

Using plasma acute-phase protein concentrations to interpret nutritional biomarkers in apparently healthy HIV-1-seropositive Kenyan adults

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Inflammation influences the assessment of nutritional status. For example, inflammation reduces plasma retinol concentrations and vitamin A deficiency is overestimated. Conversely inflammation increases plasma ferritin concentrations and Fe deficiency is underestimated. Blood samples were obtained from 163 free-living HIV-1-infected adults, not on continuous medication, anti-retroviral drugs or micronutrients, not unwell and who had not reached WHO stage IV of HIV/AIDS. We used four markers of inflammation, C-reactive protein (CRP), α 1-acid glycoprotein (AGP), α 1-antichymotrypsin and erythrocyte sedimentation rate but mainly CRP and AGP were used to separate the subjects into four groups: 'healthy' where both CRP and AGP were normal; 'incubation phase' where CRP was elevated; 'early convalescence' where AGP and CRP were elevated and 'late convalescence' where only AGP was elevated. Correction factors were calculated to remove the influence of inflammation from each biomarker and group where inflammation was present and the data are shown before and after recalculation. The correction increased median plasma retinol concentrations of the whole group from 1.16 to 1.33 μ mol/l, comparable with values (mean 1.29 μ mol/l) in HIV-negative Kenyan women. Median ferritin concentrations fell by about 50% in both sexes and the number of women with plasma ferritin concentrations $\leq 12 \mu$ g/l increased from eleven to twenty. The correction also increased plasma carotenoids and Hb but not α -tocopherol concentrations. We suggest that the method described to remove the influence of inflammation from nutritional biomarkers should be generally applicable in apparently healthy people and prevents discarding valuable data because of mild inflammation. The method does now need to be tested in other populations.

Vitamin A status: Ferritin: Carotenoids: Acute-phase proteins: HIV-1

The plasma concentrations of several important nutritional biomarkers are influenced by inflammation⁽¹⁾ including retinol^(2,3), Fe⁽⁴⁾, ferritin⁽⁵⁾, Zn⁽⁴⁾, carotenoids^(3,6,7), selenium⁽⁸⁾, pyridoxal phosphate⁽⁹⁾ and vitamin C⁽¹⁾. Markers of inflammation also show clear patterns of behaviour such that increases in C-reactive protein (CRP) occur in the first 6 h following infection and show maximum concentrations in 48 h^(10,11) but fall rapidly with the disappearance of clinical disease⁽¹²⁾ while other acute-phase proteins (APP) such as α 1-acid glycoprotein (AGP) are slower to rise but remain elevated for much longer^(10,13). These differences in APP response enabled us to use different combinations of elevated APP in a meta-analysis on fifteen studies of apparently-healthy persons to provide a method of adjusting plasma retinol to remove the effects of inflammation⁽¹⁴⁾.

Identifying sub-clinical inflammation in apparently-healthy persons with HIV is also important for nutritional intervention studies. It was recently shown that HIV-1-seropositive (HIV+) women with elevated CRP and AGP showed no

increase in serum retinol concentrations following a randomised vitamin A supplementation trial. In contrast serum concentrations increased significantly ($P = 0.02$) in women with no raised APP⁽¹⁵⁾.

The objective of this paper was to use the methods developed earlier to correct plasma retinol concentrations for the influence of inflammation⁽¹⁴⁾ on several other nutritional biomarkers in free-living Kenyan HIV+ male and female adults⁽¹⁶⁾. Some of these results have been briefly reported elsewhere⁽¹⁷⁾.

Methods

Full details on the study are described elsewhere⁽¹⁶⁾. In brief, 180 persons who tested positive for HIV-1 infection on two occasions (INNOTESTTM HIV-1/HIV-2 antibody test, Innogenetics, Ghent, Belgium) were recruited into the study. Recruitment took place in two peri-urban centres north of Nairobi: 137 individuals were recruited between February 2002 and

Abbreviations: ACT, α 1-antichymotrypsin; AGP, α 1-acid glycoprotein; APP, acute-phase proteins; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HIV+, HIV-1-seropositive; HIV-, HIV-1-seronegative.

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January 2003 at Nakuru and forty-three others between October 2002 and March 2003 at Nanyuki. Both centres are in the upper reaches of the Rift Valley Province found within the Central and Midwest Highlands of Kenya. The persons were living in their own homes and had not reached stage IV of clinical AIDS by WHO classification. Eligibility criteria for inclusion in the study were HIV positivity, non-pregnant and non-lactating if female, presenting to various HIV-support organisations or a local referral or medical facility and aged between 18 and 35 years. If persons were positive for TB, they had to have received at least 2 months' anti-TB treatment to be eligible for recruitment. Persons were additionally excluded if a concurrent illness was present which required ongoing medical intervention, presented with clinical AIDS (WHO stage IV), were already consuming micronutrients, receiving anti-retroviral therapy or any other drugs, were of no fixed address or were not willing to participate. Once recruited, the subjects were allocated to receive either a food supplement or the food plus additional micronutrients but the follow-up results will be reported elsewhere.

A blood sample was taken from persons who felt well. Persons who felt unwell were given an alternative date for blood sampling. A total of 174 persons provided a blood sample before any supplements were received. Insufficient blood or laboratory problems prevented some analyses and the results for 163 samples (men, n 56 and women, n 107) are presented in this paper. Ethical approval for the study was obtained from the Scientific Steering Committee of the Kenya Medical Research Institute, the National Ethical Review Board and the Contracts Review Committee of UNICEF East and Southern Africa Regional Office.

Biochemical methods

Hb and erythrocyte sedimentation rate (ESR) were measured on freshly collected blood in local laboratories. Hb was determined by the cyanmethaemoglobin method on 200 μ l whole blood. ESR was measured in a 4:1 trisodium citrate (109 mmol/l)–blood mixture where a 20 cm \times 2.55 mm column of diluted blood was allowed to stand for 60 min and the column of packed red cells measured to the nearest mm. CD4 and CD8 T-lymphocyte subset counts were obtained on anticoagulated whole blood (EDTA vacutainers, Becton Dickinson) within 24 h of collection using a FACSCount™ System (Becton Dickinson) at the Centre for Biotechnology, Development and Research, KEMRI, Nairobi. The FACSCount™ System was standardised daily with manufacturer's standards.

Plasma was prepared in the local laboratories and shipped frozen to Nairobi where it was stored at -20°C for up to 15 months prior to analysis. Plasma ferritin concentrations were measured at the Biochemistry Department, Kenyatta National Hospital, Nairobi. Ferritin was measured by an automated one-step sandwich enzyme immunoassay with final fluorescence detection (Mini Vidas, BioMerieux, France). Kits provided manufacturers' standards and controls and a precision between 4 and 7% was obtained.

At the end of the study, plasma was sent in dry ice to the laboratories of Professor H. Friis (Aalborg University, Denmark) to measure viral load by the method of Krarup *et al.* (18). The quantification was made in genomic equivalents

per ml blood (geq/ml). The remaining measurements were done at the laboratories of the Northern Ireland Centre for Food and Health, Coleraine. Samples were shipped in dry ice to Northern Ireland and stored at -70°C for up to a further two months before analysis. Retinol, the carotenoids and tocopherols were measured on 100 μ l plasma using liquid chromatography^(19,20). α 1-Antichymotrypsin (ACT), AGP and CRP were measured using DAKO reagents (Dako Ltd, Denmark) using a Hitachi 912 Clinical Analyzer (Roche Diagnostics Ltd, Welwyn, UK). Analysis required <100 μ l for each analysis and plasma was diluted automatically prior to analysis. Inter-assay precision of the liquid chromatography varied between 5 and 10% and was $\leq 5\%$ for each of the APP. Limits of detection for the APP were about 0.2 g/l for ACT and AGP and about 0.1 mg/l for CRP.

Data handling

Much of the data was skewed so comparison of groups or between sexes was done on log-transformed data except where indicated. In the Tables, all data are shown as medians and 25th and 75th quartiles.

As the purpose of the paper was to examine the influence of the APP on the nutritional markers, the data were grouped using the following criteria. The healthiest of the recruits (or reference group) were characterised as having normal ACT (≤ 0.4 g/l), normal AGP (≤ 1.0 g/l) and normal CRP (≤ 5 mg/l) activity. The second group is described as being in the 'incubation' phase or early stage of infection when CRP or ACT is rising but AGP is still within the normal range. In early convalescence (stage 3), elevated CRP (or ACT) and an elevated AGP occur together but in later convalescence (stage 4) only AGP is elevated. It was noted in grouping the recruits that CRP and AGP were the major determinants. An elevated plasma ACT concentration when CRP was not raised only influenced the position of three individuals. To remove the influence of inflammation from the nutritional biomarkers, correction factors were calculated by dividing the median value of the biomarker for the reference group by the respective median values for groups at stages 2, 3 and 4.

ESR was not used in the above treatment. The ESR reflects a number of changes predominantly in the viscosity of the blood accompanying an infection. Changes in viscosity can be due to changes in the permeability of the vasculature as well as the increase in proteins like fibrinogen. It is therefore less easy to link changes in the ESR to different stages in the infective process.

Role of the funding source

The study sponsors played no part in the study design, in the collection, analysis and interpretation of data, in the writing of this paper or in our decision to submit for publication.

Results

The baseline nutritional and inflammatory biomarkers obtained in the Kenyan men and women are shown in Table 1. Differences between the sexes were found for Hb and ferritin, three of the carotenoids, ESR and ACT, and the proportion

Table 1. Plasma concentrations of nutritional and inflammatory markers (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), antichymotrypsin (ACT) and α 1-acid glycoprotein (AGP)) and proportions abnormal using conventional cut-off values (Median values with 25th and 75th quartiles)

	Concentration (n 163; 56 men, 107 women)		Cut-off threshold	Reference	Number outside the threshold		P for difference between sexes*
	Median	Quartiles			n	%	
Retinol (μ mol/l)	1.16	0.87, 1.47	$\leq 0.7 \mu$ mol/l	Sommer & Davidson ⁽²¹⁾	32	20	0.95
Lutein (μ mol/l)	0.296	0.177, 0.459	NA				0.17
α -Tocopherol (μ mol/l)	14.28	6.40, 18.28	$\leq 11 \mu$ mol/l	Thurnham <i>et al.</i> ⁽²⁴⁾	6	4	0.12
γ -Tocopherol (μ mol/l)	0.57	0.43, 0.89	NA		–	–	0.44
Ferritin (μ g/l)							
Men	488	204, 1086	$\leq 12 \mu$ g/l	Worwood ⁽²³⁾	0	0	<0.001
Women	90	31, 220			11	10	
Hb (g/l)							
Men	132	117, 143	≤ 130 g/l†	UNICEF <i>et al.</i> ⁽²²⁾	25	44	<0.001
Women	116	105, 124	≤ 120 g/l		63	59	
β -Carotene (μ mol/l)							
Men	0.164	0.073, 0.248	$\leq 0.075 \mu$ mol/l	Mehendale <i>et al.</i> ⁽³⁹⁾	14	25	0.015
Women	0.300	0.119, 0.438			17	16	
α -Carotene (μ mol/l)							
Men	0.044	0.02, 0.09	NA				0.018
Women	0.080	0.033, 0.130					
Lycopene (μ mol/l)							
Men	0.063	0.024, 0.130	NA				0.024
Women	0.105	0.049, 0.189					
ESR (mm/h)							
Men	49	17.5, 82	> 20 mm/h	Dacie & Lewis ⁽⁴⁰⁾	38	68	0.001
Women	74	39, 93			90	84	
ACT (g/l)							
Men	0.35	0.27, 0.49	> 0.4 g/l	Thurnham <i>et al.</i> ⁽¹⁴⁾	24	43	0.001
Women	0.31	0.25, 0.37			22	21	
AGP (g/l)							
Men	1.05	0.73, 1.79	> 1.0 g/l	Thurnham <i>et al.</i> ⁽¹⁴⁾	33	59	0.063
Women	0.93	0.73, 1.29			46	43	
CRP (mg/l)							
All	4.97	0.86, 17.14	> 5.0 mg/l	Thurnham <i>et al.</i> ⁽¹⁴⁾	81	50	0.39

NA, cut-off values not available.

* ANOVA of log base10 values except for α -tocopherol, Hb and ACT where the data were not transformed.

† Cut-off to define moderate anaemia.

of abnormal results is shown separately for the sexes for these variables. The median concentration of plasma retinol was 1.16 μ mol/l and 20% of adults had plasma retinol concentrations $\leq 0.7 \mu$ mol/l⁽²¹⁾. Anaemia⁽²²⁾ was present in 59% of women and 10% plasma ferritin concentrations indicated deficient stores of liver Fe ($\leq 12 \mu$ g/l)⁽²³⁾. Approximately 44% of men had anaemia (≤ 130 g/l) but there were none with deficient liver Fe stores. The overall concentration of α -tocopherol in plasma was low but there were only 4% (six of 163) with values suggesting a risk of deficiency ($< 11.0 \mu$ mol/l)⁽²⁴⁾.

Pearson correlation coefficients between the different inflammatory and nutritional biomarkers are shown in Table 2. Retinol was inversely related with all four acute phase markers but the correlations were only significant with ESR and AGP. In contrast, ferritin (positively) and Hb (negatively) were significantly correlated with all four acute phase markers. It is noteworthy that ferritin was strongly correlated with the more chronic indicators of inflammation, ACT and AGP. All the carotenoids showed negative relationships with the APP; however, those with ESR were not significant while those with AGP were strongly significant. The tocopherols displayed no relationships. Inter-correlations between the APP were generally strong for ACT, AGP and CRP and less so with ESR. ESR was most strongly correlated with AGP and ACT and especially with Hb.

When the nutritional and other data were split into the four inflammation groups in accordance with the acute phase status defined in the methods, there were significant differences between the groups for all nutrients except retinol ($P=0.09$),

Table 2. Pearson correlation coefficients for the nutritional and inflammatory biomarkers (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), α 1-antichymotrypsin (ACT) and α 1-acid glycoprotein (AGP) (n 163)

Nutritional biomarkers	Inflammatory biomarkers			
	ESR	CRP	ACT*	AGP
Retinol (μ mol/l)	-0.167†	-0.038	-0.060	-0.169†
Ferritin (μ g/l)	0.170†	0.303‡	0.572‡	0.595‡
Hb (g/l)*	-0.585‡	-0.270‡	-0.235‡	-0.310‡
β -Carotene (μ mol/l)	-0.079	-0.159†	-0.190†	-0.371‡
α -Carotene (μ mol/l)	-0.133	-0.154†	-0.181†	-0.356‡
Lutein (μ mol/l)	-0.123	-0.179†	-0.115	-0.204‡
Lycopene (μ mol/l)	-0.139	-0.194†	-0.200‡	-0.306‡
α -Tocopherol (μ mol/l)*	0.059	0.014	0.095	0.062
γ -Tocopherol (μ mol/l)	0.049	-0.033	-0.033	0.008
CRP (g/l)	0.184†	–	0.538‡	0.566‡
ACT (g/l)*	0.318‡	0.538‡	–	0.797‡
AGP (g/l)	0.414‡	0.566‡	0.797‡	–

* Correlation coefficients obtained using log base10 values of all variables except those marked.

† $P < 0.05$.

‡ $P < 0.01$.

Table 3. Concentrations of nutritional markers in different stages of inflammation as defined by the acute-phase proteins (APP)*, antichymotrypsin (ACT), α1-acid glycoprotein (AGP) and C-reactive proteins (CRP) (Median values with 25th and 75th quartiles)

	Normal APP (n 66)			Incubating (n 18)			Early convalescence (n 63)			Late convalescence (n 16)			ANOVA† P
	Median	Quartiles	n	Median	Quartiles	n	Median	Quartiles	n	Median	Quartiles	n	
Retinol‡ (µmol/l)	1.33	1.00, 1.56		1.25	0.84, 1.54		1.01	0.61, 1.33		1.10	0.84, 1.41		0.090
CF (this study)				1.064			1.317			1.209			
CF§ (from the previous meta-analysis)				1.13			1.24			1.11			
Ferritin‡ (µg/l) Men n 56	166 ^a	94, 277	19	262 ^{ab}	99, 572	4	1004 ^b	448, 1200	28	540 ^{ab}	187, 938	5	<0.001
CF				0.630			0.165			0.307			
Ferritin‡ (µg/l) Women n 107	41 ^a	13, 150	47	50 ^a	20, 97	14	213 ^b	100, 386	37	107 ^{ab}	47, 208	9	<0.001
CF				0.820			0.190			0.380			
Hb (g/l) Men n 56	138 ^a	132, 156	19	130 ^a	92, 139.5	4	123 ^b	109, 123	28	158 ^a	119.5, 170	5	0.001
CF				1.062			1.122			0.873			
Hb (g/l) Women n 107	120	108, 132	47	114	94.5, 19.3	14	113	100.5, 120	37	116	100.5, 132	9	0.064
CF				1.053			1.062			1.034			
β-Carotene‡ (µmol/l)	0.320 ^a	0.175, 0.499		0.323 ^{ac}	0.181, 0.456		0.141 ^b	0.05, 0.287		0.161 ^{bc}	0.042, 0.286		<0.001
CF				0.991			2.270			1.988			
α-Carotene‡ (µmol/l)	0.103 ^a	0.044, 0.146		0.086 ^{ab}	0.035, 0.148		0.041 ^b	0.012, 0.081		0.035 ^b	0.013, 0.077		<0.001
CF				1.198			2.512			2.943			
Lutein‡ (µmol/l)	0.385 ^a	0.252, 0.519		0.344 ^{ab}	0.172, 0.468		0.220 ^b	0.116, 0.366		0.278 ^{ab}	0.207, 0.485		0.005
CF				1.119			1.750			1.385			
Lycopene‡ (µmol/l)	0.124 ^a	0.080, 0.217		0.094 ^{ab}	0.046, 0.184		0.059 ^b	0.02, 0.114		0.087 ^{ab}	0.044, 0.140		0.001
CF				1.319			2.102			1.425			
ESR‡ (mm/h)	42 ^a	18, 79		64.5 ^{ab}	22.5, 91		80 ^b	54, 99		60 ^{ab}	21, 91		<0.001
CF				0.651			0.525			0.700			
α-Tocopherol (µmol/l)	14.06	9.24, 18.10		14.10	1.12, 19.95		14.73	1.80, 19.2		13.71	7.78, 16.9		0.885
γ-Tocopherol‡ (µmol/l)	0.56	0.35, 0.89		0.59	0.50, 0.79		0.57	0.43, 0.92		0.65	0.48, 1.05		0.463
CD4 count (cells/µl)	362 ^a	270, 680	27	210 ^{ab}	76, 375	7	154 ^b	40, 285	24	168 ^{ab}	61, 200	7	0.004
CD4:CD8 (group sizes as above)	0.42	0.14, 0.6 ^a		0.28 ^{ab}	0.09, 0.31		0.13 ^b	0.07, 0.25		0.11 ^b	0.04, 0.17		0.001
Viral load‡ (geq/ml)	2350 ^a	125, 32 000	62	5800 ^{ab}	2500, 140 000	15	24 000 ^b	2275, 207 500	60	69 000 ^{ab}	880, 210 000	11	0.005

Nutritional assessment during inflammation

CF, correction factor (derived by dividing the reference median value by the median for the respective groups with raised APP; CF were not calculated for α- or γ-tocopherol as there was no difference between the groups); ESR, erythrocyte sedimentation rate.

* Normal APP were plasma concentrations: ACT ≤ 0.4 g/l, CRP ≤ 5 mg/l and AGP ≤ 1.0 g/l. Incubating was defined as concentrations above the cut-offs indicated for ACT or CRP and an AGP concentration ≤ 1.0 g/l. Early convalescence was defined as elevated AGP and elevated ACT or CRP. Late convalescence was defined as an elevated AGP only.

† ANOVA followed by Scheffé test. ^{a,b} Mean values with unlike superscripts indicate significant difference between means of subgroups.

‡ Statistics performed on log base10 data.

§ Correction factors from reference Thurnham *et al.*(14).

|| The large difference in the numbers of men and women in the sample and in some cases e.g. plasma ferritin, the difference in concentrations between the sexes, necessitated separating the sexes to calculate appropriate CF.

α -tocopherol ($P=0.89$) and γ -tocopherol ($P=0.49$) (Table 3). Furthermore, with the exception of vitamin E, the plasma concentrations of all the nutrient biomarkers showed greatest evidence of abnormalities in the early and late convalescent groups. Correction factors are also shown in Table 3. The factors adjusted median concentrations in each inflammatory group to that of the group with no raised APP, to remove the influence of inflammation. Also shown in Table 3 are plasma viral loads, CD4 T-lymphocyte subset counts and CD4:CD8 ratios as markers of the HIV-1 severity in the specific, acute-phase groups. For all three clinical markers, the poorest results were associated with raised AGP. It should also be noted that median ESR concentrations were abnormally elevated in all groups including the reference group.

Table 4 shows concentrations of nutrient biomarkers after correction. As expected the correction procedure produces median values the same as those shown in the reference column in Table 3. In the case of retinol, the use of both sets of correction factors increased median retinol concentration from 1.16 to 1.33 (this study) and 1.31 (meta-analysis) with virtually the same interquartile range in both cases. The prevalence of plasma retinol concentrations $\leq 0.7 \mu\text{mol/l}$ fell from 20 to 13% (this study) or to 16% (meta-analysis). Correcting plasma ferritin concentrations more than halved the median concentrations of ferritin in both sexes and increased the apparent number of women in the study with Fe-deficient liver stores from eleven to twenty. Corrections to Hb only slightly reduced the apparent number of people of both sexes with anaemia. There was only one person in each group where Hb was $< 70 \text{ g/l}$. In the men, this case was removed by the correction but not so in the women. There are no universally recognised cut-off values for the carotenoids but it should be noted that corrections increased median concentrations.

Table 5 shows the corrected retinol and ferritin results for the HIV+ subjects compared with data from HIV seronegative (HIV-) persons in the Kenyan National Nutrition Survey⁽²⁵⁾

and in a report by Baeten *et al.*⁽²⁶⁾ The corrected median plasma retinol was almost identical to that reported for HIV- Kenyan women who had no raised inflammatory proteins and the proportion of low values of plasma retinol concentrations in the corrected data was lower than that found in the Kenyan Survey.⁽²⁵⁾ Corrections to plasma ferritin concentrations in the HIV+ men and women produced values more comparable with those of the HIV- adults in the community.

Food frequency data were collected (not shown) but there was no association between any of the foods and the nutritional biomarkers. Meals would be taken frequently with 'ugali' which is a thick porridge prepared from whole ground white maize. Most subjects reported eating carrots, spinach and kale frequently and these would be chopped and fried with tomatoes, oil and meat when available. The frequent consumption of vitamin A and Fe-containing food is in agreement with the high carotenoid and ferritin values in the reference group.

Discussion

In recent years workers have become increasingly aware that inflammation can alter nutritional biomarkers. To detect inflammation CRP has been used and when elevated values are found, data are usually excluded. Often a relatively high cut-off is used (about 10 mg/l) and the procedure has two disadvantages: potentially valuable data are lost and some influence of inflammation may still remain because subjects with CRP values between 5 and 10 mg/l are still included. Furthermore, workers rarely measure AGP, so any influence of chronic inflammation on the data is ignored. Alternatively, regression analyses using the change in APP to predict the change in retinol⁽²⁷⁾ or ferritin⁽²⁸⁾ have also been unsuccessfully used as individual APP do not reflect exactly the behaviour of nutritional biomarkers throughout the period of inflammation especially in apparently healthy people. In the latter correlations between APP and nutritional biomarkers are poor and changes in APP explain very little of the variance in the nutritional biomarkers⁽²⁸⁾.

Table 4. Concentrations of nutritional biomarkers after correction* for the presence of sub-clinical inflammation

Nutritional biomarker (Total <i>n</i> 163)	Uncorrected data				Corrected data			
	Median	Quartiles	Abnormal results†		Median	Quartiles	Abnormal results	
			<i>n</i>	%			<i>n</i>	%
Retinol ($\mu\text{mol/l}$)	1.16	0.87, 1.47	32	20	1.33	0.94, 1.63	22	13
Retinol‡ ($\mu\text{mol/l}$)	1.16	0.87, 1.47	32	20	1.31	0.94, 1.62	26	16
Lutein ($\mu\text{mol/l}$)	0.296	0.177, 0.459		NA	0.386	0.240, 0.563		NA
Ferritin ($\mu\text{g/l}$)								
Men	488	204, 1086		0	166	82, 198		0
Women	90	32, 220	11	10	41	17, 105	20	19
Hb (g/l)								
Men	132	117, 143	25	44	138§	126, 150	18	32
Women	116	105, 124	63	59	120§	107, 130	53	50
β -Carotene ($\mu\text{mol/l}$)								
Men	0.164	0.073, 0.248	14	25	0.218	0.124, 0.387	10	18
Women	0.300	0.199, 0.438	17	16	0.381	0.207, 0.589	13	12

* Corrected data was obtained by multiplying the uncorrected values by the respective correction factor (CF) values (see Table 3) for the appropriate acute-phase-protein group in which they were situated.

† Thresholds of abnormality are listed in Table 1.

‡ Data corrected using published correction values from reference Thurnham *et al.*⁽¹⁴⁾.

§ Hb data corrected using values generated for the individual sexes.

Table 5. Comparisons of corrected plasma retinol and ferritin concentrations of HIV-1-seropositive adults (this study) with other Kenyan data

Biomarker	This study		Comparison studies	
	Inflammation-corrected data	Other Kenyan data	Description	Source (Reference)
Plasma retinol	1.33	1.29	HIV negative, acute-phase	Baeten <i>et al.</i> (26)
Median quartiles	0.94, 1.63	1.04, 1.50	protein normal	
Plasma retinol <0.7 µmol/l (%)	13 (n 22)	20 (n 81)	Non-pregnant women at four sites near Nanyuki and Nkuru	Mwaniki <i>et al.</i> (25)
Plasma ferritin, median, men (µg/l)	166	84 (n 164)	Geometric mean, twelve districts in Central and Mid-western Highlands	Mwaniki <i>et al.</i> (25)
Plasma ferritin, median, women (µg/l)	41	30 (n 968)		
Plasma ferritin, women <12 µg/l (%)	19 (n 20)	28.6 (n 276)	Data from twelve districts in Central and Mid-western Highlands	Mwaniki <i>et al.</i> (25)

In this paper, we assume that the nutritional status of the apparently-healthy individuals who have raised APP and no clinical evidence of disease when the blood is taken, should be the same as those adults in the same community with normal acute phase status. That is, the differences in nutritional biomarkers between persons without and those with raised APP is due to the inflammatory state and not altered nutritional status. Thus in the case of vitamin A, if abnormal vitamin A status, i.e. low concentrations of plasma retinol, accompanies raised APP in the HIV+ adults, we have assumed the biochemical abnormalities are similar to those following surgery⁽²⁾, or found in malaria⁽³⁾, shigella dysentery⁽²⁹⁾ or measles⁽³⁰⁾. In all these examples plasma vitamin A concentrations reverted to the pre-infection state without vitamin A intervention when the trauma of disease or surgery was removed or disappeared. However, the HIV infection is a slow onset disease and as the disease progresses, nutritional status may deteriorate even in apparently-healthy, HIV+ people. Thus even after the effects of inflammation are removed, nutritional biomarkers may still be abnormal when compared with HIV- persons living in the same area. To investigate this point, we compared the use of correction factors generated for retinol from the data of the HIV+ adults with the effects of using the correction factors that were generated previously in mainly non-HIV+ persons⁽¹⁴⁾ and we compared the corrected results with those from HIV- persons living in the same area and found that both methods of correction produced very similar median plasma retinol concentrations. This suggested that inflammation caused by HIV in apparently-healthy persons had a very similar effect on plasma retinol concentrations to that produced by other diseases.

Thus, the importance of the method used in this paper is that it presents a way of using all biochemical data from apparently-healthy persons and reduces the interference from residual inflammation in the early phase of, or following an infection. Currently our principal support for this view is that the increase in median plasma retinol concentrations after correction was almost the same irrespective of whether we used (1) correction factors produced previously by a meta-analysis of groups comprising mainly HIV- infants or (2) correction factors generated from the data of the seropositive adults themselves. Thus although the two sources of

correction factors were very different, their effect on plasma retinol concentrations was almost the same. Undoubtedly, it will be argued that AIDS is a progressive and chronic disease and evidence suggests that as the disease progresses, seropositive persons become more and more malnourished. However, the evidence of malnutrition in patients with HIV is frequently obtained by methods which do not distinguish between biochemical abnormalities caused by malnutrition and those caused by the inflammation. In the introduction we highlighted the inhibitory effect of inflammation on the response to supplementary vitamin A⁽¹⁵⁾. Likewise a recent Cochrane review found little evidence of improvement in nutritional status of HIV+ persons following nutritional supplements⁽³¹⁾ and we suggest this is because the influence of inflammation on nutritional biomarkers has been overlooked.

However, if we examine more closely the suggestion that because HIV is a progressive disease, there will be elevated requirements for nutrients like vitamin A and, as the disease progresses, the risk or extent of vitamin A deficiency will increase. Thus those with the highest viral loads or lowest CD4 counts should be at greater risk of vitamin A deficiency than those persons whose inflammatory state is due to a more acute, self-limiting condition such as respiratory disease or diarrhoea. Table 3 confirms that those seropositive adults with raised APP had higher viral loads and lower CD4 counts and it is certainly true that the more severe the disease, the greater will be the inflammation produced and the greater the effect on nutrition biomarkers. However, in this study we are not trying to adjust the data for severity of disease but rather for the milder effects of sub-clinical inflammation. The adults in this study were not clinically unwell when the blood was taken. The distribution of subjects in Table 3 suggests that approximately half of the adults were recovering from sickness (groups 3 and 4, early and late convalescence), approximately 10% may have been incubating some illness (group 2) while 40% were well (or had not recently been ill; reference group). The correction procedure adjusts the median values of those groups with raised APP to those of the reference group. If vitamin A status was poorer in HIV+-apparently-healthy people than in those without HIV as might be inferred by a report from Kenya on retinol-binding protein metabolism⁽²⁶⁾, retinol concentrations in the reference groups would be expected to be more abnormal in those with

HIV. In fact the median retinol concentration in the reference group in this study (1.33 $\mu\text{mol/l}$) is almost the same as that reported by Baeten *et al.*⁽²⁶⁾ (1.29 $\mu\text{mol/l}$) for HIV-, APP-negative Kenyan women. If there is no difference in the median biomarkers concentrations in the reference groups, then poorer vitamin A status should be evident in those with HIV+ and inflammation and the correction factors would need to be greater to adjust their data than those for people with acute, self-limiting infections. The correction factors from the two convalescent groups of HIV+ subjects are slightly higher than those from the meta-analysis but overall correction of plasma retinol by either method produced similar results, suggesting that in apparently-healthy persons with sub-clinical inflammation, correction factors for the different acute phase groups will be very similar irrespective of the nature of disease. It would obviously have been an advantage to have recruited HIV- adults from the same area to better interpret these data, but biomarker interpretation was not the primary objective of this feeding study.

Interference with nutritional biomarkers by inflammation is primarily from two sources, dietary intake and metabolism. Plasma retinol is depressed^(32,33) and plasma ferritin concentrations⁽⁵⁾ increase through the action of cytokines while carotenoids are more probably influenced by a depression in appetite. The degree of depression in plasma carotenoid concentrations probably depends on the proximity of the infection. The inverse associations between plasma carotenoid concentrations and the APP, especially AGP, suggests that previous infection depressed dietary intake. However, there is also evidence that the production of reactive oxygen species by stimulated polymorphonuclear leucocytes can oxidatively remove carotenoids from plasma⁽³⁴⁾. Other workers have also reported inverse relationships between plasma carotenoid and CRP concentrations in apparently-healthy American adults^(6,7) and it would seem prudent, irrespective of the causes of carotenoid depression, that that workers using plasma carotenoids as markers of fruit and vegetable intakes should be mindful of the influence of inflammation.

It is interesting that plasma α - or γ -tocopherol concentrations were not influenced by inflammation. If carotenoids are sensitive to oxidative damage and they, like the tocopherols, are transported in plasma by the lipoproteins, then the tocopherols should also be exposed to the same oxidative attack. However, there is no known mechanism to repair oxidised carotenoids whereas several reductants such as vitamin C, glutathione and ubiquinone⁽³⁵⁾ as well as the hepatic α -tocopherol transfer protein⁽³⁶⁾, may prevent a fall in plasma concentrations of α -tocopherol although not all these factors can influence γ -tocopherol.

Fe deficiency in the developing world is often of equal importance to vitamin A deficiency. In the absence of inflammation, plasma ferritin concentrations are a good measure of Fe stores but unfortunately infection increases plasma ferritin, conceals the true level of Fe stores in a population and at the same time contributes to the level of anaemia (i.e. low plasma Hb) by making Fe unavailable for the synthesis of new erythrocytes. Table 3 shows that plasma ferritin concentrations were more than five (women) and six (men) times higher in the groups in which both CRP and AGP were elevated than in the reference group. However corrected values from these data were more comparable with those reported for the

community (Table 5) and increased the detection of women with low Fe stores almost two fold. Wieringa *et al.*⁽³⁷⁾ also showed the importance of correcting plasma ferritin concentrations for inflammation since in the absence of raised APP, the prevalence of Fe deficiency in Indonesian infants was 26% whereas it appeared to be only 3–10% in those groups with raised APP. It is also of interest to note that in spite of the very large differences in ferritin concentrations between the Kenyan men and women, the correction factors were approximately the same for the respective groups. This suggests that in the presence of inflammation, the increase in ferritin is proportional to the pre-inflammation ferritin concentration in the different groups. Whether these relationships will apply to the very much smaller concentrations of ferritin in infants and children needs to be determined.

Hb was less strongly associated with the inflammatory APP than ferritin, the carotenoids or retinol. Hb concentrations showed very little difference between the three groups with raised APP in comparison to the reference group (Table 3). Friis *et al.*⁽³⁸⁾ previously noted a drop of only 12.9 g/l in the mean Hb associated with HIV seropositivity in pregnant Zimbabwean women. In this study the difference between median Hb concentrations of those groups without and with inflammation varied from 5 to 9 g/l. The correction for current inflammation only increased the median Hb concentration in both sexes combined by 5 g/l and had very little effect on the numbers of cases of anaemia. The lack of effect of correcting Hb for inflammation is not surprising as the fall in Hb concentration is probably slow (days or weeks), depending as it does on both the availability of Fe and erythrocyte turnover. In contrast changes in APP status reduce retinol⁽²⁾ and increase ferritin⁽⁵⁾ in the first few hours following infection.

In conclusion, the calculated correction factors increase (or reduce) the medians of the whole group to that of the 'healthy' or reference group and thereby remove the influence of current inflammation. We suggest that identifying the group with no raised APP, or using the correction factors to remove the influence of inflammation from the whole data set, improves the measurement of true nutritional status of apparently-healthy persons in a specific community. Unfortunately, not all surveys will produce a reference group of sufficient size to calculate reliable correction factors⁽¹⁴⁾. To meet this challenge, a meta-analysis as done for vitamin A will be needed also for ferritin and any other nutritional biomarkers where inflammation is shown to have major effects.

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Contributions of authors

D. I. T. assisted in the preparation of the proposals for the study, assisted with the laboratory work in Northern Ireland and initiated the writing of this manuscript.

A. S. W. M. initiated the research, prepared the proposals and sought funds from UNICEF. She also managed the field work and laboratory work and prepared the report for UNICEF. D. L. M. assisted in the writing of the proposal, seeking funds from UNICEF, hiring staff, managing funds and solving problems. E. M. assisted in the writing of the proposal, computing the numbers needed for the study and analyses for the final report.

F. A. managed the work in the main field centre, interviewed subjects, collected food consumption data and assisted with data analysis. A. de W. assisted with the writing of the proposal, in the procurement of micronutrients and food supplies for the intervention part of the proposal and in seeking funds from UNICEF; A. de W. was, and still is, employed by UNICEF but has no conflicts of interest.

All authors assisted in the writing of the paper and have seen the final draft. None of the authors have any conflicts of interest in the writing and publication of this study.

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