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Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron

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Suggested citation: Thurnham DI, McCabe GP. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: World Health Organization. *Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010*. Geneva, World Health Organization, 2012.

Abstract

■ Many plasma nutrients are influenced by infection or tissue damage. These effects may be passive and the result of changes in blood volume and capillary permeability. They may also be the direct effect of metabolic alterations that depress or increase the concentration of a nutrient or metabolite in the plasma. Where the nutrient or metabolite is a nutritional biomarker as in the case of plasma retinol, a depression in retinol concentrations will result in an overestimate of vitamin A deficiency. In contrast, where the biomarker is increased due to infection as in the case of plasma ferritin concentrations, inflammation will result in an underestimate of iron deficiency. Infection and tissue damage can be recognized by their clinical effects on the body but, unfortunately, subclinical infection or inflammation can only be recognized by measuring inflammation biomarkers in the blood. It is therefore important to measure biomarkers of inflammation as well as of nutrition in prevalence surveys of nutritional status in apparently healthy people. The most commonly used biomarkers of inflammation are the cytokines and acute phase proteins. Cytokines have very short half-lives but the acute phase proteins remain longer in the blood, and their lifespans can be matched with the changes in plasma retinol and ferritin concentrations. Using meta-analyses to determine the mean effect of inflammation on retinol and ferritin in different stages of the infection cycle, it was possible to determine correction factors that can be used either to modify raw data to remove the effects of inflammation or to modify cut-off values of nutritional risk to use when inflammation is detected in a blood sample.

Plasma nutrients that appear to be influenced by infection and inflammation with an emphasis on vitamin A and iron and where possible a quantitative estimate of the effects

Nutrient biomarkers and inflammation

The plasma concentrations of several important nutritional biomarkers are influenced by inflammation (1), including retinol (2–4), 25-hydroxy cholecalciferol (vitamin D) (2, 4, 5), iron (6), ferritin (7), transferrin receptors (8), zinc (6, 9), carotenoids (3, 10–12), selenium (4, 13), pyridoxal phosphate (2, 4, 14), α -tocopherol and total lipids (2), and vitamin C (1, 2, 4). In addition, concentrations of the transport proteins albumin, retinol-binding protein (RBP) and transferrin are often low in children frequently exposed to infections (15) or trauma (2). In the above reports the abnormal nutrient biomarker concentration was associated with a raised concentration of the acute phase protein (APP) C-reactive protein (CRP) or other evidence of inflammation. However, in a cross-sectional survey or single time-point observation there is no way of telling from the nutrient measurement alone whether a low or abnormal value represents poor nutritional status or the effect of inflammation or both.

Children with acute infections display varying degrees of anorexia and it is customary for parents and physicians to accept a limited intake of food during infections. In the nutritionally normal child such transient malnutrition is rapidly compensated for during infection-free intervals but in the child with chronic illness or those exposed to frequent infections with associated anorexia, there is the possible risk of more long-standing malnutrition of clinical significance (15). Thus an apparently healthy child living in a region where there is a high exposure to disease may be genuinely malnourished, but there is also the possibility of subclinical inflammation and that the biomarkers used to measure nutritional status may also be influenced by disease and over- or underestimate the extent of malnutrition.

Plasma retinol, α -tocopherol, total lipids, pyridoxal phosphate, and 25-hydroxy cholecalciferol and leukocyte vitamin C concentrations

In the case of vitamin A, there are numerous reports of low retinol concentrations where inflammation is known to be, or is probably, present and where the retinol concentrations normalized when the subjects recovered without any vitamin A intervention (2, 16, 17). Louw and colleagues' (2) study is particularly important with respect to vitamin A and is described more fully later. However, these authors also reported longitudinal data on a number of other nutritional biomarkers in 26 men and women with no underlying pathology who underwent uncomplicated orthopaedic surgery. No patient fasted postoperatively for more than 12 hours and intake was considered normal within 48 hours of surgery. The authors also monitored fluid intake postoperatively and found no evidence of overhydration.

Blood concentrations were measured at baseline and at 4, 12, 24, 48, 72 and 168 hours. CRP concentrations rose slightly between 4 and 12 hours and increased maximally between 12 and 48 hours. CRP concentrations fell significantly between 48 and 72 hours but no further measurements were made until day 7 when CRP concentrations in the women were still raised. Associated with these changes in CRP, there were similar changes in plasma retinol, α -tocopherol, total lipids, pyridoxal phosphate and 25-hydroxy cholecalciferol (25-HCC), and leukocyte vitamin C concentrations in both men and women. As a group, the premorbid nutritional status of the patients was adequate and dietary intakes compared favourably with recommended dietary allowances.

The fall in leukocyte vitamin C concentrations following surgery (18) or the common cold (19) is well known and has been reported as long ago as in the 1970s. Likewise falls in plasma lipoprotein concentrations have been previously reported in connection with malaria

and can in part be ascribed to extravasation due to increased capillary permeability (20, 21). Almost all the vitamin E in plasma is incorporated within the lipoprotein fraction so changes in plasma vitamin E concentrations are probably also due to the increased capillary permeability in inflammation (see below). There are numerous reports of low 25-HCC concentrations in persons with or at risk of disease (22, 23). The association is normally considered to be indirect, i.e. a consequence of low or inadequate exposure to sunlight in persons with poor health. However, Reid and colleagues (5) recently confirmed marked depression in 25-HCC following surgery, associated with changes in inflammation, which was previously reported by Louw et al. (2).

Serum and erythrocyte folate, serum B₁₂, and plasma vitamin C concentrations

Louw and colleagues also measured, but did not show, serum and erythrocyte folate, serum B₁₂ and plasma vitamin C concentrations, and commented that although some values fell below baseline values the changes appeared random and not like CRP (2). We have previously found no evidence of any influence of inflammation on folate status in young Irish adults (Thurnham and Haldar 2007, unpublished data) but others have suggested that folate is influenced by inflammation although we are not aware of any published data (24, 25).

Low plasma vitamin C concentrations are frequently reported in elderly people, a group with a high risk of subclinical inflammation (26). Likewise, low plasma vitamin C has been reported in patients with cardiovascular disease (27), and the study concluded the apparent risk of an acute myocardial infarction associated with low plasma ascorbic acid was distorted by the acute phase response (APR). Low plasma vitamin C concentrations, however, may be a consequence of the effect of inflammation on leukocytes. Inflammation stimulates the release of leukocytes into the circulation and when the cells first emerge from the bone marrow, the new leukocytes are low in vitamin C. They dilute the apparent leukocyte vitamin C concentration in the first 2–3 days following the APR but premorbid leukocyte vitamin C concentrations are generally achieved by day 5. The new leukocytes probably acquire their vitamin C from the plasma, hence plasma vitamin C concentrations are low following myocardial infarction and in elderly people, in whom there may be increased subclinical inflammatory activity.

Carotenoids

Low plasma carotenoid concentrations in the presence of infection and inflammation have been described by a number of workers. Smoking is frequently associated with low concentrations of carotenoids (28); this may be partly a dietary effect (29) but in all studies inflammation is strongly linked to the low concentrations (10–12).

Iron

Maintenance of cellular iron homeostasis is a prerequisite for many essential biological processes and for the growth of organisms, and is also a central element in the regulation of immune function (30). The hypoferraemia following infection was described over 50 years ago (6, 31) and more recent longitudinal data have described the direct effects of the macrophage-derived cytokines on serum ferritin, transferrin receptors, iron and transferrin concentrations in humans (7). The importance of iron in the APR and the influence of inflammation on iron biomarkers will be described more fully below.

Interaction of inflammation, vitamin A and iron

Serum retinol concentrations have been shown to be positively associated with haemoglobin, haematocrit, serum iron and % transferrin saturation (for references see Strube et al. (32)).

Further work suggested that anaemia did not or only poorly responded to medicinal iron in the presence of vitamin A deficiency but was ameliorated when vitamin A was given (33, 34). These data suggested that vitamin A played a role in regulating plasma iron concentrations. However, many studies have shown the vitamin A supplementation can reduce mortality (35) and morbidity, especially in measles (36, 37). Thus vitamin A supplementation may simply reduce inflammation and in so doing promote the release of iron into the circulation (38). Strube et al. (32) carried out 2×2 experiments with vitamin A (\pm) and iron (\pm). There was no evidence of inflammation but the authors reported lower plasma iron and % transferrin saturation in marginal vitamin A deficiency and an elevation of liver vitamin A in iron deficiency. This study suggested that severe deficiencies of the two nutrients do have an impact on each other but in human studies the deficiencies are unlikely to be as severe, therefore other explanations must be sought. Nevertheless the authors concluded that providing vitamin A may be more likely than treatment with iron alone to improve the iron and vitamin A status of human populations in which both deficiency conditions coexist (32).

Indicators of infection, e.g. acute phase proteins and cytokines, and their usefulness in the measurement of inflammation

Infection

This term implies that the body's structure and/or normal metabolism have been interfered with by the entry of material recognized as foreign within the tissues. The effects of the foreign material may be very small and cause only subclinical infection, or be severe enough to cause the outward appearance of clinical signs that are the symptoms of disease. The clinical signs of disease frequently take the form of fever, raised body temperature, anorexia, headache, cough, vomiting and diarrhoea, and may be nonspecific or characteristic of a disease caused by a particular foreign organism.

Inflammation

The biochemical and physical changes in a body that are initiated in response to tissue damage or a foreign organism are termed the inflammatory response. The changes begin the moment an intruder is detected and expand exponentially to meet the perceived threat. The interaction between inflammation and the invader is responsible for the clinical signs and symptoms of disease. However, evidence of inflammation may be covert if the infection is minor or the body's immunity is particularly effective in preventing the disease and removing the cause of the infection. In addition in all infections there is a short period before clinical symptoms appear, usually 24–48 hours, and after clinical signs have disappeared, i.e. during convalescence, when evidence of inflammation may only be detected biochemically. Inflammation is essentially protective and designed to neutralize and remove the invader and repair the damage caused directly by the invader and indirectly in any ensuing conflict. However, the changes that constitute inflammation are metabolically demanding and potentially destructive. An inflammatory response should ideally not last longer than 9–10 days.

The inflammatory response

In the aftermath of injury trauma or infection of a tissue, a complex series of reactions is executed by the host in an effort to prevent ongoing tissue damage, isolate and destroy the infective organism, and activate the repair processes that are necessary to restore normal function. This whole homeostatic process is known as inflammation and the early set of reactions that are induced are known as the acute phase response. The cell most commonly associated with initiating the cascade of events during the APR is the tissue macrophage or blood

monocyte (39). Activated macrophages release a broad spectrum of mediators, of which cytokines of the interleukin 1 (IL-1) and tumour necrosis factor (TNF) families play a unique role in triggering the next series of reactions, which take place locally and distally. Locally, stroma cells, e.g. fibroblasts and endothelial cells are activated to cause the release of a second wave of cytokines that include IL-6 as well as more IL-1 and TNF. These cytokines magnify the homeostatic stimulus and potentially prime all cells in the body with the potential to initiate and propagate this homeostatic response.

The endothelium plays a critical role in communicating between the site of tissue inflammation and circulating leukocytes. The cytokines IL-1 and TNF induce major changes in gene regulation and surface expression of adhesion and integrin molecules, including intracellular adhesion molecule (ICAM). These molecules interact specifically with neutrophils and other circulating leukocytes, slow their rate of flow, initiate trans-endothelial passage and allow migration into the tissue. Alterations in vascular tone are also early features of the APR. Inflamed tissue releases low-molecular-weight mediators including reactive oxygen species, nitrous oxide and products of arachidonic acid including prostaglandins. Dilatation of and leakage from blood vessels occur particularly in postcapillary venules, resulting in tissue oedema and redness. Aggregation of platelets can stimulate the clotting cascade and the release of further molecules such as bradykinin, causing pain (39).

Within the spectrum of the systemic responses to the inflammatory cytokines, two particularly important physiological responses include effects on the hypothalamus and the liver. Within the hypothalamus the temperature set-point may be altered, generating a febrile response. In the liver, there are alterations in metabolism and gene regulation to determine the level of essential metabolites for the organism during the critical stages of stress and supply the necessary components for defence, limitation, clearing and repair of damage at the site of the initial attack. The liver response is characterized by prominent changes in most metabolic pathways and in the coordination and stimulation of the APPs (40).

Acute phase proteins

The APPs are a highly heterogeneous group of plasma proteins (**Table A2.4.1**) both in respect of the physicochemical properties as well as in respect of their biological actions, which can include anti-proteinase activity, coagulation properties, transport functions, immune response modulation and/or miscellaneous enzymic activity. However, one feature that they all have in common is a role in the function of restoring the delicate homeostatic balance disturbed by injury, tissue necrosis or infection (41).

Table A2.4.1

Characteristics of some well-known acute phase proteins in humans

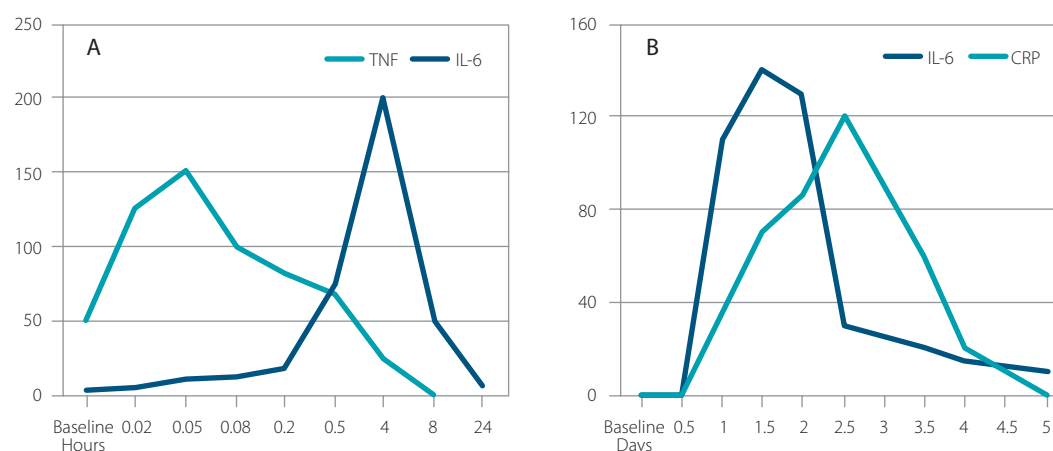
Acute phase protein	Abbreviation and synonyms	Molecular size (Mr)	Normal concentration (g/L)	Response in inflammation	
				Amount	Time to maximum
Serum amyloid A	SAA	11 000–14 000	~0.01	Increase 20–1000 fold	24–48 h
C-reactive protein	CRP	105 500	0.001		
α 1-anti-chymotrypsin	ACT	68 000	0.2–0.6	Increase 2–5 fold	4–5 days
α 1-acid glycoprotein	AGP, orosomucoid	40 000	0.6–1.0	Increase 2–5 fold	
Fibrinogen		340 000	1.9–3.3	Increase 30–60%	24–48 h
Ceruloplasmin	Co	132 000	0.3–0.4		4–5 days
Albumin	Alb	66 000	35–45	Decrease 30–60%	24–48 h
Transferrin	Tf	77 000	2.0–3.0		
Thyroxin-binding protein	Pre-albumin, transthyretin	52 000	0.3–0.4		

Data mainly from reference (40).

The production of APPs is induced and regulated by the cytokines. IL-1 and TNF enhance the expression of type 1 APPs including serum amyloid A (SAA), CRP and α -1 acid glycoprotein (AGP), while IL-6 specifically enhances production of type 2 APPs, including fibrinogen, ceruloplasmin, haptoglobin and anti-proteinases, e.g. α 1-anti-chymotrypsin (ACT). IL-6 will also synergistically enhance the effects of IL-1 and TNF in producing type 1 APP, but IL-1 and TNF have no effects on type 2 APP (39). The time courses of TNF, IL-6 and CRP in patients after surgery are shown in **Figure A2.4.1**.

Figure A2.4.1

Time course of tumour necrosis factor (TNF), interleukin (IL) 6 and C-reactive protein (CRP) after surgery (arbitrary units). (A) Systemic serum TNF concentrations rising rapidly to peak at 3 minutes in patients following limb surgery. IL-6 started to rise at 10 minutes and peaked at 4 hours (7). (B) Increase in IL-6 precedes the rise in CRP and has almost disappeared at 48 hours when CRP peaked (42).



The changes in plasma nutrient concentration that follow the onset of infection or trauma closely parallel those of CRP (2, 7). However, with the disappearance of clinical symptoms there is a sharp fall in CRP concentrations (43) but nutrient concentrations do not appear to respond so rapidly (7, 43). Therefore to detect inflammation in apparently healthy people, other inflammatory proteins that remain elevated longer than CRP are required (**Table A2.4.1**) and α 1-acid glycoprotein (AGP) is particularly useful to monitor the later stages of inflammation (44). In contrast to CRP, which rises to a maximum between 24 and 48 hours, AGP may take 3–5 days to reach a plateau (45). Furthermore CRP has a half-life of 2 days whereas for AGP, it is 5.2 days (46). Therefore on a population of apparently healthy people, the combination of the two proteins CRP and AGP will detect those who have only recently been infected, and are not yet showing clinical evidence of disease (raised CRP only), and those who have recovered and are convalescing (raised AGP with or without a raised CRP). ACT, another protein, has similar characteristics to CRP on infection (**Table A2.4.1** (47)) and was used by us previously in Pakistani preschool children (48), but CRP is more useful as it is more often measured.

The use of these proteins to identify persons with subclinical inflammation and derive correct factors to adjust retinol or ferritin concentrations is described later in this review.

Influence of infection and inflammation on plasma retinol concentrations

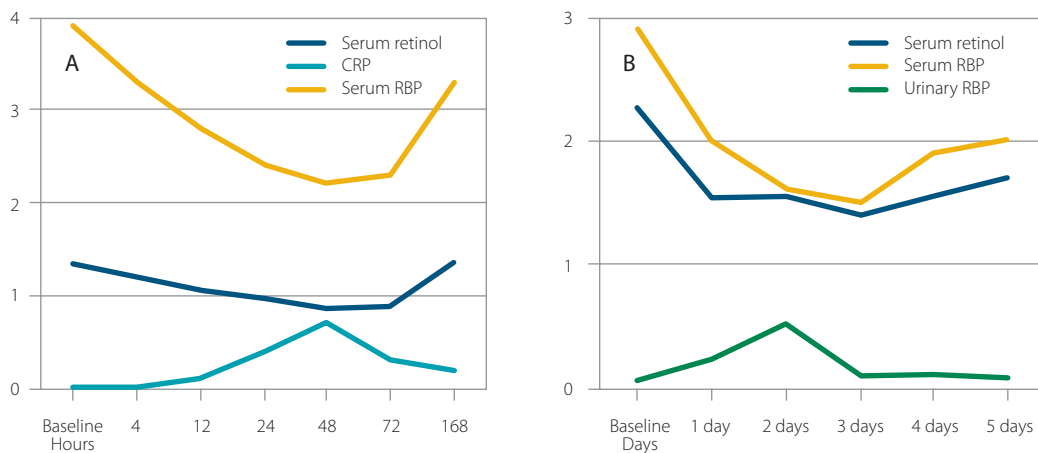
In the background papers for the Annecy Accords it was recognized that infection lowered plasma retinol concentrations, but it was argued that populations with a high prevalence of infection were also more likely to have vitamin A deficiency. Thus the cut-off of 0.7 μ mol/L for serum or plasma retinol concentrations was chosen to indicate vitamin A status irrespective of

inflammation (49, 50). However, this approach ignores the fact that there will be people in the population with inflammation but no vitamin A deficiency, who will be assessed as vitamin A deficient because of the effects of inflammation alone on plasma retinol. Thus if inflammation is ignored the prevalence of vitamin A deficiency in a population will be overestimated and interventions to reduce it may be inappropriate and unnecessary.

There are only few data where plasma retinol concentrations have been measured before, during and after a trauma. Louw and colleagues' (2) study is particularly valuable as they measured plasma retinol and CRP concentrations in 26 adult men and women who underwent uncomplicated orthopaedic surgery (**Figure A2.4.2A**). In the first 24 hours there was a 26% fall in retinol concentrations and at the height of the inflammation, plasma retinol was depressed by ~40%. The factors affecting plasma retinol concentrations are most likely to be the initial vasodilation, increasing vascular permeability and excretion of RBP and some retinol (51, 52) (**Figure A2.4.2B**) and inhibition of RBP synthesis (53). These factors will no doubt increase with the severity of the trauma so the effects on retinol will be that much greater the more severe the trauma. Thus in children admitted to hospital with severe shigellosis (17), mean plasma retinol concentration was 0.36 $\mu\text{mol/L}$, which rose to 1.15 $\mu\text{mol/L}$ on discharge without any vitamin A intervention – a mean depression of 69%.

Figure A2.4.2

Time course of retinol, C-reactive protein (CRP) and retinol-binding protein (RBP) after surgery (arbitrary units). (A) Fall in concentration of serum retinol and RBP concentrations to a nadir at 48 hours coinciding with the peak CRP concentration (2). (B) Similar data are evident for serum retinol and RBP concentrations; note the increasing appearance of urinary RBP over the first 48 hours (51).



Thus the degree of depression in plasma retinol concentrations in sickness or in patients who have undergone the trauma of surgery will depend on the severity of the trauma. In such persons it may be possible to assess the degree of depression in plasma retinol using biomarkers of inflammation, but we have not attempted this as we believe the more urgent problem was assessing the level of depression in subclinical inflammation. Workers are generally aware that retinol is depressed by disease or trauma, but the situation in apparently healthy people is generally not recognized.

Influence of infection and inflammation on iron biomarkers

Anaemia of chronic inflammation

Constant exposure to chronic inflammation is well known clinically to influence iron metabolism and result in anaemia of chronic inflammation (ACI). ACI is usually mild, that is red cells

are usually normal in colour (normochromic) and size (normocytic) and with a normal reticulocyte count, i.e. very few or no young red cells in the circulation. Red cells can occasionally be macrocytic but there is little evidence of iron deficiency (54, 55). The administration of iron to such patients by oral or parenteral routes can have little effect on serum iron or other indices of iron status, probably because iron absorption is inhibited by the inflammatory process (56) irrespective of iron status (57).

Anaemia in much of the developing world has many of the characteristics of ACI in that it is often mild (58) and there are high prevalence rates of infection and inflammation (59). That is, high exposure to and frequency of infections are probably important aetiological factors in the cause of anaemia in developing countries. Iron deficiency is also common and undoubtedly malnutrition with diets high in the iron-chelator phytate and lacking iron-rich foods such as meat are important factors, but the overriding presence of disease-inhibiting iron absorption may mean that ACI exceeds iron deficiency as the major cause of anaemia (60). The poorer the diet and the higher the frequency of infection, the greater the risk of a dietary iron deficiency.

Biomarkers of iron status include serum iron, blood haemoglobin, haematocrit, zinc protoporphyrin, serum transferrin, transferrin receptors and ferritin. These are all influenced by inflammation but as the mechanisms differ, the impact of inflammation on the biomarkers varies (**Table A2.4.2**). It is helpful to understand the mechanism by which infection influences iron biomarkers to understand why there are differences in (1) the influence of inflammation on biomarkers and (2) on the relationships between the inflammatory proteins and iron biomarkers. Work on the cytokine IL-6 and the peptide hepcidin in the past few years has provided much information on the first of these questions.

Table A2.4.2

Effects of inflammation on iron biomarkers

Blood biomarkers	Impact of inflammation		Mechanism of response
	Speed	Response	
Iron	Within 8 hours (7)	Fall by ~50% (7)	Inhibition of iron release from macrophages (61) and uptake by newly released apoferritin (7)
Haemoglobin	Slow or none	Fall by 20–30%	Insufficient iron in serum to allow normal replacement of old red cells and inhibition of erythropoiesis by TNF and IFN- γ
Haematocrit	Slow or none	Fall by 20–30%	
Zinc protoporphyrin (ZPP)	Slow	4–5-fold increase in ACI	Where there is a decreased production of haemoglobin, ZPP will increase (62)
Transferrin	Rapid in 24 hours	Fall by 20–30%	Increased vasodilation and capillary permeability
Transferrin receptors	24 hours	Fall by 30–50%	Impaired erythroid growth or inhibition of <i>TfR</i> gene expression mediated by TNF and IFN- γ (7)
Ferritin	Rapid in 8 hours	Rise paralleled CRP increase 30–100% or more	TNF, IL-6 and IFN- γ exert a stimulatory role on ferritin synthesis (7)

ACI, anaemia of chronic inflammation; CRP, C-reactive protein; TNF, tumour necrosis factor; IFN, interferon, IL, interleukin.

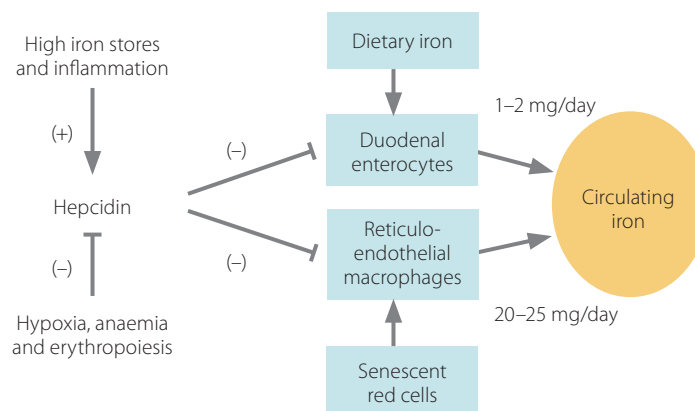
Hepcidin is a 25 amino acid peptide hormone, which acts on the small intestinal epithelium and cells of the reticuloendothelial (RE) system to limit iron delivery to the plasma (63, 64). In human hepatocytes, IL-6 induces hepcidin mRNA (61) and, as described above, the production of IL-6 is one of the earliest responses of macrophages and damaged tissues to the presence of a foreign antigen. In the past few years, the functions of hepcidin have dramatically advanced our understanding of iron metabolism (65). The hormone is produced primarily by the liver and secreted into the circulation. Its synthesis is increased in response to iron and inflammation, and reduced in response to erythropoiesis, anaemia and hypoxia (**Figure A2.4.3**).

Hepcidin is a potent antimicrobial compound as indicated by the fact that hepcidin-

mediated transcription in mice suppresses toxicity due to a single dose of lipopolysaccharide (LPS) or turpentine (67). Hepcidin acts by interacting with its receptor ferroportin, a transmembrane iron-exporter protein. Ferroportin is abundantly expressed on the cell surface membrane of RE macrophages, i.e. resident macrophages in the liver, spleen and bone marrow, and on the basolateral membrane of duodenal enterocytes. Hepcidin inhibits iron release at these sites by binding to ferroportin and the complex is internalized and degraded. This role of hepcidin explains the rapid effect of inflammation on serum iron since RE macrophages are an especially important source of iron as they enable the reuse daily of 20–25 mg iron from senescent red cells. RE iron normally undergoes rapid turnover, thus the iron retention by this cell population acutely lowers circulating iron concentrations (65, 68). Hepcidin also inhibits the uptake of 1–2 mg dietary iron from duodenal enterocytes thus chronic inflammation will gradually reduce dietary iron intake, leading to iron deficiency anaemia (IDA). Short-term effects of inflammation are unlikely to have marked effects on red cell synthesis or haemoglobin, however, long-term reductions in plasma iron will affect haemoglobin production and red cell synthesis. In addition, IFN- γ and TNF are known mediators of ACI and inhibit erythropoiesis in vitro and in vivo (69).

Figure A2.4.3

Control of iron homeostasis by hepcidin. Presence of iron stores and/or inflammation stimulates hepcidin production, which depresses iron absorption and reutilization. Hypoxia, anaemia and erythropoiesis block hepcidin production, which permits iron absorption and reutilization (65, 66).



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Inflammation and iron biomarkers

Serum iron

Most iron in the blood is present as functional iron in haemoglobin or transport iron bound to serum transferrin. There is little free or ionic iron 10^{-18} M (70), but this can rise in the presence of anoxia, physical damage to tissues or damage caused by inflammation. The large and rapid fall in serum iron concentration in response to hepcidin induction is described above. Serum iron has been shown to be depressed abruptly during the incubation period of most generalized infectious processes, in some instances several days before the onset of fever or any symptoms of clinical illness (6). In patients given endotoxin to induce therapeutic fever, depression of serum iron values began within several hours and were maximum at 24 hours. And usually the depression of iron in the prodromal period was directly related to the severity of the subsequent symptoms (31, 71). The rapidity and generalized nature of the hypoferraemia indicates it is part of the innate immune response, and is an attempt by the host to withdraw

iron and prevent its use by invading pathogens. However, the hypoferraemia is probably insufficient alone to limit bacterial growth since many bacteria can produce siderophores capable of competing effectively for the limited amount of iron available (70, 72). Other factors probably include lipocalin-2, which is secreted by immune cells on encountering invading bacteria and can limit bacterial growth by sequestering iron-laden bacterial siderophores (73) in addition to stimulating a proinflammatory influx of neutrophils to protect the host (74).

Haemoglobin and red cell synthesis

Erythropoiesis is ongoing to replace senescent red cells. As indicated in previous sections, short-term inflammation will depress erythropoiesis and the withdrawal of iron from the circulation will inhibit haemoglobin synthesis but the overall effect on the red cell mass will be minor. If the inflammation is continuous or frequent however, then the total red cell mass will fall and ACI will result.

Transferrin and per cent transferrin saturation (% Tfs)

Three consequences of inflammation affect these biomarkers. The reduction in plasma iron rapidly reduces % Tfs. The increases in vasodilation and capillary permeability rapidly reduce the concentration of transferrin in the blood (45). There is also evidence that synthesis of transferrin is slightly inhibited by cytokines TNF, IL-1 and IL-6 (7, 75), although others found no fall in the concentration of liver transferrin mRNA during the APR (40). The net effect of inflammation is to lower transferrin concentration by approximately 30% and % Tfs to ~20%.

Zinc protoporphyrin (ZPP)

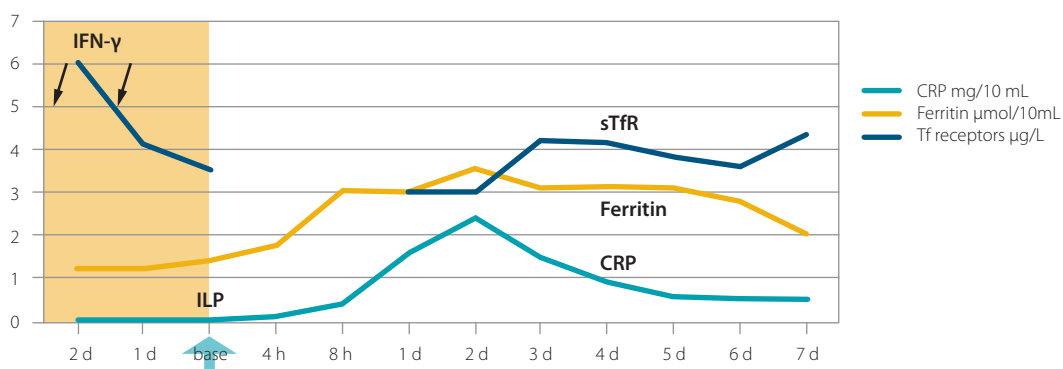
Patients with an impaired iron supply for erythropoiesis show increased ZPP concentrations despite increased storage iron in bone marrow (62). Thus inflammatory disease will increase ZPP concentrations due to its limiting effect on circulating iron and not as a specific effect on erythropoiesis.

Transferrin receptors (sTfR)

Use of phlebotomy to lower iron reserves in healthy subjects has shown that the main determinant of sTfR concentrations is iron deficiency and that sTfR concentrations rise steeply when serum ferritin concentrations fall below 15 µg/L (76). However, Feelders and colleagues also showed that sTfR concentrations were sensitive to cytokines IFN-γ and TNF, which reduced concentrations between 30% and 50%, and the authors attributed the depression to impaired erythroblast iron availability in the inflammatory response (7) (**Figure A2.4.4**). The figure

Figure A2.4.4

In patients treated with interferon (IFN)-γ on 2 days prior to isolated limb perfusion (ILP), soluble transferrin receptor concentrations (sTfR) were measured before ILP and from days 1 to 7. Following the surgery, blood was taken and C-reactive protein (CRP) and ferritin concentrations were measured at all the times shown (7).



shows that sTfR concentrations were initially depressed by the IFN- γ treatment and remained low during the 7 days following limb surgery. Other workers have also reported lower sTfR concentrations in patients with inflammation associated with malaria (8) and HIV (77).

Ferritin

It is well established that serum ferritin concentrations are proportional to liver reserves of iron but that ferritin is increased by inflammation (60, 78). The sequential changes in CRP and ferritin concentrations following the inflammatory stimulus of limb isolation in 12 patients with melanomas or sarcomas is shown in **Figure A2.4.4**. Ferritin concentrations were minimally affected by treatments with IFN- γ over 2 days before surgery but following limb perfusion there were rapid increases in systemic TNF and IL-6. Subsequently ferritin and CRP concentrations increased over the first 48 hours but then deviated (7). CRP concentrations fell but ferritin concentrations were still raised even on day 7. Others have also noted that serum ferritin concentrations parallel CRP concentrations in both acute and chronic diseases and that with recovery from illness, there is a sharp drop in CRP but not in ferritin concentrations (43). It was suggested that the persistence of ferritin might be due to a longer half-life than CRP or that erythropoiesis was still depressed (43).

Transferrin receptor/serum ferritin ratio (R/F ratio)

The R/F ratio can be used to provide an estimate of body iron and is superior to earlier estimates of body iron that relied on haemoglobin, serum ferritin, erythrocyte protoporphyrin, serum iron and total iron-binding capacity (60). However, in spite of its advantage in being obtainable from a finger-stick sample, it still requires the measurement of serum ferritin and is therefore influenced by inflammation or liver disease. The authors suggest, however, that the R/F algorithm may be modifiable by suitable markers of inflammation (60) and it is possible that the correction factors produced by the ferritin meta-analysis described in the next section (79) could be incorporated. The use of CRP only is not entirely suitable to address the problem of inflammation because of the different decay rates of CRP and ferritin (43, 80).

Outline of meta-analysis methods to calculate correction factors for retinol and ferritin

Retinol meta-analysis

As indicated in the earlier sections of this review, plasma retinol concentrations fall rapidly with the onset of infection or trauma. The extent of the fall will depend on the severity of the trauma. In the case of subclinical inflammation, however, the effects on nutritional status will be, by definition, only mild and there should be little variation between populations or between people exposed to or recovering from different diseases. This, of course, assumes that normal nutritional status will be the same in different populations and it has been generally accepted, as the same cut-offs are applied to plasma retinol concentrations regardless of population (50).

As described above, two APPs, CRP and AGP, are useful to identify those with inflammation and also characterize the temporal aspects of inflammation. We identified 15 studies with data on retinol and one or more APPs. Six of these had sufficient data in all subgroups on CRP and AGP and an additional study included ACT and AGP. ACT has similar characteristics to CRP (47) but all analyses were run with and without the latter study to ensure comparability of data. To define inflammation we used the cut-off values of >5 mg/L for CRP, >1 g/L for AGP, >0.6 g/L for ACT and >5 mg/L for SAA and then allocated the apparently healthy subjects in each study into four groups: reference (those with no raised APP), incubating (those with a raised CRP only), early convalescence (those with raised CRP and AGP) and late convalescence (those with a raised AGP only).

Two-group meta-analysis

To assess the relationship between the individual APPs and serum retinol, we first did a two-group meta-analysis (81) for CRP and AGP separately. Individuals were classed as having a normal or high value for the respective APP using the cut-offs described above. APP concentrations recorded as being below the lowest detectable level or higher than the limit of detection were classified as normal and high, respectively. We calculated the mean log retinol value of each group and the study summary, which was the difference between the mean log retinol concentration for the normal group and that for the high group. The ratios (95% confidence interval (CI)) obtained where the individual APPs were increased were 1.16 for ACT (CI 1.06–1.26, $P < 0.001$, $n = 7$), 1.19 for AGP (CI 1.10–1.29, $P < 0.001$, $n = 13$), 1.26 for CRP (CI 1.19–1.33, $P < 0.001$, $n = 5$) and 1.30 for SAA (CI 1.17–1.44, $P < 0.001$, $n = 2$). That is, the retinol concentrations were depressed on average by 16%, 19%, 26% and 30% when ACT, AGP, CRP and SAA were raised by comparison with the respective reference population.

Four-group meta-analysis

In the four-group analysis, we classified individuals from each study as reference, incubating, early convalescence or late convalescence on the basis of values of two APPs as described above. The four-group analysis resulted in comparison of six pairs of mean log retinol concentrations for each study. Each of these was summarized by study and then analysed as below.

Within-study variance

The summary statistic (effect size) was the difference between two means, and the variability associated with each summary statistic was related to sample sizes. In general, studies with a large number of samples will have smaller variability than those with small numbers. To combine the summary statistics of all the studies, traditional weights were calculated based on the inverse of the within-study variance. In this way, studies with a large variance and therefore a relatively imprecise estimate of the study summary received less weight than a study with a smaller variance. Two additional weighting methods were also examined; weights generated that were inversely proportional to sample sizes and one where the same weight was used for each study (data not shown). In general, the overall summary statistic had the smallest variability when the inverse of the within-study variance-weighting scheme was used.

In the four-group analysis, to avoid the difficulty in interpretation that would occur if different weights were computed for the comparison of each pair, weights were computed from the sum of the variances for the four groups and on the total sample size for the four groups. Although six comparisons were possible, only three had practical use for interpretation of plasma retinol concentrations in cross-sectional studies, namely the comparisons against the reference group. To estimate the variability of the overall summary statistic and to provide study to study variation, the random effects model was used for all the analyses reported as it allowed for small differences among studies and enabled the generation of valid standard deviations. The data are summarized in **Table A2.4.3**.

Table A2.4.3

Summary results from four-group meta-analysis of retinol on apparently healthy groups

Group comparison	Mean (95% CI)	P value
Reference versus incubation	1.15 (0.98–1.36)	0.09
Reference versus early convalescence	1.32 (1.12–1.55)	0.001
Reference versus late convalescence	1.12 (0.94–1.34)	0.18

Values are ratios of the geometrical mean retinol concentrations for the respective pairs from the four-group analysis. Only four of the 15 studies could be included in the final analysis ($n=4486$), i.e. there were subjects in all four inflammation subgroups.

Ferritin

The measurement of plasma ferritin concentrations to assess iron status in populations was the principal recommendation of the World Health Organization (WHO) in 2004 (82). However, it was also recognized that ferritin was a positive APP, and therefore the WHO working group recommended that ferritin measurements should be accompanied by one or more APPs to detect the presence of infection or inflammation.

Two- and four-group meta-analyses

The methods used were exactly the same as those outlined above for retinol. In the case of ferritin there were 31 studies that enabled computation of the two-group analysis and 22 studies were included in the four-group meta-analysis. The ratios (\pm 95% CI) obtained for the two-group analyses when CRP or AGP were increased were 1.50 (1.34–1.67; $P < 0.001$) and 1.38 (1.13–1.68; $P < 0.002$), respectively. The results of the four-group meta-analysis are summarized in **Table A2.4.4**.

Table A2.4.4

Ferritin ratios for respective inflammation versus reference group comparisons: four-group meta-analysis

Group comparison	Ratio (95% CI)	P value
Incubation versus reference	1.30 (1.15–1.47)	<0.001
Early convalescence versus reference	1.90 (1.51–2.37)	<0.001
Late convalescence versus reference	1.36 (1.19–1.55)	<0.001

Values are ratios of geometrical mean ferritin concentrations for the respective pairs from the four-group analysis. Results are shown for 22 studies including 5 of infants, 3 of children, 3 of men and 11 of women ($n=7848$).

Ways of using correction factors to remove the influence of inflammation from data

The cut-off of 0.7 $\mu\text{mol/L}$ was primarily intended for use with children 6–71 months of age and to indicate the point at which vitamin A deficiency begins, i.e. liver stores likely to be less than 0.07 μmol retinol/g liver ($<20 \mu\text{g/g}$). Trauma or disease rapidly depresses the concentration of retinol in the blood (2, 83, 84) and in many developing countries there can be more than 50% of apparently healthy children with elevated markers of inflammation in their blood (48, 59, 85). The concentration of plasma retinol that is below the threshold of 0.7 $\mu\text{mol/L}$ because it is depressed by inflammation does represent a risk of vitamin A deficiency, but it is potentially a biased measure of status. With the removal of inflammation, a plasma retinol concentration that is depressed by inflammation will rapidly normalize. This was beautifully illustrated by Mitra and colleagues, who reported that children admitted to hospital with shigellosis dysentery had a mean plasma retinol concentration of 0.35 $\mu\text{mol/L}$ which had reverted to normal on discharge without any vitamin A intervention (17). In dietary terms, a concentration of retinol of 0.35 $\mu\text{mol/L}$ represents severe vitamin A deficiency, but the authors pointed out that the fall in retinol concentration represented a transient decrease in plasma retinol independently of any change in liver stores.

In community studies, the Annecy Accords proposed that a vitamin A deficiency problem existed within a defined population when the prevalence of plasma retinol concentrations $<0.7 \mu\text{mol/L}$ is more than 15% (50). The cut-off was primarily intended for use in children but we found that the influence of inflammation on plasma retinol concentrations in women or preschool children did not differ (81). We subsequently applied the results outlined in the meta-analysis of vitamin A studies (81) on data from apparently healthy, human immunodeficiency virus (HIV)-1 infected Kenyan adults. That is, the data were categorized as belonging to the reference, incubation, early or late convalescence groups, and data in the inflammation groups

were multiplied by the correction factors 1.13, 1.24 and 1.11, respectively. The prevalence of low retinol concentrations ($<0.7 \mu\text{mol/L}$) in the corrected data was reduced below 15%, indicating that vitamin A deficiency was not a significant problem in that community (86). This result was in agreement with the high plasma β -carotene concentrations in the same subjects, indicating that vitamin A-rich food sources were widely available in this community. Many workers are now fully aware of the influence of inflammation on plasma retinol concentrations. However, rather than correcting plasma retinol concentrations, data exclusion is a frequent way of tackling the problem, which potentially biases data and certainly wastes valuable information.

We suggest an alternative way of compensating for the presence of inflammation in prevalence data is to adjust the cut-off used to define the risk of vitamin A deficiency. Instead of correcting individual results, the cut-off $0.7 \mu\text{mol/L}$ may be adjusted by multiplying it by the reciprocals of the correction factors used above. So for subjects in the incubation group, the cut-off would be $0.7 \times (1/1.13)$ which is approximately $0.6 \mu\text{mol/L}$. In fact the correction factors for the incubation and late convalescence groups where only one APP is elevated are very similar (Table A2.4.5). In the early convalescent group where both APPs are elevated, the cut-off would be $0.7 \times (1/1.24)$, which is $\sim 0.6 \mu\text{mol/L}$. We therefore suggest a simpler approach to correction: where one APP is elevated (either CRP or AGP), a risk of vitamin A deficiency would be $<0.6 \mu\text{mol/L}$ and where both APPs are raised, a risk of vitamin A deficiency would be assessed using $<0.6 \mu\text{mol/L}$.

The same approach is proposed for use on ferritin results. With ferritin, normal iron status is >12 or $>15 \mu\text{g/L}$ for subjects <5 and ≥ 5 years, respectively (78). After categorizing data accord-

Table A2.4.5

Using the effects of inflammation on plasma retinol and ferritin concentrations to adjust cut-off values for vitamin A deficiency and iron deficiency

	Age group	No inflammation	Influence of inflammation on plasma nutrient concentrations		
<i>Inflammation groups</i>		<i>Reference group (normal CRP and AGP)</i>	<i>Incubation group (raised CRP)</i>	<i>Late convalescence group (raised AGP)</i>	<i>Early convalescence group (raised CRP and AGP)</i>
Retinol					
Depression in plasma retinol (4 group analysis) ^a (81)		None	13%	11%	24%
Depression in plasma retinol (3 group analysis) ^b (81)		None	10%		24%
Cut-off for retinol μmol/L ^c	6–71 months (50)	0.7	0.6		0.5
Ferritin					
Increase in plasma ferritin (4-group analysis) ^a (79)		None	30%	36%	90%
Increase in plasma ferritin (3-group analysis) ^b		None	26%		83%
Calculated cut-offs for ferritin μg/L ^c	≤5 years	12	15		22
	≥5 years	15	19		27

^a The percentage differences in biomarker concentration in the three inflammation groups from the reference group for retinol or ferritin (79), respectively.

^b As above but obtained by three-group meta-analysis, where data from the incubation and late convalescence groups were combined.

^c The revised cut-offs were calculated by reducing (retinol) or increasing (ferritin) the cut-offs used for the reference groups by the respective differences in mean nutrient concentrations between reference and respective inflammation groups following the three-group meta-analysis.

CRP, C-reactive protein; AGP, α -1 acid glycoprotein.

ing to inflammation status results for subjects in the incubation, early and late convalescence groups could be either individually corrected using multipliers 0.77, 0.53 and 0.75, respectively (79), and the data then assessed using the standard cut-offs of 12 and 15 µg/L, according to the age of the subject. Alternatively, the cut-offs of 12 and 15 µg/L can be adjusted by dividing the same factors (**Table A2.4.5**). For subjects <5 years, ferritin cut-offs of 12, 15 and 23 would define iron deficiency in subjects with no evidence of inflammation, with one raised APP and with two raised APPs, respectively. For subjects ≥5 years, the corresponding ferritin cut-offs would be 15, 19 and 28 µg/L.

Conclusion

Many nutrients or nutrient biomarkers are influenced by infection and tissue damage. The effects may be present in the absence of clinical evidence of disease. APPs are relatively stable markers of infection and inflammation and changes in blood nutrient concentrations can be matched with changes in APPs. Using APPs, we have shown how the changes in retinol and ferritin associated with the different stages of the infection cycle can be quantified and correction factors produced, which can remove the influence of inflammation from the nutrient concentration in apparently healthy people.

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