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Vitamin A and retinoic acid combined have a more potent effect compared to vitamin A alone on the uptake of retinol into extrahepatic tissues of neonatal rats raised under vitamin A-marginal conditions¹

Joanna K. Hodges², Libo Tan³, Michael H. Green, and A. Catharine Ross^{*}

Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, 16802 USA

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 vs. 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 two prior kinetic studies of Sprague-Dawley neonatal rats nursed by mothers fed a VA-marginal diet (0.35 mg retinol equivalents/kg diet). On postnatal d 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 containing ~2 µCi of [³H]retinol as the tracer for VA. Total retinol concentrations and tracer levels had been measured in plasma and tissues from 1 h to 14 d after dosing. Control group data from both studies were merged prior to 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 vs. 288 times in VARA- vs. VA-treated group). Although the 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.

Conclusions—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 levels in the extrahepatic organs that continue to develop postnatally.

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^{*}Corresponding author: Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA 16802, Tel. +1 814-865-4721, Fax +1 814-865-4723, acr6@psu.edu.

²Present address: Department of Nutrition Science, Purdue University, G38 Stone Hall, West Lafayette, IN 47907

³Present address: Department of Human Nutrition and Hospitality Management, University of Alabama, 407 Russell Hall, Tuscaloosa, AL 35487

Keywords

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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 based on 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 the neonatal tissues (7).

Although knowledge about the neonatal VA metabolism is limited, prior studies of neonatal rats showed that VA retention, especially in the extrahepatic tissues such as skin, bones, and muscle, is low compared to 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 prior to catabolism (541 vs. 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), designed to create a “metabolic priming” of VA metabolism, increased the uptake of retinol into extrahepatic tissues and reduced its recycling in the supplemented compared to control neonates (10). However, these two supplements, VA and VARA, were tested in two separate studies of the supplemented vs. control group; 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 study (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, VARA or placebo in the amount equivalent to that which has been given to human infants (13). Our results build upon 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 two different supplement forms on retinol tissue uptake and distribution.

METHODS

Study design

Retinol concentration and kinetic data were obtained from two similar studies conducted in 2011 and 2013 and 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 randomized to either the VA-supplemented ($n = 52$; 33 males) or control group ($n = 52$; 30 males) in the VA study (7, 11), and the VARA-supplemented ($n = 42$; 19 males) or control

group ($n = 42$; 24 males) in the VARA study (12). In both studies, on postnatal d 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 $\sim 2 \mu\text{Ci}$ of [^3H]retinol as the tracer for VA. Plasma and organs were collected from 0.5 h to 24 d (VA study) or 14 d (VARA study) after dosing from pups sedated with isoflurane or, after postnatal d 14, asphyxiated with CO_2 . All animal procedures were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Retinol mass and tracer level were measured using ultra-performance liquid chromatography (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 using Windows version of Simulation, Analysis, and Modeling software version 3.0.8 (16).

The VA and VARA experiments differed with respect to: (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 animals/sampling time/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); (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 data prior to 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 based on the error propagation formula $SEM_{1+\dots+n} = \sqrt{SEM_1^2 + \dots + SEM_n^2}$.

Second, data from control groups in both studies (retinol concentration and tracer level measured on 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 d 18 and 24 and the resulting kinetic parameters were re-estimated based on the data observed from d 1 to 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 standard deviation was < 0.5 .

Statistical analysis

The mean total retinol (REs + unesterified retinol) concentration and tracer level vs. time were plotted and the values for control, VA-, and VARA-supplemented neonates were compared using a two-way 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 \pm SEM or the mean fraction of dose (total radioactivity

measured in plasma/organ divided by the radioactivity of the ingested dose) of $n = 4$ pups euthanized at one sampling time, which 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 d \pm SEM and compared statistically with the use of Student's t -test, with a t -statistic evaluated for significance using the table of critical values of t -distribution. Differences with $P < 0.05$ were considered significant. The percent uptake of retinol into organs was calculated using fractional transfer coefficients as the basal values.

RESULTS

Vitamin A concentration in plasma, liver, and lungs

The mean total retinol concentration on d 1 of the study was significantly higher in the liver in the VA-supplemented group compared to both the control and VARA-supplemented group ($P < 0.001$ for both), and in the VARA-supplemented group compared to control group only ($P < 0.05$) (Figure 1).

During the study, total retinol concentration in plasma was significantly higher in both supplemented groups compared to control group at 1 and 4 h after dosing (VA vs. control: $P < 0.001$ at 1 h and $P < 0.01$ at 4 h; VARA vs. control: $P < 0.001$ at 1 h and $P < 0.05$ at 4 h) (Figure 2).

Total retinol concentration in the liver was significantly higher in the VA-supplemented group compared to both other groups at all times from 4 h to 14 d, except at d 1 and 2 when it was significantly higher from the control group only (VA vs. control: $P < 0.001$ from 4 h to 4 d; $P < 0.01$ from 8 to 14 d; VA vs. VARA: $P < 0.001$ at 4 and 15 h, and 4 d; $P < 0.01$ for other) (Figure 3A). In the VARA-supplemented group, liver retinol concentration was higher compared to 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 concentration was higher in the VARA-supplemented group compared to both other groups at 8 and 24 h after dosing ($P < 0.001$ for all), and compared only to control group at 4 and 15 h ($P < 0.001$ for both) (Figure 3B). In the VA-supplemented neonates, lung retinol concentration was higher compared to both other groups during the last 6 days of the study ($P < 0.05$ at 8 and 11 d; $P < 0.001$ at 14 d) and compared to controls only between 8 h and 2 d after dosing ($P < 0.05$ at 1 d; $P < 0.01$ for other).

Tracer response in digestive organs

There were no significant differences in tracer response observed in digestive organs between the three 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 statistically significant (Figure 4B).

Tracer response in plasma, liver, and extrahepatic organs

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

The tracer level in the liver was significantly higher in the VA-supplemented group compared to the control group at 4 and 8 h after dosing ($P < 0.001$ for both), and compared to both other groups at 15 h ($P < 0.001$) and during the last 6 days of the study (VA vs. control: $P < 0.001$ for all; VA vs. VARA: $P < 0.01$ at 8 d and $P < 0.001$ for other) (Figure 6A). In the lungs, tracer level was significantly higher in the VARA group compared to 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 to both other groups during the last 6 days of the study (VA vs. control: $P < 0.01$ at 8 d, $P < 0.05$ at 11 d, and $P < 0.001$ at 14 d; VA vs. VARA: $P < 0.05$ at 14 d and $P < 0.01$ for other) (Figure 6B). Tracer level in the kidneys was significantly lower in both supplemented groups compared to the control group between 8 and 24 h after dosing (VA vs. control: $P < 0.01$ at 15 h and $P < 0.001$ for other; VARA vs. control: $P < 0.001$ 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 statistically significant.

Plasma kinetic parameters

The fractional uptake of CM-REs and 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 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, therefore, a lower recycling number of retinol between plasma and organs compared to 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 vs. VA group ($P < 0.001$ for all), whereas the uptake into the liver and kidneys was higher in the VA vs. 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 vs. VA group ($P < 0.001$ for both), whereas the uptake into the lungs, kidneys, and carcass was higher in the VA vs. VARA group ($P < 0.001$ for all).

The fractional turnover of both the CM- and RBP-derived retinol was significantly higher in the intestines in the VARA vs. VA group ($P < 0.001$ for both) (Table 4). In the liver, the turnover of CM-derived retinol was higher in the VA group ($P < 0.001$), while the turnover of RBP-derived retinol was higher in the VARA group ($P < 0.001$). In all other organs,

except the stomach, turnover of both the CM- and RBP-derived retinol was significantly higher in the VA vs. VARA group ($P < 0.001$ for all).

Percent uptake of retinol from plasma to organs

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

DISCUSSION

Previous animal studies have reported that an 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 a higher retinol concentration in plasma, liver, and lungs of the supplemented vs. control group (17). However, the primary form of supplementation used to reduce VA deficiency in low-income countries is pure VA (retinyl palmitate) (5). In order to determine 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 two treatments on retinol concentration 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 demonstrated 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 compared to 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 ones, as was their liver retinol concentration throughout the study. Finally, there was a reciprocal relationship 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 vs. VA group coincided with a significantly lower fractional uptake of RBP-ROH, and vice versa. This relationship was present in all organs except the intestines and kidneys.

Our finding of the lower organ turnover of retinol after supplementation with VARA compared to 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 demonstrated in the liver, lungs, and kidneys in both the 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 a greater retention and lower recycling of retinol back to plasma, as observed in our study.

Our second finding – the more potent effect of VARA vs. 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 prior kinetic study, which found that the residence time of retinol was 75-fold longer in the lungs of rats supplemented with retinoic acid vs. controls, compared to 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, which delivers retinol to LRAT (22, 23). STRA6 is highly expressed in all three 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 the neonatal lungs has been previously shown to increase in response to oral VARA supplementation (17, 24).

The stronger effect of VARA vs. VA on the uptake of CM-REs into the lungs vs. liver may also result from their differential effect on the activity of CYP26A1 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 to 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 it may be a result of the frequent recycling of RBP-ROH observed in this group. Although liver is the main site of RBP synthesis, approximately 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 REs (19) despite their lower expression of LRAT compared to that in 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 relationship 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 relationship 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 two studies, conducted under similar conditions regarding the age of the neonates, the maternal diet, dosing, and sample preparation, allows inference about the effect of RA

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 RA (VARA vs. VA alone). A limitation is that the two 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 RA treatment (or higher VA status) in neonates side-by-side with the absence of RA treatment (lower VA status) to confirm the results of the present study, and further explore mechanisms by which RA has the effects that are predicted from the current analysis.

In conclusion, VARA supplementation administered to neonatal rats 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 retinyl esters into several extrahepatic organs of neonates dosed with VARA, this form of supplementation may serve as a targeted treatment of low VA levels in the non-hepatic organs that continue to develop postnatally, such as lungs and intestines.

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J.K.H. conducted research, analyzed data and wrote the manuscript; L.T. conducted research; M.H.G. assisted with kinetic analysis and interpretation; A.C.R. conducted research and has primary responsibility for the final content. All authors designed research, read and approved the final manuscript. The authors also thank Dr. J.B. Green for editorial assistance. None of the authors had a conflict of interest.

Abbreviations

CM-REs	chylomicron retinyl esters
RBP	retinol-binding protein
RBP-ROH	retinol-binding protein-bound retinol
REs	retinyl esters
VA	vitamin A
VARA	vitamin A combined with retinoic acid

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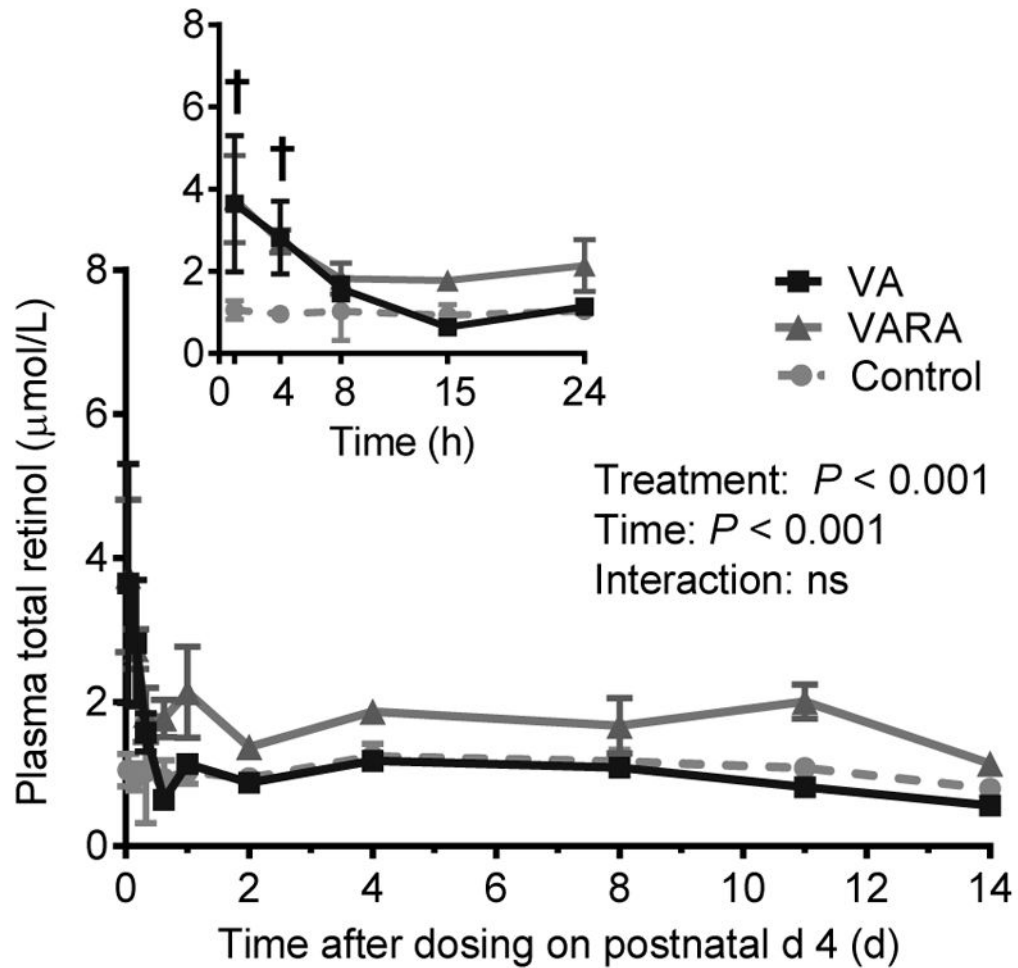


Figure 2.

Plasma total (unesterified + esterified) retinol concentration in control, VA-, and VARA-supplemented neonatal rats from 0 to 14 days after dosing on postnatal day 4. Inset shows the first 24 h after dosing. Each point represents the mean \pm SEM of $n = 7$ (control), $n = 4$ (VA) or $n = 3$ (VARA) rats. $P < 0.05$ (ANOVA); †supplemented vs. control. VA, vitamin A; VARA, VA combined with retinoic acid.

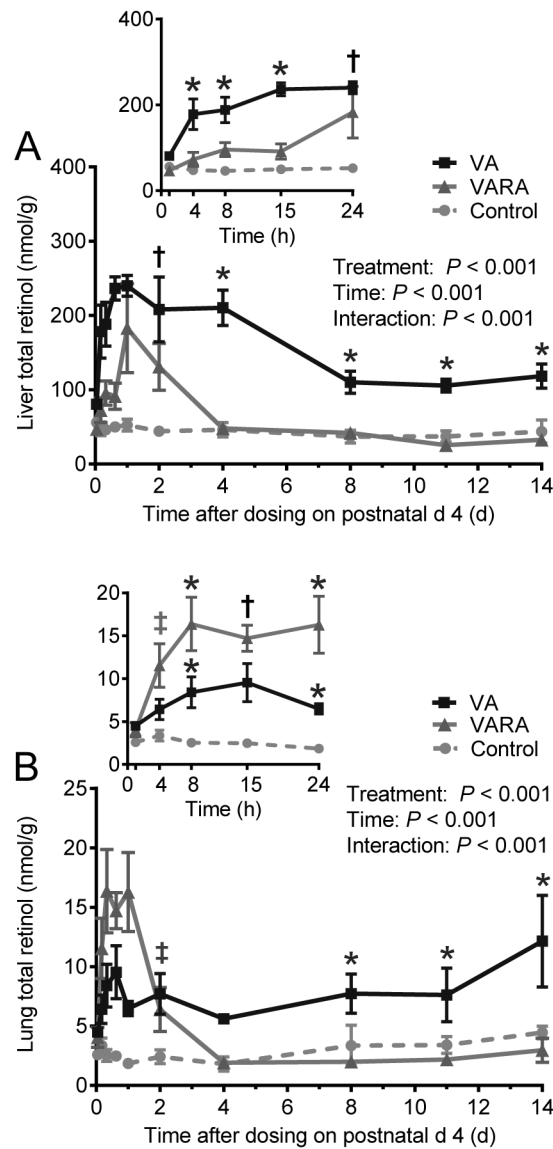


Figure 3.

Liver (A) and lung (B) total (unesterified + esterified) retinol concentration in control, VA-, and VARA-supplemented neonatal rats from 0 to 14 days after dosing on postnatal day 4. Insets show the first 24 h after dosing. Each point represents the mean \pm SEM of $n = 7$ (control), $n = 4$ (VA) or $n = 3$ (VARA) rats. $P < 0.05$ (ANOVA): *VA or VARA vs. other; †supplemented vs. control; ‡VA or VARA vs. control. VA, vitamin A; VARA, VA combined with retinoic acid.

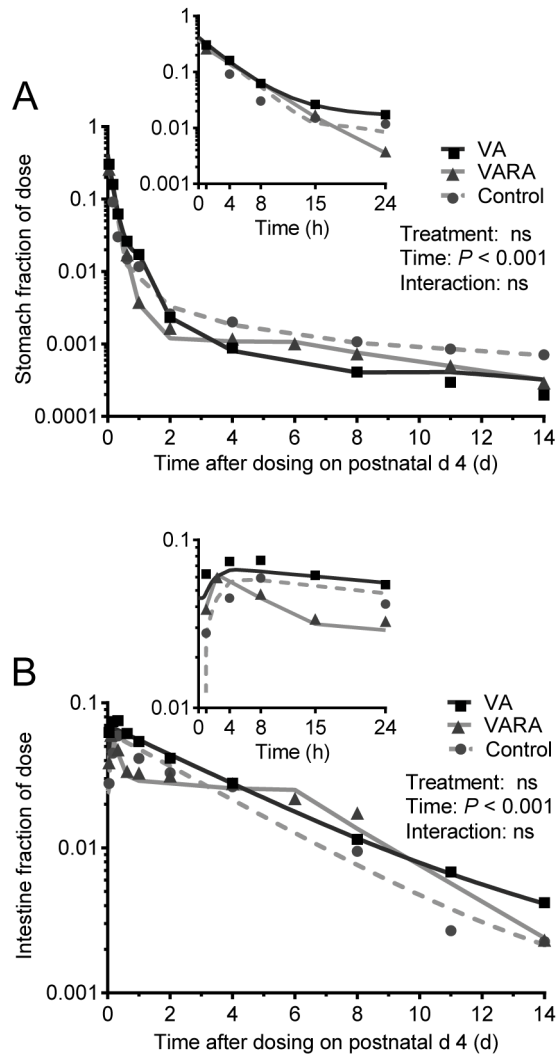


Figure 4. Stomach (A) and intestine (B) fraction of ingested [^3H]retinol dose in control, VA-, and VARA-supplemented neonatal rats from 0 to 14 days after dosing on postnatal day 4. Insets show the first 24 h after dosing. Each symbol represents the mean of $n = 7$ (control), $n = 4$ (VA) or $n = 3$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

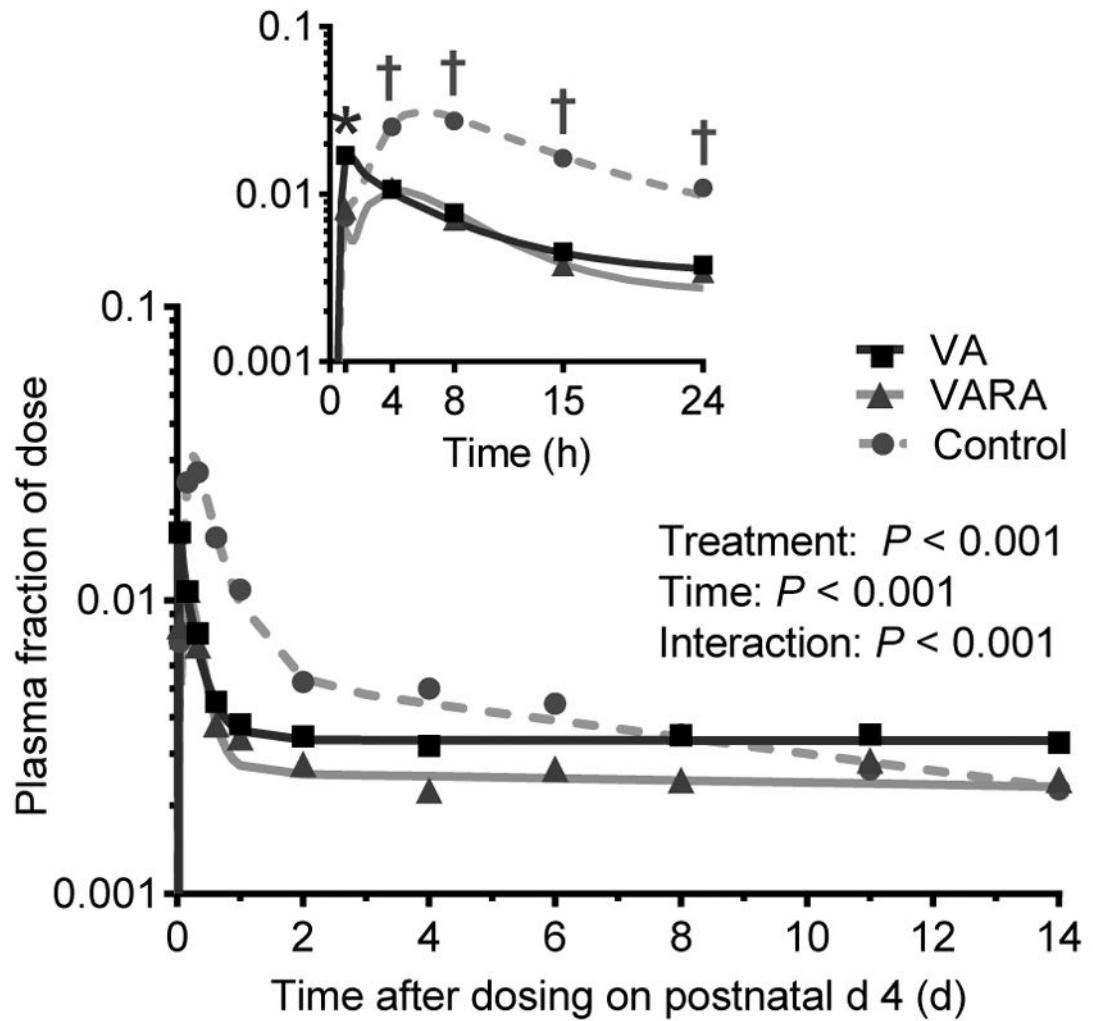


Figure 5. Plasma fraction of ingested [^3H]retinol dose in control, VA-, and VARA-supplemented neonatal rats from 0 to 14 days after dosing on postnatal day 4. Inset shows the first 24 h after dosing. Each symbol represents the mean of $n = 7$ (control), $n = 4$ (VA) or $n = 3$ (VARA) rats. $P < 0.05$ (ANOVA): *VA vs. other; †supplemented vs. control. VA, vitamin A; VARA, VA combined with retinoic acid.

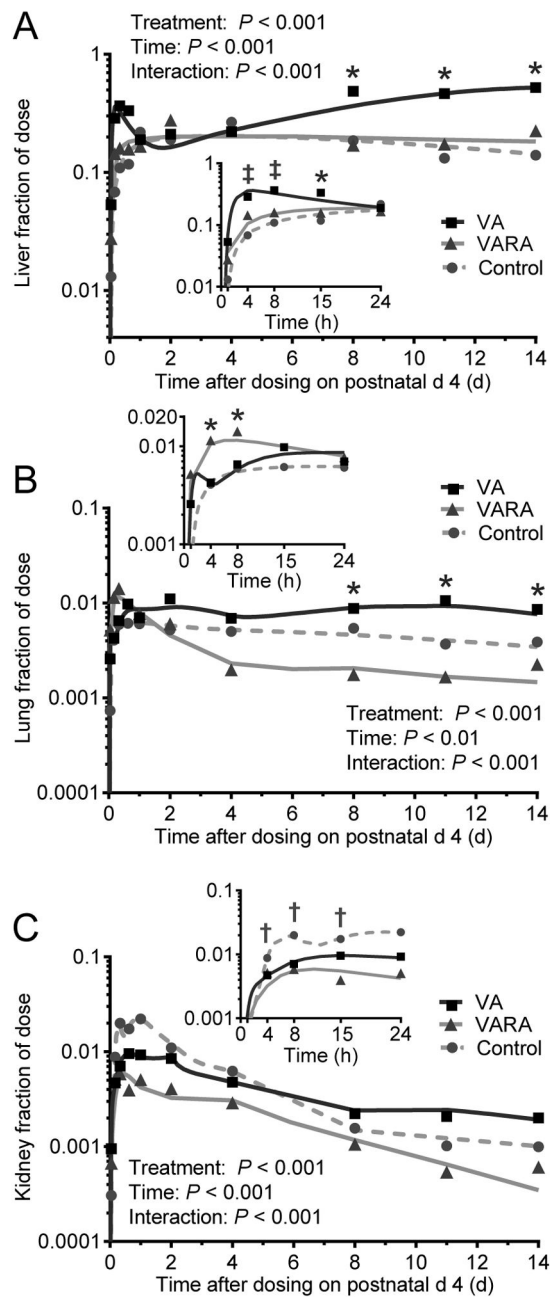


Figure 6. Liver (A), lung (B), and kidney (C) fraction of ingested [^3H]retinol dose in control, VA-, and VARA-supplemented neonatal rats from 0 to 14 days after dosing on postnatal day 4. Insets show the first 24 h after dosing. Each symbol represents the mean of $n = 7$ (control), $n = 4$ (VA) or $n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

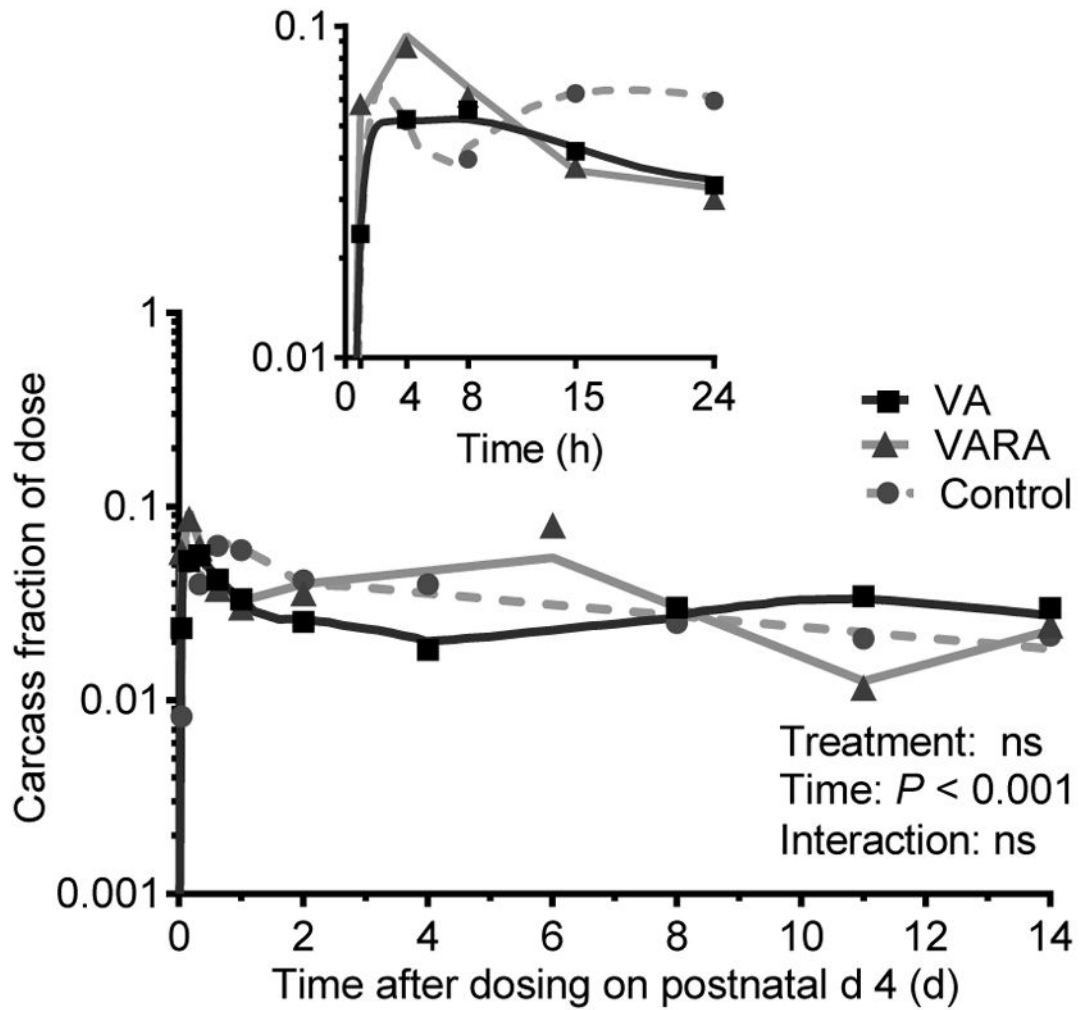


Figure 7. Carcass fraction of ingested [³H]retinol dose in control, VA-, and VARA-supplemented neonatal rats from 0 to 14 days after dosing on postnatal day 4. Inset shows the first 24 h after dosing. Each symbol represents the mean of *n* = 7 (control), *n* = 4 (VA) or *n* = 3 (VARA) rats. There were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

Table 1

Plasma fractional transfer coefficients in control, VA-, and VARA-supplemented neonatal rats dosed orally with [³H]retinol on postnatal day 4¹

Parameter	L(I,J) ± SEM		
	Control	VA	VARA
Transit of retinol through digestive system	26.9 ± 2.0 ^a	31.9 ± 13.4 ^{ab}	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 ^{ab}	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

¹CM-REs, chylomicron retinyl esters; RBP-ROH, retinol bound to retinol-binding protein; VA, vitamin A; VARA, vitamin A combined with retinoic acid.

^{a,b,c}Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$; ANOVA).

Table 2

Plasma kinetic parameters in control, VA-, and VARA-supplemented neonatal rats dosed orally with [³H]retinol on postnatal day 4¹

Parameter	Parameter description	Control	VA	VARA
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

¹VA, vitamin A.

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Fractional uptake of CM-REs and RBP-ROH from plasma to organs of control, VA-, and VARA-supplemented neonatal rats dosed orally with [³H]retinol on postnatal day 4¹

Table 3

Organ	L(I,J) ± SEM					
	Uptake of CM-REs(fraction of plasma retinol transferred/d)	VA	VARA	Control	VA	VARA
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 CM-REs, chylomicron retinyl esters; RBP-ROH, retinol bound to retinol-binding protein; VA, vitamin A; VARA, vitamin A combined with retinoic acid.

² Stomach model did not include a CM-RE uptake component.

^{a,b,c} Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$; ANOVA).

Table 4

Fractional turnover of CM- and RBP-derived retinol in organs of control, VA-, and VARA-supplemented neonatal rats dosed orally with [³H]retinol on postnatal day 4¹

Organ	L(L,D) ± SEM					
	Turnover ² of CM-derived retinol(fraction of organ retinol transferred/d)	VARA	Control	Turnover ² of RBP-derived retinol(fraction of organ retinol transferred/d)	VA	VARA
Stomach	5.8 ± 0.2 ^a	6.2 ± 0.5 ^{ab}	6.4 ± 3.0	79.5 ± 51.5	0.8 ± 0.3	0.8 ± 0.3
Intestines	0.3 ± 0.0 ^a	0.2 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.7 ± 0.0 ^b	0.7 ± 0.0 ^b
Liver	0.1 ± 0.3 ^a	1.4 ± 0.1 ^b	0.2 ± 0.1 ^a	0.1 ± 0.0 ^a	0.4 ± 0.0 ^b	0.4 ± 0.0 ^b
Lungs	0.8 ± 0.5 ^a	37.4 ± 4.6 ^b	0.2 ± 0.1 ^a	2.0 ± 0.2 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a
Kidneys	4.1 ± 0.6 ^a	21.2 ± 3.6 ^b	6.1 ± 1.1 ^a	9.4 ± 0.7 ^b	1.8 ± 0.1 ^c	1.8 ± 0.1 ^c
Carcass	26.2 ± 2.1 ^a	88.7 ± 10.8 ^b	1.1 ± 0.0 ^a	2.8 ± 0.1 ^b	0.3 ± 0.0 ^c	0.3 ± 0.0 ^c

¹ CM-REs, chylomicron retinyl esters; RBP-ROH, retinol bound to 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.

^{a,b,c} Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$; ANOVA).

Table 5

Percent uptake of CM-REs and RBP-ROH from plasma to organs of control, VA-, and VARA-supplemented neonatal rats dosed orally with [³H]retinol on postnatal day 4¹

Organ	CM-RE uptake (%)	
	Control	VA
Stomach ²	-	-
Intestines	9.2	3.7
Liver	18.1	58.6
Lungs	1.2	2.9
Kidneys	8.1	0.9
Carcass	63.4	33.9

¹CM-REs, chylomicron retinyl esters; RBP-ROH, retinol-binding protein-bound retinol; VA, vitamin A; VARA, vitamin A combined with retinoic acid.

²Stomach model did not include a CM-RE uptake component.

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