

Micronutrients in childhood and the influence of subclinical inflammation

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In the present paper biomarkers of micronutrient status in childhood and some of the factors influencing them, mainly dietary intake, requirements and inflammation will be examined. On a body-weight basis the micronutrient requirements of children are mostly higher than those of an adult, but most biomarkers of status are not age-related. A major factor that is often overlooked in assessing status is the influence of subclinical inflammation on micronutrient biomarkers. In younger children particularly the immune system is still developing and there is a higher frequency of sickness than in adults. The inflammatory response rapidly influences the concentration in the blood of several important micronutrients such as vitamin A, Fe and Zn, even in the first 24 h, whereas dietary deficiencies can be envisaged as having a more gradual effect on biomarkers of nutritional status. The rapid response to infection may be for protective reasons, i.e. conservation of reserves, or by placing demands on those reserves to mount an effective immune response. However, because there is a high prevalence of disease in many developing countries, an apparently-healthy child may well be at the incubation stage or convalescing when blood is taken for nutritional assessment and the concentration of certain micronutrient biomarkers will not give a true indication of status. Most biomarkers influenced by inflammation are known, but often they are used because they are convenient or cheap and the influence of subclinical inflammation is either ignored or overlooked. The objective of the present paper is to discuss: (1) some of the important micronutrient deficiencies in childhood influenced by inflammation; (2) ways of correcting the interference from inflammation.

Micronutrients: Children: Infants: Inflammation

Childhood malnutrition is an enormous problem in the developing world and the dietary supply of vitamin A, Fe, Zn and I is marginal to deficient in many countries. To address the situation micronutrient supplementation and food fortification programmes are being widely used to combat the problems of micronutrient malnutrition both in the developing and developed world. Assessing the scale of the problem or the success of an intervention requires nutritional biomarkers, and in the present paper factors affecting the interpretation of these biomarkers will be discussed. Children present particular problems. In comparison with adults, children have higher nutrient requirements and are highly susceptible to disease. Tests of micronutrient status have generally been established

from studies using healthy adults. While the tests are relevant, they must be interpreted with caution. Children are not adults and are often not healthy. Subclinical inflammation in apparently-healthy children can lead to misinterpretation of nutritional status and an overestimate of those with deficiencies (Brown *et al.* 1993; Thurnham *et al.* 2003). It is probably not a coincidence that the micronutrient deficiencies that are reported to be most common in developing countries, i.e. vitamin A, Fe, I and Zn, are strongly influenced by infection (Thurnham, 1997; Thurnham & Northrop-Clewes, 2004). The question that will be discussed in the present paper is how to correct for disease exposure in assessing micronutrient status.

Abbreviations: ACT, α 1-antichymotrypsin; AGP, α 1-acid glycoprotein; CRP, C-reactive protein.

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Table 1. Comparison of dietary requirements of some micronutrients in children and adult men (units/kg)

Nutrient	Child 2 years*	Adult man 19–50 years*
Vitamin A ($\mu\text{g/d}$)	35	10
Fe (mg/d)	0.8	0.12
Zn (mg/d)	0.5	0.14
I ($\mu\text{g/d}$)	7.0	2.0
Thiamin ($\mu\text{g/d}$)	0.05	0.01
Riboflavin ($\mu\text{g/d}$)	0.06	0.02
Niacin (NE/d)	0.8	0.23
Vitamin C ($\mu\text{g/d}$)	3.0	0.57
Folate ($\mu\text{g/d}$)	7.0	2.9

NE, niacin equivalents.

*Data calculated assuming 10 kg for the child and 70 kg for an adult man (Department of Health, 1991).

Principal factors influencing micronutrient status in children

Nutrient requirements and disease

On a body-weight basis, the nutrient requirements of children are higher than those of an adult. The values shown in Table 1 are calculations of nutrient requirements on a per kg basis for a child of 2 years, assuming a weight of 10 kg, and an adult man weighing 70 kg using data from the report of the UK Department of Health (1991). These data suggest that children need three to six times more nutrients per kg body weight than an adult. Higher requirements mean the risk of micronutrient deficiencies will increase where nutrient-dense foods, i.e. often those of animal origin, are expensive or unobtainable. Infants are often weaned onto starchy paps that contain low concentrations of nutrients, high concentrations of phytate that compete with the absorption of minerals and often very little vitamin A in the form of vegetables. If those problems are not enough, children are also very susceptible to disease that can both increase requirements as well as causing anorexia to reduce food intake.

Poverty, unsanitary conditions and inadequate health care encourage the spread of disease and increase exposure of infants and children to disease. Thus, it is not surprising that diarrhoea, respiratory diseases, skin diseases and malaria are common in developing countries. Children are born with very little protection against pathogens but need exposure to pathogens in order to develop their own immune defences. Infants acquire some protection from Ig and other factors in their mother's milk, and in time they acquire their own immune defences. They are, however, sick for a very large part of their early lives (Sepulveda *et al.* 1988; Filteau *et al.* 1995). Rowland *et al.* (1977) have reported that Gambian infants may spend 13% of the year with gastrointestinal disease and 10% of the year with upper respiratory tract infections. Shankar *et al.* (1999) have reported that >40% of all children aged 1–5 years in the Wosera area of Papua New Guinea are infected by *Plasmodium falciparum*-related malaria. On average children are bitten by malaria-positive mosquitos four times weekly. In such circumstances children are frequently sick until they acquire some immunity, but even when they no

longer succumb to the parasite they will still need to mount an immune response each time foreign material enters their blood stream and will consequently display the effects of subclinical inflammation in their blood and other tissues.

Low intakes of animal foods or carotene-containing foods and diets containing a high content of phytate are very likely to result in deficiencies of vitamin A, Fe and Zn. Such deficiencies may not immediately lower concentrations of biomarkers in the circulation or cause clinical evidence of disease, but tissue stores or reserves may be depleted and/or growth may be restricted by a limiting essential nutrient (Golden, 1991). In contrast, disease or stress can very rapidly lower the concentration of circulating biomarkers. Plasma retinol concentrations decrease by approximately 25% within 4 h in adults undergoing minor surgery and continue to fall over the next 3 d (Louv *et al.* 1992), while plasma Fe and Zn concentrations have been reported to fall by 50–70% in the first 24 h following experimental infections of army recruits with sandfly fever (Beisel, 1976). In addition, plasma retinol has frequently been reported to be low (Reddy *et al.* 1986; Thurnham & Singkamani, 1991) and sometimes very low (Mitra *et al.* 1998; Cser *et al.* 2004) in children with various infections, and spontaneously increases on recovery without vitamin A intervention. As infection can cause such rapid changes in the concentration of biomarkers such as plasma retinol, Fe and Zn, it is important to determine that residual alterations caused by infection are not still present in infants recruited on surveys that assess nutritional status.

In the case of plasma retinol there is also a very marked increase in concentrations with increasing age. Data from preschool children (Gregory *et al.* 1995), children and adolescents (Gregory *et al.* 2000) and adults (Gregory *et al.* 1990) living in the UK are combined in Fig. 1. A very similar picture can be constructed from US data (Ballew *et al.* 2001). Gender-related differences in plasma retinol concentrations do not appear until children reach

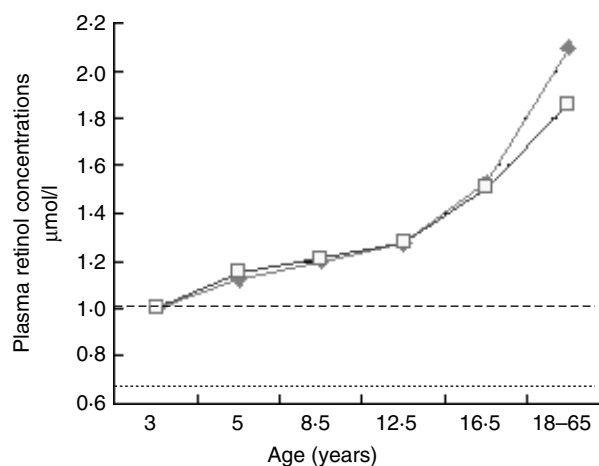


Fig. 1. Mean retinol concentrations at different ages for males and females obtained from three UK national surveys (Gregory *et al.* 1990, 1995, 2000). (◆), Males; (□), females; (.....), 0.7 $\mu\text{mol retinol/l}$; (---), 1.05 $\mu\text{mol retinol/l}$ (the two retinol concentrations represent the cut-off values used to indicate risk of vitamin A deficiency; for discussion, see text).

late adolescence and into adulthood, when concentrations in men are usually higher than those in women. Assessment of status is usually based on the proportion of subjects with a plasma retinol concentration $<0.7 \mu\text{mol/l}$. If the low retinol concentration is the result of a dietary deficiency, then at this concentration in the plasma the liver stores of retinol could be borderline at approximately $20 \mu\text{g/g}$ (De Luca *et al.* 1977). If, on the other hand, plasma retinol concentrations are low as a result of concurrent inflammation, then liver stores of vitamin A may not be at a critical state but mobilisation of vitamin A is suppressed as a result of a down-regulation of retinol-binding protein synthesis that is also a consequence of inflammation (Rosales *et al.* 1996). In terms of nutritional status both situations probably represent a deficient state for children but each situation may require a different solution to improve nutritional status. In children in areas in which malaria is heavily endemic (Shankar *et al.* 1999) and in HIV-1-positive women showing evidence of inflammation (Baeten *et al.* 2002) plasma retinol concentrations do not respond particularly well to vitamin A supplements. In contrast, in the HIV-1-positive women with no evidence of inflammation the plasma retinol concentrations show an increase in response to vitamin A supplements (Baeten *et al.* 2002).

In adults a plasma concentration of $<1.05 \mu\text{mol retinol/l}$ is sometimes used to indicate risk of vitamin A deficiency (Pilch, 1985), but at this concentration evidence suggests that liver stores of vitamin A are probably adequate. Thus, the plasma retinol concentration of $0.7 \mu\text{mol/l}$ is probably equally suitable as a criterion of risk for all groups in the community.

Measuring subclinical inflammation in the apparently-healthy child

Children who are sick would automatically be excluded from surveys in which nutritional status is being examined. Surveys recruit only apparently-healthy children and, with a few exceptions, it is generally assumed that recent disease has very little or no effect on the results. However, there is ample evidence to show that even apparently-healthy children can have elevated inflammatory markers. Rousham *et al.* (1998) have found that apparently-healthy Bangladeshi preschool children whose mothers' reported diarrhoea or fever in their child in the previous 2 weeks have elevated $\alpha 1$ -antichymotrypsin (ACT) concentrations. Fever and persistent diarrhoeas are common early symptoms in HIV-infected children (Dobosz & Marczyńska, 2004), and Filteau *et al.* (1995) have also found diarrhoea in the previous week to be associated with several elevated acute-phase proteins in preschool Ghanaian children. Panter-Brick *et al.* (2001) have reported that stunting is associated with high plasma ACT concentrations in Nepali village children even though morbidity appears to be low. Northrop-Clewes *et al.* (1994) have also reported that mean plasma ACT concentrations are elevated in $>50\%$ of apparently-healthy Gambian infants every month over 1 year. In the rainy season it was found that the mean concentration of ACT is so high that it indicates that most infants show evidence of subclinical inflammation

(Thurnham *et al.* 2000). Similarly, in an area like Papua New Guinea, or anywhere where there is high malaria transmission (Shankar *et al.* 1999), it is highly likely that subclinical inflammation is present in many individuals in the community and probably accounts for the very poor response in plasma retinol concentrations in children to four vitamin A doses of 60 mg retinol equivalent in a period of 1 year.

There are three acute-phase proteins that have been used to measure subclinical inflammation in several nutrition studies: ACT; C-reactive protein (CRP); $\alpha 1$ -acid glycoprotein (AGP; Thurnham *et al.* 2003). ACT is a good general marker of both acute and chronic disease. It rises rapidly and remains elevated for some time after clinical symptoms disappear. Plasma CRP increases rapidly within 5 h of infection. It is maximal between 24 and 48 h and falls rapidly with disappearance of symptoms. Plasma AGP concentration is slower to rise and it is unusual to detect an elevation before 48 h. It does not reach maximal concentrations until 4–5 d following infection, but there appears to be no information on how long it will remain elevated following different infections. However, any increase in AGP may also indicate that plasma retinol concentrations are still being depressed by the acute-phase response.

Correcting plasma retinol concentrations using plasma concentrations of inflammatory biomarkers

The first attempt to correct nutritional data for the influence of subclinical inflammation in order to assess the separate impacts of dietary deficiency and inflammation on plasma retinol concentrations was done using the two proteins ACT and AGP (Paracha *et al.* 2000). Plasma ACT concentrations were found to be elevated in 11% (274 of 2519) of Pakistani preschool children while elevated AGP concentrations were present in almost half the children (45%; 1141 of 2519). It is possible by using two acute-phase proteins that respond differently over the time-course of the infection to characterise three stages of subclinical inflammation, i.e. incubation and early and late convalescence. In subsequent work the use of CRP rather than ACT has been favoured, as CRP has a shorter time span of elevation than ACT; i.e. CRP identifies early convalescence better than ACT because CRP overlaps with elevated AGP concentrations for a shorter period than ACT. Fig. 2 illustrates diagrammatically how the three phases of subclinical inflammation are identified using the acute-phase proteins. Subjects in whom there are no elevated acute-phase proteins provide the reference or 'healthy' group for whom it is assumed that diet is the main factor responsible for the concentration of the biomarker. The influence of inflammation on a nutritional biomarker is determined by calculating the differences between median biomarker concentrations of the 'healthy' group and each of the groups with raised acute-phase proteins. A meta-analysis of fifteen studies, mostly of infants or children, has used these methods to calculate the depression in plasma retinol where inflammation is present and provide the factors required to normalise plasma retinol concentrations to remove the effects of inflammation

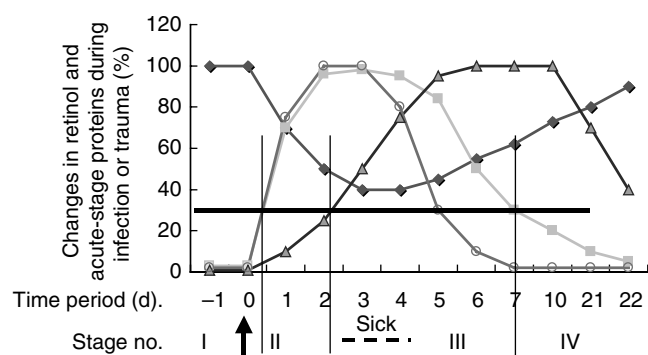


Fig. 2. Idealised behaviour of acute-phase proteins during the course of infection. Standardised changes in plasma retinol (♦), C-reactive protein (CRP; ○), α1-antichymotrypsin (ACT; ■) and α1-acid glycoprotein (AGP; ▲) in response to trauma or infection (i). (—), Threshold. The values depicting the changes should not be taken as absolute. The four stages of infection are: I, healthy or reference; II, incubation; III, early convalescence; IV, late convalescence; as indicated by abnormal elevations in CRP (or ACT) and/or AGP (see Table 2). The time period (d) shown is arbitrary and variable, except for stage II, which is unlikely to be >48 h.

(Table 2; Thurnham *et al.* 2003). Furthermore, it was pointed out that the corrections normalise the mean or median plasma retinol concentrations in all three groups in which inflammation is present, to that of the reference or healthy group. Thus, the need to do the correction will depend on the size of the reference group relative to the total population. In the industrial world there are likely to be very few subjects with elevated acute-phase proteins (<15%), this group will therefore be small and can simply be removed from the analysis. It should be noted, however, that an appropriate threshold should be used to evaluate the effects of CRP concentrations on plasma biomarkers. Clinicians use a cut-off of 10 mg/l, as this value relates better to the clinical relevance of the data. However, healthy subjects tend to display plasma CRP concentrations <5 mg/l, thus CRP values between 5 and 10 mg/l probably indicate mild inflammation and such subjects could be assigned to either groups II (incubating or preclinical) or III (early convalescence; Fig. 2), which show a correspondingly low plasma retinol concentration. Furthermore, if plasma AGP concentrations are not measured, a potentially large group of subjects in late-stage convalescence may be missed (Paracha *et al.* 2000). Thus, in any nutritional survey, especially in developing countries, information on both acute and chronic

acute-phase markers is desirable to enable a proper interpretation of the nutritional data.

Interpretation of plasma retinol concentrations in apparently-healthy HIV-1-positive adults and comparisons with HIV-negative infants

The first opportunity to test the correction factors obtained in the meta-analysis on the vitamin A studies was on baseline data from a group of apparently-healthy HIV-1-positive adults (Thurnham DI, Mburu ASW, Mwaniki DL, Muniu EM, Alumasa F & de Wagt A, unpublished results). The subjects were recruited for an intervention study to determine whether a food supplement with or without supplemental multiple micronutrients would improve the general health and well-being and quality of life of adult men and women living with HIV (Mburu *et al.* 2004). The median concentrations of plasma retinol for the group are shown in Table 3, grouped according to their acute-phase status. Following correction using the factors from the meta-analysis, median retinol concentration for the whole group was 1.31 μmol/l and the proportion of results that were <0.7 μmol retinol/l was reduced from 20% to 16%. The median plasma retinol concentration of the healthy or reference group was 1.33 μmol/l, so the meta-analysis correction factors did not completely raise the retinol concentrations in the incubation and convalescent groups to that of the reference group. However, by calculating specific correction factors from the data for the HIV-positive subjects as shown in Table 3, the median retinol concentration for the whole group is raised to that of the reference group and further reduces the number of low plasma retinol concentrations to 13%. The prevalence of low plasma retinol concentrations is very close to the 15% prevalence that would be considered a public health problem (Sommer & Davidson, 2002), but the carotenoid concentrations of the reference group (Table 4), which will be discussed later, do not suggest that vitamin A deficiency is a particular problem in this group.

Acute-phase protein-corrected plasma ferritin concentrations as a measure of iron status

Plasma ferritin concentrations for the HIV-1-positive adults are shown in Table 4. They are grouped by gender and acute-phase status. The data illustrate a very large elevation of median ferritin concentrations in both the early (5-fold) and the late (3-fold) convalescence groups in

Table 2. Characterisation of subclinical inflammation and its effect on plasma retinol concentrations

Group*	Stage of subclinical inflammation	Raised acute-phase proteins	Depression in plasma retinol concentrations (%)
I	Healthy	None	None
II	Incubating or preclinical	C-reactive protein only	13
III	Early convalescence	C-reactive protein and α1-acid glycoprotein	24
IV	Late convalescence	α1-acid glycoprotein only	11

*Group numbers define the stages of subclinical inflammation shown in Fig. 2.

Table 3. Use of correction factors to remove influence of inflammation from plasma retinol concentrations from apparently-healthy HIV-1-positive adults

	Groups separated by acute-phase proteins*			
	Reference or healthy <i>n</i> 66	Incubation* <i>n</i> 18	Early convalescence* <i>n</i> 65	Late convalescence* <i>n</i> 14
Median retinol ($\mu\text{mol/l}$)	1.33	1.25	1.01	1.10
Meta-analysis correction factors†		1.13‡	1.24‡	1.11‡
Corrected median retinol concentration for whole group using these correction factors‡			1.31 $\mu\text{mol/l}$	
Abnormal results (<0.7 $\mu\text{mol/l}$) following correction:			26/163 (16%)	
Present study correction factors§		(1.33/1.25) 1.06	(1.33/1.01) 1.32	(1.33/1.10) 1.21
Corrected median retinol concentration for whole group§			1.33	
Abnormal results following correction			22/163 (13%)	

*Subjects were allocated to the reference group unless plasma C-reactive protein concentration was >5 mg/l and/or plasma α 1-acid glycoprotein was >1.0 g/l when subjects were allocated to the incubation (only C-reactive protein elevated), early convalescence (both C-reactive protein and α 1-acid glycoprotein elevated) or late convalescence (α 1-acid glycoprotein only) groups.

†Refers to data from Thurnham *et al.* (2003).

‡Retinol data in each group multiplied by respective correction factors, data were combined and corrected median value was calculated.

§Correction factors were calculated as shown, multiplied by plasma retinol concentrations in respective groups and the data were combined to calculate the corrected median retinol.

both genders. Concentrations of plasma ferritin differ markedly between the genders and therefore separate correction factors need to be calculated, producing corrected median ferritin concentrations of 166 $\mu\text{g/l}$ and 41 $\mu\text{g/l}$ for the whole groups of men and women respectively. Both values are still higher than the corresponding mean plasma ferritin concentrations of 84 $\mu\text{g/l}$ and 31 $\mu\text{g/l}$ reported for non-HIV-positive adults living in the same area of Kenya (Mwaniki *et al.* 2001), although the corrected result in the case of the women seems quite comparable. Furthermore, the number of cases of Fe deficiency in the women was found to be increased from eleven to twenty, which is an increase of 83% as a result of correcting for inflammation.

It is interesting to compare the Kenyan adult ferritin concentrations (Table 4) with those for Indonesian infants for whom Fe-deficiency anaemia is a well-recognised problem (Wieringa *et al.* 2002). Ferritin concentrations are found to be much lower in the infants than in the adults. In the Indonesian infants the plasma ferritin concentrations were also grouped using CRP and AGP, but using less-sensitive cut-offs of 10 mg/l and 1.2 g/l respectively (Table 5). Even though the technique used was slightly less sensitive than that reported in the present paper, the elevations in ferritin associated with inflammation during convalescence are also very high. In the reference group 26% (eighty of 308) of infants were found to have Fe deficiency (ferritin <12 $\mu\text{g/l}$), whereas in the 184 infants

Table 4. Median concentrations for plasma ferritin, zinc, β -carotene and lutein and correction factors in apparently-healthy HIV-1-positive adults grouped by acute-phase status

Analyte	Whole group		Groups separated by acute-phase status*			
	Before correction	After correction	Reference or healthy	Incubation*	Early convalescence*	Late convalescence*
Median ferritin ($\mu\text{g/l}$) for men <i>n</i> †	488	166	166	262	1004	540
Correction factor	56	56	19	4	28	5
Median ferritin ($\mu\text{g/l}$) for women <i>n</i> †	90	41	41	50	213	107
Correction factor	107	107	47	14	37	9
Correction factor				0.82	0.19	0.38
Median Zn ($\mu\text{mol/l}$)§	8.8	9.4	9.3	8.6	7.9	8.4
Correction factor				1.08	1.17	1.06
Median β -carotene ($\mu\text{mol/l}$)§	0.218	0.319	0.320	0.323	0.141	0.161
Correction factor				0.99	2.270	1.988
Median lutein ($\mu\text{mol/l}$)§	0.296	0.386	0.385	0.344	0.220	0.278
Correction factor				1.119	1.750	1.385

*Subjects were allocated to the reference group unless plasma C-reactive protein concentration was >5 mg/l and/or plasma α 1-acid glycoprotein was >1.0 g/l when subjects were allocated to the incubation (only C-reactive protein elevated), early convalescence (both C-reactive protein and α 1-acid glycoprotein elevated) or late convalescence (α 1-acid glycoprotein only) groups.

†Nos. for groups in which genders are combined are shown in Table 3.

‡Specific correction factors are calculated as shown in Table 3.

§Values shown for combined gender groups, with *n* 66 (healthy), 18 (incubation), 65 (early convalescence), 14 (late convalescence).

Table 5. Influence of inflammation on iron deficiency and low plasma zinc concentrations in Indonesian infants (modified from Wieringa *et al.* 2002)

Variable	Groups separated by acute-phase status			
	Reference or healthy	Incubation*	Early convalescence*	Late convalescence*
Mean plasma ferritin ($\mu\text{g/l}$)	14.7	32.1	34.3	34.2
Fe deficient ($>12\mu\text{g Fe/l}$)				
No. relative to total	80/308	2/61	1/37	9/86
Percentage	26	3.3	2.7	10.5
Mean plasma Zn ($\mu\text{mol/l}$)	15.5	13.8	13.7	14.2
Plasma Zn $<10.7\text{ mmol/l}$	34/308	20/61	10/37	12/86
No. relative to total				
Percentage	11	33.3	27	15.1

*Subjects were allocated to the reference group unless plasma C-reactive protein concentration was $>10\text{ mg/l}$ and/or plasma α 1-acid glycoprotein was $>1.2\text{ g/l}$ when subjects were allocated to the incubation (only C-reactive protein elevated), early convalescence (both C-reactive protein and α 1-acid glycoprotein elevated) or late convalescence (α 1-acid glycoprotein only) groups.

in whom there was inflammation only twelve cases (7%) were detected. On the basis of the proportion in the reference group (26%) the corresponding number should have been forty-eight cases in the group with inflammation, i.e. approximately thirty-six cases of Fe deficiency were missed. Thus, in both the adults and the infants with very different absolute concentrations of ferritin, Fe deficiency is obscured by the presence of inflammation.

Influence of inflammation on plasma zinc concentrations

Table 4 shows the median plasma Zn concentrations for the Kenyan HIV-1-positive adults grouped according to inflammatory status. In contrast to the large change in ferritin, inflammation is only associated with approximately a 12% reduction in plasma Zn concentrations (Table 4). However, plasma Zn concentrations are low in all groups, even in the group with no inflammation, and the correction factors only reduce the prevalence of low Zn concentrations ($<10\mu\text{mol/l}$) from 75% to 59%. However, the corrected prevalence values are still higher than those reported in the Nutrition Survey (Mwaniki *et al.* 2001), in which the prevalence of low Zn concentrations for the country as a whole was found to be 46% and 52% in men and women respectively.

In the Indonesian data (Wieringa *et al.* 2002) plasma Zn concentrations in all groups are higher than those of the HIV-1-positive Kenyan adults. However, depression in plasma Zn concentrations associated with current inflammation in the Indonesian infants is also approximately 12% by comparison with the reference group (Table 5). Wieringa *et al.* (2002) used a slightly higher cut-off of $10.7\mu\text{mol/l}$, so the results are not directly comparable, but in both these studies the effects of current inflammation on plasma Zn concentrations are relatively small and other factors such as the high cereal intake and the HIV infection must be considered to explain the low Zn concentrations in the Kenyan adults.

Influence of inflammation on plasma carotenoid concentrations

Table 4 shows the plasma concentrations of β -carotene and lutein in the Kenyan adults. Concentrations of both

carotenoids in the healthy group are comparable with those found in Western populations, suggesting that vitamin A deficiency is not a problem in this group (International Agency for Research on Cancer Working Group, 1998). However, there is a large depression in both median plasma β -carotene ($>50\%$) and lutein (approximately 40%) concentrations associated with early and late convalescence but $<10\%$ during the incubation period. Plasma carotenoid concentrations are strongly linked to dietary intake, hence the relatively small effect of the incubation period on carotenoid concentrations is not surprising. Appetite is frequently depressed by disease and the low concentrations during convalescence will be at least partly a result of reduced dietary intakes during illness.

There have been a number of studies (Kritchevsky *et al.* 2000; Erlinger *et al.* 2001; Cser *et al.* 2004) that report associations between low concentrations of β -carotene and infection or stress of one form or another. In all cases inverse correlations between plasma CRP and β -carotene concentrations have been found. Low plasma β -carotene concentrations have also been shown to increase the risk for heterosexual HIV acquisition in patients with sexually-transmitted diseases in India (Mehendale *et al.* 2001) and to be associated with an increased risk of death during HIV infection in Ugandan infants (Melikian *et al.* 2001). However, the findings of Kritchevsky *et al.* (2000) and Erlinger *et al.* (2001) suggest that the association between β -carotene and disease risk is probably confounded by inflammation.

Cser *et al.* (2004), on the other hand, report the concentrations of several plasma carotenoids in children with clinical infections. They make the interesting observation that the mean provitamin A carotenoid concentration for children with infection is only 20% of that for a group of healthy controls of similar ages. In contrast, the mean concentration of non-provitamin A carotenoids for the children with infection is only half that for the healthy children. While some of this difference may be related to the different half-lives of the carotenoids (β -carotene $<12\text{ d}$ and zeaxanthin and lutein 33–61 d; Rock *et al.* 1992), the authors report that an altered dietary intake as a result of the disease would only have been effective 24–72 h before the blood was taken. The concentration

of plasma β -carotene in the sick children is strongly associated with the severity of infection and no plasma β -carotene could be detected in those children with severe and very severe infections (twenty-eight of fifty-five, 62%). These data suggest that turnover of carotenoids during inflammatory disease is accelerated. However, lutein was detected in all samples, therefore it seems unlikely that the elevated turnover rates were only a result of non-specific oxidative damage, and the possibility that the metabolism of plasma β -carotene to retinol or other retinoids during inflammation is increased cannot be excluded.

Discussion and implications

High micronutrient requirements and frequent sickness increase the risk of deficiency among infants and children in many developing countries. However, while micronutrient deficiencies may gradually deplete the body's reserves and eventually lower biomarkers of micronutrient status, the inflammatory response can very rapidly alter the same biomarkers. Even the apparently-healthy child can show evidence of inflammation, thus the net concentration of biomarkers in the blood of such children is a result of a combination of the dietary status and any lingering effects of inflammation. In the present paper data have been taken from apparently-healthy HIV-1-positive adults and using methods developed to examine the influence of inflammation on plasma retinol concentrations (Thurnham *et al.* 2003) it has been confirmed that these methods can be adapted to examine vitamin A status in the HIV-1-positive adults and that the same technique can be used to examine plasma ferritin, Zn, β -carotene and lutein concentrations. In the case of ferritin and Zn the corrected data have become much more similar to expected concentrations for those biomarkers obtained in comparable HIV-negative subjects in Kenya (Mwaniki *et al.* 2001) and the relative changes in these biomarkers in response to inflammation show similarities with a comparable analysis of data from apparently-healthy Indonesian infants (Wieringa *et al.* 2002). In the case of the carotenoids the concentrations are surprisingly low during the convalescent phase. However, it has been suggested that a reduction in plasma carotenoid concentrations is rapid in disease and much greater than would be expected from altered food intake (Cser *et al.* 2004) and/or natural turnover (Rock *et al.* 1992) alone. These results provide even stronger evidence for the questionability of studies in which infection rates or biological stresses are high and in which plasma β -carotene concentrations are used as a marker of absorption.

In conclusion, the potentially large influence of sub-clinical inflammation on plasma retinol, Zn, ferritin and the carotenoid concentrations emphasises the urgent need to monitor inflammation where these biomarkers are used to assess nutritional status. Furthermore, the association between inflammatory markers and changes in nutritional biomarkers indicates that caution should be exercised before conclusions are drawn about poor nutrition and increased disease risks. Finally, the different response to supplements by individuals with elevated as compared

with normal inflammatory markers (Baeten *et al.* 2002) needs better understanding, as it may help to improve our interpretation of or prevent future trials with adverse effects on health such as the trials with vitamin A palmitate and β -carotene (Villamor *et al.* 2005), Fe (Oppenheimer *et al.* 1986) and β -carotene (Heinonen *et al.* 1994).

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