Acute-phase protein levels, diarrhoea, *Trichuris trichiura* and maternal education are predictors of serum retinol: a cross-sectional study of children in a Dhaka slum, Bangladesh

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The objectives of the present study were to identify predictors of serum retinol concentration as well as to assess the prevalence of low serum retinol concentration, in both the whole population after correcting for the effect of serum C-reactive protein (CRP) (using multiple categories), and the healthy subgroup. A cross-sectional study of 579 apparently healthy children, aged 3–7 years from a Dhaka slum, Bangladesh, was conducted. The effects of age, gender, serum CRP and α_1-antichymotrypsin, reported morbidity (during the previous 2 weeks), *Ascaris lumbricoides* and *Trichuris trichiura* infections, parental education, wasting, stunting and underweight on serum retinol were estimated using multiple linear regression. The mean serum retinol concentration was 0·84 (SD 0·27) μmol/l. Elevated serum CRP levels, reported diarrhoea, reported nasal discharge and *T. trichiura* infection were negative predictors of serum retinol, whereas maternal education was a positive predictor. Compared with a serum CRP level of <1 mg/l, CRP levels of 2 to <5, 5 to <10 and ≥ 10 mg/l were associated with 0·12, 0·16 and 0·32 μmol/l lower serum retinol, respectively. The prevalence of low serum retinol ($<0·70$ μmol/l) fell from 31·2 % to 15·6 % in the whole population, after correcting for the effect of CRP, and was 20·1 % in the healthy subgroup (CRP <2 mg/l). The prevalence of low serum retinol was high but overestimated due to the effect of CRP. Interventions are needed to address low serum retinol in Bangladesh. Controlling diarrhoea, nasal discharge and *T. trichiura* infection and improving maternal education may be important interventions. The use of multiple categories of acute-phase proteins and cut-off values that indicate elevated levels need further research.

Vitamin A deficiency: Serum retinol: Acute-phase proteins: Children

Vitamin A deficiency is a major public health problem in many developing countries, especially among children and women of reproductive age (West, 2002). In Bangladesh, it has been estimated that 30·8 % of preschool-aged children have vitamin A deficiency (serum retinol $<0·7$ μmol/l) (West, 2002). The consequences of vitamin A deficiency include impaired immune function, increased morbidity and mortality due to infectious diseases including diarrhoea, xerophthalmia which may lead to blindness, poor growth and anaemia (Sommer & West, 1996; Stephensen, 2001). Numerous factors may contribute to vitamin A deficiency. The immediate causes are primarily an inadequate dietary intake of vitamin A and/or a poor bioefficacy of provitamin A carotenoids due to plant-based diets and low intakes of animal source foods, but repeated infections may also play a role (Bloem *et al.* 2002; Miller *et al.* 2002). The underlying socio-economic and environmental factors should, however, not be overlooked (Bloem *et al.* 2002).

Plasma and serum retinol concentrations are widely used biochemical indicators of vitamin A status to identify populations at risk of vitamin A deficiency as well as to measure the efficacy and effectiveness of interventions. However, the acute-phase response (APR), which may occur in infections and/or trauma, is associated with a depressed serum retinol concentration (serum retinol) (Filteau *et al.* 1993; Filteau, 1999; Paracha *et al.* 2000; Stephensen, 2001; Wieringa *et al.* 2002; Thurnham *et al.* 2003). As subclinical infections are common in populations at risk of vitamin A deficiency, serum retinol may not be a good indicator of vitamin A status, and the prevalence of low serum retinol may be overestimated, since the current cut-off value (World Health Organization, 1996) does not take this into account. It has been suggested that acute-phase proteins (APPs) may be used to control for the confounding effect of the APR and when assessing the prevalence of low serum retinol, by correcting individual serum retinol values and identifying the

healthy subgroup (individuals with no elevated APP) (Wieringa *et al.* 2002; Thurnham *et al.* 2003; Tomkins, 2003). To our knowledge, no studies in apparently healthy children (3–7 years) have attempted to quantify the fall in serum retinol caused by the APR by using multiple categories for serum C-reactive protein (CRP) and serum α_1-antichymotrypsin (ACT) concentrations or other APPs.

A better identification of the populations at risk of low serum retinol as well as predictors that may have a positive or negative effect on serum retinol is important for designing targeted interventions aimed at improving vitamin A status in children. We therefore conducted a cross-sectional study in apparently healthy children from a Dhaka slum with the following specific objectives: to investigate the effects of age, gender, reported morbidity, helminthic infections, parental education and anthropometric indicators on serum retinol, while controlling for APPs (CRP and ACT); to estimate the fall in serum retinol due to the effect of the APPs as well as assess the prevalence of low serum retinol, in both the whole population after correcting for the effect of CRP and in the healthy subgroup.

Materials and methods

Study population and subjects

The study was conducted between January and April 2002 in an urban slum in Mirpur, a suburb of Dhaka, Bangladesh. The slum is subdivided into camps and is characterised by a high population density, poor housing and inadequate sanitation and hygiene. The population is poor with a low literacy rate, and the prevalence of poor child health and malnutrition is high (Haque *et al.* 2003). The camps studied were established after the war of independence from Pakistan in 1971, and the majority of people are of Bihari ethnic origin (Whitaker *et al.* 1982).

This study was carried out primarily to screen children for low serum retinol with subsequent enrolment in a vitamin A efficacy trial. Children aged 3–7 years of both genders were identified from four selected neighbouring camps through a house-to-house survey by field assistants familiar with the area. Children who had received vitamin A supplements within the preceding 6 months were excluded. Severely undernourished children (weight-for-height $<70\%$ and weight-for-age $<60\%$ of the US National Center for Health Statistics/WHO international reference median) and those with serious illnesses such as clinical signs of vitamin A deficiency, measles and tuberculosis, as well as chronic diseases, were excluded and referred to Dhaka Paediatric Hospital, Dhaka Medical College Hospital or Chest Disease Hospital for diagnosis and treatment. Bitot's spots were treated twice with an oral dose of vitamin A (retinyl palmitate, 60 mg (200 000 IU)). Children (n 579) were apparently healthy, showing no signs and symptoms of acute illness such as fever, acute diarrhoea, dysentery, pneumonia and acute respiratory tract infection, at the time of blood collection.

Before carrying out the study, it was presented to and accepted by the community leaders. Written or oral informed consent was obtained from each child's parent or legal guardian. The study protocol was approved by the Research Review Committee and Ethical Review Committee, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). All children and their family members had access free of charge to the health services, including medicines, given by the physicians at the health clinic/project office. Records of diseases and medicines provided to the study children were kept. Children with serum retinol $<0.35\,\mu$mol/l received an oral dose of vitamin A (retinyl palmitate, 60 mg (200 000 IU)).

Data collection

After consent and household socio-economic status (SES) information had been obtained, faeces collection, clinical examination and anthelmintic treatment were undertaken. Later, a 2 weeks' morbidity recall interview and clinical examination including blood collection were completed on the same day.

Clinical examination

Signs and symptoms of illness in the children were obtained by history from their parents/care-givers, and they were clinically examined using a structured questionnaire by two experienced physicians. Body temperature, pulse and respiration rate were measured, and an appraisal was made based on appearance, pallor, oedema, condition of the skin, throat, heart, lungs and abdomen, and signs of vitamin A deficiency. Information on the date of the most recent intake of vitamin A capsule and immunisation status was obtained.

Height and weight were measured with the child wearing light clothing and no shoes. Height was measured to the nearest 0.1 cm using a locally made wooden height board. Weight was measured to the nearest 0.1 kg using an electronic scale (Seca, Hamburg, Germany), which was calibrated daily. Mid-upper arm circumference was measured to the nearest 2 mm using Teaching Aids at Low Cost insertion tapes (TALC, St Albans, Herts, UK). Height and mid-upper arm circumference measurements were taken twice (CV $<1\%$) and the mean used as the reported value. Weight was measured once. All measurements were performed by trained nutritionists and monitored by a senior nutritionist. The CVs of intra- and interobserver variations were $<1\%$. Z-scores (or SD scores) of weight-for-height, weight-for-age and height-for-age were calculated using the US National Center for Health Statistics/WHO international reference population (World Health Organization, 1983) and ANTHRO software (Sullivan & Gorstein, 1999). Children with weight-for-height, height-for-age and weight-for-age Z-scores <-2 were considered wasted, stunted and underweight, respectively (World Health Organization, 1983).

Blood collection

Non-fasting venous blood (4 ml) was collected from each child between 09.00 and 13.00 hours, using trace-element-free plastic syringes and stainless steel needles. Blood was injected into evacuated tubes (Venoject II; Terumo Europe NV, Leuven, Belgium), which were wrapped in aluminium foil, transported to the Nutritional Biochemistry Laboratory, ICDDR,B, and centrifuged (1000 **g** for 10 min at room temperature) within 5 h. Serum was transferred using trace-element-free pipettes into cryovials (Simport, Quebec, Canada) and immediately stored at -20°C until analysed for retinol and CRP at ICDDR,B. Serum was transported on dry ice to Denmark for analysis of ACT at the Department of Human Nutrition, The Royal Veterinary and Agricultural University.

Socio-economic status and morbidity

Eight trained female field assistants, assisted by eight local women, maintained close contact and motivated the families as well as conducted the SES and the 2 weeks' morbidity recall interviews. Data were collected through household visits, using precoded and pretested questionnaires in *Bangla*. On the day consent was obtained, SES information was collected. Only data on household size and parental education are presented here. Other SES indicators, as well as asset index and their associations with serum retinol, will be presented in a separate paper. On the day of blood collection, morbidity data were collected and the mother/care-giver was interviewed about the presence or absence of symptoms of diarrhoea, nasal discharge, cough, fever, common cold or angular stomatitis in the child during the previous 2 weeks. Symptoms of diarrhoea, nasal discharge, cough, fever and angular stomatitis were recorded separately; however, cough accompanied by fever, malaise, tiredness and sore nose with or without nasal discharge was recorded as common cold. Diarrhoea was defined as $\geq$ three loose or watery stools in a 24 h period. Physicians at the project office supervised the work and attended to sick children either at the project office or at home. To ensure good data quality, the SES and morbidity questionnaires were checked for inconsistencies just after collection. SES supervisors and physicians made spot checks and re-interviewed a random subsample of 5 % of the households. Adjustments were made and feedback given to the interviewers.

Faeces collection, faeces examination and anthelmintic treatment

A plastic container was provided by the field assistant during the collection of SES data. The parent/care-giver was asked to collect a sample of faeces from the child in the early morning of the following day. The fresh faeces sample in normal saline (0·9% w/v aqueous NaCl) was examined microscopically by direct smear, and the presence of trophozoites of *Giardia intestinalis* and *Entamoeba histolytica* was verified. In addition, 1–2 g fresh faeces were fixed in 10 % (v/v) formalin in normal saline (0·9% w/v aqueous NaCl) and later processed by a quantitative ether sedimentation technique (Hall, 1981) before microscopic examination to estimate the intensity of helminthic infections of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm, expressed as eggs/g faeces. Faeces samples were analysed at the Parasitology Laboratory, ICDDR,B, by an experienced laboratory technician.

After collecting the faeces sample, the child was given anthelmintic treatment. A chewable tablet of 400 mg albendazole (Albizol; Opsonin Chemical Industries Ltd, Dhaka, Bangladesh) was given under supervision to each child twice with an interval of 2 weeks. The second dose was given 3–5 weeks before blood collection.

Biochemical analyses

Serum retinol concentrations were measured using HPLC. Serum (100 μl) was deproteinised with methanol containing retinyl acetate (500 μg/l) (Sigma Chemical Co, St Louis, MO, USA) as an internal standard (100 μl). From this mixture, retinol was extracted twice with hexane (200 μl). The hexane layers were pooled and evaporated under N gas. The residue was redissolved in 100 μl methanol and water (95:5, v/v) as mobile phase, and 25 μl were injected on a Waters HPLC instrument using a Waters Bondapack C_{18} column and a Waters 481 detector at 325 nm, as well as a Waters 510 solvent delivery system and a Waters 746 data module (Millipore Corp, Milford, MA, USA). For quality control, a pooled human serum sample was calibrated against standard reference material (fat-soluble vitamins, carotenoids and cholesterol in human serum, 968c; National Institute of Standards and Technology, Gaithersburg, MD, USA). Three aliquots of the serum pool were analysed with each set of samples, and the retinol concentration was calculated based on the known concentration of retinol in the serum pool. Within-day and between-day CVs for retinol in the serum pool were 1·4 % and 1·5 %, respectively.

Serum CRP was measured by immunoturbidimetric assay (Hitachi 902 Automatic Analyzer; Boehringer Mannheim, Germany) using commercial kits and quality control materials (Roche Diagnostics GmbH, Mannheim, Germany). The recovery of serum CRP was 95–105 %. The accuracy was verified with each run by analysing quality controls of serum CRP of low (13·9–22·9 mg/l) and high (35·6–58·4 mg/l) levels. For low and high levels of the controls, the within-day CVs were 2·5 % and 1·0 %, respectively, and the between-day CVs 3·4 % and 1·2 %, respectively.

Serum ACT was measured by turbidimetry (Cobas Mira Plus Automatic Analyzer; Roche, Basel, Switzerland) as described by Friis *et al.* (2001*a*). The accuracy was verified by daily runs of commercial human serum protein quality controls (DAKO, Glostrup, Denmark) of low (0·21–0·29 g/l) and high (0·51–0·69 g/l) levels. Within-day and between-day CVs for ACT were 2·1 % and 3·2 %, respectively, determined by using a pooled human serum sample.

Retinol concentration was determined in serum samples from 579 children, whereas CRP and ACT concentrations were determined in serum samples from 577 and 566 children, respectively, due to insufficient serum.

Statistical analyses

Data were entered twice and cleaned in Fox Pro (Microsoft, Redmond, WA, USA) and analysed using SPSS for Windows (version 12·0; SPSS Inc., Chicago, IL, USA). Normal probability plots were used to assess whether continuous variables were normally distributed. The outcome variable, serum retinol, conformed with normality. Two-sample *t* test or one-way ANOVA with Tukey's multiple comparison *post hoc* tests were used to test for differences in means between categories. Differences in proportions were compared using chi-squared tests with the Pearson chi-squared or continuity correction test when appropriate. Multiple linear regression analysis was used to identify and estimate statistical effects of predictors of serum retinol. Variables assessed were age, gender, serum CRP and ACT, reported diarrhoea, nasal discharge, cough, fever, common cold and angular stomatitis, *T. trichiura* and *A. lumbricoides* infections, and maternal and paternal education, as well as wasting, stunting and underweight. Age and sex were forced into the model. Age (year) was a continuous variable. Dummy variables were used to assess the effects of gender, reported morbidity, the presence of helminthic

infections, anthropometric indicators, APPs and parental education. Serum CRP was arbitrarily categorised as < 1, 1 to < 2, 2 to < 5, 5 to < 10 and ≥ 10 mg/l and ACT as < 0.3, $0.3-0.4$ and > 0.4 g/l. Maternal and paternal education was categeorised as 0, $1-5$ and $6 +$ years of schooling. If helminthic infections were found to be predictors, the effect of the intensity of infection was assessed after $\log_{10}$(eggs/g faeces $+1$) transformation. Interaction between age and gender was tested.

Serum retinol concentrations < 0.70 µmol/l were considered low (World Health Organization, 1996). To calculate the prevalence of low serum retinol in the whole population corrected for the APR, three dummy variables – serum CRP, serum ACT and gender – as well as age were tested in the multiple linear regression analysis. Subsequently, the individual serum retinol concentrations were increased by the estimated fall due to the effect of CRP before categorisation.

Data were analysed using stepwise selection, and variables with $P < 0.10$ were retained in the multiple regression model. Values of $P < 0.05$ were considered significant. Residual analysis including normal distribution and homogeneity of variance of standardised residuals was carried out by investigating normal probability plots and plotting standardised residuals against predicted values.

Results

Characteristics of the children

Of the 723 children from 550 households who were mapped, consent was obtained for 688 children (twenty-four children had moved away, ten did not agree to give blood, and one did not agree for other reasons). Subsequently, sixty-five withdrew their consent before blood collection (forty-nine refused to give blood, twelve had moved away, and four withdrew for other reasons), forty-four were excluded (nineteen had taken vitamin A capsules, fourteen were severely undernourished, four had congenital malformation, two had viral hepatitis, one had Bitot's spots, one had tuberculosis, one had blast injury, and two were excluded for other reasons) and blood was collected from 579 apparently healthy children.

Helminthic infections of *A. lumbricoides* and *T. trichiura* were common (Table 1), whereas hookworm (1.2 %; *n* 7) as well as trophozoites of *Giardia intestinalis* (0.9 %; *n* 5) and *Entamoeba histolytica* (0.7 %; *n* 4) were rare. Among the 579 children, the prevalence of reported morbidity was: cough 25.4 %; nasal discharge 19.7 %; fever 19.3 %; common cold 18.0 %; diarrhoea 13.8 %; angular stomatitis 3.3 %. The mean serum retinol was 0.84 µmol/l, 31.3 % of the children had low serum retinol (Table 2), 2.6 % (*n* 15) had serum retinol < 0.35 µmol/l and 52.5 % had serum CRP ≥ 2 mg/l (Table 2). Among 296 children with serum CRP ≥ 2 mg/l, 83.5 % had serum ACT ≤ 0.4 g/l.

Bivariate analysis

The mean serum retinol and the proportion of children with low serum retinol did not differ by gender ($P = 0.18$ and $P = 0.31$, respectively) or age ($P = 0.56$ and $P = 0.61$, respectively). However, both differed by category of serum CRP (Table 3). Children with serum ACT < 0.3 and $0.3-0.4$ g/l had similar mean serum retinol ($P = 0.62$). However, those with serum ACT > 0.4 g/l had 0.15 µmol/l (95 % CI 0.06, 0.24; $P < 0.001$) and 0.13 µmol/l (95 % CI 0.04, 0.22;

Table 1. Characteristics of 579 children aged between 3 and 7 years

	Mean	SD	% (*n*)	Median	Interquartile range
Age (years)	4.8	1.4			
Boys			54.4 (315)		
Height (cm)	101.6	10.8			
Weight (kg)	14.7	3.1			
Mid-upper arm circumference (mm)	152	12			
Weight-for-height *Z*-score	−1.12	0.80			
< -2			11.9 (69)		
Height-for-age *Z*-score	−1.86	1.23			
< -2			45.1 (261)		
Weight-for-age *Z*-score	−1.92	0.92			
< -2			49.1 (284)		
Household size				5	4−7
Educational level of mother (years of schooling; *n* 560)					
0			64.6 (362)		
1−5			20.9 (117)		
6 +			14.5 (81)		
Educational level of father (years of schooling; *n* 519)					
0			51.3 (266)		
1−5			22.7 (118)		
6 +			26.0 (135)		
Helminths					
Ascaris lumbricoides					
Prevalence			57.9 (335)		
Intensity* (epg)				928	406−2326
Trichuris trichiura					
Prevalence			54.6 (316)		
Intensity* (epg)				252	128−621

epg, eggs/g faeces.
* Intensity of the helminth in infected children.

Table 2. Serum retinol, C-reactive protein (CRP) and α_1-antichymotrypsin (ACT) concentrations and proportions with low serum retinol* and different levels of CRP and ACT concentrations in 579 children

	Mean	SD	% (n)	Median	Interquartile range
Serum retinol (μmol/l)	0·84	0·27			
< 0·70*			31·3 (181)		
0·70−1·05			48·9 (283)		
≥ 1·05			19·8 (115)		
Serum CRP (mg/l; n 577)				2·1	1·0−4·2
< 1			22·9 (132)		
1 to < 2			24·6 (142)		
2 to < 5			32·0 (185)		
5 to < 10			13·9 (80)		
≥ 10			6·6 (38)		
Serum ACT (g/l; n 566)				0·29	0·26−0·34
< 0·30			51·8 (293)		
0·30−0·40			38·0 (215)		
> 0·40			10·2 (58)		

* Serum retinol concentrations < 0·70 μmol/l were considered low (World Health Organization, 1996).

$P=0.003$) lower serum retinol, respectively, than those with serum ACT < 0·3 and 0·3−0·4 g/l. In the groups with serum ACT ≤ 0·4 g/l and > 0·4 g/l, the proportions of children with low serum retinol were 28·0 % (n 142/508) and 56·9 % (n 33/58), respectively.

Children infected with *A. lumbricoides* and *T. trichiura* had 0·05 μmol/l (95 % CI 0·003, 0·09; $P=0.04$) and 0·10 μmol/l (95 % CI 0·05, 0·14; $P<0.001$) lower mean serum retinol, respectively, than those not infected. Children with reported nasal discharge had 0·09 μmol/l (95 % CI 0·04, 0·15; $P=0.001$) lower mean serum retinol than those without. Mean serum retinol tended to be lower in children with reported cough (0·04 μmol/l, 95 % CI 0·005, 0·09; $P=0.08$) and angular stomatitis (0·11 μmol/l, 95 % CI 0·01, 0·23; $P=0.08$) than in those without. There were no differences in mean serum retinol between children with and without reported diarrhoea (mean: 0·81 μmol/l, 95 % CI 0·76, 0·86 v. mean: 0·85 μmol/l, 95 % CI 0·82, 0·87; $P=0.21$); fever ($P=0.54$) and common cold ($P=0.30$). Mean serum retinol differed by category of the mother's years of schooling ($P<0.001$) with means of: 0 year, 0·81 μmol/l (95 % CI 0·79, 0·84); 1−5 years, 0·84 μmol/l (95 % CI 0·79, 0·89); 6 + years, 0·97 μmol/l (95 % CI 0·92, 1·03). Mean serum retinol also differed by category of father's years of schooling ($P=0.008$) with means of: 0 year, 0·82 μmol/l (95 % CI 0·79, 0·85); 1−5 years, 0·86 μmol/l (95 % CI 0·81, 0·90); 6 + years, 0·90 μmol/l (95 % CI 0·86, 0·94). Wasted children had 0·07 μmol/l (95 % CI 0·004, 0·14; $P=0.04$) lower mean serum retinol than those not wasted. Simi-

larly, the mean serum retinol was 0·05 μmol/l (95 % CI 0·008, 0·09; $P=0.02$) lower in stunted children than in those not stunted. In contrast, mean serum retinol ($P=0.30$) was similar in children who were underweight and not underweight.

Predictors of serum retinol

Using multiple linear regression analysis, elevated serum CRP levels, reported diarrhoea, reported nasal discharge, *T. trichiura* infection and maternal education were predictors of serum retinol (Table 4). Even though wasting was not a predictor, there was a tendency for wasting to be associated with lower serum retinol. Age, gender, serum ACT, reported cough, fever, common cold and angular stomatitis, *A. lumbricoides* infection, paternal education, stunting and underweight were not associated with serum retinol, and there was no interaction between age and gender.

Compared with serum CRP < 1 mg/l, CRP levels of 2 to < 5 and 5 to < 10 mg/l were associated with 0·12 and 0·15 μmol/l lower serum retinol, respectively, whereas ≥ 10 mg/l was associated with 0·30 μmol/l lower serum retinol (Table 4). The category serum CRP ≥ 10 mg/l was associated with a depression in serum retinol that was higher than those in the categories 2 to < 5 mg/l ($P<0.001$) and 5 to < 10 mg/l ($P=0.003$), with the latter two categories being similar ($P=0.31$). Despite similar mean serum retinol in children with and without reported diarrhoea in the bivariate analysis, diarrhoea was found to be significant in the multiple

Table 3. Mean serum retinol concentrations and proportions of children with low serum retinol concentration* by serum C-reactive protein (CRP) category (n 577)

	Serum CRP (mg/l)					P-value
	< 1 (n 132)	1 to < 2 (n 142)	2 to < 5 (n 185)	5 to < 10 (n 80)	≥ 10 (n 38)	
Serum retinol (μmol/l)	0·93 (0·89, 0·97)[a]	0·89 (0·85, 0·94)[a]	0·81 (0·77, 0·85)[b]	0·77 (0·72, 0·82)[b]	0·62 (0·55, 0·68)[c]	< 0·001
< 0·70* (%, (n))	15·9 (21)	23·9 (34)	35·7 (66)	38·8 (31)	73·7 (28)	< 0·001
0·70−1·05 (%, (n))	54·5 (72)	52·8 (75)	47·0 (87)	50·0 (40)	21·0 (8)	
≥ 1·05 (%, (n))	29·6 (39)	23·3 (33)	17·3 (32)	11·2 (9)	5·3 (2)	

[a,b,c] Values are mean (95 % CI); mean values with unlike superscript letters were significantly different ($P<0.05$).
* Serum retinol concentrations < 0·70 μmol/l were considered low (World Health Organization, 1996).

Table 4. Predictors of serum retinol concentration (μmol/l) in 579 children

	B	95 % CI	P-value
Age (years)	0·00	−0·02, 0·02	0·95
Male gender*	−0·01	−0·05, 0·03	0·63
Serum CRP (mg/l)†			
1 to < 2	−0·04	−0·10, 0·02	0·22
2 to < 5	−0·12	−0·17, −0·06	<0·001
5 to < 10	−0·15	−0·22, −0·08	<0·001
≥ 10	−0·30	−0·39, −0·21	<0·001
Diarrhoea during previous 2 weeks‡	−0·08	−0·14, −0·02	0·01
Nasal discharge during previous 2 weeks‡	−0·07	−0·12, −0·02	0·007
Trichuris trichiura infection‡	−0·08	−0·12, −0·04	<0·001
Maternal education (years of schooling)§			
1–5	0·03	−0·02, 0·08	0·30
6 +	0·12	0·06, 0·18	<0·001
Wasting‖	−0·05	−0·12, 0·01	0·09

CRP, C-reactive protein

Results are expressed as *B*, regression coefficients with 95 % CI and *P*-values. In the multiple linear regression analysis, variables (except age and male gender) with $P\geq0.10$ not shown are: serum α_1-antichymotrypsin (0·3–0·4 and >0·4 g/l); reported morbidity during previous 2 weeks (cough, fever, common cold, angular stomatitis); *Ascaris lumbricoides* infection; paternal education (1–5 and 6 + years of schooling); stunting (height-for-age *Z*-score < −2) and underweight (weight-for-age *Z*-score < −2). Model used in table: intercept: 0·98 μmol/l (95 % CI 0·89, 1·08); adjusted R^2 0·16; *n* 558 (due to missing values, in total *n* 21: insufficient serum for CRP, *n* 2; missing information for maternal education, *n* 19).

* Female gender was used as the reference category.
† Serum CRP < 1 mg/l was used as the reference category.
‡ No reported diarrhoea, reported nasal discharge and *Trichuris trichiura* infection was used as the reference category.
§ Maternal education of 0 year of schooling was used as the reference category.
‖ Weight-for-height *Z*-score < −2 was considered wasting. Weight-for-height *Z*-score ≥ −2 was used as the reference category.

regression analysis (Table 4) and was associated with 0·08 μmol/l lower serum retinol than no diarrhoea, suggesting that the result in the bivariate analysis was mediated or confounded by the other predictors in the multiple regression analysis. Reported nasal discharge was associated with 0·07 μmol/l lower serum retinol than no nasal discharge. Infection with *T. trichiura* was associated with 0·08 μmol/l lower serum retinol than no infection. *T. trichiura* assessed as intensity of infection after $\log_{10}$(eggs/g faeces + 1) transformation was also a negative predictor of serum retinol ($P=0.001$). The regression coefficient of −0·03 μmol/l (95 % CI −0·05, −0·01) corresponded to a 0·03 μmol/l decline in serum retinol per 1 unit increase in $\log_{10}$(eggs/g faeces + 1). Schooling of the mother for 6 + years was a strong positive predictor of serum retinol and was associated with 0·12 μmol/l higher serum retinol than no years of schooling (Table 4).

When serum CRP was taken out of the model, the regression coefficients of the predictors did not change considerably. At the same time, stunting ($P=0.04$) was retained as a negative predictor in the model, whereas wasting ($P\geq0.10$) was not retained, suggesting that their effects, unlike those of the other predictors, were mediated or confounded by the APR (data not shown). When anthropometric indicators were taken out of the model, the regression coefficients of the predictors did not change considerably (data not shown).

Elevated serum ACT was a predictor of serum retinol but only when serum CRP was not included in the model (intercept: 0·94 μmol/l, 95 % CI 0·85, 1·04; adjusted R^2 0·11; *n* 547). Using serum ACT < 0·3 g/l as the reference category, serum ACT of 0·3–0·4 g/l was not a predictor of serum retinol (regression coefficient −0·03 μmol/l, 95 % CI −0·07, 0·02); $P=0.28$); however, the category > 0·4 g/l was associated with 0·13 μmol/l (95 % CI 0·06, 0·20; $P=0.001$) lower serum retinol. The predictors of serum retinol in this model were similar to those found in the model with serum CRP, and the regression coefficients did not change considerably. However, stunting ($P=0.07$) was retained in the model and tended to be a negative predictor, whereas wasting ($P\geq0.10$) was not retained (data not shown).

Estimation of the effect of serum C-reactive protein for correcting the prevalence of low serum retinol

In the multiple linear regression analysis, only elevated serum CRP levels were predictors of serum retinol (intercept: 0·93 μmol/l, 95 % CI 0·89, 0·98; adjusted R^2 0·090; *n* 577), whereas serum ACT, age and gender were not. Using serum CRP < 1 mg/l as reference category, the estimated effect of serum CRP levels of 1 to < 2, 2 to < 5, 5 to < 10 and ≥ 10 mg/l on serum retinol, expressed as regression coefficients, were: −0·04 μmol/l (95 % CI −0·10, 0·02; $P=0.18$); −0·12 μmol/l (95 % CI −0·18, −0·06; $P<0.001$); −0·16 μmol/l (95 % CI −0·23, −0·09; $P<0.001$) and −0·32 μmol/l (95 % CI −0·41, −0·23; $P<0.001$), respectively. The prevalence of a low serum retinol fell markedly from 31·2 % to 15·6 % in the whole population after correction of serum retinol values for the effect of serum CRP and was 20·1 % in the group with serum CRP < 2 mg/l (healthy subgroup) (Table 5).

Discussion

Prevalence of low serum retinol

According to our results, the prevalence of low serum retinol is high (31·2 %), regardless of age and gender, among apparently healthy children in this urban slum population in Dhaka. The results indicated that vitamin A deficiency is a public health problem (≥ 15 % of the children having serum retinol concentrations < 0·7 μmol/l; Sommer & Davidson, 2002). The prevalence of low serum retinol (<0·7 μmol/l) found in this study is in accordance with the recent national estimate (30·8 %) (West, 2002) but slightly higher than the rural national value (22 %) found in preschool-aged children in Bangladesh (Helen Keller International, 1999). However, the correction of serum retinol values for the effect of APR by using serum CRP as a marker, subdivided into four categories, indicated that the prevalence of low serum retinol in the whole population (15·6 %) was halved, bordering on being a public health problem. Moreover, the identification of the healthy subgroup of children with no elevated APP, i.e. CRP < 2 mg/l in the present study, resulted in a prevalence of low serum retinol (21·1 %) that was higher than that in the whole population after correcting for the effect of CRP. However, the sample-size reduction might have made the prevalence less precise (Thurnham *et al.* 2003), and the

exclusion of children with an elevated APP might have led to sampling bias (Maqsood *et al.* 2004).

In Bangladesh, programmes to reduce vitamin A deficiency include national biannual vitamin A capsule supplementation (retinyl palmitate, 60 mg (200 000 IU)) to children aged 12–59 months with a high coverage rate (≥95 % in slums) (Bloem *et al.* 2003), and homestead food production aiming to improve the availability of micronutrient-rich foods through home gardening and recently also with the integration of animal husbandry including poultry and fish production (Bloem *et al.* 2003). In the present study, the population was not representative due to our enrolment criteria, but in light of the high prevalence of low serum retinol, additional interventions may be needed to improve low levels of serum retinol in Bangladesh.

Relationship between acute-phase proteins and serum retinol

There are no standardised cut-off values for serum CRP and ACT for assessing elevated APR, although serum CRP of ≥ 5, ≥ 8 or ≥ 10 mg/l and serum ACT of ≥ 0.4 or ≥ 0.6 g/l are commonly used. Because the relationship between changes in APP and serum retinol is non-linear, we used several categories of APP to evaluate the effect on serum retinol. In the present study, elevated APPs during subclincal infection were associated with depressed serum retinol when APPs were as low as 2 to < 5 mg/l serum CRP and > 0.4 g/l serum ACT (Friis *et al.* 2001*a,b*). This indicated that serum retinol is depressed at a much lower level of CRP than previously anticipated. Furthermore, the present study showed that the degree of depression in serum retinol seemed to be related to the category, i.e. the magnitude of serum CRP, indicating the usefulness of more than one cut-off point for CRP. We therefore suggest studies to estimate the effect of APPs using multiple categories. However, standardised cut-off values for APPs from different analytical methods are needed to compare the estimated effects of APPs from different studies and populations.

Different APPs respond differently over the time-course of the infection. Both serum CRP and ACT rise within the first 6 h of infection and reach maximum concentrations within 24–48 h, i.e. early in the infection phase (Fleck & Myers, 1985; Calvin *et al.* 1988). CRP has a rapid turnover time, and once the immediate stimulus disappears, its concentration falls, whereas ACT remains raised for some time longer than CRP (Fleck & Myers, 1985; Calvin *et al.* 1988). In the multiple regression analysis in the present study, serum CRP was more closely associated with serum retinol than was serum ACT, suggesting that CRP is a more suitable marker for depression of serum retinol in apparently healthy children.

The decrease in serum retinol due to the APR has been shown to be transitory. This is likely to be due to a transitory change in vitamin A metabolism without a change in the size of the liver vitamin A stores during mild episodes of infection. However, severe episodes or prolonged infections may diminish the liver stores (Mitra *et al.* 1998*a,c*; Stephensen, 2000). Hence, during the APR, several factors can contribute to the decrease in serum retinol (Stephensen, 2001). The mechanisms suggested include a reduction in the synthesis and secretion of hepatic retinol-binding protein and probably hepatic retinol accumulation (Rosales *et al.* 1996; Gieng *et al.* 2005); leakage of retinol-binding protein into the

Table 5. Prevalence of low serum retinol concentration* in children, correcting for the effect of serum C-reactive protein (CRP) concentrations and excluding children above different CRP cut-off values

| | Whole population (*n* 577) | | | | Children with serum CRP (mg/l)† | | | | | | | | | | | |
| | No correction | | | Corrected for the effect of serum CRP‡ | <2 (*n* 274) | | | <5 (*n* 459) | | | <8 (*n* 528) | | | <10 (*n* 539) | | |
	% (*n*)	Mean	SD	% (*n*)	% (*n*)	Mean	SD	% (*n*)	Mean	SD	% (*n*)	Mean	SD	% (*n*)	Mean	SD
		0.84	0.27			0.91	0.26		0.87	0.27		0.86	0.26		0.86	0.26
Serum retinol (μmol/l)																
<0.70*	31.2 (180)			15.6 (90)	20.1 (55)			26.4 (121)			27.3 (144)			28.2 (152)		
0.7–1.05	48.9 (282)			55.5 (320)	53.6 (147)			51.0 (234)			51.3 (271)			50.8 (274)		
≥1.05	19.9 (115)			28.9 (167)	26.3 (72)			22.6 (104)			21.4 (113)			21.0 (113)		

* Serum retinol concentrations <0.70 μmol/l were considered low (World Health Organization, 1996).
† Serum CRP concentrations ≥2 mg/l were considered elevated in the present study.
‡ The individual serum retinol concentrations (μmol/l) were increased by the estimated fall due to the effect of serum CRP before categorisation. For serum CRP levels 1 to <2, 2 to <5, 5 to <10 and ≥10 mg/l, serum retinol values of 0.04, 0.12, 0.16 and 0.32 μmol/l, respectively, were added.

extravascular space (Thurnham & Singkamani, 1991); increased urinary excretion of retinol bound to retinol-binding protein (Alvarez *et al.* 1995; Mitra *et al.* 1998*a*,*b*); and probably an increased uptake of retinol by certain tissues (Willumsen *et al.* 1997). Furthermore, reduced food intake and decreased absorption may also play a role in lowering serum retinol (Stephensen, 2001).

Relationship between morbidity, helminths, parental education and anthropometric indicators and serum retinol

In the present study, multiple linear regression analysis was used to test the associations while controlling for the effect of the APR and other predictors, but this does not necessarily elucidate causal relationships. None of the anthropometric indicators was found to be associated with serum retinol. However, reported diarrhoea and nasal discharge, but not cough, common cold, fever and angular stomatitis, were associated with lower serum retinol. This is consistent with previous studies in children showing that reported diarrhoea within the previous 2 weeks was associated with night-blindness (Stoll *et al.* 1985; Semba *et al.* 2004) and an increased risk of vitamin A deficiency (defined as serum retinol < 0·7 μmol/l) (Rosen *et al.* 1994). In contrast, other studies have shown that mean plasma retinol concentrations in children with and without reported illnesses within the previous 2 weeks did not differ (Filteau *et al.* 1993; Semba *et al.* 2000). These studies may, however, have been subjected to recall bias (Filteau *et al.* 1993; Semba *et al.* 2000), and potential confounding factors, including APR, were not taken into account. Interestingly, in one of the studies (Semba *et al.* 2000), plasma retinol concentration was significantly lower in children with nasal discharge noted on physical examination than those without. In this study, the presence as well as the intensity of *T. Trichiura* but not *A. lumbricoides* infection was negatively associated with serum retinol. *Schistosoma mansoni* but not *T. trichiura*, was found to be a negative predictor of serum retinol in Zimbabwean children after controlling for relevant factors, suggesting that high helminthic intensities may induce vitamin A deficiency (Friis *et al.* 1997).

Even if our associations were causal and not confounded, our study did not determine whether low serum retinol predisposed to the infections or the infections precipitated the development of low serum retinol (bidirectional). Vitamin A deficiency is associated with compromised integrity of the mucosal epithelial barriers of the gut (Thurnham *et al.* 2000) and respiratory tract (Chandra, 1988), as well as immune response to infections that may increase the susceptibility to diarrhoeal, respiratory and *T. trichiura* infections (Koski & Scott, 2001; Stephensen, 2001; Wieringa *et al.* 2004). On the other hand, these infections can contribute to vitamin A deficiency by previous or sustained APR, reduced retinol intake (due to anorexia and/or vomiting), malabsorption, increased retinol requirement and excretion (Stephenson *et al.* 2000; Koski & Scott, 2001; Stephensen, 2001).

Maternal but not paternal education was associated with higher serum retinol. The results indicated that the education of girls for 6 years or more is important in order to improve low serum retinol in their children. As about two-thirds of the mothers had no education, it might be worthwhile investigating whether health and nutrition education for mothers who have never attended school can also improve low serum retinol levels in their children.

Potential limitations of the study

Serum retinol is homeostatically controlled (Underwood, 1984) and, besides the APR, various factors may affect or confound the interpretation of serum retinol concentrations, including Zn deficiency (Christian & West, 1998) and protein–energy malnutrition (Bates, 1990), which interferes with vitamin A metabolism through a reduced hepatic synthesis or secretion of retinol-binding protein. Anthelmintic treatment was given in the present study in order to rule out helminthic infections as a possible confounder in a subsequent vitamin A efficacy trial. *A. lumbricoides* and *T. trichiura* infections have previously been shown to be prevalent in this slum, with more than 80 % of children being infected (Hall *et al.* 1992; Hall & Nahar, 1994).

Conclusion

The present study showed a high prevalence of low serum retinol in this group of apparently healthy children in a slum in Dhaka. After correcting for the effect of CRP in the whole population, the prevalence of low serum retinol was halved, whereas in the healthy subgroup with CRP < 2 mg/l, it was reduced by one third. Our results suggest that there is a need for interventions to address low serum retinol in Bangladesh. Elevated serum CRP and ACT levels, reported diarrhoea, reported nasal discharge and *T. trichiura* infection were negative predictors of serum retinol, whereas maternal education was a positive predictor. Thus, controlling diarrhoea, nasal discharge and *T. trichiura* infection, as well as improving the school attendance of girls for 6 years or more, may be important interventions to improve low serum retinol levels in children. Our results indicate that elevated APPs during subclinical infection markedly depress serum retinol and as a result hamper the interpretation of serum retinol. Thus, when using serum retinol, APPs must be taken into consideration. The use of multiple categories of APPs and the cut-off values that indicate elevated levels need further research.

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