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THE ONTOGENY OF HEPATIC CYTOCHROME P450 ENZYMES IN CONVENTIONAL PIGS USING ENZYME ACTIVITY AND PROTEOMICS

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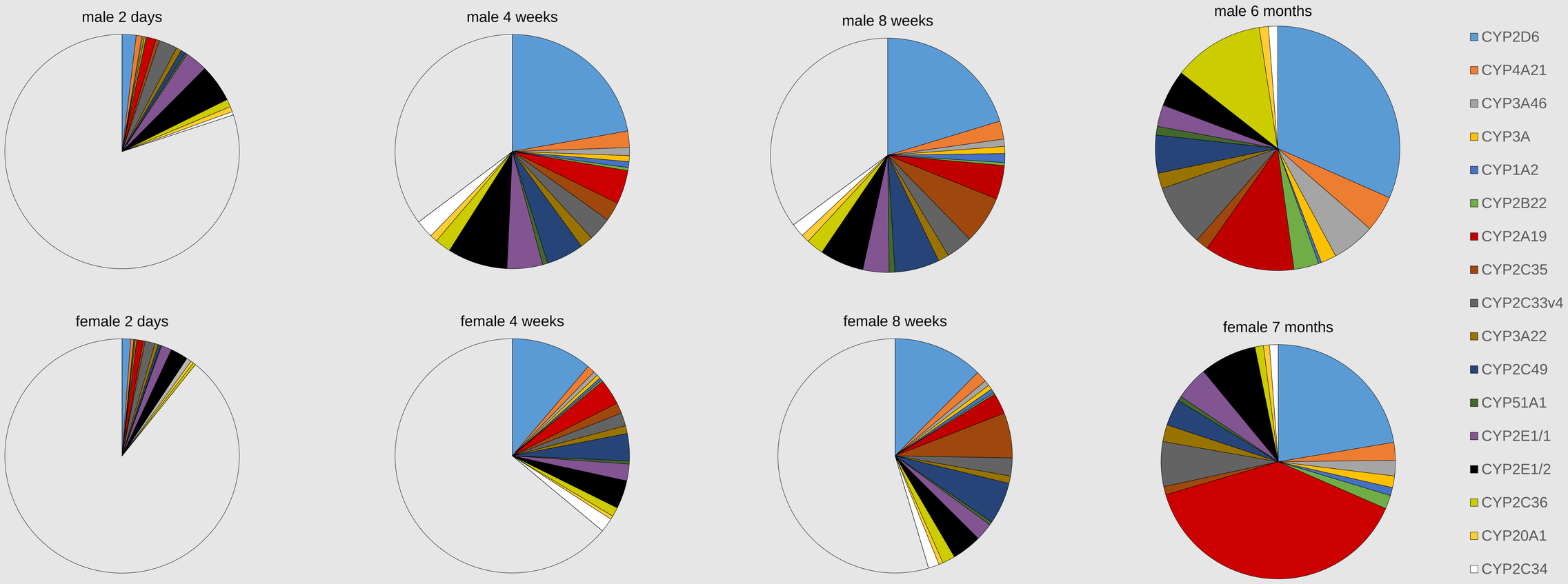
Objectives and methods

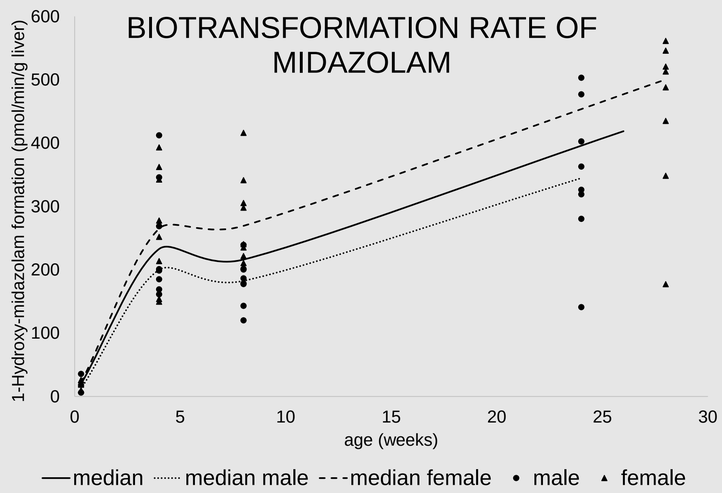
Development of appropriate animal models taking growth and maturation into account is pivotal for pediatric preclinical pharmacokinetic and pharmacodynamic (PK/PD) research. Literature reports have demonstrated a high homology between human and porcine CYP450 enzymes in adults, suggesting the pig as a suited animal model for PK/PD and safety studies (1, 2). However data regarding the ontogeny of porcine hepatic CYP enzymes are lacking. The aim of this research was to gain more insight in the development of the CYP450 enzymes by *in vitro* enzyme activity experiments and through proteomics.

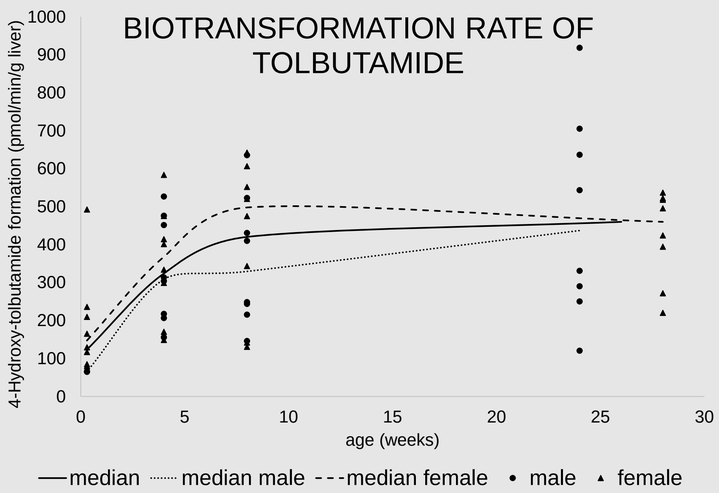
The *in vitro* CYP450 enzyme activity of the following probe substrates was measured in microsomes: midazolam (CYP3A), tolbutamide (CYP2C) and chlorzoxazone (CYP2E). The microsomes were prepared of each time 16 pigs (8 ♂ and 8 ♀, Hybrid sow x Piétrain boar) aging 2 days, 4 weeks, 8 weeks and 6-7 months. The corresponding metabolites were quantified using a validated UHPLC-MS/MS method (3). Furthermore, the microsomal protein per gram liver (MPPGL) was determined (4). In addition to these *in vitro* activity experiments, the CYP isoenzymes in the same microsomes were determined by high definition data directed analysis (HD-DDA) mass spectrometry. The data analysis was performed using Progenesis QI.

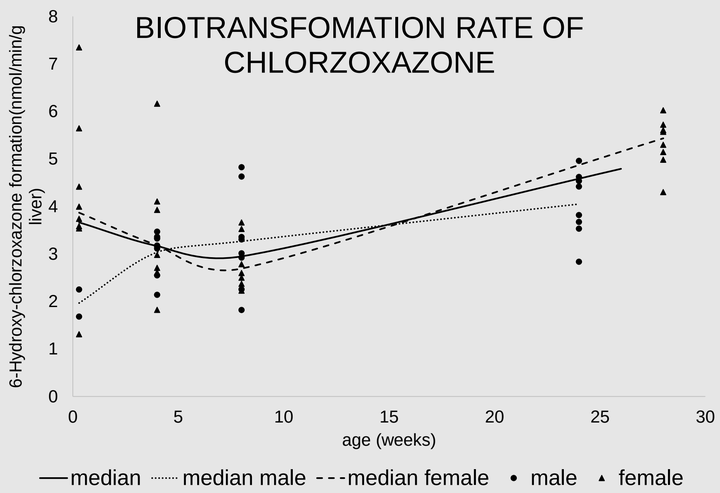
Results



A total of 20 CYP isoenzymes were identified of which 12 had 2 or more unique peptides. A pig liver pie out of the average amount of protein for the 6 and 7 months old pigs was calculated. This was considered to be 100% to recalculate the proportion of the younger age categories as shown above. At puberty significant sex differences ($p < 0,05$) were observed.







The microsomal activity of the three substrates, expressed as pmol metabolite formed per minute and per milligram microsomal protein, increased with age. Significant sex differences were observed at 8 weeks of age for the three substrates and at 6 months of age for chlorzoxazone ($P < 0,05$; data not shown). The activity per gram liver, as calculated with the MPPGL and shown above, also showed a maturation profile. However the sex differences are no longer statistically significant. The increase in microsomal activity is reflected in an increase in CYP450 proteins in the microsomes.

Conclusion

Both the absolute amount of protein and the activity per gram liver shows an increase with age, suggesting growth and maturation of the CYP450 isoenzymes. This maturation of the CYP450 isoenzymes is also observed in human, suggesting the piglet might be a suitable animal model for preclinical PK/PD research.

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