Porcine Intestinal Metabolism of Excess Vitamin A Differs Following Vitamin A Supplementation and Liver Consumption

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ABSTRACT Vitamin A is a well-established teratogen in all animal species. A number of case reports also suggest a teratogenic potential of vitamin A in humans. A possible teratogenic risk of dietary liver vitamin A intake, the kinetics of vitamin A and its metabolites in humans after intake of either a vitamin A supplement or a liver meal have been studied. Major differences were described for the kinetics of all-trans-retinoic acid (all-trans-RA), which occurred at much higher concentrations after supplementation than after liver consumption. Therefore, we investigated whether the intestine may be responsible for the differences in vitamin A metabolism after supplementation or liver feeding. We found that cytosolic fractions of porcine enterocytes oxidized retinol to all-trans-RA in vitro with a $K_m$ of 94–96 μmol/L and a $V_{max}$ of 7.9–8.6 pmol/(min · mg protein). In an in vivo approach, the portal vein and the central vein (external jugular vein) of a pig were cannulated. In two subsequent experiments, the pig was given a vitamin A supplement or liver. Plasma samples were taken from portal and central veins. Comparison of retinoid levels in these veins indicated that all-trans-RA was already formed from supplemental vitamin A in the intestine and released into the systemic circulation. Two major metabolic pathways were additionally present in the pig, leading to the formation of glucuronides of all-trans-RA and retinol itself. Our results indicate that intestinal metabolism contributes to the elevated levels of all-trans-RA in the systemic circulation after supplementation with vitamin A, but not after consumption of liver. J. Nutr. 132: 197–203, 2002.

KEY WORDS: • retinol • retinoic acid • vitamin A • liver • supplementation • intestine • metabolism

Vitamin A alcohol (retinol) and its metabolites (retinoids; Fig. 1) are essential for various biological processes, such as vision, reproduction, cell growth and differentiation, and embryonic development (1). In dermatology and oncology, retinoids are effective therapeutics (2). On the other hand, several case reports have indicated that vitamin A supplementation of 25,000 IU3 vitamin A or more by pregnant women could result in birth defects (3). The epidemiologic study of Rothman et al. (4) even suggests that intake of vitamin A at levels exceeding 10,000 IU/d may be associated with an increased teratogenic potential of vitamin A in humans. A possible teratogenic risk of dietary liver vitamin A intake, the kinetics of vitamin A and its metabolites in humans after intake of either a vitamin A supplement or a liver meal have been studied. Major differences were described for the kinetics of all-trans-retinoic acid (all-trans-RA), which occurred at much higher concentrations after supplementation than after liver consumption. Therefore, we investigated whether the intestine may be responsible for the differences in vitamin A metabolism after supplementation or liver feeding. We found that cytosolic fractions of porcine enterocytes oxidized retinol to all-trans-RA in vitro with a $K_m$ of 94–96 μmol/L and a $V_{max}$ of 7.9–8.6 pmol/(min · mg protein). In an in vivo approach, the portal vein and the central vein (external jugular vein) of a pig were cannulated. In two subsequent experiments, the pig was given a vitamin A supplement or liver. Plasma samples were taken from portal and central veins. Comparison of retinoid levels in these veins indicated that all-trans-RA was already formed from supplemental vitamin A in the intestine and released into the systemic circulation. Two major metabolic pathways were additionally present in the pig, leading to the formation of glucuronides of all-trans-RA and retinol itself. Our results indicate that intestinal metabolism contributes to the elevated levels of all-trans-RA in the systemic circulation after supplementation with vitamin A, but not after consumption of liver.

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3 1 IU = 1 International Unit vitamin A = 1.05 nmol vitamin A alcohol (retinol) and/or esters of vitamin A.

4 Abbreviations used: AUC, area under the plasma concentration-time curve; CRBP, cellular retinol-binding protein; DMSO, dimethyl sulfoxide; GI, gastrointestinal; 14-HRR, 14-hydroxy-4,14-retro-retinol; RA, retinoc acid; RAG, retinyl β-glucuronide; RAR, retinoic acid receptor; ROG, retinyl β-glucuronide; RXR, retinoid X receptor.
In addition to the liver, many tissues can convert retinol to RA including brain, kidney, spleen, pancreas, skin, testis and lung (8–11). Cytosolic alcohol dehydrogenases but also microsomal enzymes of the short-chain alcohol dehydrogenase family are involved in the first metabolic step, the oxidation of retinol to retinal (12,13). Subsequently, retinal is converted to RA either by cytosolic retinal dehydrogenases (14,15) or by cytochrome P450 isozymes (16–18). Whether small intestinal enzymes might be involved in the metabolism of excess vitamin A remains speculative. Ingested retinyl esters, the major form of orally administered vitamin A, are cleaved intraluminally to retinol, which is then absorbed into the intestinal cells (19). These esters are then reesterified to retinyl esters by the specific enzymes lecithin-retinyl acyl transferase and acyl CoA-retinol acyl transferase (20,21). Subsequently, retinyl esters are packed into chylomicrons and transferred into the lymphatic duct. Chylomicrons are reduced to chylomicron remnants in the systemic bloodstream (22). These remnants are taken up by the liver and retinyl esters are subsequently stored in stellate cells of the liver (23). The uptake and reprocessing steps in the intestine might be saturable when an excess amount of vitamin A is given as a bolus, thus leading to a faster absorption of vitamin A from a supplement compared with a liver meal. Therefore, other metabolic pathways such as oxidation or glucuronidation may contribute to vitamin A metabolism. It was demonstrated recently that cytosolic fractions of human enterocytes can convert retinol to RA in the presence of NADPH (24).

The goal of the present study was to explore the differences in the metabolic pattern of vitamin A in humans after either supplementation or liver consumption by a participation of intestinal enzymes in the oxidative processing of excessive amounts of vitamin A. Therefore, we examined by an in vitro approach whether subcellular fractions of the intestine could oxidize retinol to all-trans-RA. Additionally, an in vivo experiment was performed to examine whether excess vitamin A is metabolized to polar retinoids in the intestine and whether the metabolites generated are released into the portal vein, thus possibly contributing to systemic retinoid levels. Vitamin A metabolites were determined in plasma of the portal vein and of a systemic vein (external jugular vein, hereafter called central vein). The pig was chosen because pigs are omnivorous and their intestinal tract resembles that of humans in many aspects (25).

**MATERIALS AND METHODS**

**Chemicals and reagents.** HPLC solvents were of gradient grade quality from Merck (Darmstadt, Germany). Unless otherwise mentioned, all other chemicals and reagents were from Sigma (Deisenhofen, Germany). Dithiotreitol was supplied by Pharmacia (Freiburg, Germany). β-Glucuronidase from Escherichia coli (EC 3.2.1.31; 2 × 10² U/L) was purchased from Boehringer Mannheim (Mannheim, Germany). Retinol, all-trans-RA, 13-cis-RA and retinyl palmitate were from Sigma. Retinyl esters other than retinyl palmitate were synthesized in our laboratory. Glucuronides of retinol and all-trans-RA were kindly provided by Dr. J. A. Olson and Dr. A. B. Barua (Ames, IA). 14-Hydroxy-14,14-retinol (14-HRR) and anhydroretinol were gifts from Dr. F. Derguini and Dr. J. Buck (New York, NY). All other reference retinoids were gifts from Hoffmann-La Roche (Basel, Switzerland and Nutley, NJ).

**Isolation of porcine enterocytes and preparation of cytosolic and microsomal fractions.** Porcine small intestinal samples were obtained from a local slaughterhouse. Porcine enterocytes were isolated by the chelation-elution method according to the isolation of rat enterocytes described by Pinkus (26). The binding of enterocytes to the basal membrane was loosened by complexation with EDTA; the cells obtained were then rinsed out with phosphate buffer (pH 7.4) by repeated extension and contraction of the intestine. The isolated enterocytes were resuspended in phosphate buffer (pH 7.4) containing potassium chloride (100 mmol/L), EDTA (1 mmol/L) and dithiotreitol (1 mmol/L). Cytosolic and microsomal fractions were isolated using a differential centrifugation procedure described by Guengerich (27). The enterocytes were homogenized using an Elvehjem teflon potter and subsequent ultrasonication. The homogenate was centrifuged for 20 min at 10,000 × g, and the resultant supernatant was again centrifuged for 60 min at 100,000 × g. The supernatant of this centrifugation step (representing the cytosolic fraction of the enterocytes) was collected and frozen at −80°C until further usage. The sediment was resuspended in phosphate buffer and centrifuged for 70 min at 100,000 × g. The supernatant was discarded and the sediment (representing the microsomal fraction) was suspended in phosphate buffer (pH 7.4) containing glycerol (200 g/L) and EDTA (1 mmol/L). The protein concentration of cytosolic and microsomal fractions was determined by the bicinchoninic acid method according to Smith et al. (28).

**FIGURE 1** Structural formulas of retinol and some phase I and phase II metabolites.

**FIGURE 2** Area under the concentration-time curves (AUC) of retinol palmitate (A), 13-cis-retinoic acid (RA) (B) and all-trans-RA (C) in plasma of humans (n = 10) after intake of 500,000 IU vitamin A either as a supplement (S) or as a liver meal (L). Data adapted from Buss et al. (6).
**In vitro experiments.** The oxidative metabolism of all-trans-retinol to all-trans-RA was investigated by incubation of cytosolic or microsomal fractions of porcine enterocytes with various amounts of all-trans-retinol. In preliminary experiments, the incubation of all-trans-retinol with cytosolic fractions of porcine enterocytes was shown to be linear with respect to the duration of incubation (10–30 min) and the protein concentration (0.5–2 g/L). The incubations were performed in duplicate in a total volume of 500 μL containing NAD⁺ (2 mMol/L), potassium chloride (150 mMol/L), HEPES (20 mMol/L), dithiothreitol (2 mMol/L), cytosolic protein (2 g/L) or microsomal protein (1 g/L) and all-trans-retinol [0–750 μMol/L, predissolved in ethanol or dimethyl sulfoxide (DMSO)]. All incubations contained 1 vol % of solvent. The reactions were started with NAD⁺ and stopped after 30 min by addition of a threefold volume of isopropanol. Retinoid metabolites were determined by HPLC and UV detection as described below. The rate of all-trans-RA formation [V] was plotted vs. the substrate concentration [S] and data were fitted by nonlinear regression. \( K_S \) and \( V_{max} \) were determined from the Lineweaver-Burk plot (\(1/V\) vs. \(1/S\)).

**In vivo experiments.** The protocol for the in vivo experiments complied with NIH guidelines (29). A German Landrace pig (14 wk old; body weight, 23 kg) was supplied by the Center of Anatomy, Medical School Hannover (Hannover, Germany). After general anesthesia of the pig, the right external jugular vein was cannulated using a standard venous cannula (o.d., 2.1 mm; i.d., 1.4 mm) within a leading tube. After exteriorization, the cannula was fixed in the neck region. In a second step, a midline laparotomy was performed. In the upper jejunal mesentery a side branch of the upper mesenteric vein was exposed. Through this vein, a cannula leading to the portal vein was established. The portal vein cannula was exteriorized in the right flank of the abdomen and the laparotomy was closed by suturing the layers of the abdominal wall. The pig recovered from surgery within 3 d. Throughout the experiment, the pig was allowed to move in a wooden box (2.5 m²) without fixation during blood sampling. The pig received twice daily ~700 g of a pelleted pig maintenance diet (NAFAG No. 9103 minipig maintenance diet, NAFAG, Gossau, Switzerland, for detailed information visit http://www.nafag.ch). The pig was deprived of food for 12 h before the experiments. Food was offered again 5–6 h after dosing.

Two subsequent experiments with 4-d washout periods were performed. In the first, the pig was given a vitamin A supplement containing a retinyl palmitate oil in water emulsion (A-Mulsin, Mucos Pharma, Geretsried, Germany) mixed with a small amount of food. The total dose administered was 430,000 IU vitamin A. In the second, the pig was fed 600 g liver. The vitamin A content of the liver was determined by HPLC. The amount of liver consumed contained a total dose of 667,000 IU vitamin A. In both experiments, blood samples were taken from the portal and central veins at different time intervals up to 24 h. Plasma was prepared and stored at −80°C until analysis. Retinoid metabolites were determined in portal and central vein plasma by HPLC and UV detection as described below. Areas under the plasma-concentration time curve values (AUC) were determined by the trapezoidal method (30) for 24 h after vitamin A intake. The maximum plasma concentrations (\(C_{max}\)) and time to maximum plasma concentrations (\(T_{max}\)) are the observed values.

**HPLC analysis.** Plasma samples as well as in vitro incubation mixtures were extracted with a threefold volume of isopropanol and underwent solid-phase extraction according to a method previously described (31). A modification of the HPLC method described by Eckhoff and Nau (32) was used for determination of retinoids. This method uses a linear gradient formed from 57.5% methanol and 42.5% aqueous 60 mMol/L ammonium acetate to 95% methanol and 5% ammonium acetate within 11 min. To determine also retinol and retinyl esters in a single chromatographic run, the methanol percentage was increased to 100% at 11.2 min and further maintained at this level until 25 min (33). The starting conditions of the gradient were reached again after 26 min. With this method, separation of retinyl palmitate and retinyl oleate was not possible. Detection was performed by monitoring the UV absorbance of the eluate at 340 and 356 nm simultaneously using a Shimadzu SPD 10 AV two wavelength detector (Kyoto, Japan). Peak eluates of putative retinoid glucuronides were collected, evaporated to dryness, redissolved in buffer, subjected to hydrolysis by β-glucuronidase and the resulting retinoids were rechromatographed. The procedure was described in detail by Sass and Nau (34).

**RESULTS**

**In vitro oxidation of all-trans-retinol by subcellular fractions of porcine enterocytes.** The Michaelis-Menten kinetics of the all-trans-RA formation in cytosolic fractions of porcine enterocytes are presented in Figure 3A. The formation of all-trans-RA (dissolved either in ethanol or in DMSO) was concentration dependent. As shown in the Lineweaver-Burk plot (Fig. 3B), \(K_S\) and \(V_{max}\) of the reaction were determined to be 94–96 μMol/L and 7.9–8.6 pmol/(min · mg protein), respectively, depending on the solvent used. The incubation of microsomal fractions of porcine enterocytes with retinol did not form detectable levels of oxidative metabolites under the experimental conditions used. RA isomers other than the all-trans form were not identified. The intermediate product retinal, was detectable in trace amounts only.

**In vivo kinetics and metabolism of vitamin A in the pig from supplement or liver.** Predose levels of retinoids in plasma from portal and central veins are shown in Table 1. Only retinol and retinyl palmitate/oleate (16:0/18:1) were detected in all plasma samples. Additionally, all-trans-RA and 13-cis-RA were detected before supplementation, but only at low levels. Retinol concentrations were slightly elevated at the
13-cis-4-oxo-RA, retinoyl cis/H9252 lower in portal vein plasma than in central vein plasma,
plementation and liver feeding occurred with respect to phase veins after supplementation. Major differences between sup-
RA, 13-cis-RA (might include 9,13-di-RA, 9-
noleate and linolenate), anhydroretinol, 14-HRR, all-
including retinol, retinyl esters (palmitate/oleate, li-
several retinoid metabolites were detected in porcine plasma
in the central vein. 
tate/oleate levels were markedly lower in the portal vein than
C supplement’s availability. After liver feeding, the AUC and
max of retinol, retinyl 
max levels were comparable in central and portal
max levels were higher in portal vein than in central vein after
supplementation, all-trans-RA concentrations in the portal vein were much higher than in the central vein. The concentra-
time curve peaked at 1.75 h, then declined rapidly; there was a second peak at ~8 h, possibly reflecting entero-
hepatic circulation, whereas the all-trans-RA levels in central vein plasma were only marginally elevated at all time points (Fig. 4A). Such a difference in all-trans-RA kinetics between central and portal veins did not occur after liver feeding (Fig. 4B). On the other hand, the kinetics of 13-cis-RA did not exhibit major differences between central and portal veins or between supplementation and liver feeding (Fig. 4C and D, respectively). Glucuronidation of retinoic acid moieties to RAG also occurred. AUC of RAG in portal vein plasma were higher than those in central vein plasma, although only marginally (Fig. 4E and F, respectively). The major metabolic pathway of vitamin A, however, was the direct glucuronidation of retinol, yielding ROG. The AUC of ROG were approximately twofold higher after supplementation compared with after consumption of liver, whereas Cmax levels were five- to sevenfold higher (Fig. 4G and H, respectively). Additionally, a marked difference in ROG kinetics was obvious be-
tween portal and central veins after supplementation. ROG levels were higher in portal vein than in central vein after supplementation, but not after liver feeding.

To summarize, the major differences in metabolism of vi-
mamin A in the pig after supplementation and liver consump-
tion are illustrated in Figure 5A and B, respectively. In Figure
TABLE 1
Predose retinoid concentrations in a German Landrace pig1

<table>
<thead>
<tr>
<th>Retinoid2</th>
<th>Before supplement</th>
<th>Central vein</th>
<th>Before liver feeding</th>
<th>Central vein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retinol</td>
<td>16:0/18:1</td>
<td>All-trans-RA</td>
<td>13-cis-RA</td>
</tr>
<tr>
<td></td>
<td>nmol/L</td>
<td>1.071</td>
<td>34</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.127</td>
<td>160</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>160</td>
<td>ND</td>
<td>ND</td>
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<td></td>
<td></td>
<td>34</td>
<td>ND</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>160</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

1 Values are single measurements.
2 16:0/18:1, retinyl palmitate/oleate; RA, retinoic acid; ND, not detec-
table, detection limit 1.5 nmol/L.

TABLE 2
Area under the concentration-time curves (AUC) and maximum concentrations (Cmax) in porcine plasma of portal and central veins after either vitamin A supplementation or liver consumption1

<table>
<thead>
<tr>
<th>Supplementation (430,000 IU)</th>
<th>Liver feeding (667,000 IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoid2</td>
<td>Central vein</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
</tr>
<tr>
<td></td>
<td>nmol · h/L</td>
</tr>
<tr>
<td>Retinol</td>
<td>35,920</td>
</tr>
<tr>
<td>16:0/18:1</td>
<td>59,420</td>
</tr>
<tr>
<td>Total RE</td>
<td>99,445</td>
</tr>
<tr>
<td>All-trans-RA</td>
<td>19.3</td>
</tr>
<tr>
<td>9-cis-RA</td>
<td>ND</td>
</tr>
<tr>
<td>13-cis-RA</td>
<td>141.7</td>
</tr>
<tr>
<td>RAG</td>
<td>153.2</td>
</tr>
<tr>
<td>ROG</td>
<td>1,031</td>
</tr>
<tr>
<td>13-cis-4-oxo-RA</td>
<td>303.8</td>
</tr>
<tr>
<td>14-HRR</td>
<td>199.7</td>
</tr>
<tr>
<td>Anhydroretinol</td>
<td>187.3</td>
</tr>
</tbody>
</table>

1 Values are single measurements.
2 16:0/18:1, retinyl palmitate/oleate; RE, retinyl ester; RA, retinoic acid; RAG, retinoyl β-D-glucuronide; ROG, retinyl β-D-glucuronide; 14-HRR, 14-hydroxy-4,14-retro-retinol; ND, not detectable, detection limit 1.5 nmol/L.
shown. Again, all-trans-RA and ROG levels were higher after supplementation compared with after liver consumption.

DISCUSSION

In vitro studies have demonstrated that soluble alcohol dehydrogenases as well as other dehydrogenases can synthesize all-trans-RA (10,36–38). The formation of all-trans-RA from unbound retinol occurred in the presence of either cytosolic fractions or microsomal fractions, mainly from liver (10,17,36,37,39,40). Additionally, conversion of retinal to RA was described in presence of human cDNA-expressed P450 enzymes (18). In most tissues, retinol does not occur in its unbound form, but bound to CRBP I. The oxidation of unbound as well as CRBP I bound retinol was examined with cytosolic fractions from calf liver (41). The $K_m$ values for unbound and CRBP I bound retinol were 7.1 and 8.4 $\mu$mol/L, respectively. The $V_{max}$ value for the conversion of free retinol [15 pmol/(min · mg protein)] was higher than that for CRBP I bound retinol [10 pmol/(min · mg protein)]. Because the dissociation constant for the retinol-CRBP I complex is $10^{-8}$ mol/L and $K_m$ values were in the same range, the dissociation of the retinol-CRBP I complex should not be a prerequisite for the oxidation of retinol. It was therefore concluded that CRBP I might serve as a channel protein for retinol metabolizing enzymes (42). In the gastrointestinal (GI) tract, the functionally diverse CRBP type II exists almost exclusively (43,44). In vivo experiments in rats have shown that the molar ratio of unesterified retinol to CRBP II in the GI tract changed after oral administration of high vitamin A doses (given as retinyl acetate) from <1 in control groups to 3–19 in treated groups (45). It can therefore be concluded that large amounts of unbound retinol are present in the GI tract after administration of a vitamin A bolus. This unbound fraction of retinol might be metabolized by phase I or phase II enzymes.

In the present work, the in vitro oxidation of retinol to all-trans-RA was demonstrated in cytosolic fractions of porcine enterocytes. A $K_m$ of 94–96 $\mu$mol/L and a $V_{max}$ of 7.9–8.6 pmol/(min · mg protein) was determined. The results of the in vitro experiments indicated that retinol is a substrate for cytosolic enzymes of the porcine intestinal mucosa, although with a weak affinity. Due to the high $K_m$ of the reaction, it can be concluded that nonspecific enzymes are involved, which metabolize retinol only at high local concentrations, but with a turnover comparable to those rates described in the literature (41).

The differences in oxidative vitamin A metabolism in humans observed in the study of Buss et al. after intake of 500,000 IU vitamin A as a supplement or a liver meal raised the question whether intestinal metabolism of retinol to RA moieties and subsequent release of polar retinoids into the systemic circulation after a bolus of vitamin A as a supplement might be responsible for that phenomenon. In our in vivo experiment in a pig, amounts of vitamin A comparable to those of Buss et al. (6) were administered either as a supple-

### FIGURE 4
Kinetics of retinoids in plasma of the portal vein or central vein of a German Landrace pig after either supplementation of 430,000 IU vitamin A or consumption of liver containing 667,000 IU vitamin A.

### FIGURE 5
HPLC chromatograms of plasma samples from the portal and central veins of a pig. (A) Plasma samples were from portal and central veins 1.75 h after supplementation [at $T_{max}$ of all-trans-retinoic acid (RA)]; (B) portal vein plasma samples (at $T_{max}$ of all-trans-RA) were taken after either supplementation or liver consumption. The retinoid metabolites are as follows: 1) retinoyl β-D-glucuronide (RAG); 2) retinyl β-D-glucuronide (ROG); 3) 13-cis-RA (3); 4) 9-cis-RA; 5) all-trans-RA; and 6) 14-hydroxy-4,14-retro-retinol (14-HRR).
ment (430,000 IU) or liver (667,000 IU). It was clearly demonstrated that retinol is metabolized to all-trans-RA already in the intestine. However, contrary to the human situation, other metabolic pathways dominated, yielding glucuronides of all-trans-RA and retinol. We assume that in humans, higher amounts of all-trans-RA would have been formed intestinally than at least for all-trans-RA and ROG.

To date, in vivo experiments describing the role of the GI tract in the metabolism of vitamin A have focused mainly on the esterification of absorbed retinol and the transport of resulting retinyl esters via the lymphatic duct (46–48). Only in some experiments was the role of the GI tract in the oxidative metabolism of vitamin A studied. In ferrets, all-trans-RA was detected in the intestinal mucosa after perfusion with retinol. The in vivo experiment in a pig showed that retinol is already metabolized in the intestine to polar phase I and phase II metabolites, which are subsequently released into the portal vein. The major metabolic pathways of vitamin A in porcine intestine are summarized in Figure 6.

In summary, our study demonstrated that all-trans-RA is formed after incubation of cytosolic fractions of porcine enterocytes with retinol. The in vivo experiment in a pig showed that retinol is already metabolized in the intestine to polar phase I and phase II metabolites, which are subsequently released into the portal vein. The major metabolic pathways of vitamin A in porcine intestine are summarized in Figure 6. These findings raise the question whether a threshold of vitamin A intake exists in humans at which the intestine contributes to systemic exposure of putative teratogenic vitamin A metabolites.

**LITERATURE CITED**