

BILIARY EXCRETION OF 25-HYDROXYVITAMIN D₃ IN LAMBST.T. PHAM^{*}, H.W. SYMONDS^{**} and I.W. CAPLE^{*}

Plasma 25-hydroxyvitamin D₃ concentrations in autumn-born lambs often decrease to undetectable levels (< 2ug/l) during winter and spring in south-eastern Australia (Caple et al. 1988). As vitamin D₃ supplementation of these lambs less than 6 months of age usually does not result in improved health or growth, the value of plasma levels as an indicator of deficiency has been questioned. Since it was not known if conservation of 25(OH)D₃ in the enterohepatic circulation was able to maintain vitamin D status in lambs when plasma 25(OH)D₃ was low, studies were undertaken to examine the biliary concentrations and excretion of 25(OH)D₃ in deficient and replete lambs.

Four lambs which had been born indoors were prepared with fistulas of the gall bladder and small intestine at four months of age. The common bile duct was ligated, and bile was diverted from the gall bladder to the small intestine through external tubing connecting the fistulas. The tubing was uncoupled for collection of bile samples, and for controlled interruption to the enterohepatic circulation. Plasma and bile samples were collected for analysis of 25(OH)D₃ by high performance liquid chromatography (HPLC). Bile 25(OH)D₃ fractions were separated on sep-pak C18 cartridges and unconjugated 25(OH)D₃ identified by co-elution with labelled 25(OH)[26,27 methyl-H³] cholecalciferol by HPLC on both C18 and silica columns using water:methanol (10:90, v/v) and isopropanol:hexane (3:97, v/v) respectively.

The concentrations of 25(OH)D₃ in plasma in the four lambs remained less than 2 ug/l between 5 and 8 months after the fistulas were established. At all collections, bile concentrations of 25(OH)D₃ were higher, ranging from 7 to 310 ug/l, than those in plasma. Intramuscular injection of cholecalciferol (1200 i.u./kg) to one lamb increased plasma 25(OH)D₃ to 90 ug/l within four weeks, and during this period the concentration of 25(OH)D₃ in bile samples ranged from 200 to 1000ug/l.

In one lamb, 3.8 uCi of 25(OH)[26,27 methyl-H³] cholecalciferol was injected intravenously, and excretion of radioactivity in bile monitored at 30 min intervals for 6 hours. To maintain the enterohepatic circulation of bile salts, an equivalent amount of bile collected from another lamb was returned to the intestine. It was estimated that 7.6% of the injected radioactivity was excreted in 6 hours. In further experiments on two lambs, interruption of the enterohepatic circulation for 6 hours resulted in a decrease in bile flow (1.2 to 0.3 ml/min) and excretion of 25(OH)D₃ (0.8 to 0.4 ug/min). The decreased biliary secretion of 25(OH)D₃ during interruption of the enterohepatic circulation may have been due in part to a reduced secretion of bile salts which are important stimulants to flow and excretion of lipids in bile in sheep (Heath et al. 1970), and also to interruption in the enterohepatic circulation of 25(OH)D₃. Further studies are required to confirm that 25(OH)D₃ is conserved in the enterohepatic circulation in lambs, and to estimate the proportion of conjugated 25(OH)D₃. Unlike humans in which the enterohepatic circulation of 25(OH)D₃ is considered unimportant (Clements et al. 1984), it would seem that the lamb may maintain high concentrations of unconjugated 25(OH)D₃ in bile when there are low plasma levels.

CAPLE, I.W., BABACAN, E., PHAM, T.T., HEATH, J.A., GRANT, I., VIZARD, A., CAMERON, S. and ALLWORTH, M.B. (1988). Proc. Aust. Soc. Anim. Prod. 17:379.
HEATH, T.J., CAPLE, I.W. and REDDING, P.M. (1970). Q. Jl. exp. Physiol. 55:93.
CLEMMENTS, M.R., CHALMERS, T.M. and FRASER, D.R. (1984). Lancet 1:1376.

* Veterinary Clinical Centre, University of Melbourne, Werribee, 3030.

** Dept. Anim. Physiol. and Nutr., University of Leeds, Leeds, United Kingdom.