

Use of biomarkers of sub-clinical infection, nutrition and neonatal maturity to interpret plasma retinol in Nigerian neonates

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Using the World Health Organization criterion, the prevalence of sub-clinical vitamin A deficiency can be assessed using plasma retinol concentrations $<0.7 \mu\text{mol/l}$. However, plasma retinol can be depressed by infection; thus, the use of this criterion alone may overestimate deficiency. In the present study, we investigated the usefulness of the acute-phase proteins (APP) α_1 -antichymotrypsin (ACT) and α_1 -acid glycoprotein (AGP), plasma carotenoids and anthropometric and gestational indices to interpret plasma retinol in the blood of 192 apparently healthy Nigerian neonates collected randomly during days 1–20 postpartum. The mean weight (2.64 kg) and length (0.458 m) of the neonates and plasma concentrations (geometric mean, $\mu\text{mol/l}$) of retinol (0.54), α -carotene (0.072), β -carotene (0.076) and lutein (0.080) were low. The prevalence of vitamin A deficiency was 72%, indicating a severe public health problem. Babies who were of low birth weight ($P < 0.003$) or premature and low birth weight ($P < 0.023$) had significantly lower retinol concentrations than full-term normal weight babies. Thirty-two neonates had abnormal ACT and forty-four abnormal AGP concentrations. Positive correlations between retinol and ACT ($r = 0.186$, $P = 0.05$) and AGP ($r = 0.31$, $P = 0.0001$) during days 1–5 may be due to the increasing plasma retinol from maternal milk and a coincidental increasing capacity to synthesise APP. Subsequently, negative correlations between retinol and ACT ($r = -0.28$, $P = 0.02$) and AGP ($r = -0.29$, $P = 0.018$) from day 6 onwards reflected the continuing increase in plasma retinol, but no further increase in the APP. Overall, weight, ACT, lutein and age explained 30% of the variance in retinol, but lutein was the most significant ($r^2 = 0.18$, $P < 0.0001$). Hence, the distribution of plasma retinol concentrations in this group of neonates was more strongly linked with nutrition (via the surrogate marker lutein) than infection.

Retinol: Lutein: Acute-phase proteins: α_1 -Antichymotrypsin: α_1 -Acid glycoprotein: Nigeria: Neonate

Vitamin A deficiency (VAD) continues to be a public health problem in many countries in the tropics and subtropics. The only available national data on vitamin A status in Nigeria are from a survey carried out in 1994, in specific areas of each of the four main health zones (2836 subjects). The surveys found a prevalence of 0.2% xerophthalmia-related corneal scars in children aged 0–6 years; hence, Nigeria is listed by the World Health Organization as one of the category 1 countries having the highest risk of VAD (United Nations International Children's Emergency Fund, 1994). Results from the same survey indicated the overall prevalence of sub-clinical VAD in mothers, based on plasma retinol $<0.7 \mu\text{mol/l}$, was 9.2% (mild deficiency) but was slightly higher in the north-east of the country, where it was 14.0% (moderate deficiency) (World Health Organization, 1996). Individual studies on preschool children suggest that the problem of sub-clinical

VAD varies in different areas of the country, with a severe public health problem in eastern Nigeria (25.4%; Uzoechina & Okoro, 1994), but a more moderate situation in the south-west (11.3%; Adelekan *et al.* 1997). However, there appears to be no results available on plasma retinol in Nigerian neonates, although Coutsooudis *et al.* (1995) in Durban (South Africa) reported that 78% of neonates had plasma retinol values $<0.35 \mu\text{mol/l}$.

Plasma retinol can be used as a crude indicator of vitamin A status (particularly when concentrations are low); however, infection and/or trauma can depress plasma retinol. Therefore, the use of indices such as plasma retinol <0.7 or $<0.35 \mu\text{mol/l}$ may overestimate the prevalence of VAD (Thurnham & Singkamani, 1991). In developing countries, frequent exposure to infection is common and the prevalence of sub-clinical infection is likely to be high. Previous work has shown that α_1 -acid glycoprotein

Abbreviations: ACT, α_1 -antichymotrypsin; AGP, α_1 -acid glycoprotein; APP, acute-phase protein; VAD, vitamin A deficiency.

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(AGP) was useful in interpreting plasma retinol in Ghana (Filteau *et al.* 1995) and Pakistan (Paracha *et al.* 2000). In addition to infection, prematurity and low birth weight may influence plasma retinol concentrations, as reported by workers in Saudi Arabia (Tolba *et al.* 1998), South Africa (Coutsoudis *et al.* 1995, 2000) and the UK (Chan *et al.* 1993).

The objective of the present study was to investigate vitamin A status in Nigerian neonates. To do this, blood was collected from infants in the first few days of life and plasma retinol was measured. Concentrations of plasma carotenoids and acute-phase proteins (APP) were determined because of their potential value as indicators of vitamin A nutrition (Thurnham *et al.* 1998) or inflammatory status (Filteau *et al.* 1995; Paracha *et al.* 2000). In addition, body weight, gestational age and prematurity were also investigated because of associations with plasma retinol concentrations reported in the literature. The results obtained provide information on the prevalence of VAD in Nigerian neonates and the present report describes how various factors were used to interpret vitamin A status. Understanding the factors that influence plasma retinol concentrations in neonates is essential to developing nutritional strategies. A short report of the present study was presented at the XXth International Vitamin A Consultative Group Meeting (Adelekan *et al.* 2002).

Subjects and methods

Subjects

Blood samples were obtained from 205 neonates between July 1996 and August 1997 when *ad hoc* visits were made to the labour, postnatal and neonatal wards in an attempt to collect a representative sample of infants found in the wards. The infants were recruited at the Wesley Guild Hospital, Ilesa, Nigeria, which is part of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, in south-west Nigeria. About 3000 women per year attend the antenatal clinic at the hospital, of which approximately 70% (n 2000) deliver there. The majority (75%) of neonates were aged 0–6 d (n 152) at the time of the blood sampling and 25% of neonates were older (6–20 d). All neonates were apparently well at recruitment and were exclusively breast-fed except for twenty, who were given combined breast- and formula-feeding.

Ethical approval

Ethical approval was obtained from the Research Ethics Committee of the College of Health Sciences at the Obafemi Awolowo University, Ile-Ife, Nigeria. Each mother enrolled into the study gave informed written consent.

Blood sampling

At recruitment, 0.5–1.0 ml venous blood was drawn from the heel of each neonate into heparinised tubes. Blood samples were mixed gently on a roller mixer and then centrifuged at 1200 *g* for 5 min to separate plasma and

erythrocytes. Plasma was stored frozen at -50°C for up to 9 months before shipment on dry-ice for analyses of fat-soluble vitamins, carotenoids and APP at the Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, Northern Ireland, UK. Samples were subsequently stored at -70°C until analysis (approximately 6 months).

Determination of plasma fat-soluble vitamins

The concentrations of fat-soluble vitamins A and E and carotenoids in 0.1 ml plasma were determined by HPLC by the method of Thurnham *et al.* (1988), modified to include the measurement of lutein. Lutein in this system elutes in a combined peak with zeaxanthin. Reagents included HPLC-grade ethanol, methanol, dichloromethane, acetonitrile, heptane and hexane (E. Merck, Poole, Dorset, UK). The standards used were retinol, α -carotene, β -carotene, lycopene, tocopherol, tocopheryl acetate (E. Merck), β -cryptoxanthin and lutein (gifts from Hoffmann-La Roche Ltd, Basel, Switzerland).

Briefly, 0.1 ml plasma was mixed with 0.1 ml aqueous SDS (10 mm) and 0.2 ml internal standard (84 μmol tocopheryl acetate/l ethanol). The micronutrients were extracted into 1.0 ml heptane containing 0.5 g butylated hydroxytoluene/l. The stoppered tubes were vortexed for 5 min and centrifuged for 10 min at 1000 rpm (2500 *g*) at 10°C . The upper heptane layer (0.7 ml) was removed and dried down under N_2 at 40°C . The samples were reconstituted with 100 μl mobile phase (acetonitrile–methanol–dichloromethane (47:47:12, by vol.)) and 50 μl was injected automatically onto a 3 μm Spherisorb ODS-2 column (100 \times 4 mm; Phase Separations Ltd, Clwyd, Wales, UK), separated at a flow rate of 1.0 ml/min and the micronutrients detected using two channels of a Waters 490 detector (Millipore UK Ltd, Watford, Herts., UK). The intra-batch CV of a pooled plasma sample were found to be (%): retinol 5.8 (mean value 0.98 $\mu\text{mol/l}$); lutein 6.9 (mean value 0.46 $\mu\text{mol/l}$); α -carotene 11.2 (mean value 0.04 $\mu\text{mol/l}$); β -carotene 5.8 (mean value 0.36 $\mu\text{mol/l}$).

Determination of plasma acute-phase proteins

The concentrations of ACT and AGP were determined turbidimetrically (Dako, Ely, Cambs., UK). Plasma was diluted 1:41 with dilution buffer (S2005; Dako). ACT rabbit anti-human antibody was diluted 1:0:5:5, while the AGP antibody was diluted 1:0:13:2 with the dilution buffer. Before use, diluted antibody was mixed with reaction buffer (S2008; Dako) in the ratio 1:5, then passed through a 0.22 μm membrane filter (SLGV 025NB; Millipore UK Ltd). Standards were prepared from a serum protein calibrator (X908; Dako): the range for the ACT assay was 0.05–1.20 g/l and for the AGP assay 0.1–3.0 g/l. The sample (80 μl) was mixed with 250 μl reagent for the ACT assay, while 20 μl sample was mixed with 250 μl reagent for the AGP assay, and analysed on a Cobas Fara centrifugal spectrophotometer (Hoffman La-Roche Ltd). Intra-batch CV for ACT and AGP were 3–8%, while inter-assay CV were 6%.

The normal range for ACT in adult plasma is 0.3–0.6 g/l (Thompson *et al.* 1992). However, little data exist for neonates, although Freer *et al.* (1979) have suggested a reference range of 0.05–0.48 g/l for cord blood samples after 34–43 weeks of gestation. The adult reference range for AGP is 0.5–1.0 g/l (Fiteau *et al.* 1993). For neonates, Sann *et al.* (1981) proposed a mean plasma AGP concentration of 0.18 (SD 0.08) g/l for the first 2 d of life, rising to 0.52 (SD 0.08) g/l at 5–6 d, then remaining constant up to 1 month. Viani *et al.* (1992) agreed with the earlier workers and recommended a range of 0.14–0.50 g/l.

Statistical analyses

Data were analysed using the Statistical Package for Social Sciences (SPSS for Windows, version 9.0.0; SPSS Inc., Chicago, IL, USA). Data on fat-soluble vitamins, carotenoids and APP were skewed, and were therefore log-transformed to normalise the data and analysed using parametric statistics. Geometric mean values and inter-quartile ranges were calculated and used throughout. During descriptive analysis of the data, it was obvious that gestational age and birth weight might influence plasma retinol and the APP and their contribution was investigated using univariate ANOVA with Bonferroni *post hoc* tests. A test of homoscedasticity showed more negative residuals for the carotenoids and APP than would be expected in a normal distribution; therefore, the log-transformed data for these variables were used for correlation and regression analyses. In addition, partial correlations (which are indicated by $r_{12.3}$) were determined between each variable and retinol after controlling for the effects of age. Stepwise backward multiple regression analyses with retinol as the dependent variable was conducted to determine which variables best predicted plasma retinol concentrations in the neonates and included the following log-transformed independent variables: ACT, AGP, β -carotene, α -carotene, lycopene, lutein, β -cryptoxanthin, α -tocopherol, and non-log transformed variables: age, group, length and weight, mother's age, mother's education, mother's occupation, parity. Those variables not included in the table were not significant ($P > 0.05$).

Results

The mean age of the mothers was 28 years; 95% were married and 38% were having their first child. The largest group (48%) was educated up to secondary level. Their occupations were varied: 28% were traders, 31% wage-earners and 26% were professionally employed, while the rest were farmers, housewives or unemployed. Most of the subjects lived in urban areas. Mothers remained in hospital with their infants and feeding was on demand. Food was available for mothers within the hospital, but it was more usual for meals to be brought in for them from home; hence, many mothers continued eating their normal diet. No dietary intake records were kept. None of the mothers was given vitamin A supplements postpartum and none reported any symptoms of mastitis.

Blood samples were taken from 205 neonates, including sixteen sets of twins (in four of the sets only one twin

survived). Complete biochemical and anthropometric data were available for 192 neonates; however, data on gestational age were missing for twenty-eight subjects, because the hospital case notes were lost. The mean age of the neonates at the time of the blood sample was 5.0 (SD 3.7) d. There were no differences between gender for weight, height, any of the micronutrients or APP. Therefore, data from males and females were combined for statistical analysis.

Anthropometry

The mean weight of the neonates was 2.6 (SD 0.70) kg and the mean length was 0.457 (SD 0.048) m. However, fifty-six (34%) of the neonates were premature with a mean gestational age of 33.2 (95% CI 28.0, 36.0) weeks compared with 38.9 (95% CI 37.0, 42.6; $n = 108$) weeks at full term. The mean weights and lengths of the premature infants were significantly smaller than those of infants of normal gestational age (2.05 kg and 0.4258 m *v.* 2.88 kg and 0.4692 m respectively; $P < 0.0001$, ANOVA). Mean values for both the normal and premature infants were lower than the 50th centile on the Centres for Disease Control and Prevention (2000) paediatric growth charts (3.4 kg and 0.480 m for girls and 3.5 kg and 0.495 m for boys). Sixty-seven (35%) of the neonates were < 2.5 kg and were classified as low-birth-weight infants; forty-three (64%) of these were also premature. There was no relationship between mothers' education or occupation and birth weights (results not shown).

Relationships with age

Table 1 shows the geometric mean values of retinol, carotenoids and APP, the inter-quartile ranges (25th and 75th centiles) and, as a measure of variance, a value termed the geometric standard error. The Pearson correlation coefficient shows the relationship between each variable and age. All carotenoids and retinol showed positive correlations with age, but the APP did not.

Inter-relationships of retinol, gestational age and birth weight

The distribution of high and low retinol values according to gestational age and weight is shown in Table 2. The infants were divided into four groups defined as follows: normal gestational age (≥ 37 weeks) and normal weight (≥ 2.5 kg) (group 1); normal weight and premature (≤ 36 weeks) (group 2); low weight (< 2.5 kg) and normal gestational age (group 3); low weight and premature (group 4). Overall, neonates who had low weights had significantly lower mean plasma retinol concentrations than those whose weights were in the normal range (0.46 *v.* 0.59 $\mu\text{mol/l}$ respectively; $P < 0.003$, ANOVA). In addition, babies who were both premature and had body weight < 2.5 kg had significantly lower mean plasma retinol concentrations than those of normal gestational age and weight (0.45 *v.* 0.59 $\mu\text{mol/l}$ respectively; $P < 0.023$, univariate ANOVA; $P = 0.008$, *post hoc* Bonferroni test). If age (d after birth) and two new variables, birth-weight

Table 1. Plasma concentration of retinol, carotenoids, α -tocopherol and acute-phase proteins in Nigerian neonates and the association of age with these variables†

Variable	n	Geometric		Inter-quartile range		Pearson correlation coefficient with age
		Mean‡	SE§	25th	75th	
Retinol (μ mol/l)	192	0.538	0.020	0.386	0.735	0.238**
α -Carotene (μ mol/l)	192	0.072	0.010	0.041	0.249	0.211**
β -Carotene (μ mol/l)	192	0.076	0.11	0.033	0.281	0.247**
β -Cryptoxanthin (μ mol/l)	192	0.014	0.003	0.008	0.065	0.217**
Lutein (μ mol/l)	192	0.080	0.060	0.043	0.178	0.223**
Lycopene (μ mol/l)	192	0.0024	0.003	0.021	0.078	0.193**
ACT (g/l)	192	0.37	0.016	0.280	0.510	0.103
AGP (g/l)	192	0.60	0.030	0.410	0.995	0.083

ACT, α_1 -antichymotrypsin; AGP, α_1 -acid glycoprotein.

** $P < 0.01$.

† For details of subjects and procedures, see p. 354.

‡ Geometric mean value.

§ As a measure of variance the value termed the geometric standard error is calculated as: (antilog (mean + standard error of logged values) – geometric mean).

group (low birth weight or normal weight) and gestational group (premature or normal gestation), are put into a univariate general linear model, only age and birth-weight group had a significant effect on plasma retinol concentration ($P < 0.002$ v. $P < 0.05$ respectively). There was no interaction between age, birth weight or gestational group.

Inter-relationships of the acute-phase proteins, retinol, gestational age and birth weight

Thirty-two neonates (17%) had abnormal ACT values (> 0.60 g/l) and forty-four (23%) had abnormal AGP values (> 1.0 g/l). AGP was correlated with weight and length ($r 0.16$, $P = 0.025$ and $r 0.24$, $P = 0.001$ respectively), but ACT did not. The majority of neonates with high APP were in the group with normal weight and gestational age (ACT 44%, AGP 52%) (Table 3). APP was not correlated with any of the carotenoids (results not shown) or age. ACT and AGP strongly correlated with each other ($r 0.784$, $P < 0.0001$). Both ACT and AGP correlated weakly with plasma retinol (ACT $r 0.18$, $P = 0.009$; AGP $r 0.15$, $P = 0.039$) and in both cases the relationship was positive. Only AGP correlated with birth weight ($r 0.232$, $P = 0.004$). Elevated concentrations of both APP

showed skewed distributions with age, since 59% (AGP, twenty-six of forty-four subjects) and 63% (ACT, twenty of thirty-two subjects) of all raised values were in infants < 6 d old. That is, there were more high values in the younger infants. Sann *et al.* (1981) reported that plasma AGP concentrations rose to a peak at 5 to 6 d old in healthy full-term neonates; therefore, the Nigerian data were examined separately above and below this age. Of children < 6 d old, 76% (ninety-six of 126 subjects) had low plasma retinol results (< 0.7 μ mol/l) and, in this subgroup, the relationship with APP was positive (ACT $r 0.34$, $P < 0.0001$, results not shown; AGP $r 0.313$, $P < 0.0001$, $n 126$, Fig. 1). The proportion of low plasma retinol concentrations (< 0.7 μ mol/l) in those neonates ≥ 6 d was 62% (forty-one of sixty-six subjects) and the relationships with both APP were negative (ACT $r -0.28$, $P = 0.022$, results not shown; AGP $r -0.29$, $P = 0.018$, $n 66$, Fig. 2).

Statistical relationships

Table 4 describes the correlations among all variables with plasma retinol and with age. Weight and length, all the carotenoids, AGP, ACT and age were significantly positively correlated with retinol. There were strong positive relationships between age and the carotenoids (Table 1).

Table 2. Distribution of retinol concentrations (μ mol/l) according to gestational group and weight†† (Geometric mean values and 95% confidence intervals)

	Normal weight§		Low weight§		All groups
	Normal gestation (group 1)	Premature (group 2)	Normal gestation (group 3)	Premature (group 4)	
Mean	0.59 ^a	0.53 ^{ab}	0.49 ^{ab}	0.45 ^b	0.53
95% CI	0.22, 1.32	0.28, 1.02	0.14, 1.06	0.15, 0.94	0.24, 1.08
n	89	13	19	43	164
Mean value	0.59		0.46*		
95% CI	0.24, 1.33		0.14, 0.99		
n	125		67		

^{a,b}Mean values within a row with unlike superscript letters were significantly different (univariate ANOVA, $P < 0.023$; *post hoc* Bonferroni, $P < 0.008$). Mean value was significantly different from that of normal weight neonates (ANOVA): * $P < 0.003$.

† For details of subjects and procedures, see p. 354.

‡ Where gestational age was used in the analysis, data were only available for 164 neonates as the gestational ages of twenty-eight neonates were not recorded.

§ Normal weight ≥ 2.5 kg, low weight < 2.5 kg.

|| Normal gestational age ≥ 37 weeks, premature gestational age ≤ 36 weeks.

Table 3. Distribution of elevated and normal concentrations of α -antichymotrypsin (ACT) and α_1 -acid glycoprotein (AGP) according to gestational age and weight* (Geometric mean values and 95 % confidence intervals)

	Normal weight†						Low weight†						All groups§		
	Normal gestation (group 1)‡			Premature (group 2)‡			Normal gestation (group 3)‡			Premature (group 4)‡					
	Mean	95% CI	n	Mean	95% CI	n	Mean	95% CI	n	Mean	95% CI	n	Mean	95% CI	n
ACT \leq 0.6 g/l	0.33	0.17, 0.54	75	0.31	0.16, 0.46	11	0.32	0.08, 0.60	17	0.32	0.18, 0.57	36	0.32	0.17, 0.56	160
ACT > 0.6 g/l	0.82	0.63, 1.09	14	0.85	0.76, 0.94¶	2	0.65	0.62, 0.67¶	2	0.95	0.70, 1.15	7	0.82	0.63, 1.27	32
AGP \leq 1.0 g/l	0.49	0.14, 0.92	66	0.62	0.23, 0.95	10	0.49	0.13, 0.98	16	0.49	0.14, 0.89	37	0.50	0.14, 0.99	148
AGP > 1.0 g/l	1.30	0.98, 2.84	23	1.22	1.05, 2.03¶	3	1.34	1.11, 1.79¶	3	1.28	1.03, 2.61	6	1.36	1.00, 2.89	44

* For details of subjects and procedures, see p. 354.

† Normal weight \geq 2.5 kg, low weight < 2.5 kg.

‡ Normal gestational age \geq 37 weeks, premature gestational age \leq 36 weeks.

§ Where gestational age was used in the analysis, data were only available for 164 neonates as the gestational ages of twenty-eight neonates were not recorded; therefore, numbers shown in the 'all groups' are greater than the sum of the four columns.

|| Concentrations of ACT > 0.6 g/l and AGP > 1.0 g/l are above normal adult range.

¶ Maximum and minimum values are given because of low numbers in the group.

Further partial correlations (r_{123}) between each variable and retinol were recalculated after controlling for the effects of age. All the carotenoids, weight and ACT were still significantly positively correlated with retinol but AGP was not.

A multiple regression analysis was then conducted to determine which variables best predicted plasma retinol concentration in the neonates (Table 5). The results suggest

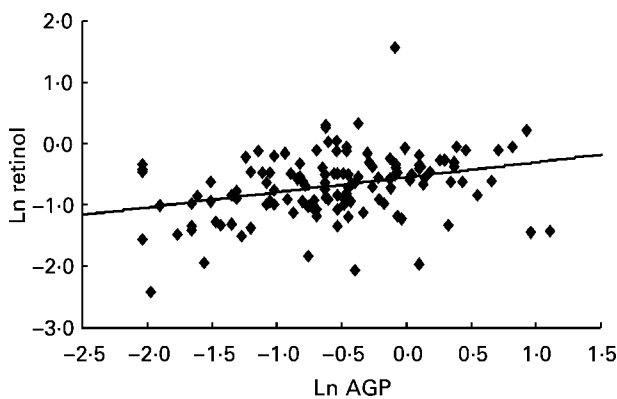


Fig. 1. Relationship between log retinol and log α_1 -acid glycoprotein (AGP) in Nigerian neonates age < 6 d (r 0.313, $P=0.0001$). For details of subjects and procedures, see p. 354.

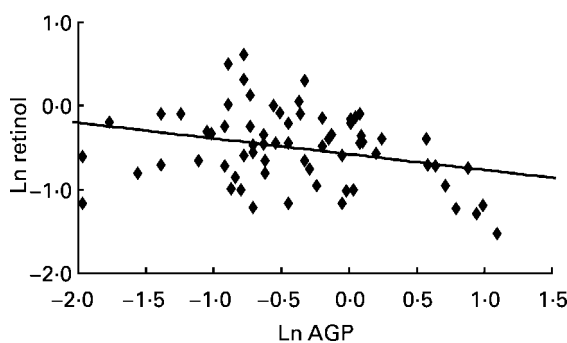


Fig. 2. Relationship between log retinol and log α_1 -acid glycoprotein (AGP) in Nigerian neonates age \geq 6 d (r 0.289, $P=0.018$). For details of subjects and procedures, see p. 354.

that plasma lutein is most strongly related to plasma retinol ($P=0.0001$) together with weight ($P=0.001$), age ($P=0.001$) and ACT ($P=0.021$), which jointly explained 30 % of the variance. The prediction equation based on Table 5 is:

predicted value for log retinol

$$= -0.679 + 0.178 (\text{weight}) + 0.172 (\log \text{ACT}) + 0.165 (\log \text{lutein}) + 0.037 (\text{age}).$$

Although the relationship between plasma retinol and the APP was not typical of that seen when inflammation is associated with depressed plasma retinol, the possible effect of inflammation on retinol was quantified using the results described by Paracha *et al.* (2000). The results of Paracha *et al.* (2000) were based on infants < 5 years of age, but a subsequent meta-analysis of fifteen studies confirmed the depressing effect of infection on retinol and proposed such a correction be performed on data for all age groups (Thurnham *et al.* 2002). Paracha *et al.* (2000) showed that if both ACT and AGP were above

Table 4. Correlation matrix describing relationships with retinol and with retinol after controlling for age†

	Retinol	Retinol with effect of age removed ($r_{12.3}$)
Age	0.238**	
Weight	0.204**	0.259*
Length	0.142*	0.180*
α -Carotene	0.352**	0.321**
β -Carotene	0.337**	0.298**
β -Cryptoxanthin	0.388**	0.357**
Lutein	0.414**	0.384**
Lycopene	0.260**	0.224**
ACT	0.186*	0.175*
AGP	0.148*	0.132

ACT, α -antichymotrypsin; AGT, α_1 -acid glycoprotein.

* $P<0.05$, ** $P<0.01$.

† For details of subjects (n 192) and procedures, see p. 354.

Table 5. Multiple stepwise regression equation with retinol as the dependent variable*†

Dependent and independent variables	B	SE B	β	Statistical significance
Constant	-0.679	0.201		0.001
Weight	0.178	0.053	0.237	0.001
ACT	0.172	0.074	0.163	0.021
Lutein	0.165	0.034	0.348	0.0001
Age	0.0371	0.011	0.250	0.001

ACT, α_1 -antichymotrypsin.* For details of subjects (*n* 164) and procedures, see p. 354 and Table 2.† Stepwise backward multiple regression analyses with retinol as the dependent variable included the following log-transformed independent variables: ACT, α_1 -acid glycoprotein, β -carotene, α -carotene, lycopene, lutein, β -cryptoxanthin and α -tocopherol, and non-log transformed variables: age, premature or not, low birth weight or not, length and weight, mother's age, mother's education, mother's occupation and parity. Those variables not included in the Table were not significant ($P > 0.05$).

normal, plasma retinol was depressed by 0.24 $\mu\text{mol/l}$, whereas if only one APP was elevated, plasma retinol was only depressed by 0.10 $\mu\text{mol/l}$. Table 6 shows the corrected retinol values for those neonates with elevated APP. Fifty-three children had elevated APP and correcting the retinol concentrations increased the mean value for this group of children from a value less than the threshold plasma retinol of 0.7 $\mu\text{mol/l}$ to one that was $>0.7 \mu\text{mol/l}$. In addition when the transient depression in plasma retinol produced by the sub-clinical infection was corrected in the most at risk group, i.e. those with plasma retinol concentrations $<0.35 \mu\text{mol/l}$ (*n* 35), 21% of these neonates moved from the high- to the low-risk group. The effect on mean plasma retinol for the whole group of neonates, however, was small, with an increase from 0.56 to 0.61 $\mu\text{mol/l}$.

Discussion

From the time of conception until delivery, the health of the fetus is dependent upon the health of the mother. Maternal health is influenced by socio-economic and nutritional status before and during pregnancy and the lack and/or excess of individual nutrients may have harmful effects on the development of the fetus (Wahid & Shadia, 1987). Fetal plasma vitamin A concentrations tend to be lower than maternal concentrations and evidence suggests that

the maternal:fetal concentrations of plasma vitamin A in healthy human pregnancies is approximately 2:1 (King, 1982). Vitamin A is transferred to the fetus during the last trimester of pregnancy and there is thought to be an association between low birth weight and low concentrations of maternal retinol (King, 1982).

The geometric mean value of plasma retinol for full-term Nigerian neonates in the present study was 0.57 $\mu\text{mol/l}$, which was low compared with a number of other studies: 0.84 $\mu\text{mol/l}$ in the USA (Shenai *et al.* 1981), 0.71 $\mu\text{mol/l}$ in the UK (Chan *et al.* 1993) and 0.74 $\mu\text{mol/l}$ in Egypt (Hussein & Ali, 2000). Even greater values were reported by Basu *et al.* (1994) and Cardonoparez *et al.* (1996) for full-term neonates in Canada, namely 1.23 and 1.38 (range 0.70–2.50) $\mu\text{mol/l}$ respectively; Godel *et al.* (1996) postulated that values between 0.7 and 2.5 $\mu\text{mol/l}$ represented a 'normal' range for newborn infants. However, this range appears to include some unusually high values, since the ranges of retinol concentrations reported by the other workers were similar to those reported by Chan *et al.* (1993), namely 0.34–1.27 $\mu\text{mol/l}$. Blood samples were taken from the neonates in our present study between 1 and 20 d postnatally and the plasma was stored for up to 9 months at -50°C and subsequently at -70°C for up to 6 months. However, we do not think that our retinol concentrations were low due to storage conditions, as work by Craft *et al.* (1988) has shown that plasma carotenoids and retinol were stable for at least 28 months when stored at -70°C . In addition, plasma retinol concentrations were not significantly different even when stored at -20°C for 15 months, although carotenoid concentrations were significantly less ($P < 0.05$) compared with plasma stored at -70°C . Our current samples were stored in small tightly stoppered tubes to reduce the possibility of dehydration and at temperatures of not more than -50°C , hence should have remained stable for at least 18 months. Therefore, the overall conclusion from the comparisons with other studies is that the mean retinol concentration was lower than would have been expected for full-term healthy neonates.

Both the premature and low-birth-weight Nigerian neonates had mean plasma retinol concentrations of 0.46 $\mu\text{mol/l}$ and this value was comparable with that reported for South African low-birth-weight and premature infants (0.45 $\mu\text{mol/l}$; Coutsoudis *et al.* 2000). However,

Table 6. Correction of plasma retinol where acute-phase proteins are elevated* (Geometric mean values and 95% confidence intervals)

Inflammatory state represented by APP (g/l)	<i>n</i>	Uncorrected retinol ($\mu\text{mol/l}$)		Corrected retinol ($\mu\text{mol/l}$)†	
		Mean	95% CI	Mean	95% CI
ACT >0.6 + AGP >1.0	23	0.54	0.22, 1.14	0.80	0.46, 1.41
ACT >0.6 + AGP ≤ 1.0	9	0.69	0.31, 0.94	0.79	0.41, 1.16
ACT ≤ 0.6 + AGP >1.0	21	0.65	0.15, 0.94	0.71	0.24, 0.96
No elevated APP, i.e. healthy subjects	139	0.54	0.23, 1.33		
All subjects	192	0.56	0.24, 1.17	0.61	0.25, 1.17

ACT, α_1 -antichymotrypsin; AGP, α_1 -acid glycoprotein; APP, acute-phase protein.

* For details of subjects and procedures, see p. 354.

† Based on the relationship between plasma retinol and APP, plasma retinol was corrected for the presence of sub-clinical infection (Paracha *et al.* 2000): where ACT ($>0.6 \text{ g/l}$) and AGP ($>1.0 \text{ g/l}$) are elevated, 0.24 $\mu\text{mol/l}$ is added to plasma retinol value. Where ACT or AGP are elevated, 0.1 $\mu\text{mol/l}$ is added to plasma retinol value.

the mean values were low compared with other studies, e.g. preterm US infants (0.56 $\mu\text{mol/l}$; Shenai *et al.* 1981) and preterm Egyptian infants (0.65 $\mu\text{mol/l}$; Hussein & Ali, 2000). Furthermore, Shenai *et al.* (1981) concluded that plasma retinol concentrations $<0.57 \mu\text{mol/l}$ in 1-d-old premature infants indicated vitamin A depletion. Low weight, particularly if coupled with prematurity, has been found to be associated with lower retinol concentrations and in the present study we found a significant correlation between plasma retinol and gestational age even after including the effect of the neonates' age (i.e. age at which the plasma sample taken) in the analysis. Chan *et al.* (1993) also found a correlation between vitamin A concentration and gestational age and suggested gestational age must be taken into account when interpreting vitamin A levels.

The question under consideration was to what extent low plasma retinol values represented inadequate maternal nutrition or the consequences of infection or trauma. In the present study 71% of the neonates had retinol values $<0.70 \mu\text{mol/l}$ and 18% of these had potentially severe VAD ($<0.35 \mu\text{mol/l}$), suggesting that vitamin A status was poor in many of the neonates. Several factors may affect the transport of vitamin A to the fetus: inadequate maternal stores of vitamin A, impaired transport of retinol across the placenta (Rosso, 1990; Bluhm *et al.* 1994; Rondo *et al.* 1995), impaired mobilisation of vitamin A from the maternal liver (as a result of reduced synthesis of retinol-binding protein due to infection or deficiencies of vitamin A or Zn), or decreased circulating maternal plasma retinol (as a result of maternal infection or deficiency). Likewise, low levels of the plasma vitamin A transport proteins retinol-binding protein and transthyretin in the newborn infant will depress plasma vitamin A (Surks & Oppenheimer, 1964; Kanai *et al.* 1968; Smith & Goodman, 1971; Goodman, 1984). Studies have also shown that immaturity of hepatic production of retinol-binding protein may be a factor responsible for low plasma levels of vitamin A in newborn infants (Valquist *et al.* 1975; Shenai, 1989).

Neither maternal nor infant plasma retinol-binding protein were measured in the present study as there was only a small amount of plasma, but two APP, ACT and AGP, were measured. ACT and AGP are useful markers of sub-clinical or chronic infection (Thompson *et al.* 1992). Plasma concentrations of ACT are known to increase rapidly in the first few hours following exposure to infection and reach a maximum at 24 h, while AGP rises more slowly reaching a maximum at 48–72 h (Calvin & Price, 1986). Once the clinical stage of the infection subsides, ACT decreases more rapidly than AGP (Fleck, 1989). In addition, AGP has been shown to be a good indicator of bacterial disease (Fleck & Myers, 1985) and is certainly capable of responding to infection in neonates, as shown by Philip & Hewitt (1983), where mean values of 0.26 (SEM 0.01) and 0.81 (SEM 0.01) g/l were obtained in uninfected and infected neonates respectively. Alt *et al.* (1984) also found slightly higher concentrations of AGP in neonates with materno–fetal infection (0.56 (SEM 0.003) g/l). Twenty-three percent of the Nigerian neonates had AGP concentrations $>1.0 \text{ g/l}$ suggestive of exposure to infection post-natally or *in utero*.

In our present study, the median ACT concentration was 0.37 g/l and only ACT contributed significantly to the variance in plasma retinol in the multiple linear regression analysis (r^2 3%). Very little data on ACT concentrations in neonates exist, and the only reference quotes a mean concentration in cord blood of 0.22 (range 0.05–0.48) g/l from eighty-one infants delivered after 34–43 weeks of gestation (Freer *et al.* 1979). However, more work has been done on plasma AGP in neonates and the strong correlation between AGP and ACT in the infant plasma suggests that the two proteins are controlled by similar processes. Median AGP (0.60 g/l) values in the Nigerian infants were within the accepted normal ranges for adults (0.5–1.0 g/l), but possibly outside the normal neonatal range (0.14–0.50 g/l) suggested by Viani *et al.* (1992). Nevertheless, the proportion with sub-clinical infection may well have been underestimated because of immaturity of APP synthesis in the livers of Nigerian neonates. Sann *et al.* (1981) reported that healthy neonates at full-term had plasma AGP concentrations of 0.18 (SD 0.08) g/l for the first 2 d, rising to 0.52 (SD 0.08) g/l at 5–6 d of life, then remaining constant for up to 1 month. This observation may explain the positive association between plasma retinol and both APP in those infants $<6 \text{ d}$ of age (Fig. 1). However, the Nigerian infants were all apparently healthy; therefore, the positive correlations with the APP observed during days 1–5 were probably due to the increasing plasma retinol from the maternal milk and a coincidental increasing capacity to synthesise APP, which peaked at about 5 d. Subsequently, the negative correlations shown in Fig. 2 (day 6 onwards) reflected the continuing increase in plasma retinol, but no further increase in APP. An underestimation of sub-clinical infection may be the reason why attempts to correct plasma retinol using the procedure outlined by Paracha *et al.* (2000) made almost no difference to the mean retinol value for the group.

Infants are born with very low reserves of vitamin A and are dependent on milk for their supply in the early months of life. The cross-sectional data suggest that plasma retinol and the carotenoid concentrations were increasing steadily during the period of development (0–20 d) and, for the majority, the source of vitamin A was breast-milk. Milk provides both pre-formed retinol, as well as carotenoids and pro-vitamin A carotenes. All infant plasma carotenoid concentrations correlated individually with retinol and with age, and the correlations with age suggest that the breast-milk was supplying an adequate amount of these nutrients. In the multiple linear regression, lutein was singularly the most important carotenoid in explaining neonatal plasma retinol. The association between lutein and retinol in the neonates suggests that a significant proportion of the increase in retinol was derived from green vegetable sources. β -Carotene is the main source of vitamin A in vegetables, but an unknown amount of β -carotene will be converted to retinol during absorption. Lutein is also present in most leafy green vegetables and in approximately equal amounts to β -carotene (Thurnham *et al.* 1997). Lutein is not a pro-vitamin A carotenoid and, as far as is known, there is far less metabolism of non-pro-vitamin A carotenoids than pro-vitamin A carotenes. Thus, because

lutein is consumed at the same time as β -carotene, when vegetables are consumed, it is a useful surrogate marker for the intake of β -carotene.

The relationship between lutein and retinol in the Nigerian neonates may nevertheless be considered surprising since the major source of the β -carotene for many, if not all, of the mothers in this community will have been red palm oil, which contains very little (if any) lutein. However, we have previously reported a close correlation between increases in plasma concentrations of retinol and lutein in Pakistani infants (mean age 10–14 months) at a time when there was a seasonal increase in the availability of fruits and vegetables (Thurnham *et al.* 1997). Furthermore, we have shown that concentrations of plasma lutein in Irish neonates correlate with maternal plasma lutein at birth; thus, maternal vegetable intake during gestation influences neonatal lutein values (Jewell *et al.* 2000). In addition, the major dietary source of vitamin A for both the Nigerian neonates and the Pakistani infants was mothers' milk. Jewell *et al.* (2000) and others (Khachik *et al.* 1997) have shown that the concentration of lutein in breast milk is often higher than β -carotene. Hence, the provision of lutein is strongly linked to the maternal intake of fruits and vegetables, and this may be the reason for the strongest correlation of the biomarkers examined in the breast-fed infants being between lutein and retinol.

In conclusion, weights and lengths, and plasma retinol, were low in the neonates and may have been a consequence of poor maternal nutrition. We suggest that the unexpected positive correlation between the APP and plasma retinol in neonates in days 1–5 inclusive might have been a consequence of the increasing hepatic synthetic capacity to produce APP and a steady increase in plasma retinol from breast-milk over the period. The cross-sectional data suggest the concentration of retinol increased rapidly following birth and was more strongly linked with nutrition than with the presence of infection. The small number of neonates with elevated APP levels may reflect the trauma of birth, but these had only a minimal influence on plasma retinol in contrast to dietary factors like lutein, a surrogate marker of vitamin A nutrition.

In Nigeria and other populations where VAD is an important public health problem, we suggest prophylactic vitamin A supplements should be given to infants and preschool children (0–59 months) and pregnant and postpartum women according to the new World Health Organization recommendations (Ross, 2002). In addition, where possible a programme of nutritional and health education should be given to schoolchildren and to all pregnant women. However, as there is very little antenatal care, specific education to pregnant mothers is currently not possible.

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