7 Summary

Kinetic of vitamin A resorption in blood plasma and vitamin A excretion in urine of dogs
88 pages; 20 figures; 10 tables; 80 citations

Dogs and other species of the order *Carnivora* transport vitamin A in blood plasma not only as retinol but predominantly as lipoprotein bound retinyl esters. In humans and rats lipoprotein bound retinyl esters are only observed postprandial or as a consequence of an excessive vitamin A intake leading to severe signs of hypervitaminosis A. In contrast, the occurrence of retinyl esters in the blood plasma of canines is not associated with any sign of vitamin A intoxication possibly by the excretion from retinol and retinyl esters, which are bound to Tamm-Horsfall protein (THP) in the urine. But there are possible different mechanisms in the regulation of urinary vitamin A excretion, because other carnivores like felides or mustelides, that also share a high concentration of retinyl ester in the blood plasma, do excrete no or low levels of vitamin A with their urine. Therefore, the present study was performed to investigate the effect of a single oral dose of vitamin A on the vitamin A concentration in blood plasma and their subsequent excretion in urine of dogs. We also examined general aspects of the vitamin A metabolism in order to provide more insight into the peculiarities of vitamin A metabolism in dogs.

Eight male beagle dogs were given a single oral dosage of vitamin A of 3000 retinyl equivalents (RE) / kg body weight (BW) after a 16 hours overnight fast. The vitamin A supplement was administered through a syringe directly in the back of the mouth in form of retinyl palmitate (Ursovit® A, Bernburg, Germany) together with 5 ml cream (30% fat). Blood was sampled at 48 hours before and 1, 2, 3, 4, 6, 8, 24, 48, 72 and 96 hours after dosing. Urine samples were collected for 18 hours each day into individual bottles beneath metabolic cages until 96 hours after dosing. Plasma was separated by centrifugation. Chylomicrons and plasma lipoproteins were separated by preparative ultracentrifugation. Retinol and retinyl esters in plasma and urine were qualitatively and quantitatively examined after organic extraction by a gradient-HPLC method. RBP in plasma and urine was detected by Western blotting after protein separation on SDS-PAGE. The quantitative determination of RBP and THP in blood plasma and urine was performed by ELISA systems. Triglyceride and cholesterol concentrations in blood plasma and in lipoprotein fractions were examined using commercial test kits.

Consistent with the results of earlier studies, the predominant vitamin A metabolites in the plasma of fasting dogs were retinol and retinyl esters. After administration of 3000 RE/kg BW, plasma levels of retinol and retinyl esters increased and reached their highest values 6 hours

and 8 hours after dosing, respectively. The ratio between plasma retinyl stearate and retinyl palmitate declined significantly indicating that newly absorbed dietary vitamin A seems to be transported primarily as retinyl palmitate in the plasma of dogs. The maximum of chylomicron retinyl esters peaked already within 1 hour after vitamin A supplementation. The percentage of retinyl palmitate and retinyl stearate associated with chylomicrons was similarly high and there were no significant effects during the time of investigation. The concentration of all three retinyl esters in blood plasma as well as in chylomicron infranatant increased until 8 hours after vitamin A dosing, whereas their corresponding values in the chylomicron fraction decreased. Because of similar percentages of retinyl esters in plasma and chylomicron infranatant, additional studies are needed to clarify possible different transport mechanisms in the postprandial vitamin A response of dogs. Retinol-binding protein (RBP) was present in plasma, but never in urine. In plasma, the postprandial concentrations of RBP decreased after vitamin A dosing and did not parallel the concentration of retinol.

In confirmation to previous investigations, plasma retinyl ester were present in all three lipoprotein fractions. In fasted dogs, retinyl stearate (55-65%) was the predominant ester of vitamin A followed by retinyl palmitate (25-30%) and retinyl oleate (10%). The retinyl esters were predominantly detected in the low density lipoprotein (LDL) and very low density lipoprotein (VLDL) fraction. The maximum of retinyl esters appeared in the LDL fraction after 8 hours, in the VLDL fraction after 24 hours.

After the oral intake of 3000 RE/kg BW only 4 out of 8 dogs showed an elevation of their vitamin A concentration in urine. But the excretion of vitamin A in the “response group” was quite variable. The carrier protein for fat soluble vitamin A in urine is THP. Until 8 hours after dosing, the THP concentration significantly increased and declined continuously at 96 hours to concentrations half of baseline values. Moreover, the results indicate that urinary THP excretion has no significant correlation to urinary vitamin A excretion.

In conclusion, the study shows that a large single oral administration of 3000 RE/kg BW in healthy dogs increases the retinol and the retinyl ester concentrations in plasma. Although half of the investigated dogs excrete vitamin A in the urine above their baseline levels, a directly affection of dietary vitamin A on the urine vitamin A excretion of dogs is questionable. More knowledge of the THP carrier on the cellular and molecular level would be helpful to elucidate the signals and mechanisms that are responsible for the excretion of VA in the urine of dogs.