Use of the retinol-binding protein : transthyretin ratio for assessment of vitamin A status during the acute-phase response

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The ratio plasma retinol-binding protein (RBP) : transthyretin (TTR) has been proposed as a means to improve the assessment of vitamin A status of individuals with concurrent infection or inflammation. We have measured RBP and TTR in stored sera from South African children who had accidentally ingested kerosene. Samples were collected from these children in hospital when suffering acute inflammation and respiratory distress, and from them and neighbourhood control children 3 months later. Vitamin A status was defined by modified relative dose response (MRDR) tests of liver retinol stores at 3 months and by serum retinol concentration both when children were ill and when they were well. Illness was defined as either being in hospital or, at follow-up, as having a raised plasma α_1-acid glycoprotein (AGP) level. The RBP : TTR value was significantly decreased by both illness and low liver retinol stores. When the effects on RBP : TTR of illness and vitamin A stores were considered together for the 3-month follow-up samples, only vitamin A status significantly decreased the value. We calculated sensitivity and specificity of the RBP : TTR ratio against established measures of vitamin A status using a cut-off value of 0·3 for RBP : TTR and standard cut-off values for MRDR (0·06) and plasma retinol (0·7 μmol/l). Compared with MRDR, RBP : TTR had sensitivities of 76 % and 43 % and specificities of 22 % and 81 % to detect vitamin A deficiency in hospitalized and well children respectively. Compared with plasma retinol, sensitivities were 88 % and 44 % and specificities were 55 % and 64 % in hospitalized and well children respectively. Only for the case of clinically well children with biochemical evidence of subclinical inflammation did sensitivity (62 % and 100 % against MRDR and plasma retinol respectively) and specificity (100 % and 60 % against MRDR and retinol) approach useful levels for an assessment tool. Overall, although a trend supporting the theory behind the use of the RBP : TTR for assessment of vitamin A status in infection was observed in the current study, the ratio did not provide adequate sensitivity and specificity to be a useful assessment tool.

Vitamin A: Retinol-binding protein: Transthyretin

The public health importance of adequate vitamin A status, especially for women and children, is now well recognized. In order to plan and monitor programmes to improve vitamin A status, it is essential to have reliable means for assessing status. Considerable research efforts have been applied towards this aim and the WHO has provided guidelines for assessing vitamin A status for various purposes and at different resource levels (World Health Organization, 1996). Several problems remain, one of which is assessing vitamin A status in individuals with concurrent systemic infection or inflammation. The acute-phase response to infection or trauma results in a decrease in plasma retinol concentration, a commonly used measure of vitamin A status, to levels which could be considered deficient; however, these generally return to normal on resolution of the acute-phase response (Ramsden *et al.* 1978; Coutsoudis *et al.* 1991; Willumsen *et al.* 1997) and thus do not necessarily indicate vitamin A deficiency or a need for vitamin A interventions. Infection may confound interpretation of plasma retinol data not only in clinical situations but also among apparently healthy children in community-based studies (Filteau *et al.* 1993).

There has been a recent controversy concerning the use of the plasma ratio retinol-binding protein (RBP) : transthyretin

Abbreviations: AGP, α_1-acid glycoprotein; CRP, C-reactive protein; MRDR, modified relative dose response; RBP, retinol-binding protein; TTR, transthyretin.

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(TTR; prealbumin) for assessing vitamin A status of infected individuals (Donnen *et al*. 1998; Rosales & Ross, 1998). This method is based on extensive evidence that levels of plasma retinol and RBP are highly correlated (Ramsden *et al*. 1978; Gamble *et al*. 1997) and both decrease in parallel during vitamin A deficiency whereas TTR is unaffected by vitamin A status (Navab *et al*. 1977). Infection and trauma result in decreases, not only of retinol, but also of RBP and TTR which are both negative acute-phase reactants (Milland *et al*. 1990). Therefore, in theory, the ratio RBP : TTR should decrease during vitamin A deficiency but not during an acute-phase response (except with concurrent vitamin A deficiency), thus permitting distinction between these two conditions. The ratio RBP : TTR has been shown to be useful for assessing vitamin A status in rats subjected to acute inflammation and in children with measles (Rosales & Ross, 1998). However, recently the ratio was found not to reflect vitamin A status of children recovering from severe malnutrition (Donnen *et al*. 1998). Further studies are needed to determine for which subjects this marker can be useful. Therefore, we have conducted additional analyses on samples previously collected from South African children both when they were acutely ill following accidental kerosene ingestion and when they were well (Willumsen *et al*. 1997).

Kerosene ingestion is a common cause of accidental childhood poisoning in South Africa and results in a serious, but usually sterile, systemic inflammation with fever and changes in plasma proteins and retinol, and in urinary RBP and neopterin which are characteristic of acute-phase responses (Willumsen *et al*. 1997; Filteau *et al*. 1998; Simmank *et al*. 1998). We considered it a useful model of inflammatory changes in vitamin A metabolism among children from a population known to have marginal vitamin A status (South African Vitamin A Consultative Group, 1996). There was no evidence that kerosene ingestion induced long-term changes in vitamin A status or health (Willumsen *et al*. 1997). Vitamin A supplements were not given, since at the time the study was conducted there were concerns that vitamin A supplements might aggravate respiratory symptoms such as are associated with kerosene ingestion (Stansfield *et al*. 1993).

Materials and methods

Design and subjects

The study design and subject characteristics have been reported in detail (Willumsen *et al*. 1997; Simmank *et al*. 1998). The study was approved by the ethical review committees of the University of Witwatersrand, Johannesburg, South Africa, and the Institute of Child Health, London, UK. Briefly, children resident in Soweto and aged 1–5 years who were admitted to Baragwanath Hospital following accidental ingestion of kerosene were eligible. Blood samples were collected from children on admission (6 (SD 6·7) h after kerosene ingestion) and the following morning. After discharge, case children and a neighbourhood control, recruited at the first follow-up visit, were examined for morbidity symptoms every 2 weeks for 3 months. At 3 months children's liver vitamin A stores were assessed by the modified relative dose response (MRDR) test (Tanumihardjo *et al*. 1996). Previous work reported results for forty-seven case and forty-five control children; since insufficient serum remained for some samples, the present work reports results for thirty-eight children during hospitalization and for thirty-two case and thirty-four control children at the 3-month follow-up. Table 1 provides descriptive details for this subset of the originally recruited group.

In order to compare RBP : TTR data between sick and well children, illness was defined in two ways: (1) acute illness in hospital, (2) raised α_1-acid glycoprotein (AGP; $>0{\cdot}75$ g/l; Filteau *et al*. 1994) levels among either case or control children at the 3-month follow-up. AGP data, rather than C-reactive protein (CRP) data were used for these

Table 1. Descriptive details of children hospitalized after kerosene ingestion (cases) and case and control children at the 3-month follow-up†

(Mean values and standard deviations, or geometric mean values and 95 % confidence intervals for thirty-eight case children in hospital and for thirty-two case and thirty-four control children at follow-up)

	Cases			Controls		
	Mean	SD	95 % CI	Mean	SD	95 % CI
Sex (*n* females/*n* males)	8/30			15/19		
Age (months)	23·5	11·7		25·5	10·6	
Weight for age (Z score)	−0·53	1·58		−0·90	0·91	
Height for age (Z score)	−0·56	1·51		−1·20	1·23	
Hospital serum retinol (μmol/l)	0·40		0·32, 0·50			
Hospital serum AGP (g/l)	0·70		0·58, 0·85			
Hospital serum CRP (mg/l)	5·6		4·3, 7·3			
Follow-up serum retinol (μmol/l)	1·03		0·88, 1·20	1·19		1·05, 1·35
Follow-up serum AGP (g/l)	0·74*		0·62, 0·88	0·55		0·48, 0·64
Follow-up serum CRP (mg/l)	0·64		0·37, 1·09	0·75		0·47, 1·20
MRDR	0·106	0·052		0·093	0·052	

AGP, α_1-acid glycoprotein; CRP, C-reactive protein; MRDR, modified relative dose response.
Mean value was significantly different from that for the control group: * $P=0{\cdot}013$.
† Except for hospital admission biochemical data, all values refer to those children who returned for follow-up; the six cases who did not have follow-up data were all boys.

analyses since too few children had raised CRP levels at follow-up. Since kerosene ingestion did not affect vitamin A stores (Willumsen *et al*. 1997), we considered MRDR values at 3 months to represent vitamin A stores at earlier time points as well and combined case and control children for analyses at 3 months.

Laboratory analyses

Serum samples were stored at −70° until analysis. Most laboratory analyses were conducted within several months of sample collection (December 1994 to January 1996) with the exception of the RBP and TTR measurements which were conducted in December 1997. The laboratory technicians were blind to whether follow-up samples were from case or control children but the numbering system was such that samples from children in hospital could be distinguished from those obtained at follow-up. Biochemical analyses for didehydroretinol and retinol by HPLC, and for the acute-phase proteins AGP and CRP by ELISA, have been described previously (Willumsen *et al*. 1997). For the HPLC analyses, interassay CV for a normal adult plasma sample were 5·5 % for didehydroretinol and 6·4 % for retinol, and for ELISA assays were 19 % for CRP and 36 % for AGP. The CV for AGP was higher than we normally obtain for this assay for unknown reasons and will have decreased the reliability of AGP for use as an indicator for illness in children at follow-up. However, it was not possible to repeat AGP analyses since samples had by this point been frozen and thawed several times and many were depleted.

TTR was measured by turbidimetry using a COBAS Fara autoanalyser (Roche, Welwyn Garden City, Herts., UK) and a method designed for this machine. The assay standard was from Behring (Hoechst Roussel, Milton Keynes, Bucks., UK) and the interassay CV for a normal adult plasma sample was 14 %.

In view of previous debate about our RBP assessment (Filteau & Willumsen, 1998; Rosales, 1998), samples were re-analysed for RBP. For this purpose, a pre-measured 20 μl portion of each sample was shipped on solid CO_2 to New York where it was diluted and RBP was measured by a radioimmunoassay employing rabbit anti-human RBP generated by Blaner (1990). The method and the excellent correlation between RBP results and plasma retinol levels have been reported in detail elsewhere (Blaner, 1990; Gamble *et al*. 1997). The within-assay CV for the method was 6·8 % and the between-assay CV was 8·4 %. Previous work with this assay (Friedman *et al*. 1986) gave a mean molar retinol : RBP value of 1·006 for 302 healthy adult Americans. The new values tended to be higher than the old ones previously reported (Willumsen *et al*. 1997) and the regression equation relating the old and new values for RBP was: $\text{new} = 0{\cdot}47 \times \text{old} + 8{\cdot}43$ (n 148, $P < 0{\cdot}0001$ for both slope and constant).

Statistical analyses

Standard statistical analyses (i.e. ANOVA, correlation, χ^2) were conducted using SPSS 6.13 (SPSS Inc., Chicago, IL, USA). Retinol and protein data were log-transformed in order to normalize distributions so means presented are geometric. Child age and sex were not significantly correlated with outcome measures so they were not included in further analyses.

RBP : TTR values were compared between children subdivided according to their MRDR results (normal vitamin A status if MRDR $< 0{\cdot}06$, deficient if MRDR $\geq 0{\cdot}06$; Tanumihardjo *et al*. 1996) and to whether they were sick or well, as defined earlier. MRDR and sickness were considered first as single factors and then together in a factorial design. For comparisons between sick and well children, parametric statistics were used only for the 3-month data divided by AGP level. Parametric tests, for example, paired t tests, could not be used to compare across time points since controls had no hospital data. For time point comparisons, Kruskal–Wallis tests were used and, where significant, were followed by a series of comparisons between pairs of time points by the Mann–Whitney U test.

We calculated the sensitivity and specificity of the RBP : TTR ratio as an assessment tool for vitamin A status compared with the commonly used methods of MRDR and plasma retinol. We used the usual cut-off values for MRDR (0·06) and plasma retinol (0·7 μmol/l) for children and, since an appropriate cut-off value was not obvious from our data (Fig. 1) we followed Rosales & Ross (1998) and used RBP : TTR $< 0{\cdot}3$ to indicate deficiency.

Results

Concentrations of RBP and TTR and their ratio are shown in Table 2 for children at all time points. TTR concentrations were not depressed even on the morning after admission when the acute-phase changes were maximal (Willumsen *et al*. 1997) as compared with values when children were generally well (3-month follow-up). RBP concentrations, on the other hand, were significantly lower than values for healthy subjects at admission and lower still the next morning. Thus, RBP : TTR values largely reflected the RBP concentrations at the three time points. When children at the 3-month follow-up were subdivided into sick and well according to whether they had raised or normal serum AGP levels, there were no significant group differences for RBP, TTR or RBP : TTR; the pattern reflected the little variation in TTR and the non-significant decrease in RBP when AGP was increased.

The RBP : TTR values were also compared with measures of vitamin A status (Fig. 1). There was considerable overlap of RBP : TTR values among children with high or low values of MRDR or serum retinol. RBP : TTR was correlated with serum retinol at each of the three time points (results not shown). However, since inflammation is known to decrease plasma retinol concentration and since our data showed some influence of illness on RBP : TTR, we attempted to control for inflammation by calculating partial correlation coefficients, controlling for concurrent AGP and CRP concentrations. The partial correlation coefficients between log values of retinol and RBP : TTR were significant at admission (r 0·46, $P = 0{\cdot}005$, n 33) and the next morning (r 0·51, $P = 0{\cdot}005$, n 27) but not at 3 months (r 0·25, $P = 0{\cdot}055$, n 58). If the case and control children were considered separately at 3 months, partial correlation coefficients were

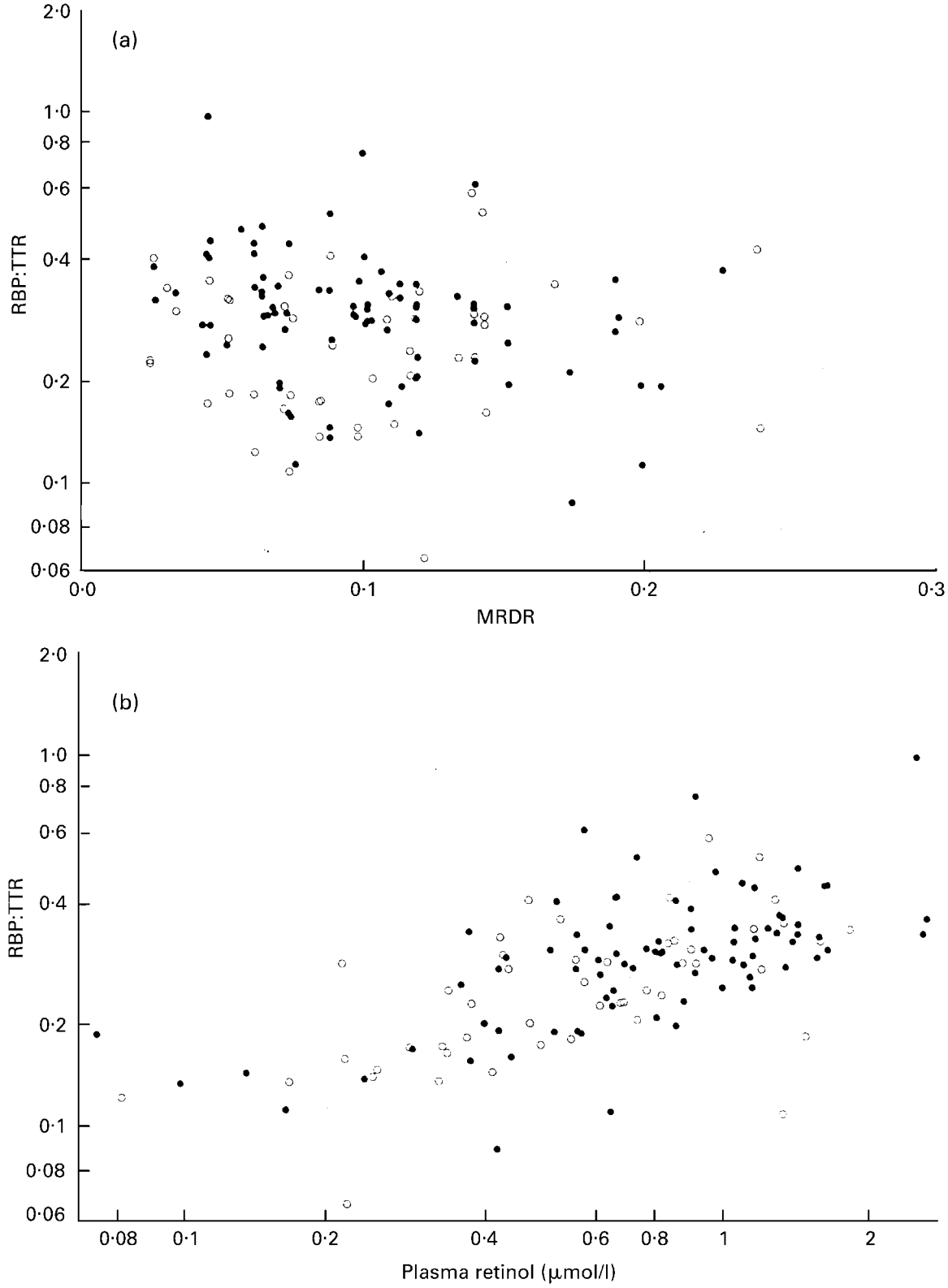

Fig. 1. Relationship between measures of vitamin A status ((a), modified relative dose response, MRDR; (b) plasma retinol concentration) and values for the ratio retinol-binding protein : transthyretin (RBP : TTR) in children with raised (○; $>0{\cdot}75$ g/l) or normal (●; $\leq 0{\cdot}75$ g/l) α_1-acid glycoprotein levels at all time points together (n 133). MRDR data at 3-month follow-up were used for comparison with RBP : TTR results in hospital also. Logarithmic axis scales have been used for data that were log-transformed in analyses.

Table 2. Serum retinol-binding protein (RBP) and transthyretin (TTR) concentrations and RBP : TTR values in children hospitalized for kerosene ingestion (cases) and in cases and controls (combined) at 3 months follow-up‡
(Geometric mean values and 95 % confidence intervals)

		RBP (mg/l)		TTR (mg/l)		RBP : TTR	
	n	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI
Admission	38	13·5**	11·5, 15·9	143	131, 157	0·25***	0·21, 0·28
Next morning	32	9·2***†††	7·6, 11·2	122†	111, 135	0·20***†††	0·17, 0·23
3 months	66	16·7	15·6, 18·0	134	125, 144	0·33	0·30, 0·35
AGP ≤ 0·75 g/l	45	17·5	16·2, 18·8	135	124, 148	0·34	0·31, 0·37
AGP > 0·75 g/l	18	15·5	13·2, 18·2	135	122, 150	0·30	0·26, 0·34

AGP, α_1-acid glycoprotein.
Mean values were significantly different from those for 3-month follow-up: ** $P < 0·01$, *** $P < 0·001$ (Mann–Whitney U test).
Mean values were significantly different from those on admission: † $P < 0·05$, ††† $P < 0·001$ (Mann–Whitney U test).
‡ Geometric means (95 % CI) are shown for information but parametric statistics were used only for comparisons of 3-month data divided by AGP level (no significant differences). Kruskal–Wallis tests were used to compare data from different time points and, where significant ($P < 0·0001$), were followed by a series of comparisons between pairs of time points by the Mann–Whitney U test.

lower (results not shown), probably as a result of reduced sample size. Since case and control children really did not differ at follow-up, we considered them together to increase statistical power.

MRDR values were used to distinguish children with normal or deficient liver retinol stores and RBP : TTR values were compared between groups (Table 3). At all three time points the value was lower in the group with deficient liver stores but, probably because of small sample sizes for the time points in hospital, the difference was significant only at the 3-month follow-up. When the RBP : TTR data were analysed in a factorial design, including effects of vitamin A stores (MRDR) and raised AGP simultaneously, only the effect of vitamin A stores was significant. The value of the ratio was decreased when MRDR was high (deficiency) but was unaffected by inflammation (Table 4).

We calculated the sensitivity and specificity of RBP : TTR as an assessment tool for vitamin A status compared with the commonly used methods of MRDR and plasma retinol (Table 5). We used the usual cut-off values for MRDR (0·06) and plasma retinol (0·7 μmol/l) for children and, since an appropriate cut-off value was not obvious from our data (Fig. 1) we followed Rosales & Ross (1998) and used RBP : TTR < 0·3 to indicate deficiency. Calculations were done for data from the two time points in hospital to represent a clinical population. In addition, since we are particularly interested in vitamin A status in field conditions, rather than in the clinical situation, we calculated sensitivities and specificities for children at the 3-month follow-up, both with the group as a whole and subdivided according to AGP level where available. Sensitivities and specificities were similar in the subgroup with normal (≤ 0·75 g/l) AGP to those in the group as a whole and were higher in the subgroup with subclinical illness as indicated by raised AGP. We repeated calculations based on a new RBP : TTR cut-off value of 0·22 derived from results for Brazilian children (Gamble *et al*. 1999) and found that sensitivities and specificities against retinol or MRDR results did not improve and, in fact, generally became lower (results not shown).

Discussion

The present study and those conducted by Rosales & Ross (1998) and Donnen *et al*. (1998) all analysed RBP : TTR values in relation to vitamin A status using samples that had been collected previously for other purposes associated with vitamin A metabolism and function. The studies had several important similarities: each analysed plasma from similar numbers of young African children both during acute illness and at a later time. The studies differed in several important respects as well. The other workers collected follow-up samples at fairly short intervals after serious illnesses compared with the time course of acute-phase depression in plasma retinol (Ramsden *et al*. 1978), that is, 2 weeks after presentation with measles (Rosales & Ross, 1998) or 1 week after hospitalization for severe malnutrition (Donnen *et al*. 1998). In contrast, we studied children 3 months after

Table 3. Retinol-binding protein : transthyretin values in children with normal or deficient liver retinol stores on admission to hospital after kerosene ingestion, the next morning and at 3-month follow-up
(Geometric mean values and 95 % confidence intervals)

	Normal (MRDR < 0·06)			Deficient (MRDR ≥ 0·06)		
	Mean	95 % CI	*n*	Mean	95 % CI	*n*
Admission	0·26	0·19, 0·35	5	0·24	0·21, 0·28	32
Next morning	0·24	0·19, 0·31	4	0·19	0·16, 0·23	27
3 months	0·38	0·32, 0·45	16	0·31*	0·29, 0·34	49

MRDR, modified relative dose response.
Mean value was significantly different from that for the normal MRDR group: * $P = 0·022$ (*t* test).

Table 4. Serum retinol-binding protein : transthyretin values in children with normal or deficient vitamin A stores and normal or high values for α_1-acid glycoprotein (AGP) (a marker for inflammation) at 3-month follow-up after hospitalization*

(Geometric means and 95 % confidence intervals)

	Normal (MRDR < 0·06)			Deficient (MRDR ≥ 0·06)		
	Mean	95 % CI	*n*	Mean	95 % CI	*n*
AGP ≤ 0·75 g/l	0·38	0·29, 0·50	10	0·33	0·30, 0·35	35
AGP > 0·75 g/l	0·36	0·32, 0·40	4	0·28	0·24, 0·34	13

MRDR, modified relative dose response.

* Geometric means (95 % CI) are presented for illustrative purposes. In the ANOVA the effect of MRDR category was significant ($P = 0·048$) but AGP category ($P = 0·31$) and the interaction ($P = 0·65$) were not.

hospitalization when they had completely recovered from an acute accidental injury (Simmank *et al*. 1998), although eighteen of sixty-three children for whom results were available had raised AGP concentrations indicating the presence of mild or subclinical illnesses typical of young children in low-income countries (Filteau *et al*. 1993). Both the other studies were randomized, placebo-controlled trials of vitamin A supplementation and treatment was used as an important indicator of vitamin A status, whereas we did not give the children vitamin A. We defined vitamin A status primarily by MRDR results whereas the other two studies relied on a combination of retinol and CRP, two plasma components which are generally significantly correlated during illness. In summary, all three studies had different strengths and weaknesses in their design as regards investigating the usefulness of RBP : TTR values as an assessment tool.

We consider that a major strength of our study is the way the results were analysed. We elected to analyse our results according to standard criteria for any proposed new nutritional assessment tool. For such a tool to be useful it should be sensitive to deficiency of the relevant nutrient (and in some cases to excess also); it should be specific to status of the nutrient of interest and not be commonly affected by other factors; and it should be feasible to conduct in the situations where it is needed. The use of RBP : TTR has been suggested as a way around the serious problem of the lack of specificity of serum retinol level under conditions of infection which are common among populations where vitamin A deficiency is a problem. Our findings showed that illness lowers the RBP : TTR value but not always significantly. Inflammation in rats and children decreased RBP : TTR in other studies also (Donnen *et al*. 1998; Rosales & Ross, 1998). This decreased value occurs both because of the relatively modest, compared with RBP, changes in TTR during inflammation and because in both children (present study) and rats (Rosales *et al*. 1996) the acute-phase changes in RBP precede those in TTR. The decrease in the value of the ratio during inflammation may have contributed to the very poor specificity of the ratio for assessing status of children in hospital following kerosene ingestion. The specificity improved when clinically well

Table 5. Sensitivity and specificity of the retinol-binding protein : transthyretin (RBP : TTR) value compared with the modified relative dose response (MRDR) or plasma retinol concentration for detecting vitamin A deficiency in children hospitalized after kerosene ingestion (cases) and in cases and controls at 3-month follow-up*

	MRDR < 0·06	MRDR ≥ 0·06	Retinol > 0·7 μmol/l	Retinol ≤ 0·7 μmol/l
In hospital				
RBP : TTR > 0·3	2	14	11	6
RBP : TTR ≤ 0·3	7	45	9	42
	sensitivity 76 %;	specificity 22 %	sensitivity 88 %;	specificity 55 %
Follow-up, all				
RBP : TTR > 0·3	13	28	36	5
RBP : TTR ≤ 0·3	3	21	20	4
	sensitivity 43 %;	specificity 81 %	sensitivity 44 %;	specificity 64 %
Follow-up, normal AGP				
RBP : TTR > 0·3	7	23	26	4
RBP : TTR ≤ 0·3	3	12	13	2
	sensitivity 34 %;	specificity 70 %	sensitivity 33 %;	specificity 67 %
Follow-up, high AGP				
RBP : TTR > 0·3	4	5	9	0
RBP : TTR ≤ 0·3	0	8	6	2
	sensitivity 62 %;	specificity 100 %	sensitivity 100 %;	specificity 60 %

AGP, α_1-acid glycoprotein.

* Numbers represent the number of children in each vitamin A status subgroup, that is normal or raised MRDR, or normal or low plasma retinol. Sensitivity and specificity refer to the 2 × 2 table above each pair of percentages. Samples in hospital are from both time points, admission and the next morning, together. AGP data were missing for a few samples so the numbers are reduced when follow-up samples were subdivided by AGP below or above 0·75 g/l.

children only were assessed, but at great loss of sensitivity. Only for the case of clinically well children with biochemical evidence of subclinical inflammation did sensitivity and specificity approach useful levels for an assessment tool. There was considerable overlap of RBP : TTR values among children with varying serum retinol or MRDR results which meant that selection of different cut-off values for RBP : TTR, retinol or MRDR would not be likely to improve the usefulness of RBP : TTR for assessing vitamin A status. We accept that neither MRDR nor plasma retinol makes a gold standard against which new assessment tools can be conclusively evaluated but there is no gold standard for vitamin A status other than liver biopsy.

The previous workers did not explicitly address the issue of sensitivity and specificity, although Donnen *et al*. (1998) implied that they were poor based on the lack of difference in RBP : TTR values in vitamin A-supplemented compared with placebo-treated children. Rosales & Ross (1998) focused on subgroups of individuals in hospital for whom information on plasma retinol and CRP, as well as RBP and TTR was available. They found that RBP : TTR was decreased in association with low retinol and high CRP levels only in children who had not received vitamin A. It is possible that RBP : TTR will prove a useful assessment tool where acute-phase protein concentrations are known, as was seen in our subgroup of clinically well children with subclinical inflammation as indicated by raised AGP. However, we did not find it useful in the hospitalized group as a whole and it should be noted that where vitamin A deficiency is common, the international agencies recommend giving vitamin A to children at any contact with health services, without necessarily measuring the child's status first (World Health Organization/United Nations Children's Fund/International Vitamin A Consultative Group, 1988).

During hospitalization but not at follow-up, the partial correlations between retinol and RBP : TTR, controlling for inflammation as measured by AGP and CRP, were significant. We believe this illustrates the lack of ideal acute-phase protein to aid interpretation of low retinol concentrations during the acute-phase response (Filteau, 1999). Although during acute inflammation CRP changes greatly and rapidly together with retinol, it is not raised as often as is AGP in reasonably healthy children in low-income countries (Willumsen *et al*. 1997). AGP, on the other hand, does not rise as rapidly or as dramatically as does CRP such that normal inter-individual variation can obscure acute-phase changes. In our study, AGP was not significantly increased during the acute illness (Willumsen *et al*. 1997). Thus, acute-phase proteins can aid interpretation of retinol data during illness but cannot completely solve the interpretative problems.

Initially we were interested in the idea of using RBP : TTR for vitamin A assessment in part because we thought it might be easier and cheaper in field studies in resource-poor situations. Proteins can often be measured using techniques which require equipment less expensive and less difficult to maintain than the HPLC needed for retinol. The techniques may also require smaller blood samples, e.g. fingerprick, than does retinol, and many proteins are fairly stable and can be collected, stored and analysed as dried blood spots on filter paper (Cordon *et al*. 1991; Ahluwalia *et al*. 1998; R Beesley, A Al Serouri and SM Filteau, unpublished results). However, the drawbacks of poor sensitivity and specificity of RBP : TTR outweigh the potential benefits for cost and feasibility. We accept that the high assay CV for AGP, compared with CV for other analytes measured, is a limitation of the present study and may have led to some misinterpretation of results. Nevertheless, although a promising idea for which the theory has been at least partly confirmed in animal models and children, our data do not support the use of RBP : TTR to distinguish the low serum retinol level of infection from that of vitamin A deficiency.

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