Impact of disease on markers of micronutrient status

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It is becoming increasingly well recognized that, although nutritional status of micronutrients is determined principally by diet, there are factors other than biological diversity which determine the concentration of a micronutrient in the blood in relation to the dietary intake.

In those halcyon days when nutrition was still 'simple', i.e. back in the 1930s–1950s, there were many investigators who worked on depletion–repletion experiments to determine nutritional requirements and at the same time obtained information on the relationships between dietary intake and biological markers of micronutrient status. These studies produced the 'nutritional bibles' of the 1960s (Interdepartmental Committee on Nutrition for National Defense, 1963; Sauberlich *et al.* 1974) which have guided workers in assessing micronutrient status and, with a few minor modifications, many of the criteria are still in use today. However, the big drawback of these studies was that most were done on young fit men and women who were monitored closely throughout depletion and at the first clinical sign of deficiency or disease, the subjects were usually replenished, to determine the amount of nutrient required to restore normality. Hence, the information obtained related to an unnatural situation where a healthy subject was deprived of a single nutrient.

The unnatural basis for these experiments was well recognized. Numerous workers point out that diets are not usually deficient in a single nutrient and healthy subjects do not get deficiency diseases. Nevertheless, the information on thresholds of acceptability of blood biomarkers obtained by these unnatural experiments continues to be used in the real world and information extrapolated from them to derive dietary intakes. However, the dangers of this blind acceptance of dietary predictions are starting to become apparent. Epidemiologists and others are drawing conclusions on diet–disease relationships from information of blood biomarkers. For example, the enthusiasm for β-carotene intervention studies arose from consistent information which suggested that because smokers had lower levels of plasma β-carotene, this suggested that dietary intake of β-carotene was also low (Connett *et al.* 1989). However, this was done without a real understanding of what factors other than diet influence plasma β-carotene in smokers. However, if a particular disease or traumatic condition can itself influence the relationship between the blood biomarkers and diet, then diet may not be a factor in the aetiology of the disease at all. It is becoming more and more apparent that disease does influence blood nutrient levels in various ways. These factors have to be recognized and ways found to discriminate between the influence of disease and diet on blood biomarkers.

VITAMIN A

Retinol or vitamin A is probably the best example of a nutrient influenced by disease. It is well accepted that the circulating concentration of retinol is not related to dietary intake, except when the amount in the blood is low (approximately $<0 \cdot 7$ μmol/l; Olson, 1984). Nevertheless, it appears that at all concentrations the amount in the blood is influenced by diseases. We first realized this when studying patients with malaria from two different

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socio-economic groups and their respective controls (Thurnham & Singkamani, 1991). Irrespective of the difference in circulating retinol between the two groups without infection, the influence of malaria was similar, producing almost the same absolute reduction in retinol levels between the respective groups (Table 1).

This particular study of malaria was cross-sectional and we were uncertain of the pre-infection retinol values. However, a longitudinal study by Reddy *et al.* (1986) clearly demonstrated a fall in circulating retinol during infection with measles; the levels returning to the pre-measles levels after infection without vitamin A supplementation (Table 2).

The study by Reddy *et al.* (1986) clearly demonstrated that clinical disease reduces plasma retinol and such work illustrates that any conclusions drawn about vitamin A status on the basis of plasma retinol in clinical disease can at best be only tentative. However, a runny nose, loose stools or mild respiratory symptoms may be so common as to be regarded as normal. Do they also influence blood nutrient levels? Subclinical or occult disease is widespread in subgroups of the population in both the First and Third Worlds. In a recent malaria survey in the eastern part of India, workers found 30 % of the children (1–11 years) showed blood-smear positivity for malaria and, while 75 % of these showed no clinical evidence of disease (Ghosh *et al.* 1995), there was evidence of abnormality with three acute-phase proteins: albumin ($P < 0.05$) ferritin ($P < 0.05$) and caeruloplasmin (not significant). Likewise Filteau *et al.* (1993) found 57 % (twenty-eight of forty-nine subjects) of Ghanian children (6–59 months) who had abnormally raised levels of the acute-phase protein, α_1-acid glycoprotein, to be asymptomatic.

Table 1. *Retinol concentrations (µmol/l) in malaria patients and control subjects in rural and urban communities in Thailand* (Data from Thurnham & Singkamani, 1991)

(Values are medians and ranges)

Group		Malaria	Control	Difference
Urban	*n*	24	27	
	Median	1.17^a	1.76^b	0.59
	Range	0.61–2.97	0.66–3.33	
Rural	*n*	21	20	
	Median	0.50^c	1.14^a	0.64
	Range	0.10–1.20	0.17–2.15	

[a,b,c] Median values with unlike superscript letters were significantly different ($P < 0.001$ by Kruskall-Wallis; $P < 0.05$ by Scheffe group test).

Table 2. *Influence of measles on serum retinol and retinol-binding protein (RBP) concentrations*

(Values are means with their standard errors)

Group	Retinol* (µmol/l)		RBP* (µmol/l)		Retinol : RBP
	Mean	SE	Mean	SE	
Pre-infection (*n* 117)	0.73	0.03	1.19	0.29	0.61
During infection† (*n* 153)	0.47	0.02	1.00	0.33	0.47
Post-infection (*n* 108)	0.89	0.02	1.19	0.29	0.68

*Recalculated from original data of Reddy *et al.* (1986) using molecular weights of 245 and 21 000 for retinol and RBP respectively.

†Values recorded during infection were significantly different from both pre- and post-infection values for both retinol and RBP.

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Table 3. *Sensitivity and specificity of conjunctival impression cytology (CIC) in relation to plasma retinol in various studies*

Reference	Country	Retinol threshold (μmol/l)	Sensitivity (%)	Specificity (%)
Natadisastra *et al.* (1988)	Indonesia*	< 0·70	61·8	88·5
Gadomski *et al.* (1989)	Guatemala*	< 0·70	26	81
Stolzfus *et al.* (1993)	Indonesia*	< 0·52	40	89
Carlier *et al.* (1991)	Senegal†	< 0·35	39·7	70
Carlier *et al.* (1992)	Senegal†‡	< 0·35	74·0	48
Chowdhury *et al.* (1996)	India†	< 0·70	90·6	100
		< 0·35	100	59

*Used CIC technique.
†Used CIC with transfer technique.
‡Best results following evaluation of different ways of classifying abnormal CIC results.

Plasma levels of circulating retinol are controlled by the level of retinol-binding protein (RBP; Kanai *et al.* 1968). As this is a negative acute-phase protein (Rosales *et al.* 1996), concentrations of plasma retinol will fall in disease, possibly to minimize urinary losses of retinol. Retinol losses during infection and in severe disease can be as high as half the recommended daily allowance (Stephensen *et al.* 1994). RBP and retinol probably appear in the urine as a consequence of the increased endothelial permeability (Areekul, 1988; Gosling *et al.* 1991) which accompanies infection and increases macromolecule exchange between the plasma and the extracellular fluid compartments and also the potential for increased losses of plasma proteins in the urine (Sandberg *et al.* 1985; Shearman & Gosling, 1988). In a well-fed person, a temporary decline in circulating retinol may not be too crucial, but the problem arises when the patient is not well-nourished at the outset and disease becomes chronic.

In Third World situations where people are living in unhygienic conditions and food is frequently contaminated with pathogens, disease is common and consequently levels of circulating retinol may be dangerously low for long periods. Low levels of retinol are probably responsible for the abnormalities in ocular epithelium which have been reported by many workers (Carlier *et al.* 1992; Nathanail & Powers, 1992; Stolzfus *et al.* 1993; Chowdhury *et al.* 1996). Ocular abnormalities have been demonstrated using conjunctival impression cytology (CIC) and results obtained by CIC have been compared with plasma retinol levels to assess sensitivity and specificity of CIC to measure vitamin A status. While some workers report good agreement between the two methods (Chowdhury *et al.* 1996; Table 3), other workers in different environments have not obtained good agreement (Natadisastra *et al.* 1988; Gadomski *et al.* 1989; Carlier *et al.* 1991, 1992; Stolzfus *et al.* 1993; Table 3). The probable reason for the lack of agreement is that while plasma retinol responds very rapidly to changes in vitamin A intake, the re-appearance of goblet cells in the ocular epithelium may take 3–8 weeks (Wittpenn *et al.* 1986).

CIC is a relatively non-invasive technique but it involves a certain amount of subjectivity and it is difficult to perform in young children (Nathanail & Powers, 1992; Chowdhury *et al.* 1996). In contrast, the fact that the level of retinol is determined by the disease activity raises the possibility that retinol may be evaluated by comparison with an independent disease marker, i.e. an acute-phase protein. Filteau *et al.* (1993) have reported correlations between plasma retinol and α_1-acid glycoprotein (r – 0·35) and retinol and serum amyloid A (r – 0·20). Sensitivity and specificity, however, were not as good as CIC when compared with the results of Chowdhury *et al.* (1996), but, of course, acute-phase

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Table 4. *Sensitivity and specificity of raised α_1-acid glycoprotein* as an indication of the effect of disease on retinol levels*

(Data from Filteau *et al.* 1993)

Retinol threshold (μmol/l)	Sensitivity (%)	Specificity (%)
< 0·35	86	36
< 0·70	72	40

*An abnormal α_1-acid glycoprotein is defined as > 1 g/l (Filteau *et al.* 1993).

proteins are synthesized in response to a stress. Different stresses undoubtedly influence different proteins in different ways and it is probably unlikely that any acute-phase protein will behave exactly like RBP. However, the acute-phase protein will give some indication of whether disease might be having any influence on the retinol levels (Table 4).

VITAMIN E

In the same way that vitamin A is influenced by its transport protein in disease, there is evidence that vitamin E is influenced by the lipoproteins (Das *et al.* 1996). The lipoproteins, however, are larger molecules than RBP so the disease has to be severe enough to increase endothelial permeability sufficiently to influence circulating lipoprotein concentrations.

Malaria is a sufficiently severe disease to reduce plasma vitamin E concentrations (Thurnham *et al.* 1990; Das *et al.* 1996; Adelekan *et al.* 1997), but the fact that vitamin E : cholesterol in malaria-infected patients is not different from that of matched controls in studies done in three countries, Thailand (Thurnham *et al.* 1990), Nigeria (Adelekan *et al.* 1997) and India (Das *et al.* 1996), suggests that malaria influences vitamin E via the lipoproteins and not directly (Table 5). Of course, it is widely held that vitamin E is an important antioxidant and that in a disease like malaria, vitamin E would be under intense pressure from oxidizing free radicals both from the parasites and the inflammatory response of the disease. However, even in patients with severe malaria, as defined by World Health Organization (1990) criteria, vitamin E : cholesterol remained normal (Das *et al.* 1996; Table 5).

It would seem unlikely, therefore, that a subclinical condition, i.e. an increased risk of IHD, would influence plasma vitamin E. However, there is evidence from the World Health Organization MONICA (monitoring trends and determinants of cardiovascular disease) studies that lipid-corrected plasma vitamin E is significantly lower in those countries where the risk of IHD is higher (Gey *et al.* 1991). Likewise, Riemersma *et al.* (1991) have reported that persons with newly-diagnosed angina had significantly lower vitamin E than age-matched controls. It is well established that subclinical heart disease is associated with abnormal acute-phase markers e.g. low albumin (Phillips *et al.* 1989; Kuller *et al.* 1991; Gillum & Makuc, 1992), elevated fibrinogen (Yarnell *et al.* 1991) and leucocyte counts (Sipe, 1985), thus it is possible that subclinical disease may in some way reduce the available vitamin E. Furthermore, there is also some evidence which suggests that vitamin E supplementation was of benefit in preventing new attacks of IHD in a secondary prevention trial (Stephens *et al.* 1996). Nevertheless, a study from my own laboratory, which examined blood from 200 randomly-selected subjects in Belfast and Toulouse, found absolutely no difference in either vitamin E or vitamin E : cholesterol between the

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Table 5. *Influence of malaria on vitamin E status based on studies done in India, Nigeria and Thailand*

(Values are medians and ranges except for data from Nigeria where values are means and standard deviations)

Country	Variable		Malaria: Severe	Malaria: Mild	Non-infected	Statistical significance of difference between groups (ANOVA) $P <$
India*						
	α-Tocopherol (μmol/l) :	Median	7·33[a]	11·53[b]	17·71[c]	0·001
		Range	2·61–18·42	3·26–24·08	6·48–28·08	
	T : C (μmol/mmol) :	Median	4·61	4·63	5·15	NS
		Range	1·34–7·20	1·75–7·86	1·80–8·93	
Nigeria†						
	α-Tocopherol (μmol) :	Mean	8·70		14·54	0·001
		SD	3·92 (*n* 46)		4·66 (*n* 19)	
	T : C‡ (μmol/mmol) :	Mean	4·05			
		SD	2·12 (*n* 35)			
Thailand§ : Urban						
	α-Tocopherol (μmol/l) :	Median	13·28 (*n* 22)		18·59 (*n* 13)	0·05
		Range	9·01–23·5		15·69–54·37	
	T : C (μmol/mmol) :	Median	4·98		4·26	NS
		Range	2·73–6·67		3·43–7·46	
Rural						
	α-Tocopherol (μmol/l) :	Median	9·85 (*n* 20)		12·71 (*n* 15)	0·05
		Range	3·00–17·22		8·79–17·93	
	T : C (μmol/mmol) :	Median	4·80		4·58	NS
		Range	1·59–11·30		2·66–7·03	

T : C, α-tocopherol : cholesterol.

[a,b,c] Data were transformed (log base 10) for analysis and mean values with unlike superscript letters were significantly different between groups ($P < 0·05$).

*Data from Das *et al.* (1996) in which there were fifty children in each group.

†Data from Adelekan *et al.* (1997).

‡Cholesterol was not measured in all patients or any of the controls.

§Data from Thurnham *et al.* (1990). Data were collected from two groups of subjects : the urban group were from Chiang Mai, the rural group from a district approximately 120 km south-east of Chiang Mai.

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Table 6. *Changes in leucocyte ascorbic acid during infection with the common cold* (Data from Hume & Weyers, 1973)

(Mean values and standard deviations; values are expressed as nmol/10^8 leucocytes, where 90 nmol/10^8 leucocytes is equivalent to 16 μg ascorbate/10^8 leucocytes)

	Subject no.					
Day of study	1*	2	3	4	Mean	SD
Pre-cold	140	106	96	114	114	19
1	60	55	–	59	58	53
2	48	46	87	–	60	23
3	83	67	93	84	82	11
4	123	90	97	119	107	16
5	160	–	–	83	–	–
10	152	126	97	121	124	23

*Subject developed the cold while consuming 200 mg vitamin C/d.

two centres in either men or women (Thurnham *et al.* 1994). These centres were MONICA study centres and were chosen because there is a three- to fourfold difference in IHD risk between them.

VITAMIN C

I will deal with vitamin C relatively briefly as I recently reviewed the peculiar relationship between vitamin C and trauma (Thurnham, 1994). Briefly, disease appears to have a major impact on vitamin C in the blood. In the 1960s and early 1970s it was shown that trauma rapidly reduced leucocyte ascorbic acid (LAA; Irvin *et al.* 1978; Vallance, 1986). The study of Hume & Weyers (1973) shows the precipitous fall in LAA on day 1 following the onset of symptoms of the common cold (Table 6) and the subsequent behaviour over the next 5 d when pre-infection LAA concentrations were usually regained. In contrast, or as a consequence, plasma ascorbate appears to remain low throughout the period of infection (Thurnham, 1994).

The mechanism behind the fall in LAA is again associated with the acute-phase response and the leucocytosis, which is one of the very early effects of the acute-phase response (Sipe, 1985). Of the new leucocytes, 70 % are neutrophils and these emerge from the bone marrow containing low levels of vitamin C. *In vitro* experiments have shown that neutrophils avidly take up vitamin C in a process which is active, temperature-dependent (with a maximum at 40°) and accelerated a further 2·5 times if the neutrophils are antigen-stimulated (Moser & Weber, 1984). The reason for the uptake of vitamin C is not known. There are three possible explanations: (1) to improve the antioxidant defence of the new neutrophil, (2) to act as a means of transporting the vitamin C to a tissue site where inflammation and tissue damage is occurring or (3) to remove a potential pro-oxidant from the plasma.

Of the three possible explanations, I favour the third one. The first possibility seems least likely since if newly-emerging neutrophils require vitamin C for the maintenance of their integrity, why is it not acquired during production? In fact, a recent report demonstrated that the production of oxygen free radicals by neutrophils from patients who recently had an acute myocardial infarction was depressed by vitamin C supplements (Herbaczynska-Cedro *et al.* 1995). The authors suggested that this would benefit the patient, since various measures of myocardial injury appeared to be positively correlated to

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neutrophil excitation, but one wonders what would happen if the patient developed an infection during treatment. Likewise, if neutrophils were needed to transport vitamin C to the tissues, why are they not prepared in the bone marrow ready for this function? In contrast, vitamin C is known to be a pro-oxidant in the presence of Fe. At times of health the amount of free Fe in the blood will be at a minimum since there is adequate binding capacity in plasma in the form of transferrin. At times of trauma, however, free Fe in the form of bleomycin-detectable Fe, has been reported (Buffinton *et al.* 1986; Halliwell *et al.* 1988; Beare & Steward, 1996). In addition, caeruloplasmin is increased to favour the production of Fe^{3+}, the high Fe-binding capacity protein, apoferritin, is increased and receptor activity in the reticulo-endothelial system is stimulated to promote Fe uptake (Herbert *et al.* 1996). Vitamin C would counter the effects of caeruloplasmin and leach Fe from ferritin so this is a further reason why plasma vitamin C concentrations would decrease in response to disease. The nature of the disease or the influence of disease severity in reducing plasma vitamin C are not known. However, there is evidence that subclinical disease (Bates *et al.* 1982, 1983) or mild trauma, such as smoking (Kallner *et al.* 1981), depresses plasma vitamin C, as discussed elsewhere (Thurnham, 1994).

WATER-SOLUBLE B-VITAMINS

The majority of the B-vitamins are present in the tissues in the form of co-enzymes. Riboflavin, thiamin and niacin are closely involved in mitochondrial functions and oxidation–reduction mechanisms, pyridoxine is closely associated with protein turnover and folate and cyanocobalamin with DNA replication, cell turnover and haemopoiesis. These functions may be strongly influenced by disease. Anorexia may stimulate a need for alternative fuels for energy needs which are obtained by tissue catabolism. Fever raises the BMR by 10 % for every 1° rise in temperature (Du Bois, 1948). Hyperplasia of cells of the immune system will stimulate DNA turnover. While it is apparent that requirements for these nutrients may be increased by the disease, there are only a limited number of studies which have measured the impact of disease on biomarkers of nutritional status of the different nutrients.

Riboflavin

One of the best methods of assessing riboflavin status is the erythrocyte glutathione reductase (*EC* 1.6.4.2; EGR) test in which the level of *in vitro* stimulation of EGR by the co-enzyme of riboflavin, is assessed as an activity coefficient (AC; Tillotson & Baker, 1972). The degree of co-enzyme saturation of EGR is determined by the availability of riboflavin at the time of erythropoiesis, but as the erythrocyte is permeable to riboflavin, riboflavin status can be influenced by a sudden influx of riboflavin into the circulation. One of the consequences of tissue breakdown and a negative N balance is a release of tissue co-enzymes into the circulation and an apparent increase in riboflavin status (Bates *et al.* 1981; Bamji *et al.* 1987).

Thiamin

Beriberi is one of those deficiency diseases which appears to be increased by an infection or fever (Platt, 1958). As indicated previously, fever will increase thiamin requirements (Du Bois, 1948) and, of course, this vitamin is of fundamental importance in the metabolism of glucose to provide energy via the Krebs cycle. There is also experimental

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evidence to suggest that the cytokine, which triggers anorexia during infection, alters food selection in favour of carbohydrate in preference to fat or protein (Macdonald *et al.* 1993).

Thiamin status is measured either by the erythrocyte transketolase (*EC* 2.2.1.1; TKL) assay (Brin, 1964) or by erythrocyte thiamin (Finglas, 1993). As in effect almost all erythrocyte thiamin is present as thiamin pyrophosphate and bound to transketolase, both methods are measuring the same component. The TKL assay is probably more sensitive than erythrocyte thiamin but apo-transketolase is unstable both *in vivo* and *in vitro*. Consequently, a chronic thiamin deficiency is less likely to be detected by the TKL assay, but the greater or longer the deficiency, the more difficult it is to measure total erythrocyte thiamin accurately.

MINERALS

Plasma concentrations of metal ions are frequently measured as markers of nutritional status, but their usefulness is questionable. Infection is commonly associated with a fall in plasma Zn and an increase in plasma Cu (Graham *et al.* 1991). The fall in Zn is a relatively rapid effect of infection but its function is not known. Plasma Zn represents less than 1 % of total body Zn in comparison with 60 % in the muscles and 20–30 % in the bone (Aggett & Favier, 1993). Thus, in catabolic conditions like infection, severe malnutrition or increased bone turnover, plasma Zn concentrations may be restored and normalized.

Plasma Cu can also be increased by tissue breakdown (Chevion *et al.* 1993), but the principal cause of the increase in plasma Cu with infection is the increased production of the acute-phase protein, caeruloplasmin (Koj, 1974). As indicated elsewhere, the ferroxidase (*EC* 1.16.3.1) activity of caeruloplasmin may be particularly important for the conversion of Fe^{2+} to Fe^{3+} to assist the uptake and removal of Fe from plasma during infection (Thurnham, 1994). The increase in caeruloplasmin, therefore, has little relationship with Cu status.

Iodine deficiency is a major public health problem in the Third World. Iodine is necessary for the synthesis of thyroid hormones and frequently these hormones are used as functional markers of iodine status. However, infection has pronounced effects on thyroid hormones, since they are transported in the blood by the negative acute-phase protein, transthyretin (TTR). The synthesis of TTR is markedly suppressed by infection (Ramsden *et al.* 1978; Dickson *et al.* 1982), thus thyroid hormone levels measured in communities where infection is common should be interpreted with caution. Whether such measurements can be better assessed using other acute-phase proteins to detect infection, as suggested for retinol, remains to be investigated.

The last mineral I want to discuss is Se. Se is an integral part of the enzyme glutathione peroxidase (*EC* 1.11.1.9; GPx) which catalyses the reduction of peroxides (Rotruck *et al.* 1973), but is also involved in thyroid metabolism (Arthur & Beckett, 1994). Both metabolic functions are likely to be influenced by infection. There is some evidence to suggest that GPx activity in the lungs can be induced by exposure to elevated oxygen stress as, for example, in rats exposed to increased amounts of ozone (Chow, 1976). However, I know of no evidence that infection will induce similar changes, and tissue culture experiments suggest that superoxide dismutase (*EC* 1.15.1.1) activity is the more critical enzyme in coping with oxygen radicals.

CONCLUSIONS

Infection has pronounced effects on markers of micronutrient status. In particular, plasma retinol, α-tocopherol and ascorbate are reduced by trauma. The effects can be linked to the

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acute-phase response and the changes may represent mechanisms designed to protect the host or his micronutrient reserves in the short term. The reductions in retinol and vitamin E can be associated with the reductions in their plasma transport proteins, while vitamin C levels are inversely related to the leucocytosis in trauma. Catabolic states may increase the availability of certain B-vitamins or minerals in the blood and produce an apparent improvement in status. The effects of infection on mineral status are variable. Changes in acute-phase proteins, for example the increase in Cu-containing caeruloplasmin or the reduction in thyroid hormone-transporting transthyretin, are direct responses to infection, and care should be taken where these markers are used to interpret nutritional status.

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