRF1, TFAM, CS, and Foxo1. (B and C) Effect of valine on oxygen consumption (O_2 pMol/min) under (B) basal and (C) peak metabolic conditions.

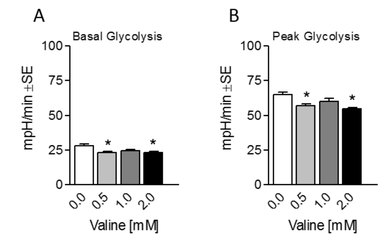


Figure 4. Effect of valine on glycolytic metabolism (mpH/min) under (A) basal and (B) peak metabolic conditions.

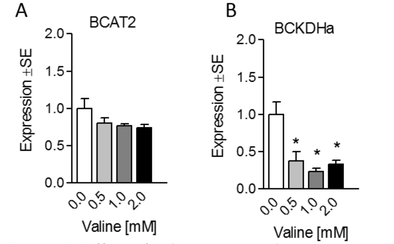


Figure 5. Effect of valine on myotube expression of BCAA-catabolic enzymes including (A) BCAT2 and (B) BCKDHa.

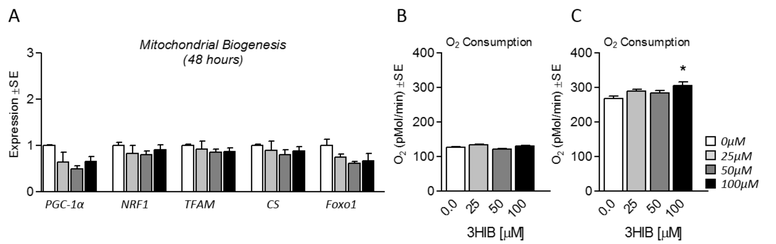


Figure 6. Effect of 3HIB on mitochondrial biogenesis and metabolism. (A) Effect of 3HIB treatment on myotube mRNA expression of PGC-1α, NRF1, TFAM, CS, and Foxo1. (B and C) Effect of valine on oxygen consumption (O_2 pMol/min) under (B) basal and (C) peak metabolic conditions.

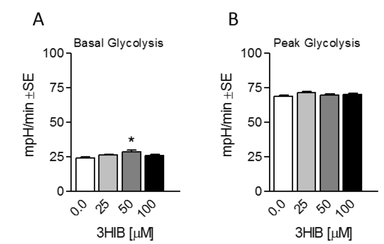


Figure 7. Effect of 3HIB on glycolytic metabolism (mpH/min) under (A) basal and (B) peak metabolic conditions.

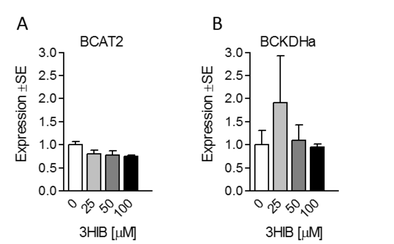


Figure 8. Effect of 3HIB on myotube expression of BCAA-catabolic enzymes including (A) BCAT2 and (B) BCKDHa.

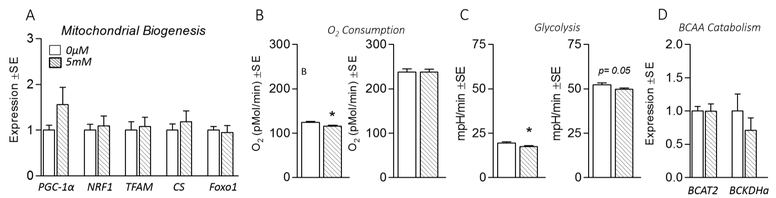


Figure 9. Effect of 3HIB treatment at 5mM for 48 hours on mitochondrial biogenesis, (B) basal and peak mitochondrial metabolism, (C) basal and peak glycolytic metabolism, and (D) BCAA catabolic gene expression.

REFERENCES

- A. Mardinoglu, S. Gogg, L.A. Lotta, A. Stancakova, A. Nerstedt, J. Boren, M. Bluher, E. Ferrannini, C. Langenberg, N.J. Wareham, M. Laakso, U. Smith, Elevated Plasma Levels of 3-Hydroxyisobutyric Acid Are Associated With Incident Type 2 Diabetes, EBioMedicine, 27 (2018) 151-155.
- C. Jang, S.F. Oh, S. Wada, G.C. Rowe, L. Liu, M.C. Chan, J. Rhee, A. Hoshino, B. Kim, A. Ibrahim, L.G. Baca, E. Kim, C.C. Ghosh, S.M. Parikh, A. Jiang, Q. Chu, D.E. Forman, S.H. Lecker, S. Krishnaiah, J.D. Rabinowitz, A.M. Weljie, J.A. Baur, D.L. Kasper, Z. Arany, A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance, Nat Med, 22 (2016) 421-426.
- C. Liang, B.J. Curry, P.L. Brown, M.B. Zemel, Leucine Modulates Mitochondrial Biogenesis and SIRT1-AMPK Signaling in C2C12 Myotubes, J Nutr Metab, (2014) 239750.