Vitamin A and Retinoic Acid Combined Have a More Potent Effect Compared with Vitamin A Alone on the Uptake of Retinol into Extrahepatic Tissues of Neonatal Rats Raised under Vitamin A–Marginal Conditions

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Abstract

Background: Vitamin A (VA; retinol) supplementation is widely used to reduce child mortality in low-income countries. However, existing research suggests that supplementation with VA alone may not be optimal for infants.

Objective: We compared the effect of VA with VA combined with retinoic acid (VARA) on retinol uptake and turnover in organs of neonatal rats raised under VA-marginal conditions.

Methods: Secondary analysis was conducted on data obtained from 2 previous kinetic studies in Sprague-Dawley neonatal rats nursed by dams fed a VA-marginal diet (0.35 mg retinol equivalents/kg diet). On postnatal day 4, pups had been treated with a single dose of VA (6 μg/g; n = 52; VA study), VA + 10% retinoic acid (6 μg/g; n = 42; VARA study), or placebo (canola oil; n = 94; both studies), all of which contained ~2 μCi [^{3}H]retinol as the tracer for VA. Total retinol and tracer concentrations were measured in plasma and tissues from 1 h to 14 d after dosing. Control group data from both studies were merged before analysis. Kinetic parameters were re-estimated and compared statistically.

Results: VARA supplementation administered to neonatal rats within a few days after birth resulted in a lower turnover of retinol in the lungs, kidneys, and carcass and less frequent recycling of retinol between plasma and organs (100 compared with 288 times in the VARA- compared with the VA-treated group). Although VA supplementation resulted in a higher concentration of retinol in the liver, VARA supplementation led to a higher uptake of postprandial retinyl esters into the lungs, intestines, and carcass.

Conclusion: Given the relatively higher retinol uptake into several extrahepatic organs of neonates dosed orally with VARA, this form of supplementation may serve as a targeted treatment of low VA concentrations in extrahepatic organs that continue to develop postnatally. *Curr Dev Nutr* 2017;1:e000265.

Introduction

Despite a modest reduction in the prevalence of vitamin A (VA; retinol) deficiency in low-income countries, it is still very common in South Asia and sub-Saharan Africa, where it affects 44–48% of children (1). VA supplementation has been widely used as a cost-effective strategy to reduce all-cause mortality among children on the basis of the positive findings of multiple intervention trials conducted in this age group (2–4). However, the same intervention conducted in neonates (<1 mo old) did not bring the expected positive outcome (4–6), possibly due to a limited VA storage capacity of neonatal tissues (7).

Although knowledge about neonatal VA metabolism is limited, previous studies in neonatal rats showed that VA retention, especially in the extrahepatic tissues such as

skin, bone, and muscle, is low compared with that in adult rats (7, 8). Moreover, oral supplementation with VA delivered during the first few days after birth resulted in an extensive recycling of retinol between plasma and tissues before catabolism (541 compared with 12–13 times normally observed in adult rats) (8, 9). On the other hand, a slightly modified form of VA supplement, VA mixed with 10% retinoic acid (VARA), which is designed to create a "metabolic priming" of VA metabolism, increased the uptake of retinol into extrahepatic tissues and reduced its recycling in supplemented compared with control neonates (10). However, these 2 supplements, VA and VARA, were tested in 2 separate studies in the supplemented and control groups; therefore, the extent to which VARA may be superior to VA in improving retinol tissue stores is currently unknown.

In this secondary analysis, we combined data from the VA and VARA studies (7, 11, 12) and used the model-based compartmental analysis to compare the concentration and kinetics of retinol in several organs of neonatal rats dosed with either VA or VARA or placebo in the amount equivalent to that which has been given to human infants (13). Our results build on previous knowledge of the neonatal VA metabolism and may help inform the most effective treatment of VA deficiency in neonates by comparing the effect of 2 different supplement forms on retinol tissue uptake and distribution.

Methods

Study design
Retinol concentration and kinetic data were obtained from 2 similar studies conducted in 2011 and 2013, which are described in detail in previous reports (7, 11, 12). Briefly, Sprague-Dawley pregnant female rats (Charles River Laboratories) were fed a VA-marginal (0.35 mg retinol equivalents/kg) AIN-93G purified diet (Research Diets) (14) to render them in VA status similar to that of mothers in low-income countries. Their newborn pups were randomly assigned to either the VA-supplemented (*n* = 52; 33 males) or control (*n* = 52; 30 males) groups in the VA study (7, 11) and to either the VARA-supplemented (*n* = 42; 19 males) or control (*n* = 42; 24 males) groups in the VARA study (12). In both studies, on postnatal day 4, pups received an oral dose of either VA [6 μg retinyl palmitate/g body weight (VA study)], VARA [6 μg retinyl palmitate/g body weight + 10% retinoic acid (VARA study)], or placebo [canola oil (both studies)]. All doses (VA, VARA, and placebo) contained ~2 μCi [^{3}H]retinol as the tracer for VA. Plasma and organs were collected from 0.5 h to 24 (VA study) or 14 (VARA study) d after dosing from pups sedated with isoflurane or, after postnatal day 14, killed with carbon dioxide. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Retinol mass and tracer concentrations were measured by using ultraperformance LC (Acquity UPLC System; Waters) and liquid scintillation counting (Beckman Coulter), respectively, according to a protocol described earlier (7, 11, 15). In both studies, kinetic analysis was performed by using the Windows version of Simulation, Analysis, and Modeling software (version 3.0.8; http://www.winsaam.org) (16).

The VA and VARA experiments differed with respect to the following: *1*) sample collection times (VA study: 0.5, 1, 4, 8, and 15 h,

and 1, 2, 4, 8, 11, 14, 18, and 24 d after dosing; VARA study: 1, 2.5, 4, 6, 8, 11, and 15 h and 1, 2, 4, 6, 8, 11, and 14 d after dosing), *2*) number of rat pups per sampling time according to group (VA study: *n* = 4; VARA study: *n* = 3), *3*) organs collected [VA study: stomach, intestines, liver, lungs, kidneys, brain, adipose tissue, and the remaining carcass (muscles, bones, and connective tissue); VARA study: the same as in the VA study; however, the carcass comprised skin, adipose tissue, and brain (organs dissected and analyzed individually in the VA study)], and *4*) retinol mass analysis (retinol mass was measured only in the liver, lungs, and plasma in the VARA study, precluding the comparison of retinol concentrations in other organs). To address these differences, the following adjustments were made to the data before statistical comparison. First, data for the skin, adipose tissue, brain, and carcass from the VA study were summed in order to match the VARA study carcass, and the resulting SEMs were recalculated on the basis of the following error propagation formula:

$$SEM_{1+\ldots+n} = \sqrt{SEM_1^2 + \ldots + SEM_n^2} \qquad (1)$$

Second, data from control groups in both studies (retinol and tracer concentrations measured at 1, 4, 8, and 15 h and 1, 2, 4, 8, 11, and 14 d after dosing) were combined to create a single comparison group. Third, models of retinol kinetics from the VA study were truncated by removing data for days 18 and 24 and the resulting kinetic parameters were re-estimated on the basis of the data observed from day 1 to day 14. The parameter estimation process followed the same steps as described in previous reports (10–12). Briefly, parameters were adjusted to obtain the best fit of the model-calculated plot to the observed data and the final values were generated through a weighted nonlinear regression analysis. The parameters were considered well identified if the sum of squares from regression analysis was $<10^{-5}$ and the parameter fractional SD was <0.5.

Statistical analysis
The mean total retinol (REs + unesterified retinol) and tracer concentrations over time were plotted and the values for the control and VA- and VARA-supplemented neonates were compared by

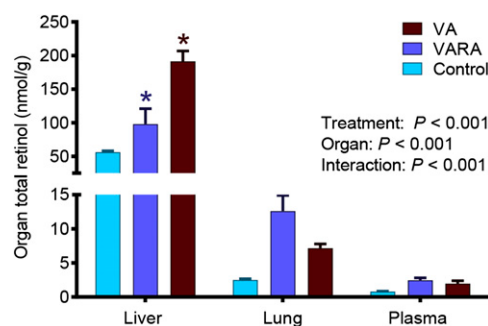**FIGURE 1** Mean total (unesterified + esterified) retinol concentrations in the liver, lungs, and plasma of control and VA- and VARA-supplemented neonatal rat pups on day 1 after dosing on postnatal day 4. Values are means ± SEMs; *n* = 42 (control), *n* = 24 (VA), or *n* = 18 (VARA) rat pups. *VA or VARA compared with the other groups, *P* < 0.05 (ANOVA). VA, vitamin A; VARA, VA combined with retinoic acid.
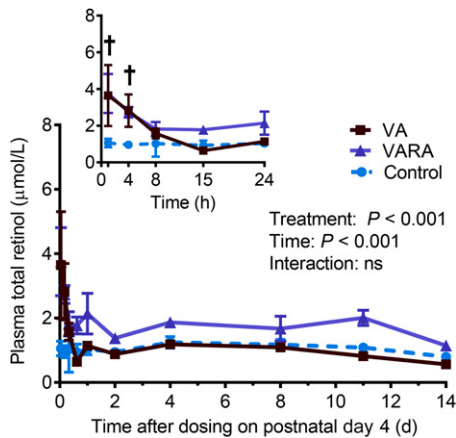
FIGURE 2 Plasma total (unesterified + esterified) retinol concentrations in control and VA- and VARA-supplemented neonatal rat pups from 0 to 14 d after dosing on postnatal day 4. The inset shows the first 24 h after dosing. Each point represents the mean ± SEM of $n = 7$ (control), $n = 4$ (VA), or $n = 3$ (VARA) rat pups. [†]Supplemented groups compared with control, $P < 0.05$ (ANOVA). VA, vitamin A; VARA, VA combined with retinoic acid.

using a 2-factor ANOVA (treatment and time) with Bonferroni correction for multiple comparisons (GraphPad Prism version 5.0). Plotted values were expressed as the mean total retinol concentration ± SEM or the mean fraction of dose (total radioactivity measured in plasma or organ divided by the radioactivity of the ingested dose) in $n = 4$ pups that were killed at one sampling time. The sample size of $n = 4$ was sufficient to detect differences of $P < 0.05$ between treatment groups for the primary outcome, irreversible loss of retinol (9). The fractional transfer coefficients [L(I,J)s] were expressed as the mean fraction of tracer in compartment (J) transferred to compartment (I) per day ± SEM and compared statistically with the use of Student's t test, with a t-statistic evaluated for significance by using the table of critical values of t-distribution. Differences with $P < 0.05$ were considered significant. The uptake of retinol expressed as a percentage was calculated by using fractional transfer coefficients as the basal values.

Results

VA concentration in plasma, liver, and lungs

The mean total retinol concentrations on day 1 of the study were significantly higher in the liver in the VA-supplemented group compared with both the control and VARA-supplemented groups ($P < 0.001$ for both) and in the VARA-supplemented group compared with the control group only ($P < 0.05$) (**Figure 1**). During the study, total retinol concentrations in plasma were significantly higher in both of the supplemented groups than in the control group at 1 and 4 h after dosing (VA group compared with the control group: $P < 0.001$ at 1 h and $P < 0.01$ at 4 h; VARA group compared with the control group: $P < 0.001$ at 1 h and $P < 0.05$ at 4 h) (**Figure 2**). Total retinol concentrations in the liver were significantly higher in the VA-supplemented group compared with both other groups at all

times from 4 h to 14 d, except at days 1 and 2 when they were significantly higher compared with the control group only (VA group compared with the control group: $P < 0.001$ from 4 h to 4 d; $P < 0.01$ from 8 to 14 d; VA group compared with the VARA group: $P < 0.001$ at 4 and 15 h and 4 d; $P < 0.01$ for other times) (**Figure 3**A). In the VARA-supplemented group, liver retinol concentrations were higher than in the control group at 1 and 2 d after dosing ($P < 0.001$ at 1 d and $P < 0.01$ at 2 d).

In the lungs, total retinol concentrations were higher in the VARA-supplemented group than in both other groups at 8 and 24 h after dosing ($P < 0.001$ for all) and higher than in the control group at 4 and 15 h ($P < 0.001$ for both) (Figure 3B). In the VA–supplemented neonates, lung retinol concentrations were higher than in both other groups during the last 6 d of the study ($P < 0.05$ at 8 and 11 d; $P < 0.001$ at 14 d) and higher than in controls
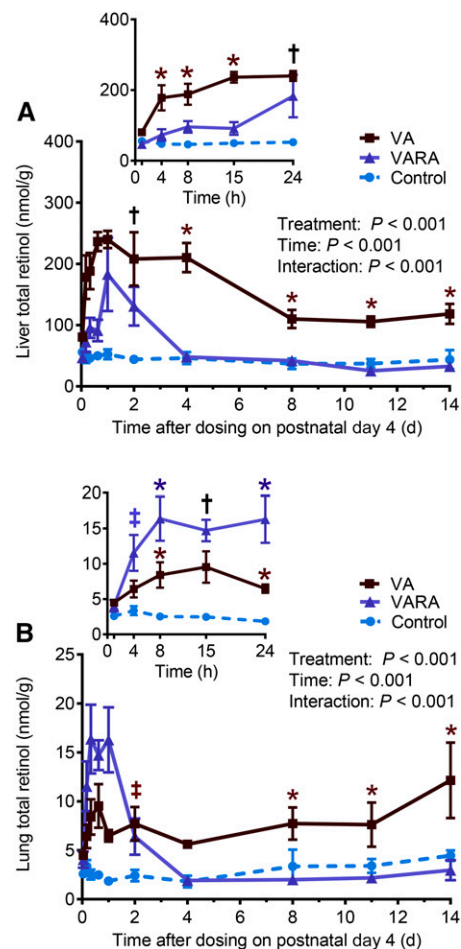
FIGURE 3 Liver (A) and lung (B) total (unesterified + esterified) retinol concentrations in control and VA- and VARA-supplemented neonatal rat pups from 0 to 14 d after dosing on postnatal day 4. Insets show the first 24 h after dosing. Each point represents the mean ± SEM of $n = 7$ (control), $n = 4$ (VA), or $n = 3$ (VARA) rat pups. *VA or VARA group compared with the other groups; [†]supplemented groups compared with control; [‡]VA or VARA group compared with control [all $P < 0.05$ (ANOVA)]. VA, vitamin A; VARA, VA combined with retinoic acid.

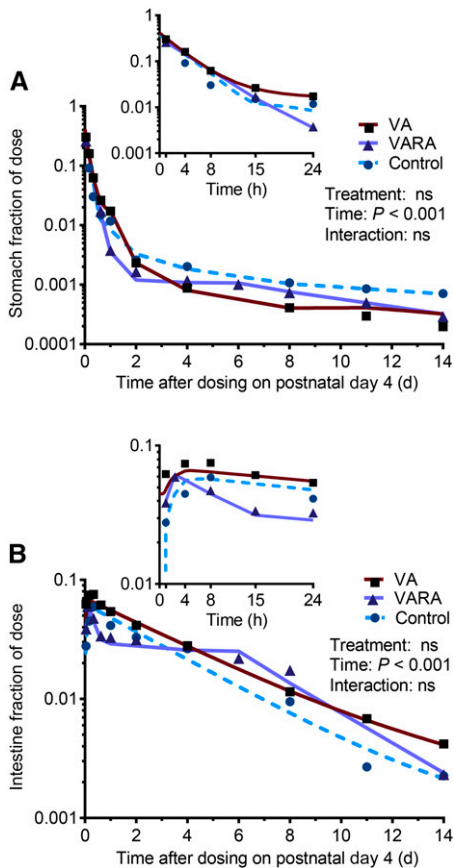**FIGURE 4** Stomach (A) and intestine (B) fractions of ingested [³H] retinol dose in control and VA- and VARA-supplemented neonatal rat pups from 0 to 14 d after dosing on postnatal day 4. Insets show the first 24 h after dosing. Each symbol represents the mean of $n = 7$ (control), $n = 4$ (VA), or $n = 3$ (VARA) rat pups. There were no significant differences between groups. VA, vitamin A; VARA, VA combined with retinoic acid.

between 8 h and 2 d after dosing ($P < 0.05$ at 1 d; $P < 0.01$ for other times).

Tracer response in digestive organs

There were no significant differences in tracer response observed in digestive organs between the 3 groups (**Figure 4**). The tracer appeared to leave the intestine more rapidly in the VARA group between 4 and 15 h after dosing, but the difference was not significant (Figure 4B).

Tracer response in plasma, liver, and extrahepatic organs

The tracer concentrations in plasma were significantly higher in the VA-supplemented group than in both other groups at 1 h after dosing (VA group compared with the control group: $P < 0.01$; VA group compared with the VARA group: $P < 0.05$) (**Figure 5**). Between 4 and 24 h after dosing, both supplemented groups showed a significantly lower tracer concentration than the control group ($P < 0.001$ from 4 to 15 h; $P < 0.05$ at 24 h).

The tracer concentrations in the liver were significantly higher in the VA-supplemented group compared with the control group

at 4 and 8 h after dosing ($P < 0.001$ for both) and compared with both other groups at 15 h ($P < 0.001$) and during the last 6 d of the study (VA group compared with the control group: $P < 0.001$ for all; VA group compared with the VARA group: $P < 0.01$ at 8 d and $P < 0.001$ for other times) (**Figure 6**A). In the lungs, tracer concentrations were significantly higher in the VARA group compared with both other groups at 4 and 8 h after dosing ($P < 0.001$ at 4 h and $P < 0.05$ at 8 h) and in the VA group compared with both other groups during the last 6 d of the study (VA group compared with the control group: $P < 0.01$ at 8 d, $P < 0.05$ at 11 d, and $P < 0.001$ at 14 d; VA group compared with the VARA group: $P < 0.05$ at 14 d and $P < 0.01$ for other times) (Figure 6B). Tracer concentrations in the kidneys were significantly lower in both supplemented groups compared with the control group between 8 and 24 h after dosing (VA group compared with the control group: $P < 0.01$ at 15 h and $P < 0.001$ for other times; VARA group compared with the control group: $P < 0.001$ for all) (Figure 6C). In the carcass, there were no significant differences between groups (**Figure 7**). The tracer appeared to peak higher in the VARA group at 4 h after dosing, but the difference was not significant.

Plasma kinetic parameters

The fractional uptake of chylomicron retinyl esters (CM-REs) and retinol-binding protein–bound retinol (RBP-ROH) from plasma to organs and the irreversible loss of retinol from organs were not different in pups that received either the VARA or VA dose (**Table 1**). However, VARA supplementation resulted in a significantly greater fractional release of RBP-ROH from the liver to plasma ($P < 0.001$) and a significantly lower recycling of retinol-binding protein (RBP)–derived retinol from organs back to plasma ($P < 0.05$). The VARA-supplemented group also showed a longer transit time of retinol in plasma, a higher fractional catabolic rate of plasma retinol, and

FIGURE 5 Plasma fraction of ingested [³H]retinol dose in control and VA- and VARA-supplemented neonatal rat pups from 0 to 14 d after dosing on postnatal day 4. The inset shows the first 24 h after dosing. Each symbol represents the mean of $n = 7$ (control), $n = 4$ (VA), or $n = 3$ (VARA) rat pups. *VA group compared with the other groups; †supplemented groups compared with control [all $P < 0.05$ (ANOVA)]. VA, vitamin A; VARA, VA combined with retinoic acid.

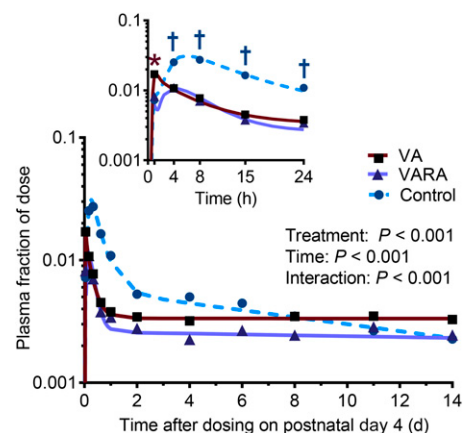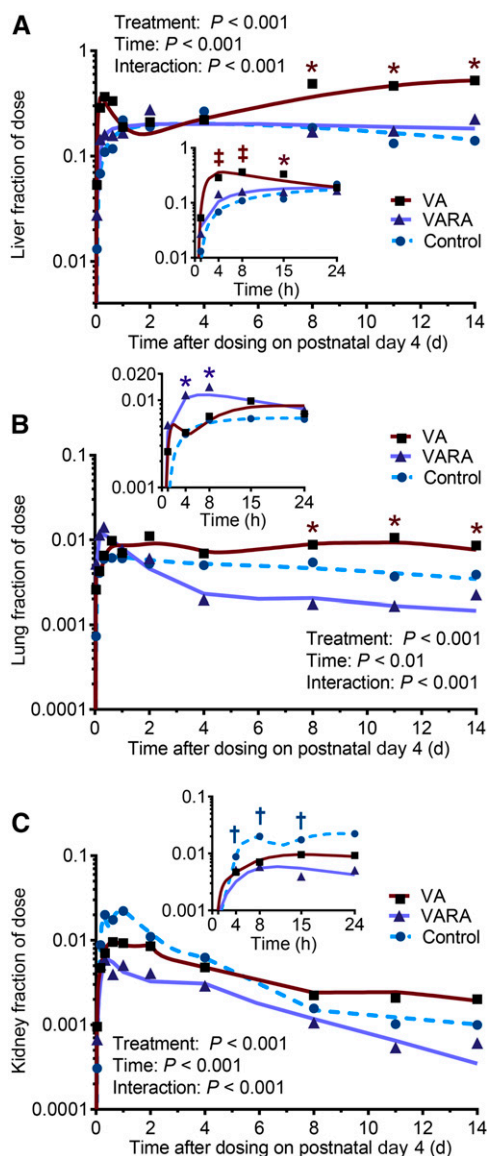**FIGURE 6**   Liver (A), lung (B), and kidney (C) fractions of ingested [^{3}H]retinol dose in control and VA- and VARA-supplemented neonatal rat pups from 0 to 14 d after dosing on postnatal day 4. Insets show the first 24 h after dosing. Each symbol represents the mean of $n = 7$ (control), $n = 4$ (VA), or $n = 3$ (VARA) rat pups. *VA or VARA group compared with other groups; †supplemented groups compared with control; ‡VA or VARA group compared with control [all $P < 0.05$ (ANOVA)]. VA, vitamin A; VARA, VA combined with retinoic acid.

therefore a lower recycling number of retinol between plasma and organs than did the VA-supplemented group (**Table 2**).

Organ kinetic parameters

The fractional uptake of CM-REs into the intestines, lungs, and carcass was significantly higher in the VARA group compared with the VA group ($P < 0.001$ for all), whereas the uptake into the liver and kidneys was higher in the VA group compared with the VARA group ($P < 0.001$ for both) (**Table 3**). The fractional uptake of RBP-ROH

into the intestines and liver was significantly higher in the VARA group compared with the VA group ($P < 0.001$ for both), whereas the uptake into the lungs, kidneys, and carcass was higher in the VA group compared with the VARA group ($P < 0.001$ for all).

The fractional turnover of both the chylomicron- and RBP-derived retinol was significantly higher in the intestines in the VARA group compared with the VA group ($P < 0.001$ for both) (**Table 4**). In the liver, the turnover of chylomicron-derived retinol was higher in the VA group ($P < 0.001$), whereas the turnover of RBP-derived retinol was higher in the VARA group ($P < 0.001$). In all other organs, except for the stomach, the turnover of both the chylomicron- and RBP-derived retinol was significantly higher in the VA group compared with the VARA group ($P < 0.001$ for all).

Percentage of uptake of retinol from plasma to organs

The percentage of uptake of CM-REs from plasma was highest in the liver in the VA group and in the carcass in the VARA group (**Table 5**). The opposite was found for RBP-ROH. The percentage of uptake of RBP-ROH was highest in the carcass in the VA group and in the liver in the VARA group.

Discussion

Previous animal studies reported that oral supplementation of neonatal rats with VA and a small proportion of retinoic acid (VARA) resulted in a greater uptake of retinol into extrahepatic tissues (10) and higher retinol concentrations in plasma, liver, and lungs of the supplemented group than in the control group (17). However, the primary form of supplementation used to reduce VA deficiency in low-income countries is pure VA (retinyl palmitate) (5). To determine

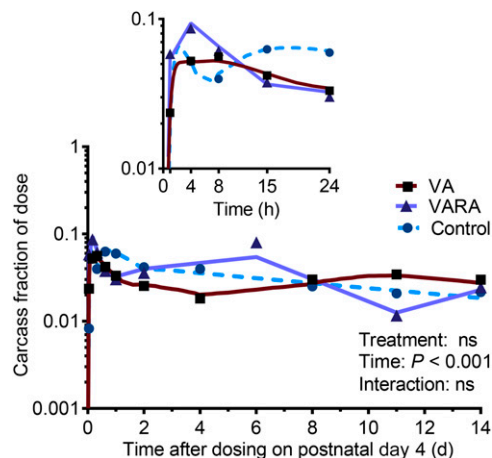**FIGURE 7**   Carcass fraction of ingested [^{3}H]retinol dose in control and VA- and VARA-supplemented neonatal rat pups from 0 to 14 d after dosing on postnatal day 4. The inset shows the first 24 h after dosing. Each symbol represents the mean of $n = 7$ (control), $n = 4$ (VA), or $n = 3$ (VARA) rat pups. There were no significant differences between groups. VA, vitamin A; VARA, VA combined with retinoic acid.

TABLE 1 Plasma fractional transfer coefficients in control and VA- and VARA-supplemented neonatal rat pups dosed orally with [³H]retinol on postnatal day 4[1]

Variable	Fractional transfer coefficients [L(I,J)]		
	Control group	VA group	VARA group
Transit of retinol through digestive system	26.9 ± 2.0[a]	31.9 ± 13.4[a,b]	11.5 ± 1.2[b]
Uptake of CM-REs from plasma to organs	567 ± 95.2	508 ± 135	647 ± 105
Release of RBP-ROH from liver to plasma	2.5 ± 0.1[a]	4.0 ± 0.2[b]	5.5 ± 0.1[c]
Uptake of RBP-ROH from plasma to organs	29.8 ± 0.8[a]	245 ± 184[a,b]	169.4 ± 3.2[b]
Recycling of RBP-ROH from organs to plasma	0.3 ± 0.0[a]	1.2 ± 0.1[b]	0.8 ± 0.0[c]
Irreversible loss of retinol from organs	0.06 ± 0.00[a]	0.01 ± 0.01[b]	0.01 ± 0.00[b]

[1]Values are means ± SEMs. Means within a row without a common superscript letter differ, $P < 0.05$ (ANOVA). CM-RE, chylomicron retinyl ester; RBP-ROH, retinol-binding protein–bound retinol; VA, vitamin A; VARA, vitamin A combined with retinoic acid.

whether the supplementation with VARA offers additional benefits in terms of improved uptake and retention of VA in tissues, we compared the effects of these 2 treatments on retinol concentrations and kinetics in organs of neonatal rats raised under VA-marginal conditions to simulate the low VA status common in areas where supplementation is practiced.

Our results showed that the recycling number of retinol, or the number of times an average molecule of retinol moves between plasma and organs before irreversible disposal, was lower in the VARA group than in the VA group due to the lower turnover of retinol in most organs in this group. We also found that the fractional uptake of CM-REs into the lungs, intestines, and carcass was significantly higher in the VARA-supplemented pups, whereas the uptake into the liver and kidneys was higher in the VA-treated pups, as were their liver retinol concentrations throughout the study. Finally, there was a reciprocal relation between the uptake of CM-REs and RBP-ROH in both the VARA- and VA-supplemented pups: a significantly higher fractional uptake of CM-REs in the VARA group compared with the VA group coincided with a significantly lower fractional uptake of RBP-ROH, and vice versa. This relation was present in all organs except for the intestines and kidneys.

Our finding of the lower organ turnover of retinol after supplementation with VARA than with VA may be attributed to the synergistic effect of VA and retinoic acid on the activity of lecithin-retinol acyltransferase (LRAT), an enzyme responsible for the esterification of retinol for long-term storage. This phenomenon was previously shown in the liver, lungs, and kidneys in both neonatal

and adult rats (17–20). However, given the presence of LRAT in other organs, including intestine, skin, and adipose tissue (18, 19), it is likely that this effect occurs throughout the body. The simultaneous induction of LRAT by VARA in multiple organs may lead to greater retention and lower recycling of retinol back to plasma, as observed in our study.

Our second finding, that is, the more potent effect of VARA than of VA on the uptake of CM-REs into the intestines, lungs, and carcass but not into the liver, may be explained by a relatively greater sensitivity of LRAT in these organs. This explanation is consistent with a previous finding that the expression of LRAT after oral treatment with retinoic acid was 6- to 9-fold higher in the lungs and only 2-fold higher in the liver (20). It is also consistent with a previous kinetic study, which found that the residence time of retinol was 75-fold longer in the lungs of rats supplemented with retinoic acid than in controls, compared with 14-fold longer in the liver (21). The effect of VARA in the lungs, intestines, and carcass may be further enhanced by the presence of the stimulated-by-retinoic acid 6 receptor (STRA6), a cell membrane transporter for RBP-ROH that delivers retinol to LRAT (22, 23). STRA6 is highly expressed in all 3 of these organs, especially during development (17, 24, 25), but it is absent in the liver (26), although an alternate form, RBP4 receptor 2 (27), might have a similar function. Furthermore, the expression of STRA6 in neonatal lungs has been previously shown to increase in response to oral VARA supplementation (17, 24).

The stronger effect of VARA than of VA on the uptake of CM-REs into the lungs compared with the liver may also result

TABLE 2 Plasma kinetic parameters in control and VA- and VARA-supplemented neonatal rat pups dosed orally with [³H]retinol on postnatal day 4[1]

Variable	Description	Control group	VA group	VARA group
Plasma residence time	Average time a molecule of retinol spends in plasma over multiple transits	4.8 h	28.3 h	14.4 h
Plasma transit time	Average time a molecule of retinol spends in plasma during a single transit	48.6 min	6.0 min	8.4 min
Organ transit time	Average time a molecule of retinol spends in organs during a single transit	2.6 d	20 h	28 h
Fractional catabolic rate	Fraction of plasma retinol utilized irreversibly per day	5.0	0.8	1.7
Recycling number	Average number of times a molecule of retinol recycles through plasma before irreversible disposal	5	288	100

[1]VA, vitamin A; VARA, vitamin A combined with retinoic acid.

TABLE 3 Fractional uptakes of CM-REs and RBP-ROH from plasma to organs in control and VA- and VARA-supplemented neonatal rat pups dosed orally with [^{3}H]retinol on postnatal day 4[1]

	Fractional transfer coefficients [L(I,J)], fraction of plasma retinol transferred/d					
	Uptake of CM-REs			Uptake of RBP-ROH		
Organ	Control group	VA group	VARA group	Control group	VA group	VARA group
Stomach[2]	—	—	—	2.0 ± 0.9	10.0 ± 5.5	0.6 ± 0.3
Intestines	39.3 ± 8.4[a]	19.1 ± 3.1[b]	327 ± 15.5[c]	0.01 ± 0.01[a]	0.04 ± 0.01[b]	9.8 ± 0.3[c]
Liver	77.3 ± 3.4[a]	304 ± 15.6[b]	223 ± 3.7[c]	6.7 ± 1.0[a]	17.0 ± 0.5[b]	28.1 ± 1.0[c]
Lungs	5.1 ± 0.2[a]	15.0 ± 1.1[b]	32.6 ± 0.3[c]	0.2 ± 0.1[a]	4.6 ± 0.4[b]	0.1 ± 0.0[a]
Kidneys	34.8 ± 1.6[a]	4.9 ± 0.4[b]	1.4 ± 0.1[c]	3.2 ± 0.1[a]	6.8 ± 0.5[b]	3.0 ± 0.1[a]
Carcass	271.6 ± 12.7[a]	176 ± 11.7[b]	438 ± 6.3[c]	7.7 ± 0.1[a]	196 ± 6.5[b]	7.2 ± 0.1[c]

[1] Values are means ± SEMs. Means within a row without a common superscript letter differ, $P < 0.05$ (ANOVA). CM-RE, chylomicron retinyl ester; RBP-ROH, retinol-binding protein–bound retinol; VA, vitamin A; VARA, vitamin A combined with retinoic acid.
[2] The stomach model did not include a CM-RE uptake component.

from their differential effect on the activity of cytochrome P450 (CYP)26A1 and CYP26B1 in these organs. Both CYP26A1 and CYP26B1 oxidize retinoic acid irreversibly, converting it to its inactive metabolites (28). However, the CYP26A1 variant is most prominently expressed in the liver where it is highly responsive to retinoic acid. In fact, its expression shows the largest fold increase due to the treatment with retinoic acid compared with any other VA–metabolizing enzyme (28, 29). In contrast, the CYP26B1 variant is more highly expressed in the lungs and is somewhat less sensitive to retinoic acid (24). Therefore, it is possible that a large dose of VA combined with retinoic acid induces its own catabolism in the liver to a greater extent than it does in the lungs, resulting in a lower accumulation of hepatic retinol. This hypothesis agrees with our finding of a lower retinol concentration and a higher turnover of RBP-ROH in the liver in the VARA group.

As for the higher uptake and concentration of retinol in the kidneys of the VA-supplemented pups, we speculate that this may be a result of the frequent recycling of RBP-ROH observed in this group. Although the liver is the main site of RBP synthesis, ~50% of the circulating retinol is sequestered in the kidneys, filtered into urine, reabsorbed, and released back into the circulation after binding to newly synthesized RBP in the cells of the proximal convoluted tubules (30). Moreover, kidneys have a considerable capacity for storing retinyl esters (19) despite their

lower expression of LRAT compared with the lungs or liver (18). This may explain the high turnover of RBP-derived retinol observed in the kidneys of the VA-supplemented pups.

Finally, our finding of the reciprocal relation between the uptake of CM-REs and RBP-ROH may reflect a highly regulated, organ-specific activity of LRAT, which ensures that a relatively high uptake of CM-REs is followed by a lower uptake of RBP-ROH, and vice versa. The reason why this relation was absent from the intestines and kidneys could be the constitutive high expression of LRAT in the intestines (18, 31, 32) and its narrow range of expression in the kidneys (17).

The design of our study has both strengths and limitations. As strengths, the comparison of the 2 studies, conducted under similar conditions with regard to the age of the neonate pups, the maternal diet, dosing, and sample preparation, allows inference about the effect of retinoic acid administration, as a surrogate for higher VA status, on the trafficking, tissue distribution, and metabolism of retinol. The results suggest that postprandial retinol metabolism is indeed influenced and suggest greater involvement of the intestines, lungs, and carcass in the removal of plasma retinol due to the presence of retinoic acid (VARA compared with VA alone). A limitation is that the 2 studies were not conducted concurrently, although to the best of our abilities, important procedural protocols were the same for both studies. Nonetheless, future research should test retinoic acid treatment (or higher

TABLE 4 Fractional turnover of chylomicron- and RBP-derived retinol in organs of control and VA- and VARA-supplemented neonatal rat pups dosed orally with [^{3}H]retinol on postnatal day 4[1]

	Fractional transfer coefficients [L(I,J)], fraction of organ retinol transferred/d					
	Turnover of chylomicron-derived retinol[2]			Turnover of RBP-derived retinol[2]		
Organ	Control group	VA group	VARA group	Control group	VA group	VARA group
Stomach	5.8 ± 0.2[a]	6.2 ± 0.5[a,b]	5.0 ± 0.1[b]	6.4 ± 3.0	79.5 ± 51.5	0.8 ± 0.3
Intestines	0.3 ± 0.0[a]	0.2 ± 0.0[a]	7.9 ± 0.3[b]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.7 ± 0.0[b]
Liver	0.1 ± 0.3[a]	1.4 ± 0.1[b]	0.2 ± 0.0[a]	0.2 ± 0.1[a]	0.1 ± 0.0[a]	0.4 ± 0.0[b]
Lungs	0.8 ± 0.5[a]	37.4 ± 4.6[b]	0.7 ± 0.0[a]	0.2 ± 0.1[a]	2.0 ± 0.2[b]	0.1 ± 0.0[a]
Kidneys	4.1 ± 0.6[a]	21.2 ± 3.6[b]	0.1 ± 0.0[c]	6.1 ± 1.1[a]	9.4 ± 0.7[b]	1.8 ± 0.1[c]
Carcass	26.2 ± 2.1[a]	88.7 ± 10.8[b]	5.8 ± 0.1[c]	1.1 ± 0.0[a]	2.8 ± 0.1[b]	0.3 ± 0.0[c]

[1] Values are means ± SEMs. Means within a row without a common superscript letter differ, $P < 0.05$ (ANOVA). RBP, retinol-binding protein; VA, vitamin A; VARA, vitamin A combined with retinoic acid.
[2] Turnover included both retinol released back to plasma and retinol metabolized irreversibly in an organ, due to an assumption inherent in uncoupling the organs from the rest of the system to model them individually.

TABLE 5 Percentage of uptake of CM-REs and RBP-ROH from plasma to organs of control and VA- and VARA-supplemented neonatal rat pups dosed orally with [³H]retinol on postnatal day 4[1]

	CM-RE uptake, %			RBP-ROH uptake, %		
Organ	Control group	VA group	VARA group	Control group	VA group	VARA group
Stomach[2]	—	—	—	10.1	4.3	1.2
Intestines	9.2	3.7	32.0	0.1	0.0	20.1
Liver	18.1	58.6	21.8	33.8	7.3	57.6
Lungs	1.2	2.9	3.2	1.0	2.0	0.2
Kidneys	8.1	0.9	0.1	16.2	2.9	6.1
Carcass	63.4	33.9	42.9	38.9	83.6	14.8

[1] CM-RE, chylomicron retinyl ester; RBP-ROH, retinol-binding protein–bound retinol; VA, vitamin A; VARA, vitamin A combined with retinoic acid.
[2] The stomach model did not include a CM-RE uptake component.

VA status) in neonates side-by-side with the absence of retinoic acid treatment (lower VA status) to confirm the results of the present study and to further explore mechanisms by with retinoic acid has the effects that are predicted from the current analysis.

In conclusion, VARA supplementation administered to neonatal rat pups raised under VA-marginal conditions decreased retinol turnover in the lungs, kidneys, and carcass and attenuated the frequent recycling of retinol observed in VA-treated neonates. Although VA supplementation resulted in a higher concentration of retinol in the liver, VARA supplementation increased the uptake of CM-REs into the intestines, lungs, and carcass to a greater extent than did VA. Given the relatively higher uptake of postprandial REs into several extrahepatic organs of neonates dosed with VARA, this form of supplementation may serve as a targeted treatment of low VA concentrations in nonhepatic organs that continue to develop postnatally, such as lungs and intestines.

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