Regulation of Lactate and Protein Lactylation by Prenatal Hypoxia

Shikhar Gupta[1], Ethan Gadra BS[2], Ana Cristancho MD PhD[2,3]

[1]School of Arts and Sciences C. 2026, University of Pennsylvania, Philadelphia, PA
[2]Division of Child Neurology, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA
[3]Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Introduction

- Prenatal hypoxia is a condition defined by a decrease in uterine oxygen level affecting fetal development and raising the risk of perinatal and infant mortality.[1]
- 23% of newborn mortality can be attributed to prenatal hypoxia.[2]
- A markedly increased metabolite after prenatal hypoxia is profound acidosis, linking hypoxia to metabolism.[3]
- Lactate levels were shown to consistently rise following hypoxic damage in a serum-based study of primate models.[4]
- Recently discovered that lysine lactylation (Kla) is a post-translational modification for histones, the proteins important for organizing the genome and dictating which genes are accessible to transcription factors.[5]
- The presence of protein lactylation in prenatal hypoxia has not been previously studied.

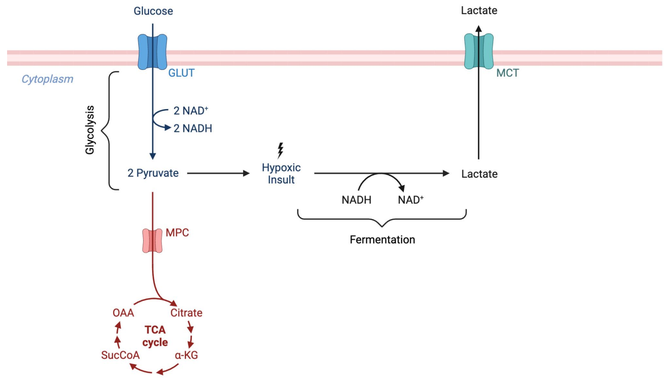


Figure 1. Mechanism of Lactate Production Following Hypoxic Insult

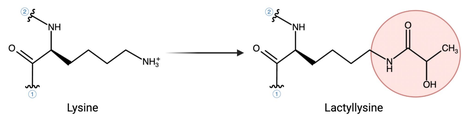


Figure 2. Mechanism of Protein Lactylation

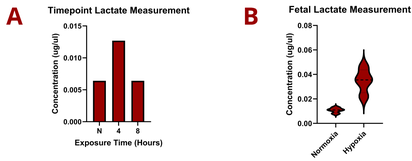
Figures created using Biorender

Methods

- Prenatal Mouse Model [6]
  - Pregnant female mice at E 17.5 acclimated at 21% inspired oxygen for 1-2 minutes. Oxygen concentration in chamber decreased to 5% inspired oxygen over 30 minutes. 5% inspired oxygen level maintained for 8 hours
  - Fetal cortex dissected immediately after hypoxia or control normoxia (pregnant animals maintained at 21% inspired oxygen for 8 hours)
- Metabolomics
  - Free Lactate measured using YSI 2900 Series Biochemistry Analyzer
  - Immunoblots run on 12% Bis-Tris Gels, probed with Anti-L-Lactyllysine Rabbit pAb for Kla detection

Results

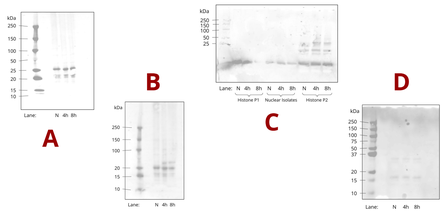
Figure 3. Intracellular lactate increased following hypoxic exposure.



(A) Timepoint analysis of lactate concentration in adult mice

(B) Lactate concentration in normoxic and hypoxic fetal mice

Figure 4. Development of a western blot Kla detection protocol.



(A) 120 minute transfer; probed for Kla

(B) 90 minute transfer; probed for Kla

(C) Histone protocol 1, nuclear isolates, histone protocol 2; probed for H3

(D) Histone protocol 2; probed for Kla

Discussion

- There is an increase in intracellular lactate in fetal mice that were exposed to 8 hours hypoxia
- A timepoint difference in lactate concentration exists in adult mice
- 90 minute transfer, 12% bis-tris gels, and histone extracts produce best blots for Kla detection

Future Directions

- Repeated fetal measurements with more litters
- Multiple timepoint measurements of lactate concentrations
- Finalizing histone-specific western blot protocol

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