Kinetic Analysis Shows That Iron Deficiency Decreases Liver Vitamin A Mobilization in Rats 1,2

Jing-Tsz Jang, Joanne Balmer Green, John L. Beard and Michael H. Green 3
Nutrition Department, The Pennsylvania State University, University Park, PA 16802

ABSTRACT In view of evidence that nutritional status of iron and vitamin A may affect the other nutrient’s metabolism, we used model-based compartmental analysis to examine effects of iron deficiency on whole-body vitamin A dynamics in rats. Weanling male Sprague-Dawley rats were fed the AIN93G diet with 2.5 nmol retinyl palmitate/g and either 45 [control (CN)] or 4 μg/g Fe [iron-deficient (ID)] for 8 wk. ID rats consumed food ad libitum; CN rats were food-restricted so that their body weights were the same as ID rats. Two rats/group were killed; liver vitamin A was determined and used for vitamin A balance calculations. [3H]Retinol-labeled plasma was administered intravenously to remaining rats, and 27 serial blood samples were collected for 7 wk. At killing, plasma vitamin A was 0.52 ± 0.12 (ID, n = 5) vs. 1.34 ± 0.12 μmol/L (CN, n = 6; P < 0.001), and liver vitamin A was 809 ± 94 (ID) vs. 112 ± 24 nmol (CN, P < 0.001). Plasma tracer data were fit to a three- or four-compartment model using the Simulation, Analysis and Modeling computer program and kinetic parameters were calculated. Vitamin A transfer rate between the retinyl ester storage pool [14 ± 3 (ID) vs. 24 ± 4 nmol/d (CN), P < 0.05] and plasma was lower in ID rats. Vitamin A remained longer in the body [44 ± 11 (ID) vs. 22 ± 3 d (CN), P < 0.05]. Adjusted mean disposal rate was lower in ID (10.0) than CN rats (19.9 nmol/d), as was estimated vitamin A absorption efficiency [58% (ID) vs. 76% (CN)]. Our results suggest that iron deficiency inhibits mobilization of vitamin A stores and may decrease the absorption and irreversible utilization of vitamin A.

KEY WORDS: • iron deficiency • vitamin A kinetics • simulation, analysis and modeling • rats

Iron and vitamin A undernutrition, both prevalent in developing countries, are recognized as important global public health problems. Worldwide, about 2 billion and 200 million people are at risk of iron- and vitamin A deficiencies, respectively (WHO 1995), and the two conditions often coexist (Trowbridge et al. 1993, Yip 1994). For example, epidemiological studies in Central Ethiopia (Wolde-Gebru et al. 1993) and Bangladesh (Ahmed et al. 1996) found significant positive correlations between serum retinol and hemoglobin (r ~ 0.2). In addition, Molla et al. (1993) found three times as many cases of low vitamin A status among children with low (vs. normal) hemoglobin. In animal models, Roodenburg et al. (1996a, 1996b) found that marginal vitamin A deficiency could produce mild anemia and interfere with body iron mobilization. Others have shown that iron deficiency is associated with lower plasma retinol concentration and increased liver vitamin A (Amine et al. 1970, Rosales et al. 1999, Staab et al. 1984) and an increased molar ratio of hepatic retinyl esters to retinol in male Sprague-Dawley rats (Rosales et al. 1999). The latter was correlated with blood hemoglobin level (r^2 = 0.65, P < 0.001). Taken together, these results suggest that nutritional status of each of these nutrients may affect the other’s metabolism.

To further investigate the effect of iron deficiency on vitamin A metabolism, we used model-based compartmental analysis (Green and Green 1990a) to compare whole-body vitamin A dynamics in control vs. iron-deficient (ID)4 rats. Based on earlier work, we hypothesized that decreased plasma retinol levels in ID rats may be due to a decrease in the movement of vitamin A between liver and plasma, and/or an increase in vitamin A disposal rate.

MATERIALS AND METHODS

Animals and diets. Male Sprague-Dawley rats (n = 16) were obtained at 22 d of age (~46 g) from Harlan Sprague-Dawley (Indianapolis, IN). Rats were housed individually under controlled environmental conditions (lights on 0600–1800 h, temperature 25°C). Distilled deionized water was provided continuously. Animal procedures were approved by The Pennsylvania State University’s Animal Care and Use Committee.

Upon arrival, rats were divided into two groups: ID and control (CN). Rats were fed a modification (Borel et al. 1991) of the AIN93G diet (Reeves et al. 1993) in which corn starch replaced sucrose. The diet contained 2.5 nmol retinyl palmitate (Sigma Chem-

2 Supported by a grant from the Inter-College Graduate Program in Nutrition, The Pennsylvania State University.
3 To whom correspondence should be addressed.
4 Abbreviations used: CN, control; FCR p, plasma fractional catabolic rate; fdo, fraction of the injected dose; ID, iron-deficient; f(I,J), fractional transfer coefficient; Ri(J) transfer rate; RBP, retinol-binding protein; TBV, total blood volume; TMMP-retinol, all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraen-1-ol.

0223-316X/00 $3.00 © 2000 American Society for Nutritional Sciences.
tional, St. Louis, MO) per gram and either 4 (ID) or 45 μg iron per gram (CN) as ferrous sulfate. ID rats had free access to food, but CN rats were food-restricted as described by Rosales et al. (1999) so that they were not maintained at the body weight of ID rats.

Preparation of \(^*\)H\)retinol-labeled plasma. As described in detail by Green and Green (1990b), two weaning male Sprague-Dawley rats (Harlan) were fed the CN diet described above but without vitamin A for 8 wk to deplete liver vitamin A stores. Rats were anesthetized with diethyl ether. A dispersion (0.5 mL containing ~37 MBq \([\text{^*}\text{H}]\) of \([11,12-\text{^*}\text{H}]\) retinyl acetate (specific radioactivity 888 GBq/μmol; donated by Hoffmann-La Roche, Nutley, NJ) in Tween 40 (Sigma) was slowly injected into an exposed jugular vein. After 100 min, rats were reanesthetized, and blood was collected from the abdominal aorta into heparinized syringes. Plasma presumably containing \([\text{^*}\text{H}]\)retinol in the retinol-binding protein (RBP)/transretin complex (Green et al. 1985) was pooled, stored under an atmosphere of nitrogen at 4°C and used for in vivo kinetic studies within 3 d.

Kinetic studies. After 8 wk of dietary treatment, two rats from each group were killed (see below) to estimate the initial values for plasma and liver retinol, as well as blood hematocrit and hemoglobin. For the kinetic study, remaining rats were anesthetized with methoxyflurane (Fitran Moore, Washington Crossing, NJ), and an external jugular vein was exposed by blunt dissection. A weighed aliquot of \([\text{^*}\text{H}]\)retinol-labeled plasma (~0.6 g containing ~37 kBq of \([\text{^*}\text{H}]\) was injected and anesthesia was removed. Serial blood samples (n = 27) were collected based on a geometric progression from 10 min after dose administration until the rats were killed 48 d later. The time for killing was chosen based on previous kinetic studies (Green et al. 1987, Green and Green 1990b). At each time point, a blood sample (≤0.2 mL) was obtained from a caudal vein into tubes containing disodium EDTA (final concentration ~8 μmol/L). Plasma was separated and aliquots were frozen at −20°C under an atmosphere of nitrogen for determination of radioactivity and, in some cases, retinol concentration.

After collection of the final caudal vein blood sample on d 48, rats were deeply anesthetized with CO₂; blood was collected by open chest heart puncture into heparinized syringes and analyzed for hematocrit and hemoglobin. Aliquots of plasma from the caudal blood samples were frozen and stored at −20°C for later analysis of tritium and vitamin A content. Livers were excised, blotted, weighed and divided for iron and vitamin A analyses and frozen at −20°C for later analyses. The remaining carcass was weighed and frozen for analysis of radioactivity.

Iron indices. Hemoglobin and hematocrit were analyzed monthly to monitor iron status. Caudal vein blood samples were collected into heparinized microcapillary tubes (VWR Scientific, Willow, OH) and centrifuged to determine hematocrit using a Micro-Hematocrit Capillary Tube Reader (Lancer; Brunswick Company, St. Louis, MO). Hemoglobin concentration was measured in whole blood (10 μL) by the colorimetric cyanmethemoglobin method (procedure No. 525; Sigma). Liver nonheme iron was measured after acid hydrolysis of ~0.3 g samples of frozen liver, using standard spectrophotometric techniques (Torrance and Bothwell 1980) with ferrozine as the color reagent.

Plasma radioactivity and retinol. To determine radioactivity, serial plasma samples (20 to 100 μL) and aliquots of the injected doses were extracted (Green and Green 1990b, Thompson et al. 1971) from 50% ethanol into hexane containing 5 mg/L of BHT (Sigma). Solvent was evaporated and extracts were solubilized in scintillation solution (ReadyOrganic; Beckman Instruments, Fullerton, CA). Samples were counted (model LS-3801; Beckman Instruments, Irvine, CA) twice to a final 2-sigma error of 1.0% or for a maximum of 720 min. Sample net counts per minute were converted to disintegrations/min (dpm) using an external standard method.

To determine plasma retinol concentration, a known amount of internal standard \([\text{[trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraen-1-ol]}](\text{TMMP-retinol}), donated by Hoffmann-La Roche, Basel, Switzerland, was added to selected serial plasma samples. Samples were extracted with hexane as described above. Solvent-free extracts were resuspended in methanol and analyzed by reverse-phase HPLC (model 1050; Hewlett-Packard, Wilmington, DE), using a 3 μm Supelcosil LC-18 column (Supelco, Bellefonte, PA) with methanol/water (90:10, v/v) at 1.5 mL/min as the mobile phase. Peaks were detected by UV absorbance at 325 nm. Retinol masses were calculated by an internal standard method using a Hewlett-Packard 1050 Chemstation and standard curves for retinol and TMM-retinol.

Liver retinol and radioactivity. Aliquots of freeze-dried liver (3 × 0.15 g) were spiked with TMM-retinol and saponified in ethanolic KOH with pyrogallol (Sigma) (Green et al. 1985, Thompson et al. 1971). Lipids were extracted into hexane containing BHT. Aliquots of the extracts were taken for retinol analysis by HPLC and tritium measurement by liquid scintillation spectrometry as described above for plasma.

Carcass radioactivity. Tritium in carcasses was analyzed using the method described by Adams et al. (1995). In brief, carcasses were ground, and aliquots (5 × ~1.5 g) were extracted using hexane/isopropanol/sodium sulfate. Solvent-free extracts were analyzed for radioactivity as described above.

Compartamental model. model-based compartmental analysis (Green and Green 1990a, 1990b) was used to calculate kinetic parameters based on kinetic analysis of the plasma tracer response curves for individual rats. First, fraction of the injected dose remaining in plasma was calculated from the observed dpm/mL at each time and the estimated plasma tracer concentration at time zero \([= \text{dpm injected/estimated plasma volume, where plasma volume (mL) = total blood volume (TBV) × (1-hematocrit), and TBV = 0.0675 × body weight (g)}\) (Wang 1959). Fraction of the injected dose \([\text{fdose}]\) in liver and carcass was also calculated, as was fdose that was irreversibly lost at the end of the kinetic study \([= 1 - (\text{plasma fdose + liver fdose + carcass fdose})]\).

To model a developing whole-body vitamin A kinetics as viewed from the plasma, we used the approach outlined by Adams et al. (1995) and Kelley and Green (1998). In this approach, processes with similar kinetics are lumped in the same compartment. First, data on fdose in plasma vs. time for each rat were fit to a three- or four-component multiexponential equation. The sum of the y-intercepts (equal to the plasma fdose value at time zero) was used to adjust the estimated value for plasma volume. Model-based compartmental analysis in the Windows 95 version (WinSaAM) of the Simulation, Analysis and Modeling (SAAM) computer program (Berman and Weiss 1978) was used to find a good fit between observed and model-predicted values. Then, weighted, nonlinear regression was done using WinSaAM to estimate the model parameters \([\text{fractional transfer coefficients or } L(I,J)s, the fraction of compartment J's tracer or tracee transferred to compartment I per day; see Green and Green (1990b) for more details}] and statistical uncertainties for these parameters. A fractional standard deviation \((\text{SD/mean}) = 0.05\) was used as the weighting factor for each datum. The fit between observed and model-predicted data was deemed acceptable and satisfactory by visual inspection of the simulated data plot and by statistical analysis, including the weighted residual sum of squares from nonlinear regression analysis and the estimated fractional SD for each kinetic parameter.

Then the model-predicted \(L(I,J)s\) and the estimated plasma retinol pool size \((\text{nmol})\) were used to calculate other parameters in a steady-state solution to the model using WinSaAM. After calculating kinetic parameters for individual rats, means and SD were calculated for each group. Calculated parameters were compartment \([M(I)]\) or the model-predicted retinol content in compartment \(I\); transfer rates \([R(I,J) = M(I) × L(I,J)]\) or the amount of retinol transferred from compartment \(J\) to compartment \(I\) per unit time; mean transit or turnover time \([T(I,1)]\); or the fraction of the fractional transfer coefficients leaving compartment \(I\), or the mean of the distribution of times a retinol molecule entering compartment \(I\) spends there during a single transit before leaving reversibly or irreversibly; mean residence time in plasma \([T(1,1)]\), or the total time an average retinol molecule spends in plasma before irreversibly leaving plasma after entering the system via plasma; \(T(1,1)\) is equal to the area under the curve (AUC<sub>p</sub>) for plasma tracer response vs. time to infinity; system residence time \([T(\text{SYS})]\), or the total time a retinol molecule spends in the system before irreversible loss; recycling number \([r(1) = T(1)/T(1)[r(1)]]\), or the number of times
Iron and vitamin A status of control (CN) and iron-deficient (ID) rats in the vitamin A kinetic study

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>CN</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A intake</td>
<td>1.2 nmol/d</td>
<td>1.4 nmol/d</td>
</tr>
<tr>
<td>Plasma ROH, mmol/L</td>
<td>112.2 ± 23.5</td>
<td>114.2 ± 27.6</td>
</tr>
<tr>
<td>Liver ROH, nmol</td>
<td>442 ± 525</td>
<td>470 ± 630</td>
</tr>
</tbody>
</table>

1 Data are means ± SD. Student's t test was performed by the Data Analysis function in Microsoft EXCEL 5.0 to test for significant differences between the ID and CN groups. An F test (Landaw and DiStefano 1984) was used to determine the minimum number of compartments that was compatible with all of the kinetic data where:

\[ F = \frac{[(WRSS_{n-1} - WRSS_n)/2]/(WRSS_n/d_f)]}{(1/n)} \]

WRSSn is the minimum weighted residual sum of squares for the fit of the order n model, and df is degrees of freedom (N-2n where N is the number of data points and n is the number of parameters in the n-order model). If the model with fewer compartments (n-1) is correct, then the value for the F statistic with (2, df) degrees of freedom is not significant (P > 0.05) when comparing the order n to the order n-1 model. Model complexity was increased only when it resulted in a statistically significant reduction in WRSS. An alpha level of 0.05 was used as the significance limit for all statistical analyses.

RESULTS

Animal outcome. One ID rat died unexpectedly after 13 wk of dietary treatment; thus results for that group are based on five rats. By restricting food intake of CN rats, we were successful in matching their body weights to those of rats in the ID group: at the beginning of the kinetic study (8 wk of dietary treatment), body weights were 217 ± 6.3 (CN) and 217 ± 6.3 g (ID); 7 wk later, they averaged 283 ± 6.5 and 281 ± 28 g. Due to the lower food intake, mean daily vitamin A intake of CN rats (24.3 ± 0.2 nmol/d) was significantly lower than that of ID rats (29.5 ± 1.2 nmol/d; P < 0.001).

Iron and vitamin A status. Iron status was significantly affected by diet at the beginning (data not shown) and end of the kinetic study (Table 1). After 15 wk of dietary treatment, hematocrit was 61% lower, hemoglobin was 80% lower and liver iron was 75% lower in ID vs. CN rats. Vitamin A status was also significantly affected by diet (Table 1). Plasma retinol concentrations in ID rats were ~40% those of CN rats at both the beginning and end of the kinetic study. In contrast, liver vitamin A levels in ID rats were ~2.7 and ~7.2 times those of CN rats at the beginning and end of the kinetic study, respectively. Based on these results, we estimate that CN rats were in a slightly negative liver vitamin A balance during the kinetic study, whereas ID rats were in a positive balance (Table 1).

Kinetic data and compartmental model. Figure 1 shows the fit of [3H]retinol remaining in plasma vs. time after dose administration, and dose irreversibly lost, for one representative rat from each group. We fit each rat’s plasma tracer response data to a three- and four-component exponential equation and used an F test (Landaw and DiStefano 1984) to determine which resulted in a better fit. According to this criterion, a three-component equation was adequate for all control rats. Among the ID rats, data for one were adequately fit by a three-component equation, whereas the others required four components. As in our previous work (Green et al. 1987, Green and Green 1994), we postulate that these results indicate that three or four compartments are needed to describe whole-body vitamin A kinetics as viewed from the plasma space. In such models (Fig. 2), plasma retinol (compartment 1) exchanges with vitamin A in both a fast turning-over extravascular pool (compartment 2) and a slower turning-over extravascular pool (compartment 3); in the four-compartment model, vitamin A in compartment 3 exchanges with vitamin A in a second storage pool (compartment 4), which turns over more slowly than vitamin A in compartment 3. We hypothesize that compartments 3 and 4 correspond, respectively, to vitamin A stored in the liver and extrahepatic tissues, whereas compartment 2 may be retinol in interstitial fluid, retinol filtered by the kidneys and more rapidly turning-over intracellular retinol pools. Input of dietary vitamin A is shown into compartment 1, which is also the site of tracer introduction; vitamin A output is from compartment 3 since that provided the best fit to the data.

After comparing characteristics of the plasma tracer response curves in CN vs. ID rats (Fig. 1), the following differences are evident. First, the bend between 1 and 10 h (which is primarily related to tracer leaving the vascular bed and some...
recycling from the fast turning-over pool to plasma) (Green and Green 1994) was similar in ID and CN rats. After 1 d, the fraction of injected [3H]retinol in plasma was lower in ID than CN rats. This indicates that iron deficiency decreased [3H]retinol recycling from the slow turning-over pool(s) to plasma, because of the smaller plasma retinol pool, the larger vitamin A liver store in ID rats and/or a decrease in mobilization. Finally, the fractional irreversible utilization of vitamin A, represented by the terminal slope of the curves, was higher in CN than ID rats.

Recovery of radioactivity in liver at the end of the kinetic study was significantly higher in ID than CN rats (27.4 ± 3.1 vs. 3.1 ± 0.9% of the injected dose; P < 0.0001). Recovery of tritium in carcass was not affected by iron deficiency (10.0 ± 0.7 [ID] vs. 12.2 ± 2.7% [CN]).

**Kinetic parameters.** WinSAAM was used to calculate parameters (fractional transfer coefficients) describing the three- and four-compartment models (Table 2). Comparable parameters were not significantly influenced by low iron status. Values for several of the parameters [L(2,1), L(1,2), L(3,1) and L(0,3)] are similar to those determined in previous studies of vitamin A kinetics done in this laboratory (Green and Green 1994). Because different model structures were required to fit data for some rats in the ID vs. CN groups, only some of the fractional transfer coefficients may be compared between groups. In the four-compartment model, compartment 3 interacts with compartments 1 and 4 and with output (compartment 0), but only with compartments 1 and 0 in the three-compartment model (Fig. 2). Therefore, kinetic parameters affected by compartment 3, including L(1,3) and L(0,3), have distinctly different meanings in the two models and are not comparable. In addition, parameters related to compartment 4 [L(4,3) and L(3,4)] only appear in the four-compartment model. For other parameters, the significance of differences was compared using data from all rats in the same group.

Table 3 lists the estimated and model-predicted compart-

![FIGURE 2 Proposed models for vitamin A kinetics in rats. Compartments are represented by circles, the asterisk indicates the site of injection of [3H]retinol-labeled plasma, the triangle indicates the site of sampling and U(1) indicates input of retinol to plasma from diet. Upper panel: three-compartment model. Compartment 1 represents plasma retinol, compartment 2 is a fast turning-over extravascular pool of retinol and compartment 3 is a slow turning-over extravascular pool of retinol, including retinyl esters. Lower panel: four-compartment model. For some rats, compartment 4, a slower turning-over extravascular pool, was also required. Presumably this contains mainly stored retinyl esters.](image)

### TABLE 2

<table>
<thead>
<tr>
<th>L[I, J], d⁻¹</th>
<th>CN²</th>
<th>ID¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(2, 1)</td>
<td>9.89 ± 1.85</td>
<td>10.3 ± 2.2 (11.4)</td>
</tr>
<tr>
<td>L(1, 2)</td>
<td>6.47 ± 0.73</td>
<td>7.89 ± 1.65 (8.10)</td>
</tr>
<tr>
<td>L(3, 1)</td>
<td>3.87 ± 0.41</td>
<td>4.05 ± 0.48 (3.73)</td>
</tr>
<tr>
<td>L(1, 3)</td>
<td>0.0650 ± 0.0052</td>
<td>0.0517 ± 0.0205 (0.0241)</td>
</tr>
<tr>
<td>L(0, 3)</td>
<td>0.0493 ± 0.0057</td>
<td>0.0619 ± 0.0224 (0.0316)</td>
</tr>
<tr>
<td>L(4, 3)</td>
<td>0.163 ± 0.086</td>
<td>0.0900 ± 0.0445</td>
</tr>
<tr>
<td>L(3, 4)</td>
<td>0.0057</td>
<td>0.0205 (0.0241)</td>
</tr>
</tbody>
</table>

¹ Fractional transfer coefficients [L[I, J]] are the fraction of compartment J's tracer or trace transferred to compartment I per day.
² Data are means ± SD for CN rats (n = 6). The three-compartment model is shown in Figure 2.
³ Data are means ± SD for ID rats whose data were fit by a four-compartment model (Fig. 2); n = 4. The value for the ID rat fit by a three-compartment model is shown in parentheses.

Table 3 lists the estimated and model-predicted compart-

### TABLE 3

| Compartment masses and plasma retinol fractional catabolic rate predicted by models for vitamin A metabolism in control (CN) and iron-deficient (ID) rats¹ |
|-----------------|------|------|
| M(I), nmol      | CN²  | ID³  |
| M(1)            | 11.1 ± 1.5 | 7.7 ± 1.8 (9.6) |
| M(2)            | 17.0 ± 3.7 | 10.1 ± 2.2* (13.6) |
| M(3)            | 379 ± 72 | 319 ± 187 (644) |
| M(4)            | 465 ± 53 | 465 ± 53 |
| M(3) + M(4)     | 379 ± 72 | 619 ± 51** (644) |
| M(TOTAL)²       | 407.2 ± 75.8 | 709 ± 50** (667) |

¹ Compartment masses [M(I, nmol)] were calculated from the plasma retinol concentration and the adjusted estimate of plasma volume [M(1)] or predicted by the compartmental models [M(2), M(3) and M(4)].
² M(TOTAL) is the sum of M(I) or the total amount of exchangeable vitamin A in the system.
³ Data are means ± SD for CN rats; n = 6. The three-compartment model is shown in Figure 2.
⁴ Data are means ± SD for ID rats whose data were fit to a four-compartment model (Fig. 2); n = 4. The value for the ID rat fit by a three-compartment model is shown in parentheses. *P < 0.05; **P < 0.0001 for CN vs. ID group.
levels of RBP are also reduced ID rats (Rosales et al. 1999). In the earlier reports by Amine et al. (1970) and Staab et al. (1984) in which rats were allowed free access to food, food intake, vitamin A intake and body weights were lower in ID than CN rats, presumably as a consequence of the impaired appetite that accompanies low iron status (Beard et al. 1995). Since we restricted food intake of CN rats in the current study, body weights were the same as in ID rats. Although vitamin A intake was 20% higher in ID than CN rats, plasma vitamin A concentration and pool size were lower (61 and 30%, respectively) in ID rats compared to the normal levels seen in CN rats. These results indicate that impaired growth and reduced appetite are not the cause of decreased plasma vitamin A concentration in ID rats.

In this study, we found some differences between liver vitamin A content determined by HPLC analysis (Table 1) and the model-predicted steady-state estimates of vitamin A stores (Table 3). In control rats, the predicted storage vitamin A pool [M(3)] was 3.4 times the liver vitamin A determined by HPLC. Although early data on plasma fraction of the injected dose vs. time determine M(3), and while CN rats apparently had more vitamin A in the liver at that time than later in the kinetic study (Table 1, t vs. tt), this only accounts for ~1/3 of the discrepancy. Further, results for radioactivity recovery indicate that the majority of vitamin A in CN rats was in extrahepatic sites (“fido carcasses”), rather than in the liver (“liver”)). That may also account for some of the difference and indicates that compartment 3 in the model for food-restricted CN rats represents almost 200 nmol of extrahepatic vitamin A as well as liver stores. Since these rats were food-restricted and very lean, it is not likely that these stores are in adipose tissue. For ID rats, our HPLC results indicate that there was 1.2 times more vitamin A in liver than was predicted by the model. The underestimation by the model may be due to the positive vitamin A balance in ID rats.

### TABLE 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CN</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(2, 1)</td>
<td>111 ± 33</td>
<td>80 ± 25 (110)</td>
</tr>
<tr>
<td>R(1, 2)</td>
<td>111 ± 33</td>
<td>80 ± 25 (110)</td>
</tr>
<tr>
<td>R(3, 1)</td>
<td>42.8 ± 6.7</td>
<td>31.0 ± 7.0* (35.9)</td>
</tr>
<tr>
<td>R(1, 3)</td>
<td>24.4 ± 3.9</td>
<td>14.0 ± 3.2* (15.5)</td>
</tr>
<tr>
<td>R(4, 3)</td>
<td>41.0 ± 17.2</td>
<td>41.0 ± 17.2</td>
</tr>
<tr>
<td>R(3, 4)</td>
<td>7.0* (35.9)</td>
<td>7.0* (35.9)</td>
</tr>
<tr>
<td>U(t)</td>
<td>18.4 ± 3.0</td>
<td>17.0 ± 4.0 (20.4)</td>
</tr>
<tr>
<td>FCRp 2.99</td>
<td>1.66 ± 0.13</td>
<td>2.22 ± 0.20* (2.12)</td>
</tr>
</tbody>
</table>

1 Transfer rates [R(I, J)] nmol/d are the amount of vitamin A transferred from compartment J to compartment I each day; plasma fractional catabolic rate [FCRp, d⁻¹] is the fraction of the plasma retinol pool utilized irreversibly each day.

2 Data are means ± SD for CN (n = 6). The three-compartment model is shown in Figure 2.

3 Data are means ± SD for ID rats (n = 4) whose data were fit to a four-compartment model (Fig. 2). The value for the ID rat fit by a three-compartment model is shown in parentheses. *P < 0.05 for CN vs. ID group.

### TABLE 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CN</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₁, h</td>
<td>1.79 ± 0.29</td>
<td>1.72 ± 0.36 (1.58)</td>
</tr>
<tr>
<td>t₂, h</td>
<td>3.76 ± 0.48</td>
<td>3.18 ± 0.87 (2.96)</td>
</tr>
<tr>
<td>t₃, d</td>
<td>8.83 ± 0.89</td>
<td>6.65 ± 6.60 (17.94)</td>
</tr>
<tr>
<td>t₄, d</td>
<td>14.6 ± 10.5</td>
<td>14.6 ± 10.5</td>
</tr>
<tr>
<td>t₁, d</td>
<td>0.607 ± 0.050</td>
<td>0.454 ± 0.041* (0.473)</td>
</tr>
<tr>
<td>tSYS, d</td>
<td>22.1 ± 2.7</td>
<td>43.7 ± 11.2* (32.8)</td>
</tr>
<tr>
<td>t₁</td>
<td>7.26 ± 0.79</td>
<td>5.47 ± 0.95* (6.17)</td>
</tr>
<tr>
<td>t₁, d</td>
<td>2.99 ± 0.44</td>
<td>8.23 ± 3.22* (5.24)</td>
</tr>
</tbody>
</table>

1 Parameters are mean transit time [t(I)] or the mean of the distribution of times a retinol molecule spends in compartment I during a single-transit; mean residence time [t(I)] or the mean of the distribution of times a retinol molecule spends in compartment I before irreversibly leaving compartment I; system residence time [tSYS] or the total time a retinol molecule spends in the system before irreversible loss; recycle number [n(I)] or the number of times on average a retinol molecule cycles back to plasma before irreversible loss; recycling time [tR(I)] or the time it takes for the average retinol molecule leaving plasma to cycle back.

2 Data are means ± SD for CN (n = 6). The three-compartment model is shown in Figure 2.

3 Data are means ± SD for ID rats (n = 4) whose data were fit to a four-compartment model (Fig. 2). The value for the rat whose data were fit to a three-compartment model is shown in parentheses. *P < 0.05 for CN vs. ID group.

### DISCUSSION

In agreement with previous studies, we found that dietary iron deficiency causes a reduction in plasma retinol levels (Amine et al. 1970, Rosales et al. 1999, Staab et al. 1984) and an elevation of vitamin A levels in liver of male Sprague-Dawley rats (Amine et al. 1970, Staab et al. 1984). Plasma
Another possible reason for this underestimation in ID rats might be that there exists some hepatic vitamin A which turned over so slowly that it was not detected by kinetic analysis over 48 d.

It is not clear why data for one ID rat required three compartments in the vitamin A model while the remaining four ID rats had four compartments. It is interesting that, for the ID rat with only three compartments (which also had the lowest liver vitamin A and the highest plasma retinol among the ID rats), the kinetic characteristics of compartment 3, such as the pool size [644 nmol] and turnover time [t(3)=17.9 d], are similar to the parameters for compartments 3 + 4 in the other ID rats [storage pool size 691 nmol and t=21.2 d]. This similarity suggests that vitamin A in the slow turning-over pools in all of ID rats had similar kinetic behavior, although in most of the rats, it was identified as two separate slow turning-over pools.

Vitamin A balance was estimated to be ~1.4 in CN and +6.8 nmol/d in ID rats (Table 1); that is, there was a slow depletion of liver vitamin A in CN rats and hepatic vitamin A accumulation in ID rats during the kinetic study. The non-steady state disposal rate R(0,3) calculated from U(1)-vitamin A balance was 19.8 nmol/d (CN) vs. 10.2 nmol/d (ID). These values are compatible with the results for radioactivity recovery. Based on a regression model developed earlier (Kelley and Green 1998), the disposal rate for ID rats is appropriate for their plasma retinol pool size. Thus an increased disposal rate did not lead to a decreased plasma retinol concentration but rather a reduction in vitamin A irreversible utilization was secondary to the reduced plasma retinol pool size. We do not know what, if any, effects this has on vitamin A function.

Although ID rats consumed more vitamin A than CN rats, the amount of vitamin A entering the system [U(1)] was similar in the groups. By estimating the absorption efficiency of vitamin A in rats from the daily intake of vitamin A and U(1), it appears that vitamin A absorption may be impaired ID rats (58% [ID] vs. 76% [CN]). The value for CN rats is the same as that measured directly in lymph duct-cannulated rats (Allen et al. 1994). Indirect evidence for impaired vitamin A absorption in ID rats is also shown by the data of Rosales et al. (1999) in which fed 3 μg Fe/g had a liver vitamin A pool of ~310 nmol compared to ~525 nmol in weight-matched, food-restricted CN rats.

Our models predict that movement of vitamin A between plasma and liver [R(3,1) and R(1,3), Table 4] is lower in ID rats; a dramatic reduction (~50%) was seen in R(1,3). It is possible that hepatic vitamin A accumulation in these ID rats is the consequence of impaired release of hepatic vitamin A into plasma due to a decrease in activity of one or more of the retinyl ester hydrolases. The lower vitamin A mobilization from liver may lead to the reduced size of the plasma retinol pool. It is not known if any of the hepatic retinyl ester hydrolases are iron-dependent enzymes or if activity is depressed by low-iron status. It is also possible that this response is not specific to vitamin A but may be part of a “malnutrition/disease signal” that down-regulates homeostatic systems for many nutrients.

In conclusion, our results confirm that plasma retinol level is reduced in ID Sprague-Dawley rats. Our kinetic analysis indicates that the reduction in plasma retinol concentration is the consequence of a decreased mobilization of vitamin A from liver, rather than an increased irreversible loss of vitamin A. Our results indicate that, while liver vitamin A can accumulate in iron deficiency, irreversible utilization and vitamin A absorption are reduced. Further research is needed to determine whether the reduced plasma retinol levels in iron deficiency have any metabolic consequences. Future work is also required to clarify how iron deficiency results in a reduction in liver vitamin A mobilization and vitamin A accumulation in liver.

LITERATURE CITED


Green, M. H., Green, J. B. & Lewis, K. C. (1987) Variation in retinol utilization


