Chronic Vitamin A Status and Acute Repletion with Retinyl Palmitate Are Determinants of the Distribution and Catabolism of all-trans-Retinoic Acid in Rats

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Abstract

The relation between vitamin A (VA) nutritional status and the metabolism of all-trans-retinoic acid (RA) is not well understood. In this study, we determined the tissue distribution and metabolism of a test dose of [3H]-RA in rats with graded, diet-dependent, differences in VA status. The design included 3 groups, designated VA-deficient, VA-marginal, and VA-adequate, with liver total retinol concentrations of 9.7, 35.7 and 359 nmol/g, respectively, (P < 0.05), and an additional group of VA-deficient rats treated with a single oral dose of retinyl palmitate (RP) 20 h before the injection of [3H]-RA. Plasma, liver, lung, and small intestines, collected 30 min after [3H]-RA, were analyzed for total [3H], unmetabolized [3H]-RA, polar organic-phase metabolites of [3H]-RA, and aqueous phase [3H]-labeled metabolites. In all groups, [3H]-RA was rapidly removed from plasma and concentrated in the liver. VA deficiency did not prevent the oxidative metabolism of RA. Nevertheless, the quantity of [3H]-RA metabolites in plasma and the ratio of total [3H]-polar metabolites to unmetabolized [3H]-RA in liver varied directly with VA status (VA-adequate > VA-marginal > VA-deficient, P < 0.05). Moreover, supplementation of VA-deficient rats with RP reduced the metabolism of [3H]-RA, similar to that in VA-adequate or VA-marginal rats. Liver retinol concentration, considered a proxy for VA status, was correlated (P < 0.05) with [3H]-RA metabolites in liver (R² = 0.54), plasma (R² = 0.44), lung (R² = 0.40), intestine (R² = 0.62), and all combined (R² = 0.655). Overall, the results demonstrate close linkage between dietary VA intake, hepatic storage of VA, and the degradation of RA and suggest that measuring plasma retinoid metabolites after a dose of RA may provide insight into the metabolism of this bioactive retinoid by visceral organs. J. Nutr. 137: 63–70, 2007.

Introduction

Previous work has shown that retinoic acid (RA), an oxidation product in the physiological metabolism of retinol, can support most of the physiological functions attributed to vitamin A (VA) (1). Additionally, studies of retinoid homeostasis have demonstrated that exogenous RA can “spare” retinol, reducing the rate of retinol catabolism and altering tissue retinol storage in an organ-specific manner (2,3). However, few studies have addressed the converse relation, namely is VA status a determinant of RA metabolism in vivo? This study was designed to provide insight into the potential relation between VA nutritional status and the metabolism of the principal active metabolite of retinol, all-trans-RA, using the rat as a model.

When VA is consumed in excess of requirements it is stored as retinyl esters, primarily in the liver, from which it can be mobilized, distributed to extrahepatic tissues (4), and used as a substrate for the production of oxidative metabolites (Fig. 1) (5). Investigations of retinol kinetics have established that retinol turnover and utilization is directly affected by VA intake and, consequently, by VA status (6,7). Retinol utilization was decreased to ~10% of control in rats with low VA stores than in their VA-sufficient counterparts (7). Kinetic analysis also revealed that the uptake, utilization, and storage of retinol by specific tissues in rats were directly influenced by VA status (8–10). These results suggest that VA status is a determinant of the distribution and utilization of retinol during VA supplementation. Furthermore, these studies suggest that VA status may affect the kinetics of other retinooids, such as RA, in a similar manner; however, the relation between VA status and the distribution and metabolism of RA has yet to be fully delineated.

Understanding the metabolism of RA as a function of VA status is of physiological importance because this active metabolite of retinol regulates numerous processes, including cell proliferation and differentiation, embryonic development, and immune function (11,12). Additionally, isomers of RA are used therapeutically in the treatment of skin disorders (13), acute promyelocytic leukemia (14,15), and other cancers (16). Moreover, RA may possess immune adjuvant properties (17,18),
suggested that treatment with RA might reduce the severity of certain infectious diseases. However, the effect of the host’s VA status on the metabolism of RA is not well understood. In view of these observations, this study was designed to determine how RA distribution and metabolism are regulated as a function of VA status, and whether repletion of VA-deficient rats with retinyl palmitate (RP), delivered as an oral dose similar to the amount and type of VA supplements given to young children (19,20), alters the distribution and/or metabolism of RA. In these studies, we determined the distribution and metabolism of a small, minimally perturbing dose of $[^3H]$-RA in rats fed diets to create conditions that represent a wide span of VA status known to be relevant to human health (21,22): VA deficiency, marginal VA status, and VA adequacy, as well as VA deficiency treated with RP.

**Materials and Methods**

**Animals and diets.** All procedures for animal care and use were approved by the IACUC of The Pennsylvania State University. Female Sprague-Dawley rats with litters of 12 female pups (purchased from Charles River Laboratories) were fed a VA-deficient diet from the time of birth. When the rat pups were 21 d old, they were weaned in pairs and fed a nutritionally complete AIN-93G diet (24), modified to contain either no VA ($n = 15$), 0.412 mg VA (VA-marginal; $n = 6$), or 7.43 mg VA (VA adequate; $n = 9$) of RP per kilogram of diet (Research Diets). Twenty hours prior to the experiment, 6 VA-deficient rats were given an oral dose of 1.67 mL of RP prepared in $~25–30$ $\mu$L of canola oil (VA-deficient + RP; $n = 6$). Finally, 3 VA-adequate rats were pair fed to 3 VA-deficient rats for 14 d to ensure that any VA-deficiency-related anorexia did not affect the results.

**Experimental design.** When the rats were between 60 and 66 d of age, they were individually anesthetized by isoflurane-oxygen inhalation and 0.15 mL/100 g body weight (BW) of albumin-bound $[^3H]$-RA, prepared as described below, was injected into the exposed left common iliac vein (25). The incision was closed with a sterile surgical staple and the rats were allowed to recover from the anesthesia. Rats were killed 30 min postinjection by carbon dioxide asphyxiation. Blood was drawn from the vena cava into heparinized syringes and the liver, lung, spleen, and small intestine were quickly removed, weighed, and aliquots frozen in liquid nitrogen for storage at $-80^\circ$C before subsequent analysis. Because RA is rapidly metabolized, it is important to collect tissues quickly after a lipid carrier because no nonradioactive RA was added, followed by 5 mL of Tween 20 (Sigma-Aldrich), 0.9 mL of 0.1% rat serum albumin (Calbiochem), diluted in sterile PBS, and 0.10 mL of whole rat plasma. The final concentration of RA in the dose was 0.023 $\mu$mol/L, and the dose delivered to each rat was standardized at 0.15 mL (33 pmol RA)/100 g BW. This resulted in a minimally perturbing dose of RA, estimated to transiently elevate plasma RA by $~3.7 \mu$g RA/(0.012 $\mu$mol)/L plasma. Following the preparation and administration of each dose, aliquots were analyzed by HPLC (29), which showed $>97\%$ $[^3H]$-RA (data not shown).

**Determination of tissue radioactivity.** Aliquots of 0.5 mL of individual plasma, or 0.5 g of liver, lung, and small intestine samples were extracted using a modification of the method of Folch et al. (30,31), as described previously (25). The procedure resulted in a washed organic phase and an aqueous phase (25). Aliquots of the injected dose, along with the organic and aqueous phases from each tissue, were analyzed by liquid scintillation spectrometry (25).

**Separation of RA from polar organic metabolites (4-oxo- and 4-hydroxy-retinoic acid).** Reverse-phase solid phase extraction was employed to separate the parent compound, $[^3H]$-RA, from its oxidation products, mainly $[^3H]$-4-oxo- and $[^3H]$-4-hydroxy-RA (32,33) in the organic phase of the liver extracts, as described previously (25). Briefly, 300 $\mu$L ($~50$ mg tissue) of Folch-washed organic phase along with all-trans-RA and 4-oxo-RA standards were dried to completeness under argon and reconstituted in 1.25 mL of HPLC-grade acetonitrile-water (65:35, vol:vol) containing 10 $\mu$mol/L of acetic acid. Following column conditioning, the samples were loaded onto the column and the polar metabolites were eluted first in 3 $\times$ 4 mL of acetonitrile-water (65:35, vol:vol), while all-trans-RA was eluted with 3 $\times$ 4 mL of acetonitrile-water (80:20, vol:vol) (25). The elution rate was maintained at $~1$ mL/min with negative pressure, using an elution vacuum apparatus (Supelco). After collection, the samples were dried to completeness and analyzed by liquid scintillation spectrometry as described previously (25).

**Retinol assay.** Plasma and liver total retinol were measured in aliquots of plasma or the organic extract of liver. Plasma and liver samples were pooled prior to retinol analysis, such that each retinol concentration data point presented represents either 2 (VA-marginal and RP groups) or 3 (VA-deficient and VA-adequate groups) rats. Pooled samples were saponified and total retinol was quantified by HPLC with UV detection. Trimethylmethoxyphenyl-retinol was used as internal standard (34).

**Real time PCR.** Total RNA was extracted from the same pooled liver samples that were used for retinol assays, using TRIzol Reagent (Invitrogen), and quantified by UV spectrophotometry. CYP26A1 mRNA levels were quantitatively assessed by RT-PCR in Penn State’s Nucleic Acid Facility using an ABI 7700 (Perkin-Elmer) cycle system. The endogenous 18S RNA transcript was used as the internal control. Briefly, the PCR mixture was assembled using the TaqMan PCR core reagent kit along 10 $\mu$mol/L CYP26 forward primer (5'-AGTGATGGGGCGCGGATAAT-3') and 10 $\mu$mol/L CYP26 reverse primer (5 '-TGCCTAGCACCACCACCGGT-3'). The PCR cycling program was run for 1 cycle of 50$^\circ$C for 2 min, 95$^\circ$C for 10 min, followed by 40 cycles of 95$^\circ$C for 15 s, 60$^\circ$C for 1 min, and then 1 cycle of 25$^\circ$C for 2 min. RNA samples were assembled in duplicate. For comparison between runs and with previous experiments, a sample of a pool of control rat liver RNA, C-1, and a sample of a pool of RA-induced rat liver RNA, C-2, were run with each set of samples. The results are expressed relative to C-1, equal to 1 before log transformation and 0 after log transformation. The data obtained from the RT-PCR were log$_{10}$ transformed prior to statistical analysis.

**Statistics.** All results are expressed as means $\pm$ SEM. One-way ANOVA (SuperANOVA, Abacus Concepts) was used to test for the effect of dietary VA level on $[^3H]$-RA metabolism. When warranted, data were
then tested by Fisher’s Protected LSD test to determine which groups differed significantly ($P < 0.05$). Simple regression was performed on pooled group data with $P < 0.05$ considered significant. When necessary, data were log_{10} transformed prior to analysis.

Results

VA status affects plasma and liver retinol concentrations and hepatic CYP26A1 mRNA expression. All rats appeared in good health. No signs of anorexia developed and, at the time of testing, the VA-deficient group did not differ in body weight from the VA-adequate group, nor did the pair-fed VA-adequate rats differ from non-pair-fed VA-adequate rats (Table 1). However, the relative weight of the liver was significantly lower in the VA-deficient and VA-deficient+RP rats than in VA-adequate and VA-marginal rats. In contrast, the relative weight of the lung was slightly but significantly higher in the VA-deficient rats than in the VA-adequate and VA-marginal rats (Table 1). There was no difference due to VA status in the relative weight of the small intestine.

Plasma and liver retinol concentrations (Table 1) were measured as indicators of VA status. Plasma retinol concentration was ~10-fold greater in VA-adequate rats than in VA-deficient rats, whereas VA-marginal and VA-deficient+RP rats did not differ from VA-adequate rats. Liver retinol was ~38, 9, and 6 times greater in VA-adequate rats than in VA-deficient, VA-marginal, and VA-deficient+RP rats, respectively ($P < 0.05$, Table 1).

Hepatic CYP26A1 mRNA expression was measured, as previous work has shown that its level differs in a sensitive manner with VA status (35). CYP26A1 was significantly lower ($P < 0.001$) in the VA-deficient group compared with the other groups by ANOVA (Table 1). Although the VA-marginal, VA-deficient+RP, and VA-adequate groups did not differ significantly, this may have been an artifact of ANOVA, as the mean CYP26 mRNA expression in the VA-adequate group was ~6.5 and 5 times those in the VA-marginal and VA-deficient+RP groups, respectively, and all groups differed significantly when analyzed by unpaired t test ($P < 0.05$).

VA deficiency increases total $^{3}$H radioactivity from $^{3}$H-RA in plasma but decreases the proportion of $^{3}$H-aqueous phase metabolites. In all groups, >96% of the injected dose was eliminated from plasma within 30 min (Fig. 2). However, total $[^{3}$H]-retinoids in plasma were higher in VA-deficient rats. VA status affected both $[^{3}$H]-aqueous and $[^{3}$H]-organic phase metabolites of RA ($P < 0.005$). $[^{3}$H]-Aqueous phase metabolites increased as a function of VA-status (VA-adequate > VA-marginal > VA-deficient; Fig. 2). Conversely, the amount of organic phase $[^{3}$H]-labeled compounds recovered in the plasma of VA-deficient rats was greater than in the other groups ($P < 0.01$, Fig. 2). The amount of aqueous and organic phase $^{3}$H in the plasma of VA-deficient+RP rats was similar to the amount in VA-marginal and VA-adequate rats, but $[^{3}$H]-aqueous phase metabolites in the plasma of VA-deficient+RP rats were 150% of VA-deficient rats ($P < 0.05$). Thus, VA status and acute retinol repletion were determinants of both the total amount of $^{3}$H and the amount of aqueous phase $^{3}$H recovered in plasma 30 min after injection of $[^{3}$H]-RA.

The total amount and distribution of $[^{3}$H]-retinoids in liver differs with VA status. The liver of VA-adequate rats contained ~14% of the injected dose of $[^{3}$H]-RA as total $[^{3}$H]-retinoids (Fig. 3A). The amount of total $[^{3}$H]-retinoids recovered in the liver was ~150 and 250% of VA-adequate rats in VA-marginal and VA-deficient rats, respectively. VA status affected both $[^{3}$H]-aqueous ($P < 0.001$) and $[^{3}$H]-organic phase ($P < 0.001$) metabolites in liver. $[^{3}$H]-aqueous phase metabolites increased as a function of VA status (VA-adequate > VA-marginal > VA-deficient; Fig. 3A), whereas, in contrast, $[^{3}$H]-organic phase metabolites decreased as a function of VA status (VA-adequate < VA-marginal < VA-deficient; Fig. 3A). Aqueous phase $^{3}$H in the liver of VA-deficient+RP rats was similar to that in VA-marginal rats, but lower than in VA-adequate rats and higher than in VA-deficient rats (Fig. 3A). However, the amount of organic phase $^{3}$H was similar in VA-deficient+RP rats and VA-adequate rats, but differed from VA-marginal and VA-deficient rats ($P < 0.01$, Fig. 3A).

To further characterize the fate of injected $[^{3}$H]-RA, the proportion of unmetabolized $[^{3}$H]-RA and $[^{3}$H]-labeled polar retinoids was determined in the organic phase of liver samples from individual rats. Similar to aqueous phase radioactivity, unmetabolized $[^{3}$H]-RA decreased as a function of VA status (VA-adequate < VA-marginal < VA-deficient; Fig. 3B). In

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>VA-deficient</th>
<th>VA-marginal</th>
<th>VA-adequate</th>
<th>VA-deficient+RP</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>234.7 ± 12.3$^a$</td>
<td>280.7 ± 16.0$^b$</td>
<td>245.67 ± 5.4$^b$</td>
<td>243.5 ± 13.6$^b$</td>
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<td>Liver, % of BW</td>
<td>3.49 ± 0.14$^a$</td>
<td>3.98 ± 0.154$^b$</td>
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<td>3.55 ± 0.07$^a$</td>
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<td>Lung, % of BW</td>
<td>0.52 ± 0.02$^a$</td>
<td>0.46 ± 0.02$^a$</td>
<td>0.47 ± 0.01$^a$</td>
<td>0.50 ± 0.02$^a$</td>
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<td>Small Intestine, % of BW</td>
<td>2.20 ± 0.04</td>
<td>2.11 ± 0.05</td>
<td>2.21 ± 0.03</td>
<td>2.22 ± 0.04</td>
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<td>Plasma retinol$^2$, log_{10}</td>
<td>-0.91 ± 0.03$^a$</td>
<td>0.09 ± 0.003$^b$</td>
<td>0.09 ± 0.03$^b$</td>
<td>0.13 ± 0.03$^b$</td>
</tr>
<tr>
<td>Mean, μmol/L</td>
<td>(0.12)$^a$</td>
<td>(1.24)</td>
<td>(1.26)</td>
<td>(1.38)</td>
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<td>Liver retinol$^2$, log_{10}</td>
<td>0.87 ± 0.26$^a$</td>
<td>1.53 ± 0.10$^b$</td>
<td>2.57 ± 0.05$^a$</td>
<td>1.74 ± 0.06$^a$</td>
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<tr>
<td>Mean, nmol/g</td>
<td>(9.7)</td>
<td>(35.7)</td>
<td>(379)</td>
<td>(55.9)</td>
</tr>
<tr>
<td>CYP26A1 mRNA$^{2,4}$, log_{10}</td>
<td>-2.49 ± 0.51$^a$</td>
<td>-0.21 ± 0.17$^b$</td>
<td>0.60 ± 0.09$^b$</td>
<td>-0.10 ± 0.02$^b$</td>
</tr>
<tr>
<td>Mean, relative expression</td>
<td>(0.003)</td>
<td>(0.617)</td>
<td>(3.981)</td>
<td>(0.794)</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SEM, $n = 9$ (VA-deficient and VA-adequate) or $n = 6$ (VA-marginal and VA-deficient+RP). Means in a row with superscripts do not differ, $P > 0.05$. $^2$ Data were log_{10} transformed prior to statistical analysis. $^3$ Values in parentheses are the antilog of plasma retinol (μmol/L), liver retinol (nmol/g), and CYP26A1 mRNA expression. $^4$ Data are relative to a VA-adequate liver sample.
contrast, total [3H]-polar metabolites in liver increased as a function of VA status (VA-adequate > VA-marginal > VA-deficient; Fig. 3B). Moreover, the amount of total [3H]-polar metabolites was similar for VA-deficient + RP and VA-marginal rats, whereas the amount of unmetabolized [3H]-RA was similar for VA-deficient + RP and VA-adequate rats. Taken together, VA status and acute repletion with retinol affected both the distribution and the metabolism of RA in the liver.

**RA metabolism and distribution in extrahepatic tissues is affected by VA status.** Less than 0.75% of the injected dose of [3H]-RA was recovered in the lungs of VA-adequate rats (Fig. 4). Total [3H] was lower in the lungs of VA-deficient rats than in VA-adequate rats (P < 0.05), but was not different from the VA-marginal rats. VA status affected (P < 0.001) [3H]-aqueous phase metabolites in lung, which increased as a function of VA status (VA-adequate > VA-marginal > VA-deficient; Fig. 4). Aqueous phase [3H] was greater in the lung of VA-deficient + RP rats than in VA-deficient, VA-marginal, and VA-adequate rats (P < 0.05), whereas organic phase [3H] in the VA-deficient + RP group was similar to that in VA-marginal and VA-adequate rats, but different from VA-deficient rats (P < 0.05, Fig. 4). Thus, unlike the other tissues examined, the lungs of the VA-deficient + RP rats contained ~25% more [3H]-aqueous phase metabolites than the VA-adequate rats. Approximately 4.5% of the injected dose of [3H]-RA was recovered in the small intestine of VA-adequate rats 30 min postinjection (Fig. 5), with less in the VA-deficient and VA-marginal groups. VA status affected [3H]-aqueous phase radioactivity (P < 0.03) in the small intestine, and [3H]-aqueous phase metabolites increased as a function of VA status (VA-adequate > VA-marginal > VA-deficient; Fig. 5). In contrast, the amount of [3H]-organic phase metabolites were similar. Aqueous phase [3H] in the small intestine of VA-deficient + RP rats was similar to that in VA-deficient, VA-marginal, and VA-adequate rats, whereas organic phase [3H] was greater than in the VA-deficient and VA-marginal rats (Fig. 5).

**Liver retinol concentrations and hepatic CYP26A1 mRNA expression are correlated with increased metabolism.** Pooled group data for liver, lung, plasma, and small intestine [3H]-metabolites were compared with hepatic retinol concentrations (Fig. 6), as a proxy for VA status, and to CYP26A1 mRNA expression as a biochemical indicator of RA responsiveness (Table 2), using linear regression analysis. As observed previously (35), CYP26A1 mRNA expression was significantly correlated with hepatic retinol concentration (Table 2). The amount of [3H]-metabolites recovered from the liver, lung, small intestine, and plasma were strongly correlated with hepatic retinol, with the amount of metabolites increasing linearly in

![Figure 2](https://example.com/figure2.png)  
**Figure 2** Total radioactivity and [3H]-aqueous phase metabolites in plasma of female Sprague-Dawley rats fed diets with different amounts of VA. Plasma was collected 30 min after injection of [3H]-RA (33 pmol RA mass) and [3H] in organic-phase and aqueous-phase extracts was determined. Values are means ± SEM, n = 9 for VA-deficient (VAD) and VA-adequate (VAS) and n = 6 for VA-marginal (VAM) and VA-deficient + RP (VAD + RP) groups. Different letters above bars within panels indicate significant differences, P < 0.05.

![Figure 3](https://example.com/figure3.png)  
**Figure 3** Total radioactivity (A) and total [3H]-metabolites (B) in liver of female rats fed different amounts of VA 30 min after injection of [3H]-RA. Liver was collected 30 min after injection of [3H]-RA (33 pmol RA mass) and [3H] in organic-phase and aqueous-phase extracts was determined (A). Reverse-phase solid phase extraction (SPE) was employed to separate the parent compound [3H]-RA from [3H]-labeled in the organic phase of the liver extracts (B). Values are means ± SEM, n = 9 for VA-deficient (VAD) and VA-adequate (VAS) and n = 6 for VA-marginal (VAM) and VA-deficient + RP (VAD + RP) groups. Different letters above bars within panels indicate significant differences, P < 0.05.

![Figure 4](https://example.com/figure4.png)  
**Figure 4** Total radioactivity and [3H]-aqueous phase metabolites present in lung of female rats fed different amounts of VA. Values are means ± SEM, n = 9 for VA-deficient (VAD) and VA-adequate (VAS) and n = 6 for VA-marginal (VAM) and VA-deficient + RP (VAD + RP) groups. Different letters above bars within panels indicate significant differences, P < 0.05.
relation to increasing hepatic retinol and CYP26 levels (Fig 6). The strongest correlation was observed in the lung, where the amount of metabolites recovered in the VA-deficient, VA-marginal, and VA-supplemented groups were directly proportional to hepatic retinol concentrations ($R^2 = 0.873$, $P < 0.001$).

**Discussion**

Under normal physiological conditions, plasma RA turns over rapidly in both humans and rats (36–39) and subsequently appears as various polar metabolites, such as 4-hydroxy-RA, 4-oxo-RA, and retinoyl $\beta$-glucuronide (26,32,40). In this study, we found that RA distribution and metabolism is altered in response to decreasing VA intake, despite similar plasma retinol concentrations, indicating that VA stores are a major determinant of RA metabolism in all tissues examined (Fig. 6).

The liver, lung, and small intestine are intimately involved in retinol and RA homeostasis (41–43). Therefore, we examined each organ for aqueous and organic-phase radioactivity to determine whether VA status affects RA metabolism. The amounts of VA provided in the diets used in our study were selected to create a wide range of hepatic VA stores (44–46), as in previous studies, and resulted in an $\sim$35-fold range of total retinol concentrations, from $<10$ nmol/g in the VA-deficient group to $>375$ nmol/g in the VA-adequate group (Table 1). The amount of aqueous-phase radioactivity recovered in the liver, lung, and small intestine was lowest in the VA-deficient rats and highest in the VA-adequate rats. Similarly, the ratio of total $[\text{H}]$-labeled polar metabolites to $[\text{H}]$-RA in the liver, lung, and small intestine was highest in VA-adequate rats and lowest in VA-deficient rats, indicating that VA-adequate rats catabolized more RA than VA-deficient rats. Moreover, RA metabolism increased in the liver, lung, and small intestine in proportion to hepatic retinol concentrations, indicating that VA stores are a major determinant of RA metabolism in all tissues examined (Fig. 6).

The liver, lung, and small intestine are involved in the uptake, recycling, and metabolism of RA by the liver, lung, and small intestine are dependent upon VA stores and that each organ is involved in the regulation of RA homeostasis.

### Table 2: Correlation of CYP26A1 mRNA expression and tissue-specific $[\text{H}]$-RA-metabolites in VA-deficient, VA-marginal, VA-adequate, and VA-deficient+RP rats$^{1,2}$

<table>
<thead>
<tr>
<th>CYP26A1 mRNA</th>
<th>$R^2$</th>
<th>$P$-value</th>
</tr>
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<tbody>
<tr>
<td>Liver metabolites</td>
<td>0.550</td>
<td>0.006</td>
</tr>
<tr>
<td>Lung</td>
<td>0.477</td>
<td>0.013</td>
</tr>
<tr>
<td>Lung, excluding RP group</td>
<td>0.726</td>
<td>0.004</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.507</td>
<td>0.009</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.413</td>
<td>0.024</td>
</tr>
<tr>
<td>Total metabolites</td>
<td>0.507</td>
<td>0.009</td>
</tr>
</tbody>
</table>

$^1$ Because CYP26A1 mRNA was determined on pools of liver tissue from 2 or 3 rats/group, data for metabolites was averaged for the same rats ($n = 3$) and the VA-deficient and VA-adequate and $n = 2$ group for VA-marginal and VA-deficient+RP prior to performing linear regression analysis. Data represent $[\text{H}]$-aqueous phase metabolites (liver, lung, small intestine, and plasma) and $[\text{H}]$-aqueous phase metabolites (liver). Total metabolites represents the sum of the individual organs.

$^2$ CYP26A1 mRNA levels were determined by real-time PCR and normalized to a reference sample of VA-adequate liver. Data were log$_{10}$ transformed prior to regression analysis.

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**Figure 5** Total radioactivity and $[\text{H}]$-aqueous phase metabolites present in small intestine of female rats fed different amounts of VA. Values are means ± SEM, $n = 9$ for VA-deficient (VAD) and VA-adequate (VAS) and $n = 6$ for VA-marginal (VAM) and VA-deficient+RP (VAD+RP) groups. Different letters above bars within panels indicate significant differences, $P < 0.05$.
In contrast to the lung and small intestine, the livers of VA-marginal and VA-deficient rats contained ~30 and 130% more of the injected dose, respectively, than the VA-adequate rats, suggesting that uptake and/or retention of RA in the liver is greater as VA status declines, which is consistent with greater conservation of RA. Conversely, VA status may be inversely related to the recycling of RA from liver to plasma. On the basis of these results, we speculate that 2 related processes could be working in concert to cause the observed increase in liver radioactivity and decrease in the metabolism of RA in VA-deficient rats and, to a lesser extent, in VA-marginal rats. First, previous work has shown that the ratio of apo- to holo-cellular retinol binding protein (CRBP) affects retinol metabolism (47–50), for as the ratio of apo-CRBP to holo-CRBP increases, retinol storage is inhibited, while retinyl ester hydrolysis is increased. If the converse is true, retinol storage will increase while retinyl ester mobilization will decrease (49,50). Our results for RA metabolism suggest there may be a regulatory mechanism parallel to that for retinol. Specifically, in the case of VA-deficient rats, we hypothesize that the ratio of apo-cellular retinoic acid binding protein (CRABP) to holo-CRABP is high, resulting in a decrease in hepatic RA metabolism and secretion/recycling into plasma, while increasing the residence time of RA in liver. In fact, the ratio of apo-CRABP to holo-CRABP is lower in VA-adequate rats than VA-marginal or VA-deficient rats, both RA metabolism and the excretion of polar and aqueous-phase RA metabolites into bile may be increased (26,40,51). Second, the expression of CYP26A1, an RA-inducible enzyme that catalyzes the oxidation of RA (46,52,53), is directly related to chronic VA intake and VA status, assessed as liver retinol concentration (35). In our studies, CYP26A1 mRNA was highest in the liver of VA-adequate rats and decreased as VA status (liver retinol) declined (Table 1) and CYP26A1 mRNA expression was directly correlated with increased metabolism (Table 2). Additionally, studies in F9 teratocarcinoma cells showed that CRABP expression and RA metabolism are correlated (54). Thus, changes in the ratio of apo- to holo-CRABP, as demonstrated in F9 cells, and differences in the level of expression of CYP26A1 due to VA status, may contribute importantly to the regulation of hepatic RA metabolism in intact rats, which in turn may maintain normal cellular and whole-body levels of RA.

VA supplementation is now used extensively in developing countries to prevent nutritional VA deficiency, which is associated with diminished disease resistance (55), prolonged infection (56), and increased mortality (57,58). Although VA supplementation is known to increase plasma retinol if liver VA stores have been depleted and to increase the storage of VA in the liver and extrahepatic tissues in proportion to the oral dose (59), it is unknown whether the metabolism of RA is affected, either acutely or over a long term, by VA supplementation. To determine whether VA supplementation affects the distribution and metabolism of RA acutely, we compared VA-deficient rats to VA-deficient+RP rats given a single oral dose of RP 20 h before the test dose of [3H]-RA. In the plasma and organs examined we observed 2 distinct trends with regards to RA homeostasis. First, the total amount of radioactivity, both unmetabolized and metabolized, that was recovered in the plasma, liver, lung, and small intestine of the VA-deficient+RP rats was similar to that recovered from VA-adequate rats. This suggests that VA supplementation results very quickly in the resetting of the homeostatic mechanisms that control RA metabolism. Secondly, the amount of [3H]-metabolites of RA recovered from tissues was significantly greater in VA-deficient+RP rats than in VA-deficient rats. Moreover, [3H]-metabolites were nearly identical in VA-deficient+RP rats and VA-marginal rats, except in the lungs, where aqueous phase metabolites were greater in the VA-deficient+RP rats than in VA-marginal rats. We speculate that the decrease in total radioactivity and the increase in RA metabolism in VA-deficient+RP rats was caused by the rapid increase in hepatic VA following supplementation (Table 1), which could have resulted in a substantial increase in the production of RA from retinol. Thus, providing an acute retinol supplement to VA-deficient rats restored plasma retinol concentrations to normal, and increased hepatic stores of VA, but the metabolism of RA was still compromised, suggesting that a complete restoration of VA homeostasis was not achieved within the 20 h following supplementation with RP.

In conclusion, the results of this study demonstrate that VA status affects both the organ distribution and metabolism of RA in rats, and suggest that the uptake, recycling, and utilization of RA are altered in a tissue-specific manner in response to decreasing stores of VA. Furthermore, the administration of RP to VA-deficient rats resulted, in a 20-h interval, in increased hepatic retinol and a pattern of [3H]-RA metabolism similar to that in VA-adequate rats. To our knowledge, this is the first study to show that both the distribution and metabolism of RA are directly affected by VA status and retinol repletion. Our results on the regulated expression of CYP26A1 in liver concur with those of Yamamoto et al. (35) and, together, suggest that molecular mechanisms capable of controlling whole-body RA homeostasis respond sensitively, both to chronic differences and acute changes in VA intake, to maintain normal physiological concentrations of RA. Although our study focused mainly on the metabolism of RA in the liver, it is interesting that difference in plasma [3H]-RA metabolites could be detected as a function both of VA status and retinol repletion. It may be possible that plasma retinoid metabolites would be useful as indicators of tissue retinoid metabolism. Previously, oxidized metabolites of retinol in plasma were correlated with VA status in lactating sows (60). We speculate that the measurement of plasma metabolites of RA following an acute RA dose could be used as a sensitive indicator of VA status in humans.

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Literature Cited


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