Muscle Weakness and Altered Fiber Type In Sheep Hypophosphatasia is Associated With Diminished Activity and Compromised Kinematics

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Objectives

1. Determine the effect of reduced ALPL activity in HPP sheep on skeletal muscle structure and mitochondrial indices: 
   - Fiber type distribution
   - Mitochondrial area and number
   - Oxidative respiration (by high-resolution respirometry)

2. Determine if HPP sheep exhibit a waddling gait characteristic of human HPP: 
   - Overall daily activity levels (by Fitbit color monitoring)
   - Motion kinematics (by Vicon motion capture)

Summary and Conclusions

- HPP sheep have altered muscle fiber type and diminished mitochondrial area and respiratory efficiency.
- HPP sheep exhibit a waddling gait, indicated by an increase in spinal sway and slower stride, correlated with diminished serum ALPL activity.
- The similarity of altered gait and muscular phenotype in HPP sheep to humans demonstrates the utility of this novel model in understanding the etiology of HPP.
- Compromises in mitochondrial energetics, along with an altered fiber type in HPP sheep provide the first mechanistic insight into the etiology of muscle weakness in HPP.
Hypophosphatasia (HPP) is a mineralization disease associated with a waddling gait and muscle weakness.

- Heritable metabolic bone disease
  - Inborn error of metabolism
  - Low serum alkaline phosphatase (ALPL) activity
- >250 mutations
- Wide range in severity
- Wide range in severity of characteristics:
  - Defective mineralization
  - Rickets/osteomalacia
  - Muscle weakness + waddling gait

Index Patient Radiographs at 3 yrs. Note flared metaphysis at the joints (arrows) as well as bowed femur (arrowhead) that lead to a waddling gait which is associated with muscle weakness.
Novel HPP Sheep Demonstrate Low Serum ALPL Activity, Aberrant Gluteal Muscle Fiber Size and Disorganized Inner Mitochondrial Cristae

A. Sheep ALPL gene with CRISPR/cas9-generated mutation.
B. Serum ALPL activity decreased in c.1077C>G Heterozygous (Het), Homozygous (Hom) compared to WT * p<0.05.
C. Brightfield microscopy of gluteal muscle from F0 cohort show an aberrant gluteal muscle fiber size
D. TEM analysis of F0 gluteal muscle shows disorganized inner mitochondrial cristae (*) and fatty infiltration (#).

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Objectives

1. Determine the effect of reduced ALPL levels in HPP sheep on gluteal skeletal muscle structure and mitochondrial indices:
   - Fiber type distribution
   - Mitochondrial area and number
   - Oxidative respiration (by high resolution respirometry)

2. Determine if HPP sheep exhibit a waddling gait characteristic of human HPP:
   - Overall daily activity levels (by 24 hr Fitbark collar monitoring)
   - Motion kinematics (by Vicon motion capture)
HPP Sheep Gluteal Skeletal Muscle Have Altered Myosin Heavy Chain Fiber Types

A. Indirect Immunofluorescence with primary antibodies against 3 MHC Muscle Fibers: Type 1 Slow-Twitch Oxidative Muscle, Type 2a Intermediate Fast-Twitch Oxidative Fibers, and Type 2x Fast-Twitch Glycolytic Fibers was performed, quantifying Average percentage of total intensity for each fiber type using Image J.

B. Evaluation of % fiber type area expressed as Mean Gray Value (MGV)/Total Tissue Area was measured at 2 and 6 months of age, demonstrating increases in Type 1 and 2a oxidative fibers and a decrease in Type 2x Glycolytic fibers in HPP Homozygous Sheep (N= 5 Wt, 3 Het, 3 Hom).
HPP Sheep Gluteal Muscle Has Smaller Mitochondrial Area and Diminished Mitochondrial Efficiency

A. TEM analysis of 13 mo old gluteal muscle at 36,000X shows disorganized, smaller mitochondria.

B. Quantification in 4300 µm² tissue area shows significantly reduced mitochondrial area in HPP sheep.

C. High resolution respirometry of gluteal muscle tissue demonstrates a significant decrease in mitochondrial capacity and efficiency *p<0.05. N=13 (7WT, 2Het, 4Hom).
Preliminary Fitbark analysis of one homozygous male and one wild type male at 13 months. 24 hour monitoring was performed to determine average Distance Traveled/Day and average Caloric Expenditure/Day. The HPP sheep showed diminished overall activity levels (3.74 vs 4.55 miles/day) and caloric usage (2750 vs 3053 Kcal/day) as compared to the wild type sheep.
A. Schematic of anatomical locations of 52 fiduciary markers on sheep for motion capture analysis. The markers at the Thoracic and Sacral spine locations allowed the measurement of spinal sway by the distance the marker deviated from the central line surrounding these 2 locations. The marker path (yellow line), and deviation from these markers was measured (red line).

B. The integrated data show the distance away from center on the X axis, and the 95% confidence intervals show HPP sheep (blue shading) have increased spinal sway at both thoracic and sacral spine compared to WT (red shading).

C. Overhead videos from representative Homozygous HPP and WT sheep
HPP Sheep Have a Waddling Gait and Slower Stride

A. Stride velocity was calculated per foot and was positively correlated to serum ALPL activity, *p<0.05.
B. Hoof path analysis demonstrated that the spinal sway (blue track) is associated with splayed front Left (red) and right (green) hoofpaths in the HPP mutant as compared to WT.
C. Forward view motion capture videos from representative Homozygous HPP and WT sheep.
Summary and Conclusions

- HPP sheep have altered muscle fiber type and decreased mitochondrial area and respiration efficiency.
- HPP sheep exhibit a waddling gait, indicated with an increase in spinal sway and slower stride, correlated with diminished serum ALPL activity.

- The similarity of altered gait and musculoskeletal phenotype of HPP sheep to humans demonstrates the utility of this novel model in understanding the etiology of HPP.
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