Interrelationship between vitamin A, iodine and iron status in schoolchildren in Shoa Region, Central Ethiopia

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A total of 14740 schoolchildren in seven provinces of Shoa Administrative Region in Central Ethiopia were surveyed for the prevalence of goitre, xerophthalmia and anaemia. Haemoglobin and packed cell volume were assessed in 966 children in one province while an in-depth study was conducted on 344 children in the same province and two others. Goitre, xerophthalmia (Bitot's spots) and clinical anaemia were observed in 34·2, 0·91 and 18·6% respectively of the children. Most biochemical variables were within the normal range while those of haemoglobin (Hb), mean corpuscular Hb concentration (MCHC) and urinary I excretion were lower, and mean corpuscular volume, mean corpuscular Hb (MCH), and immunoglobulins G and M were higher. Hb was strongly correlated with retinol, ferritin, MCHC, MCH, packed cell volume and erythrocyte count while retinol formed a triad with transthyretin (TTR) and retinol-binding protein (RBP) which were all correlated with one another. Total and free thyroxin and total and free triiodothyronine were positively correlated as were the concentrations of the total and free hormones. Thyrotropin (TSH) was negatively correlated with total and free thyroxin and positively correlated with free triiodothyronine. Thyroxin and triiodothyronine in both free and combined forms were all correlated with thyroxin-binding globulin which in turn was negatively correlated with the triad retinol, RBP and TTR. The triad was also negatively correlated with C-reactive protein. Urinary I excretion was positively associated with total thyroxin and negatively associated with TSH. The anaemia found was not nutritional in origin but due to the effect of infestation with intestinal parasites and malaria.

Vitamin A: Iodine: Iron: Childhood

The three most important nutritional problems in developing countries are those due to deficiency of vitamin A, I and Fe. Every year as a result of vitamin A deficiency 250000 children go blind and another 250000 have their eye-sight impaired, and at least 100000 of those children die within a few weeks (Grant, 1991). Possibly even more children are affected because there is strong evidence that vitamin A deficiency increases morbidity from respiratory and gastrointestinal diseases and overall mortality (Anonymous, 1990). An estimated 800 million people in the world are at risk from I deficiency, with 190 million suffering from goitre, 3 million from overt cretinism and millions more from some degree of intellectual deficit (Grant, 1991). Anaemia is prevalent in many parts of the world, particularly in developing countries, with infants, young children and pregnant women, the latter two groups being most vulnerable because of their increased physiological requirements not only for Fe but also for folic acid (DeMaeyer & Adiels-Tegman, 1985).

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In areas where intestinal parasitic infestations and malaria prevail the problem is exacerbated resulting in decreased work performance, higher morbidity and mortality during pregnancy, increased risk of infection, subnormal mental performance and behavioural changes (International Nutritional Anemia Consultative Group, 1977).

As in most developing countries, Ethiopia harbours all three nutritional problems (Interdepartmental Committee of Nutrition for National Defense, 1959; Hofvander, 1968; Wolde-Gebriel *et al.* 1991, 1993*a*). In our earlier papers care was taken to obtain representative samples of the population in the country. However, in the study reported in the present paper the purpose is to examine the interrelationships between the three nutritional conditions. Understanding such interrelationships enables the generation of hypotheses which can be tested in further studies such as intervention studies in humans and by using appropriate animal models. The areas in which the study was carried out were those chosen for a series of in-depth studies.

MATERIALS AND METHODS

Study sites and subjects

Schools, chosen at random, located along the main roads between Addis Ababa and the district and provincial towns of Shoa Administrative Region were surveyed. The baseline survey comprised two phases in which a total of 14740 schoolchildren (8779 males and 5961 females) from forty-four schools in seven provinces were clinically assessed in the first phase, while a more comprehensive study involving 344 boys with further measurement of biochemical and anthropometric variables was carried out in the second phase. In one of the provinces (Chebona-Gurage) haemoglobin (Hb) and packed cell volume measurements were carried out on the 966 schoolboys of the total 3725 boys seen during the first phase of the survey who were found to have pale tongues and buccal membranes, indicating anaemia. Informed consent in writing was obtained from Party, Health and Education Offices at Regional and Provincial level as well as from the school directors, while verbal consent was obtained from parents.

Physical examination

The physical examination comprised the following: examination of the eyes for signs of xerophthalmia which were classified as recommended by the World Health Organization (1982); palpation of the thyroid gland for the presence of goitre which was graded according to the method recommended by Delange *et al.* (1986); and assessment of anaemia on the basis of paleness of buccal membranes and tongue.

Biochemical tests and analytical methods

All analyses were carried out in duplicate. After transfer to the field laboratory, Hb was determined according to the cyanmethaemoglobin method of van Kampen & Zijlstra (1961) and packed cell volume (PCV) was determined using the microhaematocrit centrifuge. In the second phase, erythrocytes (RBC) were counted by two senior technicians with many years of experience from Atat Hospital. Mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and mean corpuscular Hb concentration (MCHC) were estimated from haemoglobin, PCV, and RBC values.

Also in the second phase, an additional 15 ml blood was collected using the Vacutainer system (Venoject; Terumo, Belgium) without anticoagulant. Serum was separated within a few hours after collection and stored in deep-freezes of the local health institutions. They were then transported in cold boxes with cooling elements to the laboratories of the Ethiopian Nutrition Institute (ENI) where they arrived frozen and were stored at $-20°$. All

analyses were done in ENI except retinol, retinol-binding protein (RBP), transthyretin (TTR), immunoglobulins and C-reactive protein (CRP) which were done in The Netherlands. The specimens for the analyses in The Netherlands were sent by air in cold boxes with dry ice and then stored at $-20°$ until analysed. All analyses on serum which were done in Ethiopia were carried out within 6 months of collection, while those done in The Netherlands were done within 2 years. Serum retinol was estimated by HPLC as described earlier (Wolde-Gebriel *et al.* 1993*b*) while RBP, TTR, transferrin, immunoglobulin A, G and M (IgA, IgG and IgM) and CRP were analysed by immunonephelometry using reagents and equipment supplied by Behringwerke AG, Germany, as described by Fink *et al.* (1989). Albumin was measured using the bromocresol green method of Doumas *et al.* (1971). Free and total triiodothyronine (FT3 and TT3), free and total thyroxin (FT4 and TT4), thyrotropin (TSH) and ferritin were measured by radioimmunoassay using kits supplied by Amersham International UK, and thyroxin (T4)-binding globulin (TBG) using the enzyme-linked immunosorbent assay (ELISA) method with kits supplied by Boehringer Mannheim, Germany. The TT4/TBG quotient, defined as ten times TT4 divided by TBG both expressed in nmol/l, was used to classify subjects as defined by Boehringer Mannheim as follows: hypothyroid < 3·4, borderline 3·4–3·9, euthyroid 3·9–6·5, borderline 6·5–7·7, hyperthyroid > 7·7. Total Fe-binding capacity (TIBC) and serum Fe were measured as described by Schade *et al.* (1954).

Casual urine samples were collected and urinary I excretion was determined using the Sandell–Kolthoff reaction (Sandell & Kolthoff, 1937). Specimens of food and drinking water were collected from some of these areas for the analysis of their I content as described previously while in some specimens of drinking water microbiological tests were also carried out.

Statistics

The significance of differences was compared using Chi-square and Mann–Whitney for pair testing and Kruskal–Wallis for multiple-variable testing as the data were not normally distributed. For examining correlations, data were checked for normal distribution and those with skewness values outside the range of -1 to $+1$ were regarded as skewed and the data were transformed to natural logarithms before correlation analysis.

RESULTS

Clinical

Goitre. The mean total prevalence for males and females of gross goitre in all schools was 32·7% (Table 1). In the seven provinces the prevalence ranged from 16·4% in Selale to 49·1% in Merhabete. The prevalence in females (42·2%) was significantly higher ($P < 0·001$) than that in males (26·2%) and the higher prevalence in females was found when all schools were taken individually or aggregated by province.

Xerophthalmia. As shown in Table 1, the average of mean prevalence values for males and females of Bitot's spots was 0·91%. In the group as a whole, 1·4% of the children had corneal scars which were attributed to measles in 41·1%, 'mitch' (an Amharic word used to describe a vague and ill-defined febrile illness) in 34·7%, trauma in 13·9%, non-specific eye illness in 6·9%, chickenpox in 1·5% and was unknown in 1·9% of the children. More males (71·3%) had corneal scars than females (28·7%).

Anaemia. Clinical anaemia was observed in 18·6% of the children examined (Table 1) and the prevalence was higher in boys (20·9%) than in girls (15·1%). This difference was observed in the age-groups 6–10 years and 11–14 years but not in those aged 15–18 years (Table 2).

Table 1. *Prevalence of goitre, xerophthalmia and anaemia among schoolchildren in seven provinces of Shoa Region, Ethiopia.*

Sex	No. of schoolchildren examined	Prevalence (%)					
		Goitre		Xerophthalmia (Bitot's spots)		Anaemia	
		n	%	*n*	%	*n*	%
M	8779	2297	26·2	98	1·1***	1838	20·9***
F	5961	2518	42·2***	36	0·6	902	15·1
M+F*	14740	4815	32·7	134	0·9	2740	18·6

* The prevalence for the sexes combined, expressed as a percentage, was not corrected for the unequal number of males and females in the sample studied.

Prevalence values were significantly higher than those for the opposite sex: *** $P < 0{\cdot}001$.

Table 2. *Prevalence of goitre, xerophthalmia and anaemia by sex in schoolchildren in seven provinces of Shoa Region, Ethiopia*

Sex		Goitre grade					Xerophthalmia			Anaemia		
		0	IA	IB	II	III	Absent	Bitot's spots	Corneal scar	Absent	Present	Total
M	*n*	6482	1588	631	77	1	8537	98	144	6941	1838	8779
	%	73·8	18·1	7·2	0·9	0·0	97·2	1·1	1·6	79·1	20·9	100·0
F	*n*	3443	1330	991	190	7	5867	36	58	5059	902	5961
	%	57·8	22·3	16·6	3·2	0·1	98·4	0·6	1·0	84·9	15·1	100·0

Clinical chemistry

In children examined in Chebona-Gurage in the first phase of the study the median Hb concentration (mmol/l) and PCV values (with 25th and 75th percentiles respectively) were 7·63 (7·14 and 8·19; *n* 966) and 0·40 (0·38 and 0·41; *n* 966) respectively. The median concentrations of the biochemical variables measured in the selected population of the second phase of the baseline survey are shown in Table 3. There were five (1·5%) children with concentrations of retinol and RBP below 0·35 μmol/l, indicative of vitamin A deficiency (World Health Organization, 1982). The percentage of children with Hb levels below 7·45 mmol/l, indicative of anaemia, was 65·4 while PCV was less than 0·35 in 21·2, ferritin was less than 10 μg/l in 1·2, serum Fe was less than 10·74 μmol/l in 28·8 and RBC count was less than 4·12 million/mm^3 in 44·8 of the children. Using the multiple criteria of Hb, PCV and serum Fe values as suggested by International Nutritional Anemia Consultative Group (1985), 48·4% of the children were below the cut-off points for the three variables. The MCV and MCH median values were within the upper range while 24·4 and 11·3% of the children had values of MCV and MCH respectively which were above the normal cut-off points. IgA, IgG and IgM were higher than the upper limit of the normal range in 8·1, 87·7 and 88·3% of children respectively. Values for TT4/TBG quotient, TT3, TT4 and TSH were above normal limits in 6·0, 28·2, 3·6 and 5·4% of the children respectively, suggestive of hyperthyroidism (Boehringer Mannheim, Germany and Amersham International, UK). Hypothyroidism was observed in 8·6, 1·8 and 14·4% of the children based on the TT4/TBG quotient, TT4 and TSH levels respectively.

Table 3. *Median values for biochemical variables of schoolboys from seven provinces of Shoa Region, Ethiopia in the second phase of the study**

Biochemical variables	No. examined	Median	25th & 75th percentiles	Normal range	Source
Serum albumin (μmol/l)	344	558	522–601	507–797	Jelliffe & Jelliffe (1989)
Transthyretin (μmol/l)	344	3·72	3·22–4·19	1·82–7·27	Behringwerke AG, Marburg, Germany
Retinol-binding protein (μmol/l)	344	0·93	0·71–1·16		See values for retinol (μmol/l)
Transferrin (g/l)	334	2·56	2·28–2·88	2·95 (2·0–4·0)	Behringwerke AG, Marburg, Germany
Retinol (μmol/l)	344	1·00	0·77–1·22	< 0·35 deficient 0·35–0·69 low 0·70–1·05 adequate > 1·05 normal	ICNND (1963)
Total Fe-binding capacity (μmol/l)	265	51·1	45·7–55·6	< 70·44	INACG (1985)
Ferritin (μg/l)	264	44·5	27·0–64·2	> 10	
Serum Fe (μmol/l)	265	11·87	9·59–14·59	> 10·74	
Transferrin saturation (%)	265	23·4	18·4–28·9	> 16	
Haemoglobin (mmol/l)	344	7·14	6·67–7·85	> 7·45	
Packed cell volume	344	0·38	0·35–0·41	> ·35	
Erythrocyte count (million/mm^3)	162	3·52	3·18–3·75	4·12–5·57	Hellemam *et al.* (1973)
Mean corpuscular volume (fl)	162	103	96–113	83·5–103·1	
Mean corpuscular haemoglobulin (pg)	162	32·4	30·3–35·7	28·3–35·7	
Mean corpuscular haemoglobulin concentration (mmol/l)	162	30·3	28·9–33·6	19·9–22·9	
Free triiodothyronine (pmol/l)	174	7·55	6·47–8·32	3·30–8·20	Amersham International UK
Free thyroxin (pmol/l)	173	11·58	8·85–14·00	9·36–25·00	
Total triiodothyronine (nmol/l)	342	2·21	1·92–2·56	0·80–2·69	
Total thyroxin (nmol/l)	342	103	87–118	61·8–164·7	
Thyrotropin (μIU/ml)	343	2·95	1·99–4·10	1·00–5·50	
Thyroxin-binding globulin (nmol/l)	231	236	195–276	168–324	Boehringer Mannheim, Germany
Urinary I excretion (μg/g creatinine)	229	6·60	1·27–19·91	> 50	Hetzel (1987)
Immunoglobulin A (g/l)	334	2·05	1·62–2·65	0·74–3·25	Behringwerke AG, Marburg, Germany
Immunoglobulin G (g/l)	334	19·8	17·5–22·0	7·3–15·1	
Immunoglobulin M (g/l)	334	2·31	1·80–2·82	0·68–1·50	
C-reactive protein (mg/l)	334	0·00	0·00–1·01	< 0·005	

ICNND, Interdepartmental Committee on Nutrition for National Defense; INACG, International Nutritional Anemia Consultative Group.
* For details of procedures, see pp. 594–595.

The median concentrations of protein, vitamin A and Fe variables by study sites grouped by altitude and major staple foods consumed are compared in Table 4. Serum albumin, transferrin, TIBC, ferritin, PCV and CRP were significantly higher and RBP, retinol, serum Fe, transferrin saturation, Hb and IgM were significantly lower in the lowland cereal staple areas (Kobo and Robi) than in the lowland ensete (*Ensete ventricosum*)-consuming areas (Chebona-Gurage). TTR, RBP, retinol, Hb, PCV, IgA, IgM and CRP were significantly higher in the highland ensete-staple areas (Kembatana-Hadya) than in the lowland cereal-staple areas, while serum albumin, TTR, RBP, transferrin, Hb, PCV, IgA and CRP were also significantly higher in the highland than the lowland ensete-staple areas.

Stool examination on 344 children showed that 22·0% did not have parasites while 31·6% had hookworm, 13·1% ascaris, 7·8% amoeba cyst, 4·9% strongyloides, 0·9% schistosoma, 11·3% double parasites, and the rest (8·4%) other parasites. Malaria was reported in the lowland areas where the prevalence of anaemia was high.

The biochemical variables expressed in terms of goitre grade are shown in Table 5. Compared with those with no goitre or grade IA goitre, children with grade IB and II goitres were significantly heavier and taller (values not shown), had lower serum retinol, RBP, Fe, transferrin saturation, T4, TBG and IgM values, and higher serum ferritin, TIBC, blood Hb and PCV, and TSH values. For T4 the values for all grades of goitre were significantly different from one another. TSH concentrations were significantly ($P < 0·001$) inversely correlated with T4/TBG quotient. However, no significant correlation was observed between triiodothyronine (T3) and T4/TBG quotient (values not shown).

The correlations obtained between the various biochemical variables were used to develop the models shown in Figs. 1 and 2. In Fig. 1 Hb has been taken as the central variable. On the one hand, it was significantly correlated with putative determinants of Hb concentration, namely retinol and ferritin. The triad retinol, RBP and TTR were significantly correlated with one another, while TTR, a marker for short-term malnutrition, was correlated with albumin, a marker for long-term malnutrition. In addition, the triad was also negatively correlated with CRP, indicated in Fig. 1 by the relationship with retinol. Ferritin was correlated with transferrin saturation which in turn was correlated with serum Fe. On the other hand, Hb was significantly positively associated with variables dependent on Hb concentrations, namely PCV, MCHC, MCH and RBC count. Hb was negatively correlated with FT3, FT4, TT4 and TBG, while it was positively correlated with TSH (values not shown). PCV was also positively correlated with albumin (values not shown). Within the population as a whole, weight-for-age and height-for-age, which are measures of wasting and stunting respectively, were closely and positively associated with albumin, TTR, RBP, Hb, PCV, transferrin and TIBC. However, when the ensete-eating areas (Kembatana-Hadya and Chebona-Gurage) and the teff (*Eragrostis teff*)-eating areas (Kobo and Robi) were taken separately, these relationships for weight-for-age and height-for-age no longer held in the cereal-staple areas (except for RBP with weight-for-age) while there were stronger correlations in the ensete-staple areas (values not shown).

In Fig. 2 correlations between thyroid hormones are considered. TT4 and FT4 as well as TT3 and FT3 were positively correlated. TSH was negatively correlated with TT4 and FT4 and positively correlated with FT3. T4 and T3 in both free and combined forms were all correlated with TBG, which in turn was negatively correlated with the triad of retinol, RBP and TTR. Albumin was also positively associated with FT3 (values not shown). In the population as a whole, weight-for-age and height-for-age were negatively and strongly associated with FT3 ($-0·28$ and $-0·29$ respectively), FT4 ($-0·28$ and $-0·29$ respectively), TT4 ($-0·22$ and $-0·20$ respectively) and TBG ($-0·44$ and $-0·43$ respectively). These relationships did not hold in the cereal-staple area and only with respect to TT4 and TBG in the ensete-staple area. Urinary I excretion was positively associated with TT4 (0·20) and

Table 4. *Distribution of values of biochemical variables by staple food and high and lowland areas for schoolboys from seven provinces of Shoa Region, Ethiopia in the second phase of the study**

	(*Ensete ventricosum*)								
	Lowland cereal area (*n* 103) (A)		Lowland (*n* 162) (B)		Highland (*n* 79) (C)		Statistical significance (*P*) of differences between:		
	Median	25th and 75th percentiles	Median	25th and 75th percentiles	Median	25th and 75th percentiles	A *v.* B	A *v.* C	B *v.* C
Serum albumin (μmol/l)	587	558–616	540	507–572	587	536–609	0·000	0·519	0·000
Transthyretin (μmol/l)	3·60	3·16–3·93	3·63	3·14–4·06	4·18	3·56–4·71	0·430	0·000	0·000
Retinol-binding protein (μmol/l)	0·76	0·58–0·93	0·96	0·78–1·20	1·12	0·81–1·31	0·000	0·000	0·006
Transferrin (g/l)	2·61	2·43–2·97	2·50	2·22–2·74	2·69	2·40–2·95	0·000	0·892	0·002
Retinol (μmol/l)	0·79	0·61–0·91	1·10	0·92–1·30	1·15	0·86–1·31	0·000	0·000	0·644
Total Fe-binding capacity (μmol/l)	53·0	47·6–56·3	49·7	44·5–54·7		NA	0·003	NA	NA
Ferritin (μg/l)	58·4	34·9–68·9	36·0	23·5–53·3		NA	0·000	NA	NA
Serum Fe (μmol/l)	10·9	8·5–13·4	12·5	10·2–14·9		NA	0·002	NA	NA
Transferrin saturation (%)	21·4	15·3–25·5	24·8	19·6–31·9		NA	0·000	NA	NA
Haemoglobin (mmol/l)	6·83	6·36–7·45	6·98	6·63–7·24	8·38	8·04–8·72	0·965	0·000	0·000
Packed cell volume	0·39	0·35–0·42	0·37	0·35–0·38	0·43	0·41–0·44	0·000	0·000	0·000
Immunoglobulin A (g/l)	2·03	1·55–2·63	1·89	1·57–2·34	2·66	1·91–3·18	0·218	0·000	0·000
Immunoglobulin G (g/l)	20·2	17·4–22·3	19·6	17·6–22·5	19·6	17·4–21·1	0·455	0·215	0·522
Immunoglobulin M (g/l)	2·01	1·61–2·58	2·45	1·87–3·01	2·39	1·94–2·81	0·000	0·008	0·422
C-reactive protein (mg/l)	0·84	0·00–2·81	0·00	0·00–0·79	0·00	0·00–0·00	0·000	0·000	0·014
Weight-for-age Z-score	−1·43	−1·88 and −1·02	−1·75	−2·16 and −1·35	0·15	−0·23 and 0·75	0·000	0·000	0·000
Height-for-age Z-score	−1·26	−1·97 and −0·73	−1·56	−2·29 and −0·98	1·03	0·35 and 2·04	0·016	0·000	0·000
Weight-for-height Z-score	−0·81	−1·32 and −0·28	−0·93	−1·38 and −0·57	−0·79	−1·40 and −0·34	0·113	0·955	0·153

NA, values not available.
* For details of procedures, see pp. 594–595.

Table 5. *Median values for biochemical and anthropometric variables by goitre grade for schoolboys from seven provinces of Shoa region, Ethiopia in the second phase of the study*

Goitre grade...	0 (*n* 110–112)		IA (*n* 40–42)		IB (*n* 78–141)		II (*n* 34–49)	
	Median	25th and 75th percentiles	Median	25th and 75th percentiles	Median	25th and 75th percentiles	Median	25th and 75th percentiles
Serum albumin (μmol/l)	536^{a‡,b‡}	507–565	550^{c†,d‡}	507–579	579^{a‡,c†,e†}	536–608	608^{b‡,d‡,e‡}	565–623
Transthyretin (μmol/l)	3·67	3·11–4·12	3·70	3·16–4·12	3·72	3·27–4·25	3·78	3·15–4·25
Retinol-binding protein (μmol/l)	0·97^{a*,b*}	0·80–1·21	0·97^{c*}	0·77–1·29	0·85^{a*}	0·68–1·13	0·89^{b*,c*}	0·64–1·06
Transferrin (g/l)	2·53	2·24–2·75	2·45^{a*,b*}	2·21–2·74	2·62^{a*}	2·36–2·96	2·64^{b*}	2·45–2·94
Retinol (μmol/l)	1·12^{a‡,b‡}	0·93–1·31	1·11^{c‡,d‡}	0·95–1·28	0·87^{a‡,c‡}	0·70–1·15	0·89^{b‡,d‡}	0·66–1·14
Total Fe-binding capacity (μmol/l)	50·9	45·3–54·9	47·8^{a†,b†}	43·0–52·9	52·3^{a†}	47·0–56·6	53·6^{b†}	46·5–55·9
Ferritin (μg/l)	35·8^{a‡}	22·5–51·8	34·0^{b†}	25·0–58·6	57·8^{a‡,b†}	41·2–68·8	48·5	23·6–66·6
Serum Fe (μmol/l)	12·70^{a*,b*}	9·87–14·82	12·72^{c*,d*}	10·49–15·58	11·14^{a*,c*}	9·22–13·70	11·10^{b*,d*}	6·57–13·47
Transferrin saturation (%)	25·0^{a*,b*}	21·8–26·9	26·6^{c†,d†}	24·3–29·5	21·3^{a*,c†}	19·6–24·2	20·8^{a†,b†}	14·0–24·2
Haemoglobin (mmol/l)	6·98^{a‡,b‡}	6·61–7·28	7·00^{c‡,d†}	6·64–7·27	7·73^{a‡,c‡}	6·83–8·38	7·45^{b‡,d†}	6·52–8·30
Packed cell volume	0·37^{a‡,b‡}	0·34–0·38	0·36^{c‡,d‡}	0·34–0·38	0·40^{a‡,c‡}	0·38–0·44	0·40^{b‡,d‡}	0·38–0·43
Total triiodothyronine (nmol/l)	2·16	1·80–2·38	2·21	1·91–2·45	2·12^{a*}	1·87–2·45	2·27^{a*}	2·06–2·54
Total thyroxin (nmol/l)	110·7^{a*,b‡,c‡}	95·2–126·1	101·7^{a*}	92·7–114·5	96·5^{b‡}	81·1–113·3	94·0^{c‡}	77·2–110·7
Thyrotropin (μIU/ml)	2·70^{a†}	1·60–3·90	2·18^{b‡,c‡}	1·40–3·21	3·05^{b‡}	2·15–4·20	3·70^{a†,c‡}	2·38–4·58
Thyroxin-binding globulin (nmol/l)	NA		254^{a*,b*}	233–294	228^{a*}	186–275	235^{b*}	203–268
Urinary I excretion (μg/g creatinine)	NA		5·28	1·08–18·43	9·34	1·36–21·89	3·84	1·12–11·92
Immunoglobulin A (g/l)	1·97^{a‡}	1·56–2·32	2·01^{b*}	1·53–2·40	2·35^{a‡,b*,c*}	1·71–2·96	2·01^{c*}	1·47–2·66
Immunoglobulin G (g/l)	19·7	17·6–22·8	19·0	16·3–21·8	20·3^{a*}	17·8–22·0	19·2^{a*}	16·4–20·5
Immunoglobulin M (g/l)	2·53^{a†}	1·84–3·00	2·42^{b*}	2·05–3·01	2·22	1·82–2·74	2·00^{a†,b*}	1·60–2·69
C-reactive protein (mg/l)	0·0^{a*}	0·0–0·95	0·0^{b†}	0·0–0·62	0·0	0·0–0·97	0·12^{a*,b†}	0·0–1·72

NA, values not available.

[a,b,c,d,e] Values in the same row with the same superscript letter were significantly different: $*P < 0·05$, $\dagger P < 0·01$, $\ddagger P < 0·001$.

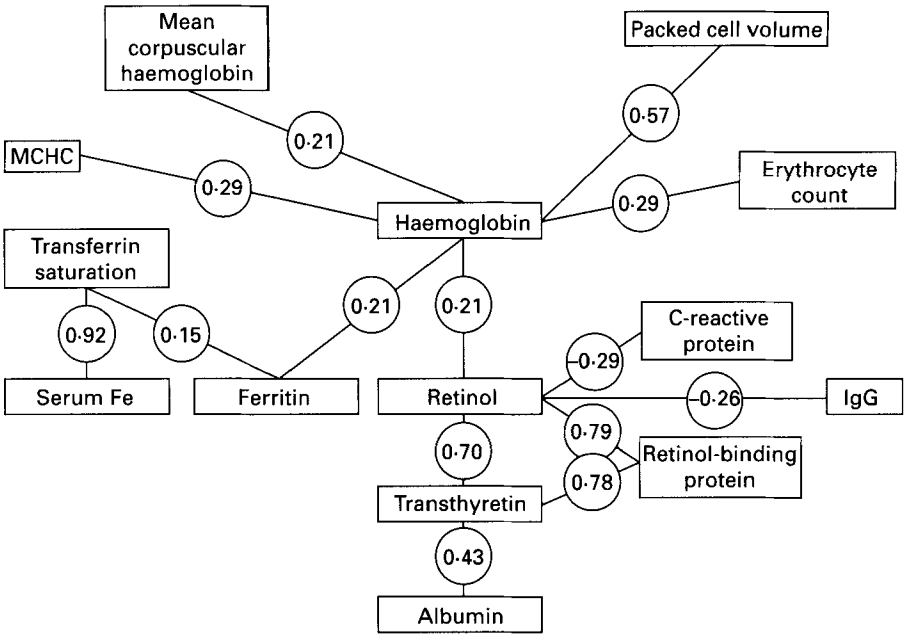

Fig. 1. Model based on correlations between variables for iron and vitamin A metabolism based on the study of Ethiopian schoolchildren from seven provinces of Shoa Region. IgG, immunoglobulin G; MCHC, mean corpuscular haemoglobin concentration. Values shown are correlation coefficients.

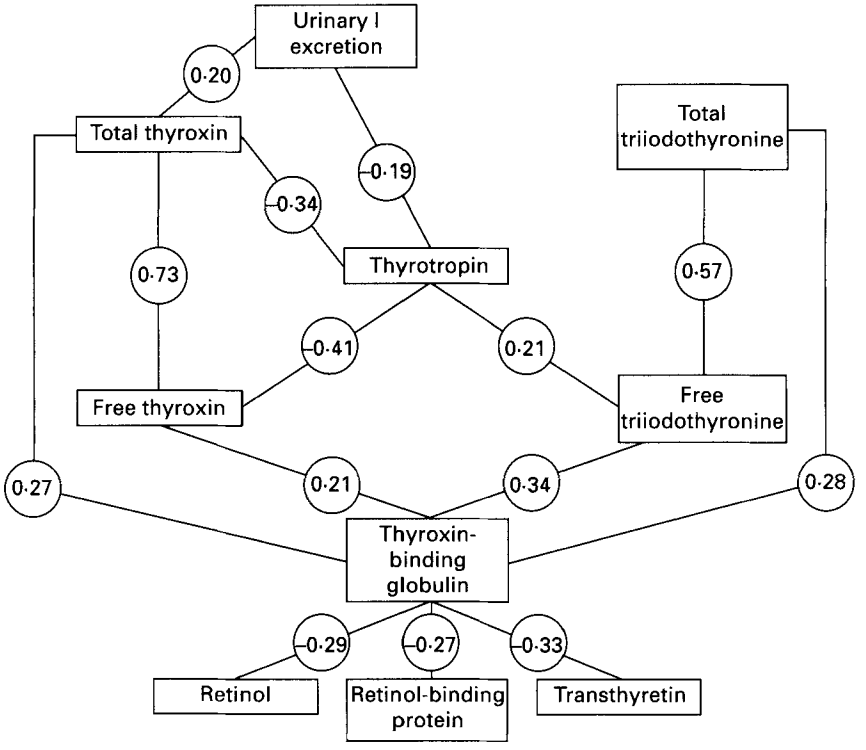

Fig. 2. Model based on correlations between variables for iodine and vitamin A metabolism based on the study of Ethiopian schoolchildren from seven provinces of Shoa Region. Values shown are correlation coefficients.

Table 6. *Iodine content of food items and drinking water from Ethiopian study areas*

	Local name	Collection site	No. of samples	I content (ng/g) Mean	I content (ng/g) Range
Type of food					
Ensete (*Ensete ventricosum*)					
Root	Amitcho	M, H†	2	42·7	0·00–85·4
Pulp sap	Bulla	M, H	1	88·5	88·5
Pulp solid matter	Kocho	M, H	5	74·3	45·8–85·3
Kale (*Brassica carinata*), boiled	Gommen	M, H	6	381·5	188·0–598·4
Maize (*Zea mays*)					
Roasted	Bequolo quolo	M, H	4	70·6	0·0–163·2
Boiled	Bequolo nefro	M, H	2	132·5	54·4–210·5
Bread	Bequolo kita	M, H	2	42·5	0·0–85·0
Sorghum (*Sorghum vulgare*), whole grain	Mashila	K, R*	2	26·2	23·7–28·6
Sweet potato (*Ipomoea batatas*), fresh	Sequar dinich	M, H	1	145·4	145·4
Teff (*Eragrostis teff*), whole grain	Teff	K, R	2	163·4	145·2–181·5
Wheat (*Triticum vulgare*), roasted	Sinde quolo	M, H	3	23·7	9·2–39·7
Wheat–maize, roasted	Sinde quolo	M, H	1	79·3	79·3
Type of water source					
Spring		H	2	3·7	2·5–5·0
Spring		M	3	7·7	2·5–12·5
Spring		K	2	9·2	6·0–12·5
Well		K	1	5·0	5·0
River		R	1	2·0	2·0
Pipe		R	1	1·7	1·7
Pipe		Z*	1	6·4	6·4

M, Mino; H, Hobicheka; K, Kobo; R, Robi; Z, Zuti.
* Yifatna-Timuga.
† Kembatana-Hadya.

negatively associated with TSH (−0·19) in the total population and the cereal-staple area but not in the ensete-staple area.

The relative dose–response test conducted on eighty children showed that four children (5·0%) had values above 14% (Flores *et al.* 1984; Solomons *et al.* 1990) while the median value was 0·00 (25th and 75th percentiles −8·25 and 5·20% respectively) indicating that the majority of the children had sufficient liver vitamin A stores.

Food and water analysis

The I content of the foods analysed varied from none in the maize and ensete samples to a mean of nearly 400 ng/g in the kale (*Brassica carinata*) samples while the I content in drinking water was low in all samples analysed (Table 6).

Microbiological assay

Fourteen samples of drinking water from the goitrous areas were analysed for bacterial contamination. All specimens showed growth of coliforms of differing intensity (Table 7). Of these specimens, six showed contamination with *Escherichia coli* and there was an

Table 7. *Bacteriological tests by study site and type of source of drinking water in Ethiopian study*

Site	Type of source†	*n*‡	Coliform count (per l)	*Escherichia coli* count (per l)	*n*§	Goitre prevalence (%)
Kembatana-Hadya						
Hobicheka	Spring	4	$3{\cdot}0\times10^{3}$–$3{\cdot}4\times10^{4}$	$5{\cdot}0\times10^{3}$	3	80·6
Mino	Spring	4	$2{\cdot}0\times10^{3}$–$7{\cdot}9\times10^{4}$	$2{\cdot}0\times10^{3}$	3	86·8
Yifatna-Timuga						
Kobo	Spring	2	$1{\cdot}4\times10^{4}$–$5{\cdot}4\times10^{5}$	$7{\cdot}0\times10^{3}$–$1{\cdot}4\times10^{4}$	2	79·9
Kobo	Well	1	$1{\cdot}7\times10^{4}$	0		79·9
Robi	River	1	$9{\cdot}2\times10^{5}$	$9{\cdot}2\times10^{5}$		37·8
Robi	Pipe	1	$1{\cdot}2\times10^{4}$	0		37·8
Zuti	Pipe	1	$0{\cdot}8\times10^{3}$	0		6·5***
Tequlena-Bulga						
Gudoberet	Spring	1	$1{\cdot}7\times10^{4}$	$1{\cdot}7\times10^{4}$		70·0
Chebona-Gurage						
Emdibir	Pipe	1	$0{\cdot}1\times10^{3}$	0		13·0
Gubre	River	1	$8{\cdot}4\times10^{4}$	0		10·5

† Source from which most of the study subjects obtained their drinking water.
‡ No. of sources from which samples were collected.
§ No. of specimens which showed presence of *E. coli*.
Values were significantly different from the goitre rates in Kobo and Robi: ***$P < 0{\cdot}001$.

indication that the high prevalence of goitre was related to the presence of *E. coli* in drinking water as the prevalence rate of goitre in Zuti was significantly lower ($P < 0{\cdot}001$) than in the other areas where the *E. coli* count was higher (Table 7).

DISCUSSION

The prevalence, sex and age distributions of gross goitre in the present study were not very different from those of schoolchildren at the national level (Wolde-Gebriel *et al.* 1993*a*). Thus, the prevalence rates of gross and visible goitre were 32·7 and 1·9% respectively in the present study, compared with 30·6 and 1·6% respectively at the national level.

The median TT3 and TSH values of our study children were higher and those of TT4 lower than the values reported for Ethiopian university and nursing students (Wassie & Abdulkadir, 1990). In I-deficient areas TT3 thyrotoxicosis due to high TT3 was reported (Hollander *et al.* 1972; Abdulkadir & Besrat, 1981) but in our study, where the goitre rate was high, deviation from the suggested reference value was not sufficient to classify the children as hyperthyroid.

In the present study the prevalence of Bitot's spots was 0·9%. This indicates that the problem is of public health importance as the prevalence is above the 0·5% cut-off point set by the World Health Organization (1982). The higher prevalence in males than in females is also similar to the findings of the national survey. The vitamin A store in liver as evidenced from the relative dose–response was satisfactory except for four children. In these four children (two from the cereal-staple areas and two from the ensete-staple areas) the serum retinol values were less than 0·35 μmol/l confirming the biological validity of the relative dose–response test (Solomons *et al.* 1990).

In the first phase of the assessment all children with indications of paleness were registered for inclusion in the second phase. Hence, 18·6% of the children examined were

diagnosed as clinically anaemic while in children selected from this group 65·4% had Hb values less than 7·45 mmol/l, which is the cut-off point set by the World Health Organization (1972); 48·4% were classed as anaemic based on multiple criteria and 21·2% had PCV values less than 0·35. Thus, assuming that all subjects with anaemia showed clinical signs, the overall prevalence of anaemia would be about 10%.

Studies carried out by others have concluded that non-nutritional factors may be responsible for the anaemia seen in parts of the country (Interdepartmental Committee on Nutrition for National Defense, 1959; Hofvander, 1968; Gebre-Medhin *et al.* 1976). In the cereal-staple areas teff is consumed and, apart from the relatively high Fe content of the grain itself, the teff as consumed contains much Fe derived from contamination with soil during threshing (Mengesha, 1966; Besrat *et al.* 1980). In the ensete-staple areas kale would provide substantial Fe in the diet.

The close correlations observed between the various biochemical and anthropometric variables in the population as a whole are sometimes artificial. Thus, in the correlations presented in Figs. 1 and 2 only those significant for the population as a whole and for the cereal- and ensete-staple areas separately have been reported and used for the basis of developing hypotheses. The artifact arises from the differences between the areas.

It is interesting to note that in Fig. 1 the strength of the relationship between serum retinol and blood Hb is as strong as the relationship between serum ferritin and blood Hb and stronger than the relationship between serum ferritin and transferrin saturation since ferritin levels provide an indication of Fe stores. A similar relationship between serum retinol and blood Hb was found by several authors (Mejía *et al.* 1977; Mohanram *et al.* 1977; Hodges *et al.* 1978; Mejía & Arroyave, 1982; Bloem *et al.* 1990). More recently, such a relationship was also observed in a cross-sectional study on the Fe status of pregnant women in West Java, Indonesia (Suharno *et al.* 1992). Studies in Asia have also shown a relationship between serum vitamin A concentration and PCV (Bloem *et al.* 1989; Suharno *et al.* 1992) but this was not seen in the present study even though Hb concentration was correlated with PCV, RBC count, MCHC and MCH. This may be because some of the anaemia is macrocytic, indicating malaria-induced deficiency of folic acid and/or vitamin B_{12}. There are several possible sites where vitamin A may exert its action: absorption of Fe from the gut, transport in serum, uptake and release of Fe in the liver, uptake of Fe by the bone marrow (which is the site of Hb synthesis) and in Hb synthesis itself. Absorption of Fe seems to be, in fact, increased in vitamin A deficiency (Sijtsma *et al.* 1993). As far as Fe transport is concerned, we found no relationship between vitamin A and transferrin concentrations in the present study or in our study of pregnant women in Indonesia (Suharno *et al.* 1992), but we did in the study of children in an area of severe vitamin A deficiency in the Hararge Region of Ethiopia (Wolde-Gebriel *et al.* 1993*b*), while others have found a negative correlation (Bloem *et al.* 1989). Uptake of Fe by the liver does not seem to be affected as it is known that vitamin A deficiency results in hepatic Fe accumulation (Staab *et al.* 1984; Sijtsma *et al.* 1993), although it may well be that release of Fe from the liver is affected. Uptake of Fe by the haematopoietic tissues could possibly be inhibited by vitamin A deficiency since we have found that Fe accumulation in the femur is lowered in vitamin A-deficient rats (Sijtsma *et al.* 1993), but it may well be that haematopoiesis itself is directly affected as Douer & Koeffler (1982) have shown that retinoic acid enhances the growth of human erythroid progenitor cells.

The association between retinol, RBP and TTR has been found in our earlier study in Hararge (Wolde-Gebriel *et al.* 1993*b*) and in Nigeria (James *et al.* 1984), and is explained by the formation of a one-for-one complex of retinol with RBP and, at low TTR levels, of the three molecules (Raz *et al.* 1969). It is only at levels of TTR above 0·7–1·0 μmol/l that its concentration is no longer linearly related to that of the other two.

In Fig. 2 several relationships highlight the mechanisms by which the body compensates for low I intake. As would be expected, TT4 is positively correlated with urinary I excretion. The relationships of TSH with urinary I excretion, TT4 and FT4 (all negative), and with FT3 (positive) are in line with the compensatory mechanism by which TSH stimulates thyroxin 5′-deiodinase (*EC* 3.8.1.4) activity resulting in the conversion of less active T4 to more active T3 (Erickson *et al.* 1982) when the availability of I for the synthesis of thyroid hormone is limited. This mechanism was reported to be responsible for the absence of sub-clinical hypothyroidism observed in goitrous areas in India and New Guinea (Kochupillai *et al.* 1973; Pharoah *et al.* 1973).

The negative association between TBG and the triad of retinol, RBG and TTR shown in Fig. 2 would explain the shift in binding of T4 from TTR to TBG when rats are made vitamin A-deficient (Oba & Kimura, 1980). However, since 90% of thyroid hormones are normally carried on TBG, it would not lend support to the hypothesis that vitamin A plays a role in the development of goitre. Such an hypothesis has been developed on the basis of interrelationships observed between vitamin A nutriture and occurrence of goitre on the Island of Krk in Yugoslavia (Horvat & Maver, 1958) and in Senegal (Ingenbleek & De Visscher, 1979). The low urinary I excretion also indicates that the goitre problem in the surveyed areas is due primarily to I deficiency rather than to other factors although, as mentioned previously, bacterial contamination of water may also play a significant role.

This and earlier studies clearly indicate that I deficiency disorders, as manifested by goitre, and vitamin A deficiency are the two major nutritional deficiency problems of public health significance in Ethiopia. Unlike in many developing countries, nutritional anaemia is not a problem of major concern to the country. The present paper also shows that the effects of the deficiencies of the various nutrients cannot be considered in isolation because they are very much interrelated.

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