Continuous Feeding Does Not Blunt Skeletal Muscle Protein Synthesis, Satellite Cell Abundance, or Lean Growth in a Neonatal Pig Model of Prematurity

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BACKGROUND
- Premature birth can lead to lower linear growth rates and altered body composition
- The specific reduction in skeletal muscle mass by prematurity may increase lifelong risk for metabolic disease
- Muscle growth requires the coordinated activation of: protein synthesis for the accretion of muscle proteins
- Proliferation and differentiation of satellite cells (adult muscle stem cells) for the addition of myofibers
- Infants who cannot feed normally can be fed with an orogastric tube on an intermittent bolus feeding or continuous feeding schedule
- In term pigs, feeding modality can affect lean growth:
  - Intermittent bolus feeding elicits a cyclical pattern of circulating insulin and amino acids that promotes skeletal muscle protein synthesis and lean growth
  - Continuous feeding elicits low and constant levels of insulin and amino acids in blood that is not conducive to maximizing anabolic signaling
- The impact of feeding modality on satellite cell abundance and myonuclear accretion in preterm pigs has not been investigated

HYPOTHESIS
- Intermittent bolus feeding will promote lean growth compared to continuous feeding in a neonatal piglet model of prematurity
- Intermittent bolus feeding will promote satellite cell abundance and myonuclear accretion compared to continuous feeding in skeletal muscle of preterm pigs

METHODS
- Animals
  - Preterm neonatal pigs delivered by Cesarean section (105 d gestation, equivalent to 30-32 week gestation for humans)
  - Initial BW = 952 ± 205 g
  - Surgeries: unilateral arterial catheter, jugular vein catheter, orogastric feeding tube
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- Diet (18% kcal/kg-d, 210 kcal/kg-d, 16 g protein/kg-d)
- Pigs fed from full panerianal to full enteral feeding over 6 d
- Treatments
  - Intermittent bolus feeding (INT; n = 30)
  - Fed every 3 h for the initial 7 d; fed every 4 h for the remaining 15 d
  - For protein synthesis analysis, INT pigs divided into postabsorbptive (INT-PP, n = 14, sampled before feeding) and postprandial (INT-PP, n = 16, sampled 60 min after feeding) groups
  - Continuous feeding (CONT; n = 14)
  - Fed at a constant rate for 22 d
  - Pigs fed every 4 h for 22 d
  - Intraperitoneal 2-deoxyglucose (BrdU, 25 mg·kg⁻¹·i.v) was injected every 12 h from D19 to D20
  - Body composition analyzed by DXA on D21
  - J-Inj, II-Phenylalanine injected on D20
  - Pigs euthanized on D22 for longitudinal dors (LD) muscle collection
- Sample analysis
  - Serial LD muscle cryosections from a subset of pigs analysed by immunofluorescence: Pax7+, laminin, and nuclei (sublaminal cell satellite cells); Pax7 is a transcription factor regulated by satellite cells
  - BMD, dystrophin, and nuclei (sublamellar BrdU and total myonuclei); BrdU labels proliferating cells
  - Fiber cross-sectional area (CSA) and minimum Feret diameter
  - Fluorescent images captured by confocal microscopy (>1000 fibers per pig)
- Plasma insulin and branched-chain amino acid (BCAA) concentrations
  - Analysis of protein synthesis for the addition of adult muscle stem cells (for the accretion of muscle proteins): the measurement of fractional protein synthesis rate by LCMS-MS
- Statistical analysis
  - Data were analyzed in SAS (mixed and generalized linear models)
  - Differences among treatment means were determined with the post-hoc Tukey test
  - Data are presented as least square means ± SE. Means with different letters are significantly different (P < 0.05)

RESULTS
- Baseline plasma insulin and BCAA concentrations were lower in the INT group compared to the CONT group
- Feeding increased plasma insulin and BCAA concentrations in the INT group in a time-dependent manner
- Plasma insulin was higher at 30 and 60 min in the INT group compared to the CONT group
- Plasma BCAA was higher at 90 min in the INT group compared to the CONT group

CONCLUSIONS
- Despite differences in plasma insulin and amino acid profiles between INT and CONT feeding, LD muscle protein synthesis was similar between feeding modalities in pigs born preterm
- Feeding modality likewise did not affect LD muscle satellite cell abundance or myonuclear accretion in pigs born preterm
- Taken together, intermittent bolus feeding did not promote lean growth more than continuous feeding in a neonatal piglet model of prematurity
- We have previously reported that the number of satellite cells is approximately 42% greater in pigs born at term at 28-30 d age compared to pigs born preterm at 14-15 d term equivalent age
- Diminished satellite cell abundance in skeletal muscle may contribute to growth faltering in premature infants

Figure 1. Plasma insulin and BCAA concentrations

Figure 2. LD muscle sublaminal Pax7+ satellite cell abundance

Figure 3. LD muscle subbassarolemmal BrdU+ and total myonuclei

Figure 4. LD muscle fiber CSA and minimum Feret diameter

Figure 5. LD muscle protein synthesis and piglet body composition