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Vitamin A status affects the efficacy of iron repletion in rats with mild iron deficiency

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In populations with vitamin A deficiency, vitamin A administration in addition to supplemental iron has been shown to further improve blood indicators of iron status. To obtain clues to associated changes at the level of organ indicators of iron status, we have attempted to mimic previous human studies in a rat model. The influence of vitamin A on selected indices of iron metabolism was studied during iron repletion of rats with mild iron deficiency combined with either vitamin A deficiency or normal vitamin A status. In the vitamin A–deficient rats, but not in those with normal vitamin A status, the administration of vitamin A raised body weight and liver weight. Vitamin A administration also raised the total iron-binding capacity and plasma iron concentrations but depressed iron concentrations in liver, kidney, spleen, and bone in the vitamin A–deficient rats. We conclude that vitamin A administration to rats with both vitamin A and iron deficiency enhances the utilization of iron. (J. Nutr. Biochem. 7:99–105, 1996.)

Keywords: vitamin A deficiency; iron deficiency; iron metabolism; iron repletion; rats

Introduction

Studies with children[1–4] and pregnant women[5] have shown that vitamin A deficiency is associated with impaired iron status, while vitamin A supplementation produced an increase in blood hemoglobin concentrations.[2,6–10] The mechanism by which vitamin A affects iron metabolism is poorly understood. Vitamin A deficiency in rats may impair erythropoiesis[11,12] but increased iron concentrations in liver[13–15] and spleen[12,13] may point to disturbed iron transport.

The administration of vitamin A in addition to supplemental iron has been found to enhance the response of serum iron concentration, transferrin saturation, and blood hemoglobin concentration in children and pregnant women with low vitamin A and iron status.[8,10] This suggests that vitamin A improves the utilization of ingested iron and affects the iron concentrations of organs. To test this hypothesis and to develop an animal model for studying the underlying mechanisms, we examined the influence of vitamin A administration on selected indices of iron metabolism during iron repletion in rats with mild iron deficiency. Because the effect of vitamin A administration may depend on the vitamin A status of the rats, we used rats with either normal or low vitamin A status. In vitamin A supplementation studies with children, growth has been found to be enhanced[7,16] which in turn might influence the observed interrelationship between vitamin A and iron metabolism. In order to mimic the situation in children, we allowed our rats to have access to feed ad libitum so that not only feed composition but also feed intake could influence growth. Any confounding effects of growth on iron parameters were assessed by using body weight as a covariate in the statistical analysis.

Methods and materials

This experiment was approved and supervised by the animal welfare officer of Wageningen Agricultural University.

Animals, diets, and housing

Seventy-two, 3-week-old male Wistar rats (Cpb:WU) were purchased from a commercial breeder (Harlan CPB, Zeist, The Neth-

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erlands). They were divided into two groups of 36 rats matched for body weight. The experimental design is illustrated in Figure 1. During the first experimental phase of 25 days (Figure 1), one group was fed a diet with adequate vitamin A (1,200 retinol equivalents [RE]/kg) and iron (35 mg/kg), The other group was fed a diet without vitamin A but with adequate iron. The diet adequate in vitamin A was formulated according to the nutrient requirements of rats (National Research Council).[17] The diets were in the form of powder and stored at 4°C until feeding.

On day 25 (Figure 1), when the rats were about 45 days old, a blood sample was taken for hematological examination and analysis of plasma retinol. All animals entered the iron depletion period and continued to receive their respective diets, except that iron was omitted (Figure 1). On days 39 and 60 (Figure 1), blood samples were taken again for hematological examination and analysis of plasma retinol.

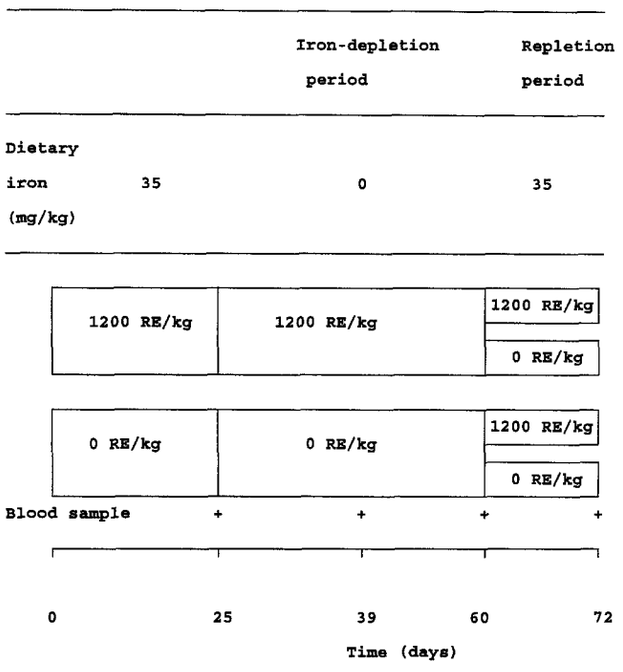

Figure 1 Experimental design: During the first experimental phase, two groups of 36 rats were fed different levels of dietary vitamin A. From day 25, when the rats were 45 days old until day 60, all rats were maintained on an iron depletion diet followed by an iron-repletion period (days 60 to 72). Simultaneously, vitamin A intake was varied so that during the repletion period there were four groups of 18 each, differing in vitamin A intake during the periods. The rats were killed on day 72 for the removal of tissues. The diets used differed in vitamin A and iron content only. In the figure the amounts are indicated as retinol equivalents (RE) and mg of iron/kg of feed. The amount of vitamin A, added to the diets as Rovimix A 500® (150 RE/mg; F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland; consisting of retinyl acetate and retinyl palmitate) was 1200 RE/kg. For the vitamin A–deficient diets, no vitamin A was added. Per kilogram of iron-sufficient diet, 174 mg of $FeSO_4 \cdot 7H_2O$ was added. No iron was added to the diet low in iron. All diets contained (g/kg feed): casein, 151; corn oil, 25; coconut fat, 25; glucose, 709.2; cellulose, 30; $CaCO_3$, 12.4; $NaH_2PO_4 \cdot 2H_2O$, 15.1; $MgCO_3$, 1.4; KCl, 1.0; $KHCO_3$, 7.7; mineral premix (iron-free), 10; vitamin premix (vitamin A-free), 12. The mineral premix consisted of (mg): MnO_2, 79; $ZnSO_4 \cdot H_2O$, 33; $NiSO_4 \cdot 6H_2O$, 13; NaF, 2; KI, 0.2; $CuSO_4 \cdot 5H_2O$, 15.7; $Na_2SeO_3 \cdot 5H_2O$, 0.3; $CrCl_3 \cdot 6H_2O$, 1.5; $SnCl_2 \cdot 2H_2O$, 1.9; NH_4VO_3, 0.2; corn meal, 9853.2. The vitamin premix consisted of (mg): thiamin, 4; riboflavin, 3; niacin, 20; D,L-calcium pantothenate, 17.8; pyridoxine, 6; cyanocobalamin, 50; choline chloride, 2000; folic acid, 1; biotin, 2; menadione, 0.05; D,L-α tocopheryl acetate, 60; cholecalciferol, 0.025; corn meal, 9,836.125.

On day 60 when the iron-repletion period started, both the vitamin A–deficient and –sufficient groups were divided into two groups of 18 rats each that were matched for body weight and hemoglobin concentration. Within each group of rats with identical dietary history, half were fed an iron-sufficient diet with 1,200 RE/kg, and the other half were given the same diet but without added vitamin A (Figure 1). After a further 12 days (day 72) all animals were killed, and blood and organs were collected for analysis.

All animals had free access to food and demineralized water. Body weight was measured once a week and feed intake twice weekly. The iron concentration of the diets as analyzed was 10 mg/kg for the low iron diets and 43 mg/kg for the diets with adequate iron. The rats were housed in groups of four in stainless steel cages with wire mesh bases (30 × 42 × 19 cm). A controlled 12 hr light/dark cycle (light on: 0600–1800 hr), temperature (20 to 22°C), and relative humidity (50 to 60%) were maintained in the animal room.

Collection of samples

Blood was collected in heparinized vials by orbital puncture while the rats were under diethyl ether anesthesia. The blood was stored at 0°C for hematological examination on the same day. Then plasma was isolated by centrifugation (10 min, 1,580*g*) and stored at –20°C until analysis except for a portion which was stored at –80°C prior to analysis of retinol. On day 72 (Figure 1), the anesthetized rats were decapitated immediately after bleeding. The left kidney, liver, spleen, and both hindlegs were removed and stored at –20°C until analysis. Organs were weighed before storage.

Chemical analyses

The hemoglobin concentration, packed cell volume, red blood cell count, and mean cell volume were analyzed with a blood cell counter (Model K-1000, Sysmex, IJsselstein, The Netherlands). Plasma iron concentrations and total iron-binding capacity were determined spectrophotometrically using a test kit (Roche Nederland, Mijdrecht, The Netherlands). Iron levels in liver, spleen, kidney, and tibia were measured by flame atomic absorption spectrometry after dry ashing of the samples. Prior to ashing, the spleen and liver were washed with saline, and the liver was homogenized as described elsewhere.[12] Tibia iron was calculated as the mean of the left and right tibia. Plasma and liver retinol were measured by reversed phase high performance liquid chromatography (HPLC) as described previously.[12]

Statistical analysis

The data for days 25 and 39 (Figure 1) were subjected to one-way analysis of variance with the feed composition as an independent variable. Contrasts with pooled variances were used for comparison of group means (Student's *t*-test). For comparison of group means of data for days 60 and 72, a multiple comparison test (Tukey's test) was used after demonstration of a significant diet effect using one-way analysis of variance. The data for day 72 were also subjected to a two-way analysis of variance with previous vitamin A intake (prior to day 60) and vitamin A supplementation during iron repletion (day 60 to 72) as independent variables. The two-way analysis of variance for all iron parameters and organ weights was done without or with body weight at day 72 as a covariate. Prior to the analysis with the covariate, we checked for

any interaction between the independent variables and the covariate. No significant interactions were found. When body weight as a covariate contributed significantly to the explained variance, the adjusted means are also given. If the variances were not homogeneous (Cochran's C-test), the data were log-transformed prior to statistical testing. A pre-set P value of 0.05 was used throughout. Data were analyzed using the of SPSS/PC + software package (SPSS Inc., Chicago, IL USA).[18]

Results

Body weight and feed intake

On days 25 and 39 there was no effect of the lack of dietary vitamin A on body weight (Table 1) and feed intake (data not shown). On day 60, body weight (Table 1) was significantly reduced in the vitamin A–deficient rats. The average feed intake per animal from days 53–60 was 13.9 g/day in vitamin A–deficient and 17.3 g/day in the vitamin A–sufficient rats (Student's t-test, $P < 0.001$; pooled SE = 0.3 g/day; $n = 9$ cages with 4 rats each).

Body weights were increased in the rats transferred from the vitamin A–deficient diet to the diet containing vitamin A during the final 12 days of the experiment. The rats deprived of vitamin A throughout the experiment did not grow during the iron repletion period (Table 1) and ate less than those in the other groups. In the vitamin A–deprived rats, the feed intake per animal, as measured on days 65 to 72, was on average 12.3 g/day ($n = 5$ cages with 4 or 2 rats) whereas it was 18.3 g/day in the other groups ($n = 15$ cages with 4 or 2 rats) given vitamin A during any phase of the experiment (Student's t-test, $P < 0.001$; pooled SE = 0.2 g/day).

Vitamin A status

On days 25 and 39, the plasma retinol concentration was reduced in the rats fed the diet without vitamin A (Figure 2). On day 60, plasma retinol concentrations were significantly reduced in the vitamin A–deficient rats (Figure 2).

The rats transferred from the diet deficient in vitamin A to the diet containing vitamin A during the final 12 days of the experiment had increased concentrations of retinol in liver (Table 2) and almost normal plasma retinol concentrations (Figure 2). In the rats transferred from the diet sufficient in vitamin A to a deficient diet, the hepatic retinol concentrations were reduced (Table 2). The group deprived of vitamin A throughout the experiment had, on day 72, low liver weights, vitamin A levels in liver less than the level of detection with the method used (Table 2), and very low plasma retinol concentrations (Figure 2).

Hematology

On days 25 and 39, there was no effect of the lack of vitamin A on the hemoglobin concentration, red blood cell count, and mean cell volume (Table 1). A small reduction in the packed cell volume was seen on day 25, but this had disappeared by day 39 (Table 1). On day 60, at the end of the iron depletion period, the hemoglobin concentration,

Table 1 Body weight and hematological characteristics at the beginning of the iron depletion period (day 25), after 14 days of iron depletion (day 39), and before (day 60) and after (day 72) iron repletion

Dietary vitamin A (RE/kg)		Body weight (g)		Hemoglobin (mmol/L)		Packed cell volume		Red blood cell count ($\times 10^{-12}$/L)		Mean cell volume (fL)	
Days 0–60		Day 25	Day 39	Day 25	Day 39	Day 25	Day 39	Day 25	Day 39	Day 25	Day 39
1,200		182.6^a	243.6^a	8.47^a	9.07^a	0.43^a	0.46^a	6.3^a	7.2^a	68^a	64^a
0		180.8^a	234.5^a	8.35^a	9.21^a	0.42^b	0.46^a	6.2^a	7.4^a	67^a	63^a
Pooled SE		3.1	3.6	0.07	0.09	0.003	0.004	0.1	0.1	0.3	0.4
Days 0–60	Days 60–72	Day 60	Day 72	Day 60	Day 72	Day 60	Day 72	Day 60	Day 72	Day 60	Day 72*
1,200	1,200	326.4^a	361.3^a	8.54^a	9.49^a	0.44^{ab}	0.48^a	7.8^{ab}	8.2^a	57^a	58^a **(58)**
1,200	0	320.8^a	357.4^a	8.53^a	9.47^a	0.43^a	0.47^a	7.6^a	8.0^a	56^a	59^a **(59)**
0	1,200	275.1^b	330.4^b	9.09^b	8.95^b	0.46^b	0.45^b	8.1^{bc}	7.6^b	57^a	59^a **(59)**
0	0	274.1^b	279.9^c	9.19^b	9.46^a	0.46^b	0.46^{ab}	8.3^c	8.3^a	56^a	56^b **(56)**
Pooled SE		6.3	7.2	0.14	0.09	0.007	0.005	0.1	0.1	0.7	0.6
Day 72: analysis of variance, P value†											
Body weight at day 72					NS		NS		NS		0.004
Previous vitamin A feeding			<0.001		0.002		0.001		0.049		NS (NS)
Vitamin A feeding during iron repletion			<0.001		0.006		NS		0.001		0.030 (NS)
Interaction			0.002		0.003		NS		<0.001		0.002 (0.005)

Values represent means and pooled SE ($n = 36$, day 25 and day 39; $n = 18$, day 60 and day 72). Values in a column not sharing the same superscript letter are significantly different ($P < 0.05$).
*When the covariate "body weight at day 72" contributed significantly to the explained variance adjusted means are given in parentheses.
†Analysis of variance with previous vitamin A feeding and vitamin A feeding during iron repletion as independent variables without or with body weight at day 72 as covariate. When the covariate contributed significantly to the explained variance, the matching P values are given in parentheses. NS = not significant.

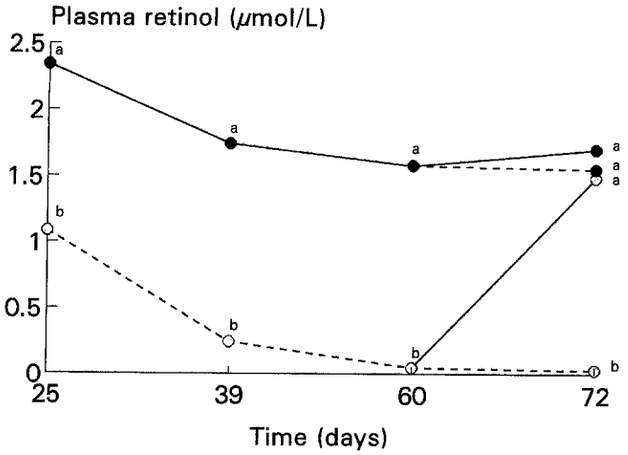

Figure 2 Plasma retinol concentrations throughout the experiment. The vitamin A–sufficient group (•) was fed adequate vitamin A (1,200 RE/kg of feed) from days 0 to 60 (Figure 1). The vitamin A–deficient group (○) received no vitamin A from days 0 to 60. From days 25 to 60, all animals were fed diets without added iron. The solid lines represent groups that received adequate vitamin A (1,200 RE/kg of feed); the broken lines correspond to the groups that received no dietary vitamin A. The number of rats per group from days 0 to 60 was 36. During the last 12 days of the experiment, each of the four groups comprised 18 animals. Statistically significant differences between groups at the same time point as based on contrasts (days 25, 39, and 60) or Tukey's multiple comparison test (day 72) are indicated by different letters ($P < 0.05$). Analysis of variance for the data from day 72 showed significant effects of body weight ($P < 0.001$) as a covariate, vitamin A intake prior to day 60 ($P < 0.001$), vitamin A administration during iron repletion ($P < 0.001$), their interaction ($P < 0.001$). The adjusted means for the levels at day 72 were 1.65, 1.50, 1.41, and 0.14 μmol/L. Values at days 60 and 72 were log-transformed prior to statistical testing.

packed cell volume, and red blood cell count were generally higher in the groups fed the vitamin A–deficient diet before iron repletion (Table 1). The mean cell volume had decreased during the iron depletion period but there was no effect of vitamin A status (Table 1). After iron repletion (day 72), hemoglobin concentrations, packed cell volume, and red blood cell count were reduced by simultaneous vitamin A administration in the vitamin A–deficient but not in the vitamin A–sufficient animals (Table 1). The mean cell volume was increased by vitamin A administration in the vitamin A–deficient animals (Table 1). The effects on mean cell volume were slightly but significantly influenced by body weight as shown by analysis of variance.

Total iron-binding capacity, plasma iron, transferrin saturation

On day 72 (Figure 1), the total iron-binding capacity and plasma iron concentration were reduced by about 20% in the animals that were deprived of vitamin A throughout the entire experiment (Table 3). Administration of vitamin A during iron repletion significantly raised the total iron-binding capacity and plasma iron in the vitamin A–deficient rats but had no effect on their vitamin A–sufficient counterparts. However, the total iron-binding capacity in the vitamin A–deficient rats supplemented with vitamin A remained below the level seen in the vitamin A–sufficient groups. After correction for body weight, the effect of vitamin A feeding on plasma iron concentrations disappeared but that on the total iron-binding capacity remained statistically significant. Transferrin saturation was not affected by the dietary regimes (Table 3).

Iron in organs

In the vitamin A–deficient animals that were supplemented with vitamin A during iron repletion, iron concentrations of liver, kidney, spleen, and tibia were significantly reduced when compared with the vitamin A–deficient rats not supplemented with vitamin A (Table 4). In the former animals, liver weight was markedly higher (Table 2) so that supplemental vitamin A produced an increase in total liver iron in the vitamin A–deficient rats. In the vitamin A–sufficient rats, the absence or presence of vitamin A in the diet during iron repletion had no effect on the iron concentrations in organs (Table 4). The iron concentrations in the spleen and tibia were highest in the group that was deprived of vitamin A throughout. Analysis of variance with body weight as a covariate revealed that the spleen and tibia iron concentrations were influenced by changes in body weight (Table 2).

Discussion

The main objective of this study was to test whether vitamin A status affects the efficacy of iron repletion in rats with mild iron deficiency. The rats with mild iron deficiency and either a low or normal vitamin A status were repleted with iron alone or with iron plus vitamin A.

At the end of the iron-depletion period (day 60), vitamin A deficiency versus normal vitamin A status had produced an increase in hemoglobin concentrations, packed cell volume, and red blood cell count. This effect of vitamin A deficiency had been reported previously.[12,13,19,20] Both water imbalance and reduced growth might have caused this hemoconcentration.[21,22] In the rats with a normal vitamin A status, there was no effect of vitamin A consumption during iron repletion on blood hemoglobin, packed cell volume, and red blood cell count. In contrast, when vitamin A was administered to the vitamin A–deficient rats during iron repletion, the values of the hematological parameters dropped significantly. This vitamin A effect does not necessarily reflect a specific influence on iron metabolism but could be secondary to overcoming the hemoconcentration.

In this study, the effect of vitamin A on feed intake and body weight may confound the effects of vitamin A on iron metabolism. The use of restricted or pair-feeding would have ruled out any such influence of differences in feed intake and body weight. From studies with children it is known that vitamin A status affects growth,[7,16,23] and thus body weight was considered a parameter of interest in our study which dictated the use of an ad libitum feeding regimen. To assess possible confounding by differences in growth, a two-way analysis of variance was also performed with body weight as a covariate. This approach served to isolate the effects on the iron metabolism of body weight from those of vitamin A feeding. Only the effects of vitamin A feeding on plasma iron concentrations were affected by body weight. The changes in other parameters of iron me-

Table 2 Liver retinol levels and liver wet weight after iron repletion (day 72)

Dietary vitamin A (RE/kg)		Liver retinol concentration (μmol/g of wet weight)*	Liver wet weight (g)
Days 0–60	Days 60–72		
1,200	1,200	0.119[a]	12.0[a] (11.1)†
1,200	0	0.081[b]	11.8[ab] (11.0)
0	1,200	0.015[c]	11.0[b] (11.0)
0	0	ND[d]	7.4[c] (9.0)
Pooled SE		0.002	0.3
Analysis of variance, *P* value‡			
Body weight at day 72		NS	<0.001
Previous vitamin A feeding		<0.001	<0.001 (<0.001)
Vitamin A feeding during iron repletion		<0.001	<0.001 (<0.001)
Interaction		<0.001	<0.001 (<0.001)

Values represent means and pooled SEs ($n = 18$). Values in a column not sharing the same superscript letter are significantly different ($P < 0.05$). ND = not detectable.
*Values are log-transformed prior to statistical testing, unadjusted means are given.
†When the covariate "body weight at day 72" contributed significantly to the explained variance adjusted means are given in parentheses.
‡Analysis of variance with previous vitamin A feeding and vitamin A feeding during iron repletion as independent variables without or with body weight at day 72 as covariate. When the covariate contributed significantly to the explained variance, the matching *P* values are given in parentheses.
NS = not significant.

tabolism can be considered rather specific effects of vitamin A. Despite possible hemoconcentration, the total iron-binding capacity and also plasma iron concentrations were reduced in vitamin A deficiency, which corroborates earlier work in both humans[1,2,5,9] and rats.[11–13] The administration of vitamin A to the vitamin A–deficient rats during iron repletion significantly raised the total iron-binding capacity and plasma iron levels. A greater increase in plasma iron as a result of vitamin A in addition to iron administration also occurred in pregnant women.[10] Perhaps, vitamin A deficiency interferes with iron transport so that absorbed iron cannot be utilized optimally. This is supported by the finding that vitamin A is necessary for the synthesis of the glycoprotein transferrin.[24] However, in the present study the increased plasma iron concentrations were also related to enhanced growth and possibly point to an indirect effect of vitamin A feeding.

This experiment with rats allowed us to study the effect of vitamin A on the distribution of iron between organs. The observed increase in iron concentrations in liver, kidney, spleen, and tibia in vitamin A deficiency is consistent with earlier studies in rats.[12,13,25] The new observation is that in rats with vitamin A deficiency, but not in those with normal vitamin A status, vitamin A administration during iron repletion reduced the concentration of iron in the liver, kidney, spleen, and tibia.

Table 3 Total iron-binding capacity, plasma iron, and transferrin saturation after iron repletion (day 72)*

Dietary vitamin A (RE/kg)		Total iron-binding capacity (μmol/L)	Plasma iron (μmol/L)	Transferrin saturation (%)
Days 0–60	Days 60–72			
1,200	1,200	91.87[a] (90.81)*	38.11[a] (36.97)*	41.47[a]
1,200	0	91.21[a] (90.29)	39.82[a] (38.84)	43.99[a]
0	1,200	84.06[b] (84.13)	38.17[a] (38.25)	45.84[a]
0	0	72.39[c] (74.31)	31.35[b] (33.39)	43.27[a]
Pooled SE		1.78	1.59	2.09
Analysis of variance, *P* value†				
Body weight at day 72		<0.001	<0.001	NS
Previous vitamin A feeding		<0.001 (<0.001)	0.010 (NS)	NS
Vitamin A feeding during iron repletion		0.001 (0.025)	NS (NS)	NS
Interaction		0.003 (0.017)	0.009 (NS)	NS

Values represent means and pooled SEs ($n = 18$). Values in a column not sharing the same superscript letter are significantly different ($P < 0.05$).
*When the covariate "body weight at day 72" contributed significantly to the explained variance, adjusted means are given in parentheses.
†Analysis of variance with previous vitamin A feeding and vitamin A feeding during iron repletion as independent variables without or with body weight at day 72 as covariate. When the covariate contributed significantly to the explained variance, the matching *P* values are given in parentheses.
NS = not significant.

Table 4 Iron concentrations in organs after iron repletion (day 72)

Dietary vitamin A (RE/kg)		Iron concentration in organs (μmol/g of dry weight)			
		Liver	Kidney	Spleen*	Tibia
Days 0–60	Days 60–72				
1,200	1,200	4.10^{ab}	4.03^{ab}	23.40^{a} **(23.88)**†	1.07^{a} **(1.08)**†
1,200	0	4.35^{ab}	4.02^{ab}	24.51^{a} **(24.92)**	1.14^{ab} **(1.14)**
0	1,200	3.81^{a}	3.70^{a}	25.02^{a} **(24.99)**	1.23^{b} **(1.23)**
0	0	4.49^{b}	4.39^{b}	44.17^{b} **(43.30)**	1.59^{c} **(1.58)**
Pooled SE		0.16	0.14	1.99	0.04
Analysis of variance, *P* value‡					
Body weight at day 72		NS	NS	<0.001	<0.001
Previous vitamin A feeding		NS	NS	<0.001 (0.007)	<0.001 (<0.001)
Vitamin A feeding during iron repletion		0.007	0.031	<0.001 (<0.001)	<0.001 (0.001)
Interaction		NS	0.013	<0.001 (<0.001)	<0.001 (0.001)

Values represent means and pooled SEs ($n = 18$). Values in a column not sharing the same superscript letter are significantly different ($P < 0.05$).
*Values are log-transformed prior to statistical testing, unadjusted means were given.
†When the covariate "body weight at day 72" contributed significantly to the explained variance, adjusted means are given in parentheses.
‡Analysis of variance with previous vitamin A feeding and vitamin A feeding during iron repletion as independent variables without or with body weight at day 72 as covariate. When the covariate contributed significantly to the explained variance, the matching *P* values are given in parentheses.
NS = not significant.

We have hypothesized[12] that in vitamin A deficiency, red blood cell synthesis is impaired. However, reduced hemoglobin concentrations as seen in humans[1–5] are masked in growing rats by hemoconcentration.[12,13] It may be that the extra iron in the spleen and tibia of the vitamin A–deficient rats is located in macrophages due to increased destruction of abnormal erythrocytes. This is supported by the report by Mejía et al.[11] that with vitamin A deficiency, the incorporation of radioactive iron was increased in the spleen but decreased in red blood cells.

In conclusion, this experiment with rats shows that supplementation of vitamin A together with iron was more beneficial in restoring normal iron status than was iron repletion alone. This can be seen from the increase in total iron binding capacity and decrease in spleen and tibia iron when animals were fed diets containing vitamin A. The effect of vitamin A only occurred when the vitamin A status of the rats was low. The present results are compatible with those from human studies[8,10] and indicate that vitamin A might interfere with plasma iron transport and the location of iron stores. The rat model described here will be useful in unraveling the underlying mechanisms.

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