Effects of N-(4-Hydroxyphenyl)retinamide on Vitamin A Metabolism in Rats (43849)

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Abstract. **Chronic administration of the anticancer drug N-(4-hydroxyphenyl)-retinamide (4-HPR) causes reductions in the plasma levels of vitamin A and its transport protein, retinol-binding protein. Here, we used model-based compartmental analysis to study effects of 4-HPR on the whole-body kinetics of vitamin A metabolism in rats. Rats (n = 8) were fed a purified diet containing vitamin A (~49 nmol retinol/day) plus 0 or ~50 μmole 4-HPR/(kg body wt · day). Plasma retinol kinetics were monitored for 35 days after intravenous administration of [^{3}H]retinol-labeled plasma. 4-HPR caused an 80% reduction in plasma retinol; after 40 days of treatment with 4-HPR, liver vitamin A levels were 2.33 times higher than those of control rats. A three compartment model, in which plasma retinol exchanges with two extravascular compartments, was required to fit data for both groups. Vitamin A input was via the central plasma compartment, while irreversible loss was via the larger extravascular compartment. The time retinol spent in plasma before reversible or irreversible exit was normal (1.7 hr) in 4-HPR-treated rats, but the rate of plasma retinol turnover was reduced, and the recycling of retinol to plasma was delayed and reduced. Vitamin A utilization was significantly lower in 4-HPR-treated rats (20 nmol retinol/day vs 42 nmol/day in controls). We conclude that 4-HPR partially blocks access and thus binding of retinol to retinol-binding protein and may therefore lead to vitamin A accumulation in certain cells.** [P.S.E.B.M. 1995, Vol 208]

N-(4-Hydroxyphenyl)retinamide (4-HPR) is a synthetic retinoid which inhibits neoplastic development in the mammary gland (1), urinary bladder (2), and skin (3) of mice. In contrast to many other retinoids, 4-HPR is not stored in substantial amounts in the liver; thus, its potential for causing hepatotoxicity is limited (4). 4-HPR has a high therapeutic index and exhibits low toxicity in rats and mice. However, in humans, chronic administration of 4-HPR is associated with reductions in the plasma levels of retinol and retinol-binding protein (RBP) (4, 5), and with dark adaptation problems (6–8).

In a recent study in our labs (9), we found that the acute iv administration of 4-HPR to rats led to declines in plasma retinol and RBP but the turnover times for these entities were similar to the plasma turnover time for labeled retinol in kinetic studies (10). This observation indicates that the 4-HPR-related decrease in plasma retinol levels is due to a decreased input of retinol into plasma. In order to further investigate the mechanism by which 4-HPR lowers plasma levels of retinol and RBP, we designed the present study to compare plasma retinol kinetics in control versus 4-HPR-treated rats. By applying the technique of model-based compartmental analysis (11), we obtained information on the rates of transfer of retinol between kinetically distinct compartments, the total traced mass of vitamin A and several other kinetic parameters which describe the dynamics of retinol metabolism in rats.

Materials and Methods

Animals and Diets. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were obtained as weanlings and housed individually in suspended, stainless steel, mesh-bottomed cages, in quarters maintained at 22°–23°C, 60% relative humidity and with a 12-hr light cycle (0700–1900 hr). Food and water were continuously available. Body weights and esti-

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mates of food intake were routinely recorded. Animal procedures were approved by The Pennsylvania State University's Institutional Animal Care and Use Committee.

Rats to be used as recipients of retinol-labeled plasma (n = 8) were fed a nutritionally adequate, purified diet (12) containing 2.63 nmol of retinol as retinyl palmitate (Sigma Chemical Co., St. Louis, MO) per gram of diet. This dietary regimen was formulated to produce marginal liver vitamin A stores (~350 nmol/liver). After 7–8 weeks, 4-HPR (700 μmol/kg diet; R. W. Johnson Pharmaceutical Research Institute, Spring House, PA) was added to the diet of four of the rats (4-HPR group) to obtain a dose of ~50 μmol 4-HPR/(kg body wt · day). This level was chosen to elicit the desired reduction in plasma retinol levels (9). Rats consumed the 4-HPR-containing diet for 5 days prior to and during the retinol turnover study. The four other rats were fed the control diet throughout the study (control group).

Preparation of Retinol-Labeled Plasma. All procedures involving vitamin A were carried out under amber light. A dispersion (1 ml containing ~18.3 MBq of [^{3}H]) of [11,12(n)-^{3}H]retinol (specific radioactivity 2.22 TBq/mmol; Amersham Corp., Arlington Heights, IL) in Tween 40 (Sigma) was injected into a vitamin A-depleted donor rat and plasma containing [^{3}H]retinol/RBP/transthyretin (TTR) was collected 100 min later (13). Plasma was stored under a nitrogen atmosphere at 4°C and was injected into recipients within 48 hr of collection.

***In Vivo* Turnover Studies.** Nonfasting recipient rats were anesthetized with methoxyflurane (Pitman-Moore, Washington Crossing, NJ) between 0930 and 1045 hr. A weighed aliquot of [^{3}H]retinol/RBP/TTR-labeled plasma (~0.4 g containing ~1 MBq of [^{3}H]) was injected into an exposed jugular vein and anesthesia was immediately removed. For determination of plasma radioactivity, serial blood samples (~250 μl, n = 22) were obtained from a caudal vein at geometrically increasing intervals from 10 min after the dose was administered until the rats were killed (35 days). This sampling schedule resulted in removal of <7% of total blood volume during the first 24 hr. Blood samples were collected into tubes containing Na_2EDTA (final concentration, ~4 μmol/l). At five sampling times (1, 8, 16, 27, and 35 days), additional blood (~100 μl) was obtained for the determination of plasma retinol concentration. Aliquots of plasma were stored at −20°C under nitrogen atmosphere for subsequent analysis.

At the time rats were to be sacrificed, they were anesthetized with methoxyflurane. The whole body was perfused with ~200 ml of Hanks' balanced salt solution (pH 7.4) that was circulated from the left cardiac ventricle to the right auricle. Livers and kidneys were isolated, blotted, weighed, frozen, lyophilized in the dark, purged with nitrogen and stored at −20°C until analyzed. Carcasses were weighed, frozen and stored until analysis.

Plasma and Tissue Analyses. All chemicals used were reagent grade or HPLC grade. Lipids in aliquots of plasma and the injected dose were extracted into hexane as previously described (13), and samples of liver and kidneys were saponified and extracted using a modification (14) of the procedure of Thompson *et al.* (15). Solvent-free lipid extracts were analyzed by liquid scintillation spectrometry after solubilization in Ecoscint O (National Diagnostics, Manville, NJ). Samples were counted twice (Model 3801; Beckman Instruments, Irvine, CA) to a final 2-σ error of 1.0%. Sample counts were automatically corrected for background and converted to Bq using an external standard method.

Retinol mass in extracts of plasma, and total (unesterified plus esterified) retinol mass in liver and kidneys, was determined by reverse phase HPLC as previously described (10) with the following changes. We used retinyl acetate as an internal standard for plasma and kidney samples, and 15,15-dimethyl retinol (12) for liver. For plasma samples and livers from the 4-HPR group, a Nova-Pak C-18 column and guard column (Millipore Corp., Milford, MA) and Model 440 ultraviolet detector (Millipore) were used, and methanol:water (90:10 v/v, 1 ml/min) was used as the mobile phase. Kidney samples were similarly analyzed except that the column used was a Resolve C-18 (Millipore). For liver samples from the control group, a Resolve C-18 column and methanol:water (95:5 v/v) were used.

Frozen carcasses were allowed to partially thaw and were then cut into ~2.5-cm cubes. Cubes were ground in a meat grinder (Chop-Rite, Pottstown, PA) that was motorized by a MAXI-TORQ split phase gear motor (Model 6K583, Dayton Electric Co., Chicago, IL). Trapped carcass residues were extruded with bread. Samples were collected into a preweighed dish, the grinder was disassembled, and each part was cleaned into the dish. The contents of the dish were mixed manually until they appeared to be homogeneous and triplicate 3-g aliquots were placed into 50-ml round-bottom tubes. Lipids were extracted using a modification of the method of Hara and Radin (16). First, 7.2 ml of isopropanol was added to each sample, and samples were allowed to incubate, with several mixings, for 1 hr. Next, 10.8 ml of hexane containing 22.7 nmol of butylated hydroxytoluene/ml was added, samples were mixed and left to incubate for 1 hr. As described in detail elsewhere (13), samples were then washed with 9 ml of aqueous sodium sulfate (0.56 M), the upper phase was removed by aspiration, and the aqueous phase was washed twice with 4 ml of hexane:isopropanol (7:2, v/v). Aliquots of the extract

were taken for analysis of radioactivity as described above.

Kinetic Analyses. For each rat, fraction of the injected dose remaining in plasma was calculated from the observed Bq/ml at each time and the estimated plasma tracer concentration at time zero (Bq injected/estimated plasma volume, where plasma volume = body weight [g] · 0.038 ml of plasma/g body wt [17]). This calculation thus took into account the slow increase in body weights during the turnover study (see Results). Fraction of the dose that was irreversibly lost was calculated as 1.0 − (fraction of the injected dose recovered at the time of killing in plasma + liver + kidneys + carcass). Thus, this variable reflects excretion of labeled vitamin A metabolites.

Then, model-based compartmental analysis (11, 13) was used to develop a model of vitamin A kinetics as viewed from the plasma space and to calculate kinetic parameters. Our aim was to develop a simple compartmental description of whole body vitamin A dynamic behavior in which many processes with similar kinetics would be lumped in the same compartment. First, to obtain information on the number of kinetically distinct compartments and to get initial estimates for model connectivities, plasma tracer data for each rat were fit to a multiexponential equation (10) using the conversational version (CONSAM) (18) of the Simulation, Analysis and Modeling computer program (SAAM) (19). Since data for all animals required at least a three-component equation, we postulated a three-compartment model, in which the central plasma retinol compartment exchanges with two extravascular compartments. A centrally exchanging (mammillary) model was chosen, since we have previously found (10) that plasma retinol mixes with at least two kinetically distinct extravascular pools. Next, model-based compartmental analysis (11) was applied to develop the simplest compartmental model that was compatible with tracer data for plasma and irreversible loss. For each rat, weighted, nonlinear regression analysis in CONSAM was used to estimate the model interconnectivities (fractional transfer coefficients or L[I,J]s; see below) and to obtain estimates of the statistical uncertainty for model parameters. A fractional standard deviation of 0.05 was assigned as a weighting factor to each datum. Next, the computer files for the four rats in each group were combined and the "multiple studies" feature of SAAM and CONSAM (20) was used to determine the group population mean and standard error of the mean for the model L(I,J)s. These mean L(I,J)s and the group mean plasma retinol pool sizes (nmol) were then used to calculate a steady state solution to the model in order to obtain estimates of other kinetic parameters (see below).

Fractional transfer coefficients (L[I,J]s) are defined as the fraction of Compartment J's tracee or tracer transferred to Compartment I per day. Retinol transfer rates (R[I,J]; nmol/day) are defined as L(I,J) · M(J), where M(J) (nmol) is the mass of vitamin A in compartment J. Model-predicted mean transit time ($\bar{t}$[I]) is the mean of the distribution of times retinol molecules entering Compartment I spend there during a single transit before leaving reversibly or irreversibly, and is calculated as the inverse of the sum of the fractional transfer coefficients describing output from Compartment I. Mean residence time ($\bar{T}$[I,J]) is the mean of the distribution of times that retinol molecules spend in Compartment I from the time of entering the system via Compartment J until leaving Compartment I irreversibly. For the plasma compartment, residence time is calculated as the area under the curve for fraction of the injected dose in plasma versus time, integrated from the time of dose injection to infinity. System residence time ($\bar{T}$[SYS]) is the sum of the individual compartment residence times. Fractional catabolic rate (FCR[I,J]) is the fraction of molecules in Compartment I that leave irreversibly per day after introduction into the system via Compartment J and is calculated as $1/\bar{T}$(I,J). Recycle number (ν[I]) is the average number of times a retinol molecule recycles through Compartment I before irreversibly exiting that compartment and is calculated as ($\bar{T}$[I]/$\bar{t}$[I]) − 1. Recycling time ($\bar{\tau}$[I]) is the time it takes for the average retinol molecule leaving Compartment I to cycle back and is calculated as ($\bar{T}$[SYS] − $\bar{T}$[I])/ν[I]. See Ref. 13 for more detailed information on methods of calculation.

Statistical Analyses. Descriptive data are presented as arithmetic group mean ± population estimate of the standard deviation. Descriptive data were statistically compared using Student's unpaired *t* test in Minitab (21) or AOV with repeated measures (plasma retinol concentration). An α level of 0.05 was used as the significance limit for statistical analyses. Values for fractional transfer coefficients (L[I,J]) are presented as population estimates of the mean ± a population estimate of the standard error of the mean (SEM). For other kinetic parameters the group mean values are presented with the SEM calculated from the individual animal models. Kinetic parameters were considered significantly different between groups if the population estimate for the mean value ±1 SEM did not overlap.

Results

Descriptive and Kinetic Data. Group mean body weights were similar in control and 4-HPR-treated rats at both the beginning (370 ± 13 and 370 ± 11 g, respectively; mean ± SD, n = 4) and end of the turnover study (452 ± 16 and 442 ± 17 g, respectively). Data on retinol masses and tracer recovery in plasma, liver, kidneys, and carcass are shown in Table I. Mean

Table I. Tissue Vitamin A Masses and Tracer Recovery[a]

	Vitamin A mass			Fraction of injected dose				
	Plasma (μmol/l)	Liver (nmol)	Kidney (nmol)	Plasma	Liver	Kidney	Carcass	Total
Control								
1	1.91	698	18.0	0.00508	0.171	0.00302	0.111	0.290
2	1.84	591	19.1	0.00507	0.153	0.00345	0.127	0.289
3	1.90	539	14.3	0.00492	0.142	0.00239	0.125	0.274
4	1.87	749	23.6	0.00531	0.173	0.00382	0.135	0.317
Mean	1.88	644	18.7	0.00510	0.160	0.00317	0.124	0.292
SD	0.03	96	3.8	0.00016	0.015	0.00061	0.010	0.0179
4-HPR								
5	0.44	1373	18.9	0.00162	0.329	0.00355	0.0857	0.420
6	0.41	1549	16.0	0.00157	0.367	0.00278	0.0737	0.445
7	0.35	1596	35.8	0.00193	0.365	0.00593	0.0663	0.439
8	0.44	1484	26.7	0.00174	0.324	0.00398	0.0897	0.419
Mean	0.41	1501	24.3	0.00172	0.346	0.00406	0.0789	0.431
SD	0.04	97	8.9	0.00016	0.023	0.00134	0.0108	0.013
$P<$	0.0001	0.0001	NS	0.0001	0.0001	NS	0.0016	0.0001

[a] Data are individual rat values, as well as mean ± SD, for plasma retinol concentrations, liver and kidney vitamin A masses, and for fraction of injected dose of [^{3}H]retinol-labeled plasma recovered in plasma, liver, kidneys, carcass, and their sum (total) for rats in the control or 4-HPR-treated groups. Data were obtained from rats sacrificed 35 days after injection of [^{3}H]retinol-labeled plasma.

plasma retinol concentrations did not change significantly during the turnover study except in one 4-HPR-treated rat in which plasma retinol increased from 0.26 μmol/l on Day 1 to 0.42 μmol/l on Day 35 of the turnover study. Plasma retinol concentrations were significantly lower in 4-HPR-treated than control rats (0.41 vs 1.9 μmol/l, respectively). At the end of the study, liver vitamin A levels (unesterified plus esterified retinol) were significantly higher in 4-HPR-treated rats (1501 nmol [85.6 ± 10.0 nmol/g wet weight] vs 644 nmol [43.4 ± 5.1 nmol/g]). Fraction of the injected dose recovered in liver was also significantly higher in 4-HPR-treated versus control rats (0.35 vs 0.16, respectively). In contrast, kidney vitamin A levels and recovery of the injected dose were not significantly different between groups. Recovery of tracer in carcass 35 days after administration of [^{3}H]retinol-labeled plasma was significantly lower in 4-HPR-treated rats (0.079 vs 0.12 for the control group). Total tritium recovered in the body at 35 days was significantly higher for the 4-HPR-treated versus control rats (43% vs 29% of the injected dose, respectively). That is, irreversible loss (excretion) was higher in the control rats.

Data on fraction of the injected dose remaining in plasma versus time after administration of [^{3}H]retinol-labeled plasma are shown in Figure 1 for one representative rat from each group. The initial rapid disappearance of label from the plasma was similar in both groups. By 6 hr, plasma disappearance curves for rats in the two groups began to diverge. Specifically, after 6 hr, the slope of the curve decreased more in control rats than in those treated with 4-HPR, indicating that there was more extensive recycling of labeled retinol to plasma in control rats. The terminal slopes were similar in the two groups (Fig. 1) (0.033/day for the control rat and 0.024/day for the 4-HPR-treated rat). That is, 2%–4% of the exchangeable vitamin A pool was irreversibly utilized each day in both groups. By 35 days, cumulative irreversible loss of label was greater in the control group (Fig. 1).

Compartmental Model and Kinetic Parameters. In the three-compartment, "working hypothesis" model shown in Figure 2, plasma retinol (Compartment 1) is the site of input for both tracer and for newly absorbed dietary vitamin A after it is processed in the liver. Retinol in Compartment 1 exchanges with retinol in two extravascular compartments. Compartment 3, the extravascular compartment with slower turnover, is the site of irreversible loss and is hypothesized to represent primarily retinyl esters located mainly in storage sites such as liver perisinusoidal stellate cells. Compartment 2, the extravascular pool with more rapid turnover, is hypothesized to contain primarily extravascular retinol.

Before arriving at this model structure, we tested models in which irreversible loss was from Compartment 2 and dietary input was into Compartment 3. Neither of these structures was compatible with vitamin A balance data nor provided an adequate fit to tracer data for plasma and irreversible loss. The model presented is reasonable in view of what is currently known about whole body vitamin A dynamics. Specifically, this model implies that newly absorbed vitamin A was transported to liver parenchymal cells and the vast majority of this retinol was secreted into the plasma bound to RBP prior to access to sites of degradation. Thus, input of newly absorbed vitamin A was into Compartment 1. Compartment 3 may contain

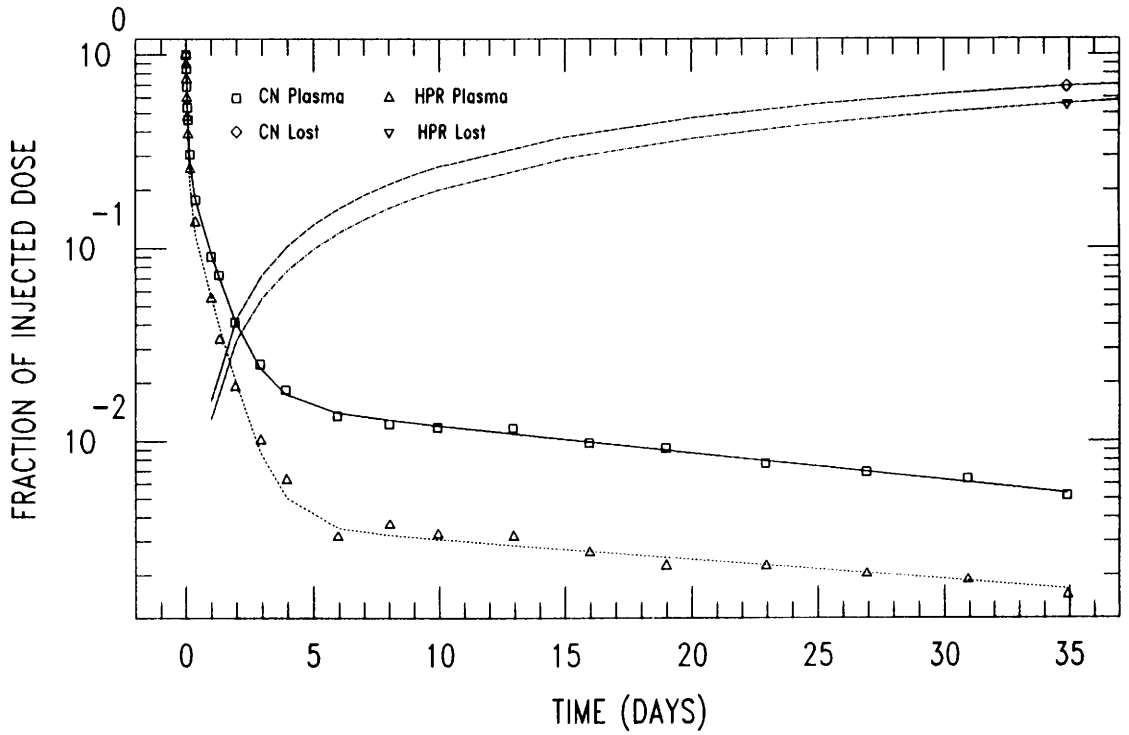

Figure 1. Shown are observed data (symbols) and model-predicted values (lines) for fraction of injected dose remaining in plasma or irreversibly lost versus time after administration of [^{3}H]retinol-labeled plasma for one representative rat from each group (control group, plasma [□] and loss [◇]; 4-HPR group, plasma [△] and loss [▽]).

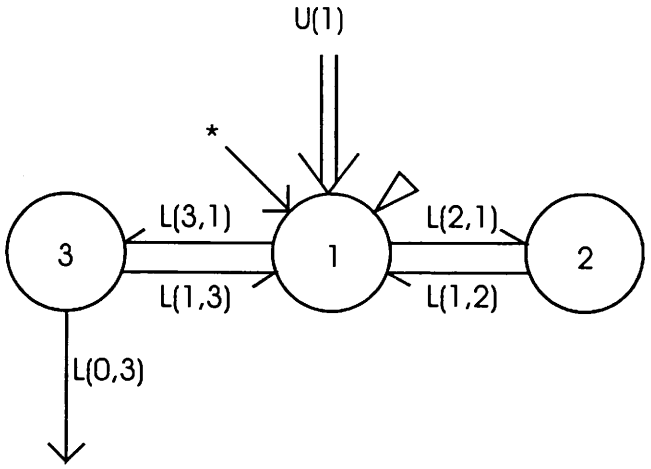

Figure 2. Proposed model for vitamin A turnover. Compartments are represented by circles. Numbers between the compartments are fractional transfer coefficients (L[I,J]; day^{-1}). The asterisk denotes the site of injection of [^{3}H]retinol/RBP/TTR-containing plasma and the triangle denotes the site of sampling. U(1) denotes input of dietary retinol into plasma via chylomicron remnants after hepatic processing.

Table II. Model-Predicted Fractional Transfer Coefficients[a]

	Control	4-HPR
L(2,1)	9.73 ± 0.75[b]	8.83 ± 0.43[b]
L(1,2)	7.58 ± 1.23[b]	4.52 ± 0.37[c]
L(3,1)	3.57 ± 0.089[c]	5.20 ± 0.14[b]
L(1,3)	0.0723 ± 0.0052[b]	0.0202 ± 0.00058[c]
L(0,3)	0.0416 ± 0.0022[b]	0.0256 ± 0.0014[c]

[a] Shown are group mean fractional transfer coefficients (L[I,J], or the fraction of Compartment J's tracee or tracer transferred to Compartment I per day] ± estimated standard error of the mean (n = 4). Means in the same row that do not share a common superscript letter are significantly different (i.e., the means ± 1 SEM do not overlap). The model is shown in Figure 2.

retinyl ester stores in exchange with retinol or other vitamin A compounds involved in degradation. These pools appear to act kinetically as a single compartment but we do not predict that retinyl ester stores are the primary site of vitamin A degradation.

Group mean values for the fractional transfer coefficients describing the proposed model are shown in Table II. The fractional transfer coefficient describing the movement of retinol out of the vascular bed and into the smaller extravascular pool with faster turnover (L[2,1]) was not significantly different between the two groups. In both groups, 9–10 pools of plasma retinol were transferred to Compartment 2 per day. The fractional transfer coefficient describing retinol transfer out of the vascular bed and into the larger extravascular pool with slower turnover (L[3,1]) was higher in the 4-HPR-treated rats. In the control group, 3.6 pools of plasma retinol transferred to Compartment 3 each day (versus 5.2 pools/day in the 4-HPR-treated rats). The fractional transfer coefficients describing recycling of retinol to plasma from both the extravascular compartments with fast (L[1,2]) and slow (L[1,3]) turnover, and irreversible loss from the

latter compartment (L[0,3]), were all lower in 4-HPR-treated rats.

Calculated mean values for kinetic parameters in control versus 4-HPR-treated rats are shown in Table III. Plasma retinol transit time ($\bar{t}$[1]) was similar in both groups (1.7–1.8 hr) and compatible with previous calculations (10, 14, 22). In contrast, plasma retinol residence time ($\bar{T}$[1,1]) was lower in 4-HPR-treated rats (0.33 vs 0.75 days for control rats). The retinol transit time for Compartment 2 ($\bar{t}$[2]) was higher in the 4-HPR group (5.3 hr) as compared with the control group (3.2 hr), while the retinol residence time for Compartment 2 ($\bar{T}$[2,1]) was lower in the 4-HPR group (0.65 vs 0.97 days in control rats). The retinol transit time for Compartment 3 ($\bar{t}$[3]) was higher in the 4-HPR group (22 days) than in the control group (8.8 days), as were $\bar{T}$(3,1) (36 days in 4-HPR group vs 24 days in the control group) and the system residence time ($\bar{T}$[SYS]) (37 days in 4-HPR group vs 25 days in the control group). The plasma retinol recycling number for the 4-HPR group (3.7) was less than half that of the control group (9.0), and the plasma retinol recycling time was almost quadrupled in the 4-HPR group (10 days) relative to the control group (2.8 days).

Plasma retinol pool sizes, model-predicted plasma retinol fractional catabolic rate, steady state estimates of compartment masses, transfer rates and vitamin A output rates are shown in Table IV. Plasma retinol pool size was significantly higher in control versus 4-HPR-treated rats (32 vs 6.9 nmol, respectively). The model-predicted retinol masses in Compartment 2 and 3 were also lower in 4-HPR versus control rats. Therefore, the total traced mass was lower in the 4-HPR group (803 nmol) compared with the control rats (1086 nmol). Our model underestimated the total traced mass of vitamin A in 4-HPR-treated rats relative to observed liver vitamin A levels and the model predicts that ~40% of the vitamin A is in exchangeable, extrahepatic pools in control rats. All transfer rates describing plasma retinol output to, and input from, the extravascular compartments were significantly lower in the 4-HPR-treated rats. The vitamin A disposal rate (irreversible loss, R[0,3]) in the 4-HPR group was only 47% that in control animals (42 nmol/day). Plasma fractional catabolic rate (FCR_p) was higher in the 4-HPR group (3.0/day) than in the control group (1.3/day).

Table III. Model-Predicted Kinetic Parameters[a]

	Control	4-HPR
$\bar{t}$(1) (hr)	1.80 ± 0.06[b]	1.71 ± 0.04[b]
$\bar{t}$(2) (hr)	3.17 ± 0.18[c]	5.32 ± 0.19[b]
$\bar{t}$(3) (days)	8.77 ± 0.16[c]	21.8 ± 0.43[b]
$\bar{T}$(1,1) (days)	0.750 ± 0.005[b]	0.330 ± 0.002[c]
$\bar{T}$(2,1) (days)	0.970 ± 0.030[b]	0.650 ± 0.010[c]
$\bar{T}$(3,1) (days)	23.5 ± 0.40[c]	35.9 ± 0.71[b]
$\bar{T}$(SYS) (days)	25.2 ± 0.41[c]	36.9 ± 0.71[b]
ν(1)	9.03 ± 0.37[b]	3.70 ± 0.11[c]
$\bar{tt}$(1) (days)	2.78 ± 0.21[c]	10.0 ± 0.62[b]

[a] All values are mean ± an estimate of the SEM (n = 4). Means for kinetic parameters that have a different superscript letter are significantly different. Parameters are model-predicted mean transit times ($\bar{t}$[I]) or the mean of the distribution of times retinol molecules entering Compartment I spend there during a single transit before leaving reversibly or irreversibly; mean residence times ($\bar{T}$[I,J]) or the mean of the distribution of times that retinol molecules spend in Compartment I from the time of entering the plasma retinol/RBP/TTR pool (Compartment J) until leaving Compartment I irreversibly; recycle number (ν[I]) or the average number of times a retinol molecule recycles through Compartment I before irreversibly exiting that compartment; and recycling time ($\bar{tt}$[I]) or the time it takes for the average retinol molecule leaving Compartment I to cycle back. The model is shown in Figure 2.

Table IV. Model-Predicted Compartment Masses, Transfer Rates, and Plasma Retinol Fractional Catabolic Rate[a]

	Control	4-HPR
M(1) (nmol)	32.3 ± 1.5[b]	6.89 ± 0.39[c]
M(2) (nmol)	41.5 ± 1.3[b]	13.5 ± 0.29[c]
M(3) (nmol)	1012 ± 15[b]	782 ± 16[c]
M(Total) (nmol)	1086 ± 16[b]	803 ± 16[c]
R(2,1) (nmol/day)	314 ± 15[b]	60.9 ± 2.2[c]
R(1,2) (nmol/day)	314 ± 15[b]	60.9 ± 2.2[c]
R(3,1) (nmol/day)	115 ± 1.3[b]	35.8 ± 0.33[c]
R(1,3) (nmol/day)	73.2 ± 1.2[b]	15.8 ± 0.28[c]
R(0,3) (nmol/day)	42.2 ± 0.30[b]	20.0 ± 0.17[c]
FCR_p (day^{-1})	1.33 ± 0.01[b]	3.02 ± 0.02[c]

[a] Data presented are the model predictions for the population means for compartment masses, transfer rates, and plasma retinol fractional catabolic rate plus a measure of their statistical uncertainty (±SEM). Data are group mean plasma retinol pool size (M[1]) and model-predicted masses (M[I]) for Compartment 2 and 3. Means for the population that have a different superscript letter are significantly different. Also listed are group mean model-predicted transfer rates (R[I,J]) or the amount of retinol (nmol) transferred from Compartment J to Compartment I per day, and the plasma retinol fractional catabolic rate ([FCR_p]) or the daily irreversible utilization of vitamin A as a fraction of the plasma retinol pool size. R(0,3) is the system disposal rate or the daily rate of irreversible utilization of vitamin A. The model is shown in Figure 2.

Discussion

Although the synthetic retinoid 4-HPR offers promise as a chemopreventive agent for human breast cancer (7), its usefulness may be limited because it also causes dramatic reductions in plasma retinol concentrations. In order to investigate the mechanism by which chronic administration of 4-HPR affects plasma retinol levels, we used compartmental analysis (11) to study the influence of this drug on plasma retinol kinetics and other parameters of vitamin A metabolism in the rat. Our finding that plasma retinol transit time was not affected by 4-HPR indicates that the observed decrease in plasma retinol concentration is not due to a more rapid turnover of vitamin A from plasma. This is compatible with our earlier results (9) on the acute

effects of 4-HPR on levels of unlabeled plasma retinol and retinol-binding protein.

We propose the following explanation of why the estimate of total traced mass was so much lower in 4-HPR-treated rats than controls. Assuming control rats were in vitamin A balance during the turnover study, then input equals disposal rate (42.2 nmol/day). If input is the same in 4-HPR-treated rats (23), but disposal rate averages 20 nmol/day (Table IV), then those rats were in a positive vitamin A balance of 22.2 nmol/day, or 888 nmol by the end of the 40 days of treatment with 4-HPR. The sum of this value and the mean liver vitamin A level for control rats (644 nmol) (Table I) is 1532 nmol, which is similar to the observed mean liver vitamin A level for 4-HPR-treated rats (1501 nmol). Thus we hypothesize that vitamin A absorption is normal in 4-HPR-treated rats, but newly absorbed vitamin A does not exchange normally with endogenous vitamin A pools.

Based on current observations and previous studies (9, 24), we propose the following mechanism of action for 4-HPR. Since absorption of diet-derived vitamin A does not appear to be affected by 4-HPR (23), we assume that input of vitamin A is normal in 4-HPR-treated rats. In control animals, most of the chylomicron retinyl esters are taken up by hepatocytes, the retinyl esters are hydrolyzed to retinol and the retinol is transported to the endoplasmic reticulum where it can bind to apoRBP for secretion as holoRBP (25). Previous work in our labs indicates that the transfer of retinol to apoRBP may be blocked by 4-HPR (9). Berni and Formelli have recently shown (26) that 4-HPR binds to RBP but the complex does not bind to TTR with the same affinity as does holoRBP. We speculate that this apparent block in hepatocytes and other cells may account for the decrease in plasma retinol levels seen in 4-HPR-treated rats. We hypothesize that the 4-HPR-related increase in liver vitamin A seen in this study is due to the accumulation of diet-derived retinol in hepatocytes and that this accumulation would lead to reesterification of retinol and storage in hepatocytes. Normally, most of the storage of liver retinyl esters occurs in perisinusoidal stellate cells (25). Thus, we hypothesize that 4-HPR may cause an abnormal storage of vitamin A in hepatocytes.

In conclusion, results of the current study indicate that chronic feeding of 4-HPR (50 μmol/[kg body wt · day]) to rats does not affect growth rate but leads to a dramatic reduction in plasma retinol levels, an accumulation of vitamin A in the liver, a decrease in vitamin A utilization and a decrease in the rate of retinol secretion into plasma. The 4-HPR-treated rats irreversibly utilized only half as much vitamin A per day compared with the control group rats but the fractional catabolism of plasma retinol was greater. This implies that, although the vitamin A disposal rate is reduced by 4-HPR treatment, there is a pool of retinol or a metabolite building up that drives irreversible loss at a rate greater than would be predicted by the plasma retinol pool size (10). Our results raise the interesting possibility that the anticarcinogenic action of 4-HPR may not be due to a direct effect of 4-HPR but rather result from an (indirect) increase in intracellular retinol or one of its metabolites. This might be especially significant in tissues such as the mammary gland which accumulates 4-HPR (7) and has the potential to obtain large amounts of vitamin A from chylomicrons. If 4-HPR administration blocked the secretion of such retinol on RBP, the extra retinol and/or a metabolite might be available for stimulating cell differentiation.

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