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The effect of retinyl palmitate added to iron-fortified maize porridge on erythrocyte incorporation of iron in African children with vitamin A deficiency

Lena Davidsson*, Pierre Adou, Christophe Zeder, Thomas Walczyk and Richard Hurrell

Laboratory for Human Nutrition, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, PO Box 474, CH-8803 Rüschlikon, Switzerland and Institut National de Santé Publique, Adjamé, PO Box 47, 01 Abidjan, Côte d'Ivoire

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Retinyl palmitate added to Fe-fortified maize bread has been reported to enhance Fe absorption in adult Venezuelan subjects but not in Western Europeans. It is not known to what extent these results were influenced by differences in vitamin A status of the study subjects. The objective of the present study was to evaluate the influence of retinyl palmitate added to Fe-fortified maize porridge on erythrocyte incorporation of Fe in children with vitamin A deficiency, before and after vitamin A supplementation. Erythrocyte incorporation of Fe-stable isotopes was measured 14 d after intake of maize porridge (2·0 mg Fe added as ferrous sulfate) with and without added retinyl palmitate (3·5 μmol; 3300 IU). The study was repeated 3 weeks after vitamin A supplementation (intake of a single dose of 210 μmol retinyl palmitate; 'vitamin A capsule'). Vitamin A status was evaluated by the modified relative dose–response (MRDR) technique. Retinyl palmitate added to the test meal reduced the geometric mean erythrocyte incorporation of Fe at baseline from 4·0 to 2·6 % ($P=0·008$, n 13; paired t test). At 3 weeks after vitamin A supplementation, geometric mean erythrocyte incorporation was 1·9 and 2·3 % respectively from the test meal with and without added retinyl palmitate ($P=0·283$). Mean dehydroretinol:retinol molar ratios were 0·156 and 0·125 before and after intake of the single dose of 210 μmol retinyl palmitate; 'vitamin A capsule' ($P=0·15$). In conclusion, retinyl palmitate added to the labelled test meals significantly decreased erythrocyte incorporation of Fe in children with vitamin A deficiency at baseline but had no statistically significant effect 3 weeks after vitamin A supplementation. The difference in response to retinyl palmitate added to Fe-fortified maize porridge on erythrocyte incorporation of Fe before and after intake of the vitamin A capsule indicates, indirectly, changes in vitamin A status not measurable by the MRDR technique. The lack of conclusive data on the effect of retinyl palmitate on Fe absorption indicates the complexity of the interactions between vitamin A status, dietary vitamin A and Fe metabolism.

Iron: Vitamin A: Retinyl palmitate: Children: Modified relative dose–response technique

Interactions between vitamin A and Fe metabolism have been demonstrated in several human studies and the current knowledge of the effect of vitamin A on haematological indicators was recently reviewed by Fishman *et al.* (2000) and by Semba & Bloem (2002). Substantial evidence exists that vitamin A deficiency is associated with anaemia and that vitamin A supplementation also can be expected to increase haemoglobin (Hb) concentrations in anaemic children and pregnant women. The mechanisms by which vitamin A deficiency induces anaemia have not been clearly demonstrated but have been suggested to include impaired differentiation and proliferation of haematopoietic cells, disturbed erythropoietin synthesis, reduced mobilisation of body Fe stores and/or effects on Fe metabolism through sequestration of Fe during the acute-phase response to infection (for reviews, see Fishman *et al.* 2000; Semba & Bloem, 2002).

In addition, interactions between vitamin A and Fe, resulting in enhanced Fe absorption in human subjects, have been suggested (Layrisse *et al.* 1997; Garcia-Casal *et al.* 1998). The enhancing effect of retinyl palmitate on Fe absorption reported in adult Venezuelan peasants was recently evaluated but not confirmed in healthy Western European adults (Walczyk *et al.* 2003). The reasons for the contradictory results reported from Latin America and Europe are unknown. Although a number of differences in the experimental design can be identified between the two study sites, all studies were based on the measurement of erythrocyte incorporation of Fe isotopes (radioactive or stable) as a proxy for Fe absorption.

Abbreviations: DR:R, dehydroretinol:retinol; Hb, haemoglobin; MRDR, modified relative dose–response; TfR, transferrin receptor.

* **Corresponding author:** Dr Lena Davidsson, fax +41 1 704 5710, email lena.davidsson@ilw.agrl.ethz.ch

However, differences in nutritional status between the study populations can be speculated to have influenced the results. Until now, no information has been available on the effect of retinyl palmitate on erythrocyte incorporation of Fe in individuals with vitamin A deficiency.

The aim of the present study was to evaluate the effect of retinyl palmitate on erythrocyte incorporation of Fe from a test meal based on maize porridge in African schoolchildren with vitamin A deficiency, before and after supplementation with 210 μmol (200 000 IU) retinyl palmitate. Nutritional status was evaluated by the modified relative dose–response (MRDR) technique and by plasma transferrin receptor (TfR) concentration, respectively, as these indicators of vitamin A and Fe status have been demonstrated not to be influenced by infections (Tanumihardjo *et al.* 1996*b*; Staubli Asobayire *et al.* 2001; Wieringa *et al.* 2002). Erythrocyte incorporation of Fe stable isotopes was measured 14 d after intake of labelled test meals (2·0 mg Fe added as ferrous sulfate), served with and without added retinyl palmitate (3·5 μmol; 3300 IU).

Subjects and methods

Subjects

The sample size was based on previous data on erythrocyte incorporation of Fe in infants (Davidsson *et al.* 1994). It was estimated that ten children would be a sufficient sample size to detect a nutritionally significant difference in erythrocyte incorporation of 50 % with 90 % power and a type I error rate of 5 %. Thirteen children were included to allow for dropouts. The study was implemented in the northern part of Côte d'Ivoire, in a region where a high prevalence (34 %) of low plasma retinol concentration (< 0·7 μmol/l) in schoolchildren, indicating vitamin A deficiency in this population group (Sommer & Davidson, 2002), had been observed in a previous survey (Staubli Asobayire, 2000). Based on an initial screening study by the Institut National de Santé Publique, Abidjan, thirteen schoolchildren living in one of the villages included in our previous study of nutritional status (Staubli Asobayire *et al.* 2001) with low plasma retinol (< 0·7 μmol/l) were invited to participate in the study. All children were enrolled in the study at the same time. Children, their parents and/or guardians and their teachers were fully informed about the aims and procedure of the study. Informed oral consent was obtained from at least one parent or guardian of each child. Oral consent was obtained from the village chief. At the end of the study, children with Fe deficiency or Fe-deficiency anaemia were treated with medicinal Fe. As distribution of vitamin A capsules is irregular in this region, all schoolchildren in the village were given a single dose of 210 μmol (200 000 IU) retinyl palmitate at the end of the study.

The study protocol was reviewed and approved by the ethical committee of the Children's Hospital, Zürich and by the Institut National de Santé Publique, Abidjan.

Test meals and study design

Individual servings of stiff maize porridge were prepared from 50 g maize flour ('polenta'; Migros, Zurich, sSwitzerland), 200 g water and 6 g sugar. Solutions of stable isotopes of Fe ($^{57}FeSO_4$ or $^{58}FeSO_4$) were added immediately before serving. Test meal A was served without added retinyl ester (retinyl palmitate) while test meal B contained 3·5 μmol (3300 IU) retinyl palmitate. Retinyl palmitate was added as water-soluble retinyl palmitate (Hoffmann LaRoche, Basel, Switzerland) as in previous studies (Layrisse *et al.* 1997; Garcia-Casal *et al.* 1998; Walczyk *et al.* 2003). A working solution of retinyl palmitate was prepared each day by mixing pre-weighed quantities of retinyl palmitate and water. Individual doses were added to the test meals immediately before consumption. Each test meal was administered twice, on four consecutive days, after an overnight fast. Test meal A (without added retinyl palmitate; labelled with ^{57}Fe) was served on days 1–2 and test meal B (with added retinyl palmitate; labelled with ^{58}Fe) on days 3 and 4. Mineral water (Volvic; Danone, Paris, France) was used for preparation of test meals and served as a drink (200 g). As one hypothesis was that retinyl palmitate could influence Fe absorption at the mucosal level, the feeding of meals 1 and 2 in a random order was considered inappropriate. All meals were fed under close supervision and no food or drink was allowed for 3 h following intake of test meals.

On day 19, the content of a vitamin A capsule (210 μmol retinyl palmitate, 200 000 IU; Hoffmann LaRoche) was squeezed directly into the mouth of each child. At 3 weeks later, a venous blood sample was drawn and labelled test meals with and without added retinyl palmitate were administered in an identical manner as during the first part of the study. The final blood sample was drawn 14 d after intake of the last test meal (day 57).

Modified relative dose–response test

Vitamin A status was evaluated by the MRDR test according to Tanumihardjo *et al.* (1996*a*) on days 18 and 57. After an overnight fast, each child was given 7·0 μmol (2·0 mg) 3,4-didehydroretinol (vitamin A_2) dissolved in oil. Doses of 3,4-didehydroretinol (225 μl) were administered directly into the mouth, using a Gilson Microman pipette with positive displacement mechanism (Gilson, Villiers-le-Bel, France). A high-fat snack (peanut butter sandwiches) was served after intake of 3,4-didehydroretinol. Venous blood samples were drawn 4–5 h later. Molar ratios of dehydroretinol:retinol (DR:R) ≥ 0·06 were used to define vitamin A deficiency.

Blood sampling and analyses

Venous blood (5 ml) was collected in EDTA-treated vacutainer tubes at each sampling. Baseline blood samples were drawn at the start of the study (day 1) and on day 40 (second baseline). In addition, blood samples were drawn on days 18 and 57 (14 d after intake of the last test meal in each series of studies). Blood samples were kept cool and protected against light until separation of plasma. Hb was determined in duplicate by the cyanomethaemoglobin method (Sigma Diagnostic Kits, St Louis, MO, USA). A three-level quality-control

material (Dia-HT-1,2,3; Diamed AG, Cressier sur Morat, Switzerland) was analysed in parallel.

Plasma samples were transported to Zürich frozen (−20°C) and protected against light until analysis. Plasma retinol was analysed by HPLC (Merck-Hitachi, Tokyo, Japan) on a reversed-phase column (Hypersil ODS RP-18 200 × 4·6 mm, 3 μm; Crom, Herrenberg-Kayh, Germany) according to Tanumihardjo *et al.* (1994*a*). A commercial reference material (NIST 968c; National Institute of Standards and Technology, Gaithersburg, MD, USA) was analysed together with the plasma samples. CV for retinol and 3,4-didehydroretinol analyses were 5 and 12 % respectively. The concentration of circulating TfR was measured by ELISA, using a commercial kit (Ramco Laboratories, Houston, TX, USA). The CV for TfR analysis was 4 %.

Stable-isotope labels

Stable-isotope solutions were prepared from isotopically enriched elemental Fe (Chemgas, Boulogne, France) by dissolution in diluted H_2SO_4. The isotopic composition of Fe solutions was determined by negative thermal ionisation–MS, using a magnetic sector instrument (MAT 262; Finnigan MAT, Bremen, Germany). Fe concentrations of the solutions were determined by isotope dilution MS against a diluted commercially available Fe standard (Titrisol; Merck, Darmstadt, Germany).

Individual doses (2·0 mg ^{57}Fe and 1·75 mg ^{58}Fe per administration) were prepared by weighing the corresponding amounts of solution into Teflon containers. Containers were flushed with Ar to avoid oxidation. Fe with normal isotopic composition ($FeSO_4$; Merck) was added to the $^{58}FeSO_4$ solution to a total content of 2·0 mg Fe per dose.

Analysis of isotopic composition of blood samples

Blood samples were analysed in duplicate for Fe isotopic composition under chemical blank monitoring. Sampling handling was done under clean laboratory conditions to reduce the risk of sample contamination. Whole blood samples were mineralised in a HNO_3–H_2O_2 mixture, using microwave digestion. Fe was separated from the matrix by anion-exchange chromatography following a solvent/solvent extraction step into diethyl ether (Beer & Heumann, 1993; Kastenmayer *et al.* 1994). All isotopic analyses were performed by negative thermal ionisation–MS according to Walczyk (1997) using a magnetic sector field mass spectrometer (MAT 262; Finnigan MAT) equipped with a multi-collector system for simultaneous ion beam detection. Because of the high enrichment of the stable-isotope labels and the low amounts of ^{57}Fe and ^{58}Fe expected to be incorporated into erythrocytes, data were normalised for the natural ^{54}Fe:^{56}Fe isotope ratio (Taylor *et al.* 1992). Relative differences in isotopic ratios between samples analysed in duplicate were 0·006 % for the ^{57}Fe:^{56}Fe isotope ratio and 0·02 % for the ^{58}Fe:^{56}Fe isotope ratio.

Calculation of iron erythrocyte incorporation

Based on the shift in Fe isotope ratios in blood samples and the amount of Fe circulating in the body, the amounts of ^{57}Fe label and ^{58}Fe label present in the blood 14 d after test meal administration were calculated. Calculations were based on principles of isotope dilution and considered that the Fe stable-isotope labels were not mono-isotopic (Walczyk *et al.* 1997). Circulating Fe was calculated based on blood volume and Hb concentration, as described by Kastenmayer *et al.* (1994). Blood volume calculations were based on height and weight according to Linderkamp *et al.* (1977). Corrections were made for the altered baseline Fe isotope ratios during the second phase of the study.

Food analysis

Fe and Ca in the test meal were analysed by atomic absorption spectrometry (SpectrAA 400; Varian, Mulgrave, Australia) using a standard addition technique after digestion in a microwave system (MLS 1200; MLS GmbH, Leutkirck, Germany) in a mixture of HNO_3–H_2O_2. Phytic acid (inositol hexaphosphate) was measured by HPLC according to Sandberg & Ahderinne (1986). CV for Fe, Ca and phytic acid analyses were 5, 3 and 4 % respectively.

Statistics

Paired *t* tests were used for the evaluation of data, i.e. for comparisons of Hb, TfR, plasma retinol concentrations and DR:R ratios at different time points as well as for comparisons of erythrocyte incorporation of Fe from test meals with and without added retinyl palmitate at baseline and 3 weeks after intake of the vitamin A capsule. In addition, paired *t* tests were used to compare erythrocyte incorporation of Fe from test meals without added retinyl palmitate at baseline and 3 weeks after intake of the vitamin A capsule as well as to compare erythrocyte incorporation of Fe from test meals with added retinyl palmitate at the two time points. Fe erythrocyte incorporation data were log-transformed before statistical analysis and are presented as geometric means +1 SD, −1 SD. Other results are presented as arithmetic means and standard deviations.

Results

Four girls and nine boys (6–13 years old) participated in the study. Fe status did not change significantly during the study. Hb concentration was 110 (SD 8) g/l at baseline and 106 (SD 11) g/l at the second baseline (day 40, $P=0·179$); six and eight children were anaemic (Hb < 110 g/l) at baseline and on day 40 (Table 1). TfR concentration was 9·3 (SD 3·3) and 8·8 (SD 2·8) mg/l at these two time points ($P=0·255$); seven children were Fe-deficient (TfR > 8·5 mg/l) (Table 1). Five children were Fe-deficient anaemic (Hb < 110 g/l and TfR > 8·5 mg/l) on days 1 and 40. Individual values for plasma retinol and MRDR are presented in Table 2. At each time point, seven to nine children had plasma retinol concentration below 0·7 μmol/l. Plasma retinol at baseline (0·659 (SD 0·182) μmol/l) was not significantly different from plasma retinol on day 40 (0·650 (SD 0·202) μmol/l; $P=0·80$). Vitamin A deficiency was clearly demonstrated as all children had elevated DR:R ratios (≥0·06) on day 18. Vitamin A liver stores were not improved significantly

Table 1. Haemoglobin (Hb) and transferrin receptor (TfR) concentrations and fractional erythrocyte incorporation from test meals with and without added retinyl palmitate at baseline and 3 weeks after intake of a vitamin A capsule (single dose of 210 μmol retinyl palmitate) in thirteen schoolchildren*

		Baseline				3 weeks after vitamin A supplementation			
				Fe erythrocyte incorporation (%)				Fe erythrocyte incorporation (%)	
ID and sex	Age (years)	Hb (g/l)	TfR (mg/l)	No retinyl palmitate added	3·5 μmol retinyl palmitate added	Hb (g/l)	TfR (mg/l)	No retinyl palmitate added	3·5 μmol retinyl palmitate added
1 F	8	104	9·4	5·4	3·8	94	9·2	1·1	1·1
2 F	6	109	13·9	6·6	6·7	106	10·4	4·9	2·8
3 F	6	110	6·3	2·4	0·8	119	4·5	1·7	1·0
4 M	7	101	9·4	4·4	4·7	112	10·4	4·0	3·2
5 M	6	118	9·1	0·8	1·2	120	9·6	1·1	0·3
6 M	8	104	9·5	4·1	2·2	91	9·6	0·7	1·1
7 M	9	118	13·0	3·2	1·3	94	14·5	1·5	1·6
8 M	12	92	16·6	2·6	1·2	96	12·4	2·3	1·6
9 M	8	123	5·9	3·3	2·9	120	6·8	3·2	1·9
10 M	13	108	7·3	8·4	4·2	107	7·6	10·7	20·4
11 M	12	120	6·0	4·6	1·5	109	6·0	7·0	3·8
12 F	10	110	7·8	4·2	4·6	114	6·6	1·1	1·9
13 M	12	112	7·3	10·8	6·5	91	6·5	1·9	2·2
Geometric mean				4·0	2·6			2·3	1·9
+1 SD				7·8	5·3			5·2	5·0
−1 SD				2·1	1·2			1·0	0·7
P value†				0·008				0·283	

ID, identification number; F, female; M, male.
* For details of procedures, see p. 338.
† *P* values based on log-transformed data and paired *t* tests.

after vitamin A supplementation (intake of a single dose of 210 μmol retinyl palmitate; 'vitamin A capsule'). Mean DR:R decreased from 0·156 (day 18) to 0·125 (day 57), but this difference was not statistically significant ($P=0·15$). On day 57, only one child had a DR:R ratio $\leq 0·06$.

Each test meal contained 0·19 mg native Fe, 20 mg Ca and 160 mg phytic acid (inositol hexaphosphate). Individual values for erythrocyte incorporation of Fe from the test meal, without and with added retinyl palmitate, are presented in Table 1. At baseline, erythrocyte incorporation of Fe was significantly lower from the test meal with added retinyl palmitate (geometric mean 2·6 *v.* 4·0 %; $P=0·008$). No significant difference in erythrocyte incorporation of Fe from test meals with or without added retinyl palmitate was observed when the study was repeated 3 weeks after the intake of a single dose of 210 μmol retinyl palmitate ('vitamin A capsule'; geometric mean 1·9 *v.* 2·3 %,

Table 2. Plasma retinol concentration and dehydroretinol:retinol (DR:R) molar ratios at baseline (days 1 and 18) and after intake of a vitamin A capsule (single dose of 210 μmol retinyl palmitate) (days 40 and 57) in thirteen schoolchildren*

		Day 18			Day 57	
ID	Day 1 plasma retinol (μmol/l)	Plasma retinol (μmol/l)	DR:R	Day 40 plasma retinol (μmol/l)	Plasma retinol (μmol/l)	DR:R
1	0·517	0·636	0·171	0·378	0·587	0·190
2	0·629	0·626	0·183	0·517	0·580	0·162
3	0·468	0·510	0·168	0·514	0·440	0·091
4	0·374	0·409	0·172	0·559	0·476	0·134
5	0·486	0·479	0·199	0·577	0·458	0·245
6	0·741	0·909	0·120	0·741	0·976	0·131
7	0·643	0·636	0·125	0·615	0·643	0·098
8	0·657	0·353	0·321	0·413	0·958	0·078
9	0·787	0·762	0·180	0·671	0·783	0·136
10	0·608	0·664	0·060	0·769	0·664	0·057
11	1·066	1·224	0·076	1·115	0·860	0·086
12	0·829	0·846	0·113	0·909	0·993	0·086
13	0·759	0·948	0·134	0·671	0·836	0·130
Mean	0·659	0·692	0·156	0·650	0·712	0·125
SD	0·182	0·243	0·065	0·202	0·201	0·052

ID, identification number.
* For details of subjects and procedures, see Table 1 and p. 338.

$P=0{\cdot}283$). Erythrocyte incorporation of Fe from test meals without added retinyl palmitate was significantly higher ($P=0{\cdot}029$) at baseline (geometric mean 4·0 %) as compared with on day 40 (geometric mean 2·3 %). No statistically significant difference was found for erythrocyte incorporation of Fe from test meals with added retinyl palmitate ($P=0{\cdot}253$) at the two time points (geometric mean 2·6 and 1·9 % respectively).

Discussion

A major finding in the present study was the different response to retinyl palmitate added to an Fe-fortified test meal on erythrocyte incorporation of Fe before and after vitamin A supplementation (intake of a single dose of 210 μmol retinyl palmitate; 'vitamin A capsule') in children with vitamin A deficiency. At baseline, the addition of retinyl palmitate to the test meal significantly reduced the geometric mean erythrocyte incorporation of Fe from 4·0 to 2·6 %. When the study was repeated 3 weeks after intake of a vitamin A supplement (210 μmol retinyl palmitate), no statistically significant difference in erythrocyte incorporation of Fe from the test meal with and without added retinyl palmitate was observed (geometric mean 1·9 *v.* 2·3 %). It is probable that the different response to added retinyl palmitate on erythrocyte incorporation of Fe from the labelled test meals was due to improved vitamin A status in the second phase of the study, although these changes were not measurable by analysis of plasma-retinol concentration or by using the MRDR technique.

The results from the present study, as well as those from our earlier study in healthy, adult Europeans (Walczyk *et al.* 2003), are not in agreement with the reports about an enhancing effect of retinyl palmitate on Fe absorption from Venezuela (Layrisse *et al.* 1997; Garcia-Casal *et al.* 1998). The significantly decreased erythrocyte incorporation of Fe from the test meal with added retinyl palmitate at baseline in the present study was surprising. However, the lack of any statistically significant effect of added retinyl palmitate on erythrocyte incorporation of Fe as observed after vitamin A supplementation (intake of a single dose of 210 μmol retinyl palmitate) in the present study is in agreement with our previous finding in adult Europeans.

In the previous human studies of the effect of added retinyl palmitate on Fe absorption reported by us (Walczyk *et al.* 2003) and by Layrisse and colleagues (Layrisse *et al.* 1997; Garcia-Casal *et al.* 1998), erythrocyte incorporation of Fe isotopes 14 d after intake was used as a proxy for Fe absorption. This technique was first established for radioactive isotopes (Cook *et al.* 1972; Hallberg & Björn-Rasmussen, 1972) and later for stable isotopes to enable studies in infants and children (Kastenmayer *et al.* 1994). The methodology is based on the assumption that a major, and constant, fraction (typically 80–90 %) of newly absorbed Fe isotopes is incorporated into erythrocytes within 14 d. Although very little is known about the mechanisms of the interactions between vitamin A status and Fe utilisation, the observation that erythrocyte incorporation of injected ^{59}Fe was significantly reduced in vitamin A-deficient animals (Mejia *et al.* 1979) is important in this context. No information is available on the incorporation rate of newly absorbed Fe into erythrocytes in individuals with vitamin A deficiency, before and after vitamin A supplementation. This measurement was not included in the present study, as the protocol would have required the injection of an additional stable isotope of Fe. The present study therefore reports erythrocyte incorporation of Fe without applying any incorporation factor to convert the values to Fe absorption.

However, it is important to stress that measurements of erythrocyte incorporation of Fe represent the final result of several stages in Fe metabolism, including absorption and transport of Fe as well as erythropoiesis *per se*. Thus, the mechanism(s) of the influence of retinyl palmitate added to the diet and/or the influence of vitamin A status on one or more of these steps cannot be studied with the technique used in the present study or in the previous studies of the influence of retinyl palmitate on Fe absorption (Layrisse *et al.* 1997; Garcia-Casal *et al.* 1998; Walczyk *et al.* 2003). Earlier studies in rats have reported contradictory data on the effects of vitamin A deficiency on Fe metabolism, indicating increased Fe absorption and retention (Amine *et al.* 1970; Roodenburg *et al.* 1994) or no difference in Fe absorption as compared with control animals (Mejia *et al.* 1979). In these animal experiments, Fe absorption was measured by whole-body counting (Amine *et al.* 1970; Mejia *et al.* 1979), measurements of faecal radioactivity (Mejia *et al.* 1979) or by the metabolic balance technique (Roodenburg *et al.* 1994). The conflicting results can probably be ascribed to differences in experimental design and/or to differences in vitamin A status of animals in the separate studies. It is interesting to note that differences in the distribution of Fe were observed between vitamin A-deficient and normal animals. For example, vitamin A-deficient animals accumulated more Fe in liver (Mejia *et al.* 1979) and spleen (Mejia *et al.* 1979; Roodenburg *et al.* 1994) than did healthy animals. Such differences in the metabolism of newly absorbed Fe could be important factors also in the present study. However, there is no information available on the distribution of Fe in different organs related to vitamin A status in man.

An additional methodological constraint in the present study was the lack of sensitive techniques to monitor changes in vitamin A status. Plasma retinol concentrations are well known to be depressed by infections (Willumsen *et al.* 1997; Wieringa *et al.* 2002) and are therefore of limited value to identify vitamin A deficiency in individuals living in poor, rural communities such as the study site in the present study. A high prevalence of elevated C-reactive protein concentrations and helminth and malaria infections has recently been reported in school children living in this region (Staubli Asobayire, 2000; Staubli Asobayire *et al.* 2001). The children participating in the present study showed no obvious signs of current infection or other health problems during the study but minor infections can be assumed to be prevalent. For practical reasons, the study population was selected based on low plasma retinol concentrations during a screening study about 6–8 weeks before the start of the present study. At baseline, eight

children had plasma retinol below 0·7 μmol/l and the number of children with low plasma retinol remained relatively stable throughout the study; seven to nine children had low plasma retinol (<0·7 μmol/l) at each blood sampling.

The usefulness of the MRDR technique to evaluate vitamin A status in children in developing countries has been demonstrated previously (Tanumihardjo *et al.* 1990, 1994*b*, 1996*a*,*b*; Wieringa *et al.* 2002). Most importantly, this status indicator has been demonstrated not to be influenced by infections (Tanumihardjo *et al.* 1996*b*; Wieringa *et al.* 2002) and the MRDR technique was therefore used in the present study. Vitamin A deficiency was indicated by elevated DR:R ratios (≥0·06) at the first MRDR test (day 18) in all children, and vitamin A liver stores had not increased significantly when re-evaluated 39 d after the intake of a single dose of 210 μmol retinyl palmitate; 'vitamin A capsule'. The mean DR:R ratio decreased slightly, from 0·156 (day 18) to 0·125 (day 57) and in only one child was the DR:R ratio normalised to <0·06 after intake of the vitamin A capsule. However, this child had a DR:R ratio of 0·06 on day 18 and the decrease in DR:R ratio (to 0·057) was thus marginal. Although the MRDR test is not a quantitative measure of vitamin A status, most children in the present study had substantially elevated DR:R ratios at baseline, clearly indicating vitamin A deficiency. Under these conditions, it can be assumed that the single dose of 210 μmol retinyl palmitate was utilised rapidly in different body compartments, but did not provide enough retinol to improve liver stores. Thus, the lack of improvement in DR:R ratios is not surprising (Tanumihardjo, 2001). No information is available on the dose of retinyl palmitate or other retinyl esters required to normalise DR:R ratios in individuals with vitamin A deficiency similar to the African children in the present study. However, the difference in response to added retinyl palmitate on erythrocyte incorporation of Fe from the labelled test meals before and after intake of a vitamin A capsule (single dose of 210 μmol retinyl palmitate) indicates changes in vitamin A status. These results highlight the need to develop sensitive techniques to better define vitamin A status at the cellular level.

In conclusion, added retinyl palmitate significantly decreased erythrocyte incorporation of Fe from the Fe-fortified test meal in children with vitamin A deficiency at baseline but had no significant influence 3 weeks after the intake of a high-dose vitamin A supplement (single dose of 210 μmol retinyl palmitate). These results indicate, indirectly, changes in vitamin A status after intake of the vitamin A supplement, although no significant improvement in vitamin A status could be detected by the MRDR technique. Intake of a single dose of 210 μmol retinyl palmitate did not improve vitamin A liver stores in the African children with vitamin A deficiency participating in the present study. More information is clearly needed on the doses of retinyl esters required to normalise DR:R ratios in children with vitamin A deficiency to optimise the health benefits of vitamin A supplementation programmes.

The results from the present study add to the contradictory information on the influence of dietary retinyl palmitate on Fe absorption as enhancing, inhibitory or as having no effect. The lack of conclusive data indicates the complexity of the interactions between vitamin A status, dietary vitamin A and Fe metabolism.

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