Placental expression and neonatal plasma concentrations of leptin in horses and ponies

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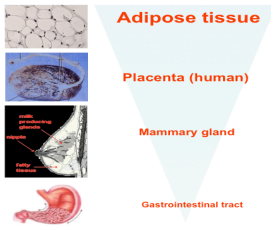
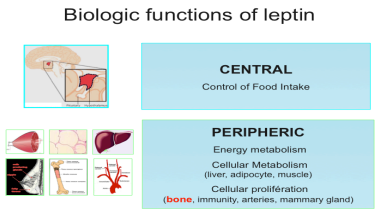


Ponies and horses are characterized by large metabolic differences, with ponies being much more hardy than horses. Leptin is a cytokine essentially produced by adipose tissue. Other sources have been described like placenta, mammary gland and stomach.
In adults, leptin concentrations are related to adipose tissue reserves and regulate food intake. Leptin is known to regulate glucose homeostasis. It also acts on bone growth and a dysregulation of this secretion might play a role in osteochondrosis (OCD). In rodent and pig neonates, leptin programs the normal development of the hypothamamic neuronal circuits involved in the regulation of food intake.
The aim of this study was to compare leptin concentrations between horses and ponies during pregnancy and in the post-natal period.

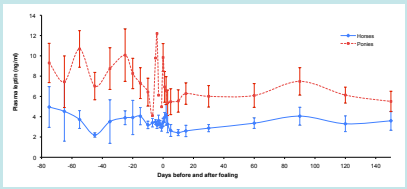


Results

➢ **In mares**, mean plasma leptin concentrations were higher before than after foaling (P<0.01) and higher in ponies than in horses (P<0.0001) (Fig 1a).
➢ **In foals**, at birth, no statistically difference was found between breeds (0.84 ±0.38 ng/ml) but leptin increased steeply in pony foals to reach significantly higher concentrations than horse foals by 2 days of age (P<0.001) (Fig. 1b).
➢ **Milk leptin** concentrations tended to be higher in horse mares on days 2 and 5 of lactation and were significantly higher in horse mares on day 10 of lactation (8.86 ±3.23 vs 5.67 ±3.03 ng/ml, P<0.01).
➢ **Fasting glycaemia** was significantly lower in pony foals at 5 months of age compared to horse foals (4.34 ±0.27 vs 6.05 ±0.11 mmol/l, P<0.002). There were no differences in insulin and glucose responses in the IVGTT. Insulin resistance, however, as calculated by the HOMA and QUICKY tests, indicated a tendency for horses to be more resistant to insulin than ponies.
➢ **In placenta**, expression of horse leptin mRNA was detected from each mare examined. Nevertheless, placental leptin mRNA levels were much lower in horse than in human, since 35 PCR cycles were used to amplify leptin in horse placenta vs 25 in human samples. Semi-quantification using household genes is currently being performed.

Figure 1: Plasma leptin in pony and horse mares and foals

a: mares



b : foals

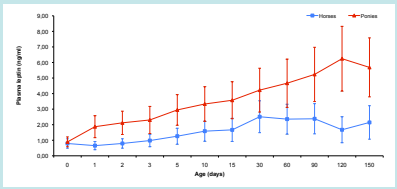
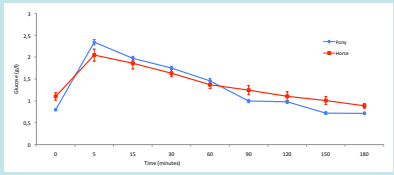
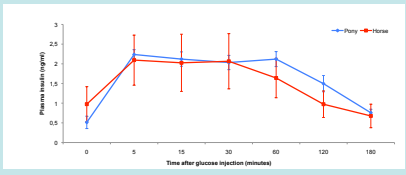


Figure 2: Plasma glucose and insulin IVGTT reponse in pony and horse foals

a: glucose



b: insulin



Conclusion

Foals from both breeds have very low leptin concentrations at birth, probably due to the lack of leptin transfer from the placenta. In contrast, the steep increase in leptin in pony foals compared to horses, despite milk higher leptin concentrations in horses, indicates that differences exist for glucose and leptin metabolism between the two breeds, although genetic and environmental effects are confounded here. More work is currently being performed to understand if these may play a role in the higher incidence of OCD in horses compared to ponies.

Materials and methods

Twenty pregnant mares (N=10 welsh ponies and N=10 saddlebred horses) were used.
Blood samples were collected from mares and foals (late gestation, perinatal period, up to 5 mo post-partum). Milk was also collected in the neonatal period. Placenta was collected from the area next to the umbilical cord within minutes of birth.
An intra-venous glucose tolerance test (IVGTT) was performed in 5-6 months old foals using 0.25 g of glucose per kg of body weight.
Plasma and milk leptin were measured by radio-immunoassay as described previously (Berg et al., 2007, J Anim Sci 85:1660). Insulin was measured by RIA using pig insulin for standards and iodination and a guinea pig antibody to porcine insulin. Results are expressed as means ± SEM.
Total RNA from placental tissues was isolated following the Trizol reagent protocol. The amount of total RNA was determined spectrophotometrically at 260 nm. Five micrograms of RNA were reverse-transcribed using 100 U of Superscript II and leptin cDNA was amplified by PCR using 2.5 U Taq Polymerase and horse oligonucleotide primers chosen accordingly to the horse leptin cDNA sequence.

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