Retinol and Retinyl Ester Responses in the Blood Plasma and Urine of Dogs after a Single Oral Dose of Vitamin A\textsuperscript{1,2}

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EXPANDED ABSTRACT

KEY WORDS: • dog • vitamin A • absorption • plasma • urine

It is well established that dietary vitamin A (VA\textsuperscript{4}) is taken up by the intestinal cells and is incorporated as retinyl esters along with other dietary lipids into chylomicrons (1). The chylomicrons are secreted into the lymphatic system and enter the circulation, where they are metabolized into chylomicron remnants by activity of lipoprotein lipase. The remnant particles contain most of the retinyl esters originally packed in the intestinal chylomicrons and are rapidly cleared primarily by hepatocytes, where they undergo hydrolysis [for review see Blomhoff et al. (2)]. As a result of these processes retinol is either reesterified for storage in lipid droplets in the stellate cells or bound to retinol-binding protein (RBP), the sole plasma transport protein for retinol. The retinol–RBP complex is secreted into circulation bound to transthyretin for delivery to peripheral tissues (3). However, dogs and other carnivores do transport VA in blood plasma not only as retinol bound to RBP but predominantly as retinyl stearete (RS) and retinyl palmitate (RP) associated with all lipoprotein fractions (4–6). In mammals other than carnivores the occurrence of lipoprotein-bound retinyl esters is observed only under conditions of acute or chronic vitamin A intoxication (7). The physiological factors and processes responsible for a lower susceptibility of carnivores to high dietary VA intake are not entirely understood. One factor protecting dogs from a VA intoxication may be the excretion of retinol and retinyl esters in the urine.

Blood was sampled into EDTA evacuated tubes at 0, 2, 3, 4, 6, 8, 24, 48, 72 and 96 h after dosing. The bottles were wrapped in aluminum foil to protect their contents from light. Chylomicrons were isolated by ultracentrifugation (15 min, 100,000 g) from 1 mL fresh plasma at density 1.006 g/mL (11). Retinol and retinyl esters in plasma and chylomicrons were determined by a gradient-HPLC method (9). RBP in plasma and urine was detected by Western blotting after protein separation on SDS–PAGE (6). THP was determined by an ELISA system. The intra-assay variability was assessed by measuring the amount of THP in six aliquots of the same urine sample run on a

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4. Abbreviations used: BW, body weight; RBP, retinol-binding protein; RE, retinyl equivalent; RO, retinyl oleate; RP, retinyl palmitate; RS, retinyl stearate; THP, Tamm–Horsfall protein; VA, vitamin A.

5. Nutrient and energy content of the basal diet per kg was as follows: metabolizable energy (12.1 MJ), N-free extract (455 g), moisture (125 g), protein (220 g), fiber (55 g), fat (50 g), ash (85 g), calcium (15 g), phosphorus (11 g), potassium (9 g), sodium (2 g), magnesium (2 g), vitamin A (15,000 IU), vitamin D\textsubscript{3} (600 IU) and vitamin E (75 mg).

single ELISA plate. The coefficient of variation (CV) at a mean concentration of 48.07 ± 2.29 μg/mL was 4.8%. The interassay coefficient was monitored by incubating three wells on each ELISA plate with an aliquot from a single urine sample. For a random sampling of 18 plates, these interassay samples yielded a THP concentration of 32.6 ± 2.32 μg/L, which represents an interassay CV of 7.1%. Triglyceride and urine creatinine were determined using a commercial test kit (Sigma, Deisenhofen, Germany).

**Statistical analysis**

Results are expressed as means ± SD. Variations in the response variable (retinol and retinyl esters in plasma and urine) were partitioned using the general linear model (GLM) procedure of SAS (version 8.0, SAS Institute, Cary, NC) for repeated-measurement design. In the case of significant differences a Scheffé post hoc test was used to determine the cause, assuming a confidence level of \( P < 0.05 \).

**RESULTS**

**Plasma VA responses**

In fasted dogs, VA was present as retinol, RO, RP and RS (Table 1). RS (49%) was the predominant form of VA followed by retinol (24%), RP (21%) and RO (6%). As a consequence of the administration of 3000 RE as RP/kg BW, plasma levels of retinyl esters reached their highest values 8 h after dosing (30.94 ± 8.72 μmol/L vs. 10.21 ± 3.45 μmol/L; \( P < 0.001 \)) and returned to baseline levels by 72 h. Plasma retinol increased slightly from 3.16 ± 0.65 to 4.99 ± 0.74 μmol/L \( (P < 0.001) \), with a maximum at 6 h, and decreased to baseline at 48 h (Fig. 1). The ratio between plasma RS and RP declined significantly \( (P < 0.01) \) from 2.60 ± 0.36 at baseline to 1.44 ± 0.14 at 3 h after the vitamin A administration. The RS/RP ratios remained unaffected until 6 h, than started to increase again and returned to baseline levels by 48 h (2.12 ± 0.21; \( P < 0.001 \)).

**Chylomicron VA responses**

The chylomicron retinyl ester responses did not parallel the increase of retinyl esters observed in plasma. After the VA load, the chylomicron RP and RS concentrations peaked at 1 h and decreased slightly by 8 h. However, there were no significant time effects on the ratios of chylomicron RS to chylomicron RP for each time point throughout the postprandial period, which ranged between 0.96 and 1.33.

**Plasma and chylomicron triglyceride response**

Plasma triglyceride concentrations peaked between 2 and 4 h at levels between 0.80 and 0.83 mmol/L and returned to baseline by 24 h (Fig. 2). The chylomicron triglyceride concentration reached a peak at 4 h \((0.16 ± 0.05 \text{ mmol/L})\), decreased slightly and increased again at 8 h \((0.25 ± 0.05 \text{ mmol/L})\).

**Urinary VA responses**

In all dogs, VA in urine was present as retinol, RO and RP, but no RS was detected. The excretion of VA was estimated by measuring the ratios of the concentrations of urinary total VA to urinary creatinine concentration (Fig. 3). No significant change in the excretion of VA could be observed during the 96 h after dosing. However, the individual excretion of retinol and retinyl esters was quite variable during the experiment. The presence of THP could be demonstrated in all urine specimens, showing a significant increase \((P < 0.001)\) in the ratio of THP to creatinine at 8 h that decreased continuously until 96 h after dosing. In contrast to plasma, no RBP was detected in urine.

**DISCUSSION**

As in other canines, VA in the blood plasma of fasting dogs was present as retinol but predominantly also as RS and RP (4,6). The retinyl esters are transported in plasma associated with all lipoprotein fractions of very low, low and high density (VLDL, LDL, HDL) \((4,12)\). This is a peculiarity of carnivores, given that in most other species, elevated retinyl ester levels in
proteinuria (16). Based on Western blot analysis, RBP can be served only during severe infections as a result of a decreased excretion of retinol but not retinyl esters in humans is observed. Therefore, it is unlikely that the loss of VA in urine of dogs is a consequence of merely glomerular filtration and decreased tubular RBP reabsorption. The renal excretion of VA seems to be a regulated secretory process by kidney tubules cells, given that the patterns of retinyl ester in plasma (predominantly RS) and the urine (nearly exclusively RP) are quite different. Furthermore, retinol and retinyl esters are associated with the Tamm–Horsfall glycoprotein (THP) that is actively secreted by the epithelial cells of distal tubules (8).

In conclusion, the results indicate that urinary THP excretion has no relationship to urinary vitamin A excretion. The excretion of vitamin A might be related to its uptake, transcellular transport, apical secretion as well as a sufficient accumulation in the kidney (6). However, both the exact mechanism of excretion as well as the consequences for vitamin A supplementation and metabolism in carnivores remain to be elucidated.


discussion

Another objective of this study was to investigate whether the postprandial increase of plasma VA may have an effect on the excretion of VA in the urine, as has been shown in dogs on diets with various amounts of VA fed for extended periods of time (9). Although all dogs in this study were fed the same basal diet and plasma VA increased simultaneously during the postprandial period, the individual urine concentrations of retinol and RP were quite variable. Maximal urine RP excretion in the postprandial period, the individual urine concentrations of retinol and RP were quite variable. Maximal urine RP excretion was observed at 8 and 48 h after VA dosing; however, if the values were calculated as the ratio of urinary VA to creatinine, no significant change in the excretion of VA in the urine was observed. Therefore, the results of this study extend the observation that the excretion of VA in the urine of dogs may not be directly affected by plasma VA levels (8). Renal excretion of retinol but not retinyl esters in humans is observed only during severe infections as a result of a decreased proteinuria during febrile proteinuria (16). Based on Western blot analysis, RBP can be excluded as a carrier for VA in urine. Therefore, it is unlikely that the loss of VA in urine of dogs is a consequence of merely glomerular filtration and decreased tubular RBP reabsorption. The renal excretion of VA seems to be a regulated secretory process by kidney tubules cells, given that the patterns of retinyl ester in plasma (predominantly RS) and the urine (nearly exclusively RP) are quite different. Furthermore, retinol and retinyl esters are associated with the Tamm–Horsfall glycoprotein (THP) that is actively secreted by the epithelial cells of distal tubules (8).

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