Thyroid Hormones and Mitochondrial Development in Skeletal Muscle of Fetal Sheep near term

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Introduction
The fetal to neonatal transition is associated with an increase in oxygen consumption¹. Skeletal muscle accounts for 30% of fetal oxygen uptake², and requires increased energy at birth as it takes on the roles of thermogenesis and locomotion. Aerobic ATP production is regulated by mitochondria (Fig.1).

In adult tissues, thyroid hormones increase mitochondrial biogenesis and oxidative metabolism through their influence on enzyme abundance, activity and proton leak⁵. In the fetus, thyroid hormones influence total oxygen consumption² as well as maturation of several fetal tissues including skeletal muscle when fetal triiodothyronine (T₃) concentration rises towards term⁶. However, whether thyroid hormones regulate any changes in fetal mitochondrial function during late gestation is unknown.

Methods
- After 11 twin-bearing Welsh Mountain ewes were anaesthetised at 105 days of gestation (d; term ~145d) one fetus was thyroidectomised (TX) and its twin was sham-operated. All procedures were carried out under the Animals (Scientific Procedures) Act 1986 after ethical approval of the University of Cambridge.
- At 129d (n=6 ewes) and 143d (n=5 ewes), the ewes were killed with an overdose of anaesthetic (200mg/kg sodium pentobarbital i.v.). Fetal plasma samples were taken before both fetuses were killed (200mg/kg sodium pentobarbital i.v.) and biceps femoris muscle samples were dissected, weighed and immediately frozen in liquid nitrogen. Fetal plasma triiodothyronine (T₃) concentrations were measured using radio-immunoassays.
- Muscle citrate synthase activity was measured spectrophotometrically as an index of mitochondrial density⁷.
- Abundance of electron transport system (ETS) complexes I-IV and ATP-synthase was measured by western blotting.
- Results are presented as mean±SEM. Data were analysed using Pearson correlation coefficient, and compared using a 2-way ANOVA followed by Tukey’s post-hoc test. P<0.05 was considered significant.

Results
Skeletal muscle citrate synthase activity was higher at 143d than 129d (Fig.2). At both ages, muscle from TX fetuses had a lower citrate synthase activity than the control muscle (Fig.2).

Combining all data, muscle citrate synthase activity showed a positive correlation with fetal plasma T₃ concentration (Fig.3).

Protein abundance of ETS complexes I-IV and ATP-synthase were lower in muscle from TX fetuses than the age-matched controls, with the difference being significant for complexes I and IV at 129d and for ATP-synthase and ETS complexes I, III and IV of 143d (Fig.4).

As an indication of protein abundance per mitochondrion, protein levels of the ETS complexes and ATP-synthase were normalised to CS activity. ATP-synthase per mitochondrion was consistently higher in muscle from TX fetuses than from the age-matched controls (Fig.5).

Conclusions
- Thyroid hormones are necessary for the normal developmental increase in mitochondrial density and components of oxidative phosphorylation in ovine biceps femoris in the lead-up to birth. Future work will determine whether the tissue respiratory function in situ correlates with these in vitro findings.
- ATP-synthase levels per mitochondrion are higher in muscle from hypothyroid fetuses, suggesting a compensatory response to maintain ATP production with fewer mitochondria.

Acknowledgements and References
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3. Larsen et al. (2012) J Physiol 590:3349