The Modified-Relative-Dose-Response Values in Serum and Milk Are Positively Correlated over Time in Lactating Sows with Adequate Vitamin A Status

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ABSTRACT The modified-relative-dose-response (MRDR) test, which requires a blood sample after dosing with 3,4-didehydroretinyl acetate (DRA), has been used to determine vitamin A (VA) status of individuals and groups worldwide. Less invasive methods using milk are in development in a swine model. Swine are a good choice for studying VA metabolism because their gastrointestinal anatomy, morphology, physiology, and VA requirements are similar to those of humans. In this study, DRA was used as a VA tracer in lactating sows to follow the metabolism of newly ingested VA. Lactating sows (n = 6) were administered 35 μmol DRA after overnight food deprivation. Blood and milk were collected at 0, 1.5, 3, 5, 7, 9, 24, and 48 h; livers were obtained at the time of killing. Samples were analyzed for didehydroretinol (DR), retinol (R), and didehydroretinyl esters (DRE). Serum DR:R was compared with that in milk and other VA indicators. DRE rapidly increased in serum, corresponding to chylomicra, whereas DR increased at a slower rate corresponding to the holo-DR:retinol-binding protein complex released from the liver. An estimated 10–20% of the dose was irreversibly lost in milk over 48 h. The mean MRDR value was 0.018 ± 0.013 at 5 h and the mean liver VA was 0.73 ± 0.21 μmol/g, both signifying sufficient stores. Milk and serum DR:R values were directly correlated (r = 0.64, P < 0.0001). Thus, DR:R values in milk may be a potential alternative to serum in determining VA status in lactating women. Future work is required in VA-deficient sows and women of varying VA status to determine DR trafficking and to compare DR:R values in milk with those in serum. J. Nutr. 136: 939–945, 2006.

KEY WORDS: vitamin A status • sows • didehydroretinol • milk fat • swine • models, animals

Vitamin A deficiency (VAD) is a major public health problem throughout the developing world. VAD is the leading cause of preventable blindness in children and a factor in increased morbidity and mortality from severe infections (1,2). Women have increased susceptibility to VAD during pregnancy and lactation (1,3,4). Evaluating the vitamin A (VA) status of individuals and populations requires sophisticated assessment methodology and adequate resources (5,6). Serum retinol (R) concentrations are commonly used, but they do not always respond to interventions (6). Developing less invasive, more responsive methods of assessing VA status in lactating women would help to determine the groups at risk of VAD. Such methods could facilitate the evaluation of the efficacy of supplementation programs targeted specifically toward lactating women.

The modified-relative-dose-response (MRDR) test has been used extensively to qualitatively assess liver VA reserves in the deficient-to-normal range in humans and animals (4,7–10). For the test, 3,4-didehydroretinyl acetate (DRA) is administered orally, hydrolyzed in the gut to 3,4-didehydroretinol (DR), and repackaged into the chylomicra (CM) as 3,4-didehydroretinyl esters (DRE). DRE are taken up by hepatic tissue in chylomicron remnants and hydrolyzed to DR. If the individual has adequate liver VA reserves, DR will be stored as didehydroretinyl acetate (DRA), has been used to determine vitamin A (VA) status of individuals and groups worldwide. Less invasive methods using milk are in development in a swine model. Swine are a good choice for studying VA metabolism because their gastrointestinal anatomy, morphology, physiology, and VA requirements are similar to those of humans. In this study, DRA was used as a VA tracer in lactating sows to follow the metabolism of newly ingested VA. Lactating sows (n = 6) were administered 35 μmol DRA after overnight food deprivation. Blood and milk were collected at 0, 1.5, 3, 5, 7, 9, 24, and 48 h; livers were obtained at the time of killing. Samples were analyzed for didehydroretinol (DR), retinol (R), and didehydroretinyl esters (DRE). Serum DR:R was compared with that in milk and other VA indicators. DRE rapidly increased in serum, corresponding to chylomicra, whereas DR increased at a slower rate corresponding to the holo-DR:retinol-binding protein complex released from the liver. An estimated 10–20% of the dose was irreversibly lost in milk over 48 h. The mean MRDR value was 0.018 ± 0.013 at 5 h and the mean liver VA was 0.73 ± 0.21 μmol/g, both signifying sufficient stores. Milk and serum DR:R values were directly correlated (r = 0.64, P < 0.0001). Thus, DR:R values in milk may be a potential alternative to serum in determining VA status in lactating women. Future work is required in VA-deficient sows and women of varying VA status to determine DR trafficking and to compare DR:R values in milk with those in serum. J. Nutr. 136: 939–945, 2006.

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The modified-relative-dose-response (MRDR) test has been used extensively to qualitatively assess liver VA reserves in the deficient-to-normal range in humans and animals (4,7–10). For the test, 3,4-didehydroretinyl acetate (DRA) is administered orally, hydrolyzed in the gut to 3,4-didehydroretinol (DR), and repackaged into the chylomicra (CM) as 3,4-didehydroretinyl esters (DRE). DRE are taken up by hepatic tissue in chylomicron remnants and hydrolyzed to DR. If the individual has adequate liver VA reserves, DR will be stored as DRE in the
liver. If liver reserves are low, DR binds to accumulated apo-retinol-binding protein (RBP) and circulates throughout the body to replenish depleted extrahepatic tissues (5,7). A blood sample is taken 4–6 h after the DRA dose and the ratio of DR to R (DR:R) is determined; DR:R is also called the MRDR value at these time points. A MRDR value ≥ 0.060 is indicative of inadequate VA status; one between 0.030 and 0.060 is considered uncertain, and a value ≤ 0.030 reflects adequate VA status (5).

Breast milk VA concentrations offer a unique opportunity to assess the status of lactating women and extrapolate to that of nursing infants. Values ≤ 1.05 µmol R/L breast milk or ≤ 8 µg (28 nmol) R/g milk fat are considered low in humans (9,10). Breast milk collection is less invasive and usually easier and more culturally acceptable than blood drawing. VA is taken up by the mammary gland from CM or holo-RBP and secreted into milk as VA esters (11–13). VA tracers were observed in rat milk (11,12) and DR was detected in human milk (Tanumihardjo, personal observation). The DR:R value from a milk sample may represent VA status if it correlates with the serum value over a broad range of liver VA reserves.

The sow model is a good choice for extrapolation to humans because of the anatomic and physiologic similarity of the gastrointestinal tracts and their use in previous VA research (14–19). Additionally, swine metabolize retinol (16–18) and DRA (19) comparably to humans. Furthermore, the use of the serum MRDR test was validated in VA-depleted piglets by comparing MRDR values with liver reserves (19). The sow model is a good approach with which to observe VA metabolism during lactation and further validate the MRDR test because liver reserves are accessible. The purposes of this study were to determine the uptake and clearance of DR as a tracer in a VA-sufficient lactating sow in blood and milk, to determine whether serum DR:R is correlated with that in milk, and to compare liver reserves of VA with the 5-h serum DR:R value.

MATERIALS AND METHODS

Animals and diet. Approval for the study was obtained from the University of Wisconsin (UW)-Madison Research Animal Resources Center. Sows were housed at the Swine Research and Teaching Center in Arlington, WI, and the UW-Madison Livestock Laboratory. The sows were crossbreeds of Large White and Landrace. Baseline characteristics and reproductive histories of the lactating sows were obtained before treatment (Table 1). The sows were fed a standard lactation diet containing 5500 IU (1667 mg) retinyl acetate/kg feed and 30 g) as previously described (17). One hour before sample collection, nursing piglets were removed to a heated creep to ensure adequate milk for collection. Blood was collected at 0, 1.5, 3, 5, 7, 9, 24, and 48 h after the dose as described previously (17). Fore and hind milk (~45 mL) were collected manually at the same time points as blood as published (16).

TABLE 1
Baseline characteristics of lactating sows administered 35 µmol DRA

| Age, y | 3.1 ± 0.9 |
| Number of parities, n | 6.2 ± 1.9 |
| Live births this parity, n | 8.0 ± 2.5 |
| Lactation time at dosing, d | 11 ± 2.6 |

1 Values are means ± SD, n = 6. 2 Sow weights were not available. The mean weight of similarly aged sows from a previous study was 222 ± 17 kg (17).

Sample collection. At ~10 d of lactation, catheters were placed into the jugular vein of nonanesthetized sows (n = 6) as previously described (17). One hour before sample collection, nursing piglets were removed to a heated creep to ensure adequate milk for collection. Blood was collected at 0, 1.5, 3, 5, 7, 9, 24, and 48 h after the dose as described previously (17). Fore and hind milk (~45 mL) were collected manually at the same time points as blood as published (16). Available livers (n = 5) were collected ~2 mo later when the sows were killed, which was arranged by the UW-Madison Livestock Laboratory. All samples were stored on dry ice until transferred to a −80°C freezer. Serum, milk, and livers were analyzed by optimizing published procedures (7,19). All analyses were performed under gold fluorescent lights to minimize photooxidation and isomerization of VA. Standard curves were generated with HPLC-purified DR and R to quantify concentrations for all samples.

Serum and milk 3,4-didehydroretinol to retinol ratio analysis. To determine the DR:R value, 200 µL serum was prepared using a standardized method (19). The only modifications were a change in the mobile phase [90:10 (v:v) methanol:water and 0.05% triethylamine (TEA)] and use of a different HPLC system [injector, Rheodyne; detector, Shimadzu SPD-10A UV-Vis; pump, Waters 600 Multisolvent Delivery System; and data processor, Shimadzu C-R7A]. The detector was set at 350 nm to optimize detection of DR.

Milk fat analysis. Fat was determined by a modified Folch extraction method (24). Briefly, 2 mL 2:1 (v:v) dichloromethane (DCM):methanol and 1 mL ethanol was added to 1 mL milk. The mixture was vortexed and centrifuged. The top layer was placed in a new test tube and 1 mL DCM and 1 mL water with 0.6% NaCl were added, followed by mixing on a vortex and centrifuging. The bottom layer was transferred back to the original storage test tube; 1 mL DCM:methanol and 1 mL ethanol were added following by mixing on a vortex and centrifuging to form a delipidated pellet. The extract was transferred to a tared test tube; 1 mL water with 0.6% NaCl was added and the sample was mixed on a vortex and centrifuged. The aqueous layer was discarded and the extract dried under argon and reconstituted with 80 µL 50:50 (v:v) methanol:dichloroethane. After mixing on the vortex and centrifuging at 1380 × g for 30 s, 60 µL was injected onto the Waters HPLC system as that used for serum DR:R analysis with the addition of a Waters 275 plus autosampler. Analysis of didehydroretinyl esters in serum. Retinyl butyrate was synthesized by a condensation reaction of retinol with butyric anhydride (Sigma Chemical) in TEA. Retinyl butyrate (40 µL; 4.7 µmol) was added to 1 mL serum as an internal standard. Samples were mixed on a vortex with 1 mL ethanol to denature the proteins followed by three 1-mL hexane extractions. The extracts were dried under argon and reconstituted with 80 µL 50:50 (v:v) methanol:dichloroethane. After mixing on the vortex and centrifuging at 1380 × g for 30 s, 60 µL was injected onto a Waters Resolve™ C18 5-µm column, 3.9 × 300 mm equipped with a guard column. The Waters HPLC system consisted of a 1525 binary pump, 717 autosampler, and 996 photodiode array (PDA) detector. A gradient system was used that optimized separation of DR, DRE, and retinyl esters (23); the only notable modification was a reduction of the TEA modifier to 0.05%. Chromatograms were generated at 350 nm.

Analysis of vitamin A in liver. Liver retinol and retinyl esters were determined in duplicate by randomly combining three 0.5-g samples and grinding with sodium sulfate (~3 g) as described (23). The extract (100 µL) was dried under argon and reconstituted with 100 µL 50:50 (v:v) methanol:dichloroethane. A 50-µL aliquot was until dose preparation. The concentration was determined by UV-visible spectroscopy (E1% = 1455 at 350 nm); 10 µg (35 nmol) in 5.1 mL was administered to each sow by mixing with 500 g feed. This dosage is 4 times the standard dose given to women (i.e., 8.8 µmol). The sows were observed to ascertain that the entire dose was consumed.

Analysis of vitamin A in breast milk. Breast milk VA concentrations offer a unique opportunity to assess the status of lactating women and extrapolate to that of nursing infants. Values ≤ 1.05 µmol R/L breast milk or ≤ 8 µg (28 nmol) R/g milk fat are considered low in humans (9,10). Milk (500 mL) was saponified as published (16,21) with minor modifications. C23-9-cis-carotene was synthesized (22) and used as an internal standard. After saponification and extraction, the residue was reconstituted with 100 µL 50:50 (v:v) methanol:dichloroethane; 25 µL was injected into the same HPLC system as that used for serum DR:R analysis with the addition of a Waters 275 plus autosampler.
Statistical analysis and pharmacokinetic calculations. Statistical analyses were performed with SAS software (version 8.2; SAS Institute). A repeated-measures ANOVA test with fixed effects was applied to determine the main effect of time using SAS PROC MIXED, which allows the treatment variances to differ among the time points. Significance between time points was determined by using least-squares mean differences. Repeated-measures models were fitted to investigate 9 variables over time: DR, R and DR:R in serum and milk, DRE in serum, and DR and R/g fat in milk. Differences were considered significant at  $P \leq 0.05$. Area under the curve (AUC) was estimated using linear trapezoidal approximation for DR in milk and serum and DRE in serum.

Noncompartmental analysis was performed for each sow with complete data at all time points ($n = 4$) using WINNONLIN (version 4.1, Pharsight). The elimination rate constant ($\lambda z$) in mmol/L $\times h$ and half-life ($t_{1/2}$) in hours were estimated via linear regression of time vs. log concentration. Extrapolation of AUC to infinity was done by adding $C_t/\lambda z$ to AUC, where $C_t$ is serum or milk DR concentration at the last sampled time point, $t$. Other parameters determined were the maximum concentration ($C_{\text{max}}$) and time of $C_{\text{max}}$ ($T_{\text{max}}$).

**RESULTS**

Serum and milk 3,4-didehydroretinol to retinol ratios. The DR:R values in serum and milk followed a similar pattern over time (Fig. 1). The serum and milk DR:R changed significantly with time ($P = 0.024$ and $0.003$, respectively). Serum DR:R correlated directly with milk DR:R ($r = 0.64$, $P < 0.0001$); however, a more than 10-fold difference was observed between values at each time point (Fig. 1). Serum DR:R values were significantly different from baseline at 3 through 9 h after dosing with DRA ($P \leq 0.01$) and these values did not differ in milk. DR:R values were significantly different from baseline at 5 through 24 h after dosing ($P \leq 0.036$) and these values did not differ. Thus, peak milk DR:R lagged behind serum DR:R. Modeling revealed that a quadratic model described the relation between response and time for both serum and milk DR:R values ($P = 0.047$ and $P = 0.004$, respectively).

Serum didehydroretinyl esters, didehydroretinol, and retinol. The appearance and disappearance of serum DRE and DR derived from the DRA dose were plotted over time (Fig. 2). The specific DRE identified in serum by PDA analysis were 3,4-didehydroretinyl-oleate, -palmitate, and -stearate and the relative percentages were 1.7 ± 1.6, 71 ± 7, and 28 ± 6%, respectively. As expected, DRE rapidly increased with time in serum ($P = 0.024$) with a sharp peak at 3 h postdose ($P < 0.0001$), corresponding to CM circulation. DRE concentrations were significantly different from baseline at 1.5 through 7 h ($P \leq 0.04$) and tended to be higher at 3 h than at 7 h ($P = 0.079$). DR, derived from DRE, was presumably released from the liver bound to RBP and increased at a slightly slower rate after dosing ($P = 0.013$). DR concentrations were significantly different from baseline at 3 through 9 h ($P \leq 0.008$) and these values did not differ. Correspondingly, the calculated $T_{\text{max}}$ for DRE was earlier than that for DR (Table 2). By 48 h, most of the sows had completely cleared detectable DR from the serum. The elimination rate constants for DR and DRE were calculated for this physiologic dose (Table 2). AUC was calculated to compare DR and DRE relative concentrations over the 48-h period and extrapolated to infinity (Table 3). Serum R concentrations did not change during the course of this study. The serum R concentration for all time points collected and analyzed ($n = 45$) was $0.91 \pm 0.18$ mmol/L.

Modeling was applied to analyze serum concentrations of DRE, DR, and R over time. A quartic model fit the data for DRE ($P = 0.0046$), explaining the rapid rise and steady decrease of DRE in serum after dosage with DRA. A quadratic model better fit the data for serum DR ($P = 0.057$), supporting the changing pattern seen in the descriptive plot (Fig. 2). A linear model fit serum R concentration over time ($P = 0.020$).

Milk didehydroretinol and retinol. Didehydroretinol response in milk from the administrated DRA dose over 48 h was...
TABLE 2

Pharmacokinetic data determined in lactating sows after 35 μmol DRA was administered orally and followed for 48 h in serum and milk.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>T&lt;sub&gt;max&lt;/sub&gt;</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>λ</th>
<th>τ&lt;sub&gt;1/2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum DR, nmol/L</td>
<td>7.5 ± 1.9</td>
<td>7.0 ± 2.5</td>
<td>0.04 ± 0.01</td>
<td>17.4 ± 4.5</td>
</tr>
<tr>
<td>Serum DRE, nmol/L</td>
<td>4.3 ± 2</td>
<td>36.5 ± 8.8</td>
<td>0.14 ± 0.18</td>
<td>8.8 ± 3.9</td>
</tr>
<tr>
<td>Milk DR, nmol/L</td>
<td>15.5 ± 9.8</td>
<td>292 ± 94.8</td>
<td>0.01 ± 0.006</td>
<td>71.8 ± 51.2</td>
</tr>
<tr>
<td>Milk DR, nmol/g fat</td>
<td>8.5 ± 1</td>
<td>4.6 ± 1.5</td>
<td>0.02 ± 0.006</td>
<td>29.7 ± 6.9</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 4.
2 C<sub>max</sub> is the concentration at T<sub>max</sub>.

TABLE 3

AUC from 0 to 48 h and 0 to ∞ for serum and milk

<table>
<thead>
<tr>
<th>Indicator</th>
<th>AUC&lt;sub&gt;0–48 h&lt;/sub&gt; (nmol/L · h)</th>
<th>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (nmol/L · h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum DR, (nmol/L) × t</td>
<td>366 ± 193</td>
<td>570 ± 275</td>
</tr>
<tr>
<td>Serum DRE, (nmol/L) × t</td>
<td>404 ± 315</td>
<td>473 ± 278</td>
</tr>
<tr>
<td>Milk DR, (nmol/L) × t</td>
<td>9790 ± 4830</td>
<td>26800 ± 21200</td>
</tr>
<tr>
<td>Milk DR, (nmol/g fat) × t</td>
<td>118 ± 57</td>
<td>212 ± 136</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 6.

A significant difference was found over time for both DR/L milk (P = 0.003) and DR/g fat milk (P = 0.0001). DR/L vs. DR/g fat were highly correlated (r = 0.957, P < 0.0001). DR/L milk was significantly different from baseline at 5 through 24 h after dosing with DRA (P = 0.0017) and these values did not differ. The same time frame was found for DR/g fat milk, i.e., difference from baseline at 5 through 24 h (P ≤ 0.0013). DR/g fat tended to be elevated at 48 h after dosing compared with baseline (P = 0.058). The calculated T<sub>max</sub> for DR/L milk and DR/g fat lagged behind the serum values (Table 2). AUC<sub>0–48 h</sub> of DR/L milk and DR/g fat were computed and extrapolated to infinity (Table 3). Modeling found the quadratic model to fit DR/L and DR/g fat over time (P = 0.0003 for both models).

To estimate the irreversible loss of DR tracer in milk over the 48-h period, a time course was generated by extrapolating DR concentration in milk every 0.5 h. Assuming that a sow produces 7.5–13.6 kg milk/d (25) with milk density equal to ~1 kg/L, 10–20% of the dose was estimated to be irreversibly lost in milk over the 48-h period. The amount of DR tracer in serum was calculated at the mean peak time point by assuming that a sow contains 9 L serum during lactation (26). Only 0.65% of the 35 μmol DR tracer was estimated to be circulating in serum, consistent with the assumption that most of the dose is stored in the liver when VA status is sufficient.

The R concentration per L milk (R/L milk) differed significantly over time (P = 0.04) due to a higher value at 24 h driven by 1 sow. Using Dixon’s criteria for outliers, this value was considered an extreme observation at the 5% test level. When this value was omitted, significance was lost. R/g milk fat was not different even with this extreme value included. Furthermore, R/L milk and R/g fat were not correlated (r = 0.16, P = 0.30). The individual sow CV for R/g fat over time were slightly lower than those for R/L milk, i.e., 23 ± 17 vs. 33 ± 12\%, respectively. A linear model appropriately fit R/L milk (P = 0.02), but neither a linear nor a quadratic model gave a good fit for the relation between concentration and time for R/g fat (P = 0.26 and P = 0.12, respectively).

Liver vitamin A reserves. Liver VA reserves (n = 5) were measured to validate the sows’ VA status. The VA concentration, which included R plus all identifiable esters, was 0.73 ± 0.21 μmol/g, indicative of sufficient status. DR and DRE were not detected in the liver at the time sampled. As the “gold” standard of vitamin A status, liver values were compared with the other VA indicators at 5 h (Table 4).

DISCUSSION

Accurate measures of VA status are vital for determining groups at risk of deficiency and for evaluating efficacy trials. Ideal indicators should be minimally invasive, require small
biological sample volumes, and be sensitive to changes in status from an intervention. Liver VA reserves are considered the "gold" standard for determining VA status (5). Because liver biopsies are generally not feasible in humans, indirect methods are required. In this study, a healthy lactating sow model was used to examine several VA status indicators over time compared with liver VA concentration. Swine are a good model for VA metabolism studies (27), and serum and liver VA concentrations are similar to human values (14–19).

The use of serum R as an indicator is limited by the capacity to distinguish marginal from adequate VA status in individuals in a satisfactory manner (5,6). The results presented here show that serum R is an uncertain indicator of VA status in lactating sows. The mean serum R concentration was <1.05 μmol/L, a value suggested to be indicative of low VA status in humans (28). In contrast, mean liver VA was 0.73 μmol/g, consistent with highly sufficient VA stores; therefore, circulating R concentrations were under "normal" homeostatic control. Breast milk collection is less invasive and usually easier than blood drawing. The samples do not have to be further processed in the field, allowing shorter preparation time than serum. Although breast milk is a potentially useful indicator of VA status at the population level, many factors affect milk VA concentration at the individual level. These include stage of lactation, time of day, last feeding episode, and milk fat content (29). R/L milk did not correlate with R/g fat in this study. Although milk R concentrations are not tightly regulated, R/g fat gave a smaller CV over time than R/L milk. R/g fat may better reflect changing VA status for individuals (29). Of note, sow’s milk VA concentration in R/L is similar to that of humans (30), but R/g fat is lower due to the generally higher fat content in sow’s milk (25).

During lactation, lipoprotein lipase (LPL) activity increases in mammary glands, allowing chylomicron retinyl ester incorporation into milk before reaching the liver in chylomicron remnants (CMrem) (11,12). The uptake of retinyl esters may vary depending on VA status as well as species. AUC differences for DR between 0 and 48 h and extrapolation to ∞ in milk were much higher than the serum value AUC differences, possibly indicating VA storage in the mammary gland. Because of repeated sampling in this study, the loss of DRE from the body in milk could be estimated. If significant loss occurs, a blood sample taken at 5 h may result in a lower MRDR value in lactating women compared with nonlactating women (30). By considering certain assumptions, 10–20% of the DR dose was irreversibly lost in milk over the 48-h period. Although this is important for stable isotope testing in which a quantitative estimate of total body VA reserves is determined based on the dose administered, it is not a drawback for a dose-response test in which a qualitative estimate of an individual’s VA status or categorical indication of a population’s status is sought (6). The mean MRDR value of 0.018 ± 0.013 at 5 h indicates normal VA status for this group of sows. In contrast, groups of lactating women suspected to have marginal VA status had mean MRDR values > 0.09 (4,31,32) with serum R concentrations (0.85–0.99 μmol/L) similar to these sows (0.91 ± 0.18 μmol/L). Thus, the argument that differences in MRDR values between groups are due to differences in serum R concentrations (33) does not fit this data.

Conflicting results will occur among indicators if the following study parameters are not considered: average body weight of subjects, estimated liver weight, amount of VA administered, estimated loss in breast milk (if applicable), and study duration (34). The use of the MRDR test in Indonesian lactating women was a sensitive indicator for assessing the improvement of VA status after an intervention with 8.4 μmol VA for 35 d (4). Similarly, Rice et al. (9) found the MRDR value to be more responsive than serum R as an indicator of VA status, but less so than casual breast milk samples. In comparison, de Pee et al. (31) found serum R to be more sensitive than the MRDR test after 3.5 mg β-carotene was administered either as a vegetable or in a wafer to lactating Indonesian women for 84 d. It is likely that the latter study would have found the MRDR value more responsive if the β-carotene administered was increased. That is, a marginal increase in VA liver reserves, which are initially depleted, may increase serum R but not change overall VA status to adequate while still resulting in positive MRDR values at follow-up (34).

Although the validity of dose-response tests has been questioned (33), such tests, when applied appropriately, offer more information about VA status than serum R concentrations alone (6). The MRDR test was validated against liver reserves in 2 animal models, i.e., swine and rats. In this lactating sow model, the mean MRDR value was normal at all time points even though serum R was low. In fact, according to Verhoef and West (33), the MRDR values in this study should have been elevated because of the underlying low serum R concentration. However, the MRDR values were similar to those that occur in well-nourished American adults over time (0.013 ± 0.003) even though serum R was almost double in that study (1.7 ± 0.5 μmol/L) (35).

The appropriate time frame for collecting blood for the MRDR test is between 4 and 6 h postdose as shown in well-nourished Americans (36,37) and groups of Indonesians suspected to have marginal VA status (32,38). This time interval was further validated and increased to 7 h using piglets (19). This study confirms this time frame because MRDR values did not change from 3 through 9 h in these VA-sufficient sows. However, this study does not support shortening the time postdose to 3 h because it is likely too soon to elicit a response in all VA-depleted individuals. Positive MRDR values at 3 h (i.e., ≥ 0.06) are a highly sensitive indicator of depleted VA reserves at the individual level but may result in more false negatives in the group as a whole.

Correlation between milk and serum DR:R values warrants further investigation in VA-deficient lactating sows and in women who have varying VA status. Even though the relationship was highly correlated, there was a large difference in the values. In comparing serum and milk DR:R values in either VA-deficient or -adequate individuals, 2 separate lines or a continuous line could result. If indeed the line is continuous, breast milk DR:R values could serve as a surrogate measure for serum values. A continuous line would result in a VA-deficient state if less DR is stored in the liver and more DR is shunted to the milk than during normal VA status. For example, extrapolating from our data in which liver reserves were known (Table 4), a serum DR:R value in a VA-sufficient woman at 0.02 would have a milk value at 0.15; a deficient woman with a DR:R of 0.09 would have a milk value of 0.7. However, if 2 parallel or separate lines result, DR in milk could be used for tracer kinetics to examine trafficking in individuals with different VA status but not as a measure of status.

Before this study, DRA was used as a tracer in rats (7) and 2 fur seals (39). This study used DRA in a lactating swine model. In summary, an oral dose of DRA undergoes enzymatic hydrolysis in the gut to DR (Fig. 4). DR is absorbed as part of micelles with dietary fat and facilitated by a transporter (40), reesterified to DRE in the mucosa, and packaged into CM. The CM are secreted into the lymphatic system and enter the general circulation. DRE can be taken up by extrahepatic tissue such as the mammary gland in which LPL activity is high (11) or cleared by the liver as part of CMrem. Once in the liver, DRE
The trafficking of an oral dose of DRA to milk and serum in a lactation model. DRA is hydrolyzed in the gut to DR (A), absorbed as part of micelles, reesterified to DRE, and packaged into CM (B). The CM are secreted (C) and enter the circulation to be taken up by hepatocytes where CPE activity is high (D) or cleared by the liver as part of CMrem (E). DRE can be stored in the liver (F) or hydrolyzed to DR, bound to RBP, and secreted into plasma (G). Once in circulation, DR-RBP is taken up by the mammary gland (H) and esterified for secretion into milk (I). DR-R in milk (J) and serum (K) are positively correlated in a vitamin A-sufficient lactating sow model.

FIGURE 4 The trafficking of an oral dose of DRA to milk and serum in a lactation model.
34. Tanumihardjo SA. Can lack of improvement in vitamin A status indicators be explained by little or no overall change in vitamin A status of humans? J Nutr. 2001;131:3316–8.


