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The use of acute phase proteins to interpret vitamin A status

A meta-analysis of data collected in apparently-healthy pre-school children and its wider applicability to other neonatal, child and maternal groups

Main authors:

David I. Thurnham [1]

George P. McCabe [2]

Christine A Northrop-Clewes [1]

Associated collaborators:

Delana A. Adelekan [3]

Anna Coutsoudis [4]

Parul Christian [5]

Omar Dary [6]

Henri Dirren [7]

Suzanne M. Filteau [8]

Champaklal C Jinabhai [9]

Demetre Labadarios [10]

Penny Nestel [11]

Anne S.W. Mburu [1]

Parvez I. Paracha [12]

Fransisco Rosales [13]

ILSI collaborators

Penny Nestel

Paula Trumbo

Suzanne Harris

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Addresses

1. Northern Ireland Centre for Diet and Health, School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, Northern Ireland
2. Department of Statistics, Purdue University, West Lafayette, IN 47907-1399, USA.
3. Department of Community Health, Faculty of Clinical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.
4. Department of Pediatrics & Child Health, University of Natal, PO Box 17039, Congella 4013, South Africa
5. Division of Human Nutrition, John Hopkins University, 615N Wolfe Street, Room 2041, Baltimore, MD 21205-2179, USA
6. INCAP, Guatemala
7. Nestec Ltd, Lausanne, CH-1000, Switzerland
8. Centre for International Child Health, Institute of Child Health, Guilford Street, WC1N 1EH, UK
9. Department of Community Health, University of Natal, Private Bag 7, Congella 4013, South Africa.
10. Department of Human Nutrition, Faculty of Medicine, University of Stellenbosch, PO Box 19063, 7505 Tygerberg, South Africa.
11. <pnestel@erols.com>
12. PO Box 981, Peshawar University, Peshawar, North West Frontier Province, Pakistan
13. Nutrition Department, The Pennsylvania State University, 126 S. Henderson Building, University Park, PA 16802, USA.

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Abstract

A cross-project analysis of information from thirteen data sets containing information on plasma retinol and acute phase proteins (APP) from almost 7000 subjects was used to determine the influence of sub-clinical infection on plasma retinol concentrations. Nine of the data sets were apparently-healthy pre-school children. Information on three acute phase proteins, α1-antichymotrypsin (ACT), α1-acid glycoprotein (AGP) and C-reactive protein (CRP) were used to assess the state of sub-clinical inflammation. Effects of elevated APP on retinol were examined singly (to determine specific effects) as well as in combination (to determine 'time-' or 'severity'-based effects). To make the best use of the data, the combined-APP approach was used to define three 'health' states viz 'healthy (no elevated APP), current sub-clinical infection (elevated AGP and ACT or AGP and CRP) or chronic sub-clinical infection (elevated AGP only). Using this method on the apparently-healthy pre-school child data showed that a chronic, sub-clinical inflammatory state was associated with a reduction in retinol of 10% whereas in a child with a current sub-clinical infection, retinol was depressed by 27%. Inclusion of the data from the other studies, which included children with slide-positive malaria and women who were HIV positive, did not appreciably alter these conclusions.

1. Introduction

Background

It is a common observation that the concentration of plasma retinol is lower in the blood of both infants and adults living in the lesser developed countries of the world. The lower concentrations are widely accepted to mean that vitamin A status is poorer in those countries than in the developed world. However, it is also well known that the level of exposure to disease is far higher in developing than in developed countries and it is now recognised that disease depresses the concentration of plasma retinol (Thurnham & Singkamani,1991; Louw *et al.*1992). Disease or trauma depresses the plasma concentration of the retinol-binding protein (RBP), reducing the circulating vitamin A and so potentially affects the supply of vitamin A to the tissues during infection. RBP is a negative acute phase protein, and its synthesis is inhibited during inflammation (Rosales *et al.*1996). It is suggested that the so-called 'negative' acute phase proteins (APR) are decreased in plasma during the acute phase response to allow an increase in the capacity of liver to synthesize the induced APRs (Steel & Whitehead,1994). However, in the case of RBP, this explanation seems a little naïve in view of the important role of vitamin A in the immune response (Semba,1998; Ross,1996) and in reducing susceptibility to infection (Fawzi *et al.*1993; Glasziou & Mackerras,1993), and others have suggested that the reduction may be a protective response to prevent losses of urinary vitamin A, which are increased at the time of fever (Stephensen *et al.*1994). The latter would appear to be particularly important since it has been shown that 50% of plasma retinol turnover is in the kidneys in the rat and there are many similarities in retinol metabolism between rat and man (Green & Green,1994)

However, fever is associated with sickness and sick persons are excluded in selecting volunteers for surveys. In this study we are not concerned with the acute effects of sickness on plasma retinol. We know that plasma retinol is lowered in sickness and the degree of effect will depend very much on the severity of the illness. The concern in this meta-analysis is to determine the extent to which the concentration of plasma retinol is reduced in apparently-healthy children but where there is nevertheless evidence of sub-clinical infection. Plasma retinol concentrations are often low even in the 'apparently healthy' infant or adult in many surveys. In lesser developed countries in particular, the inhabitants are exposed more frequently to infection and to more severe infections than subjects living in developed countries. The presence of sub-clinical infection is indicated by elevated levels of acute phase proteins in apparently-healthy individuals (Filteau *et al.*1993; Paracha *et al.*2000). Frequent exposure to infection may mean that immune mechanisms are in a constant state of activation, potentially influencing vascular permeability, which is one of the very early features of the APR (Fleck,1989), and potentially affecting the flux of plasma retinol to tissues to account for the low plasma concentrations.

Plasma retinol, even in association with its binding proteins, is still a relatively small molecule, and the increased flux between the plasma and extra-cellular fluids may lower the apparent concentration of plasma retinol and at the same time increase the availability of retinol to the tissues (Thurnham & Singkamani,1991; Willumsen *et al.*1997). Most retinol in plasma is bound in a complex with both RBP and transthyretin (TTR). However a small proportion (~4.4%) of the retinol is present bound only to RBP and this form is probably the form mainly responsible for transporting retinol to the tissues (Green & Green,1994). Small changes in vascular permeability accompanying the acute phase response will facilitate increased flux of retinol from the plasma into the tissue compartments of the body. In such a situation, the relatively low plasma retinol concentrations may incorrectly indicate an insufficiency of retinol. However, it may be possible to adjust the apparent plasma retinol concentrations by taking into account some of the other disturbances associated with the acute

phase response, particularly the changes in plasma concentrations of acute phase markers indicating sub-clinical or chronic infection.

Acute phase response

The acute phase of the inflammatory response refers to the wide ranging physiological changes that are initiated immediately after an infection or physical trauma has occurred (Steel & Whitehead,1994). The mammalian acute phase response (APR) is characterised by fever, changes in vascular permeability along with changes in the biosynthetic, metabolic and catabolic profiles of many organs (Koj,1985; Fleck,1989). The response is initiated and co-ordinated by a large number of diverse inflammatory mediators to elicit changes in target cells and tissues to promote repair processes and a return to normal function. Normally the APR only lasts a few days however in cases of recurring inflammation, some aspects of the APR may continue to promote repair or, in chronic disease, may contribute to the underlying tissues damage characteristic of such diseases.

An important target organ of the APR is the liver where there is a radical alteration of the biosynthetic profile with the synthesis of increased amounts of a characteristic range of plasma proteins. The usual acute phase protein which is measured to monitor the state of infection is C-reactive protein (CRP) which can increase a 1000-fold. Less frequently measured than CRP are serum amyloid A (SAA), which is also a major acute phase protein, and α1-acid glycoprotein (AGP, also called orosomucoid) and α1-antichymotrypsin (ACT), which do little more than double in concentration. In addition, erythrocyte sedimentation rate (ESR), which is an indirect measure of the chronic acute phase protein, fibrinogen and α1-antitrypsin (ATT), is also sometimes measured.

In a cross-sectional survey of apparently-healthy children, children can be selected either (1) in the very early or incubation phase of an infection, or (2) during the clinical stage of a very low grade infection or (3) in the convalescent stage when the clinical signs have subsided. Presumably there were will be fewer in the first stage than the last, as the incubation phase will be of limited duration, while convalescence will be longer and depend on the time taken for a child to fully recover. Likewise, the number of children in the second stage is also likely to be small, as presumably most children in the second stage of the disease will display symptoms of disease and be excluded from the survey. However, elevated plasma concentrations of acute phase proteins may assist in identifying these groups of children. Plasma concentrations of CRP and ACT are known to increase rapidly in the first 24 hr following an infection and reach a maximum at 48 hr while AGP rises more slowly reaching a maximum at 48-72 hr (Fleck & Myers,1985; Calvin & Price,1986). During the clinical stage of an infection, all acute phase proteins are likely to be increased but, once the clinical stage of infection or trauma subsides, CRP decreases rapidly but both ACT and AGP remain elevated for longer (Calvin *et al.*1988; Cruickshank *et al.*1989; Thompson *et al.*1992). Thus an elevated ACT or CRP in the absence of a raised AGP level may indicate a sub-clinical infection in the incubation phase, an increase of either ACT or CRP or both in the presence of raised AGP - the clinical stage of an infection, but an elevated AGP alone will be more likely to indicate the convalescent stage of the infection.

In this paper, data on plasma retinol and several acute phase proteins from 15 different population groups were assembled for meta-analysis. The objective of the meta-analysis was to compare plasma retinol and several acute phase proteins in data collected from apparently-healthy pre-school children and determine what conclusions could be drawn as to the influence of the sub-clinical infection on plasma retinol. The results were then compared with those from the other groups which included neonates, schoolchildren and mothers and included groups with known low grade infections (HIV) or high exposure to HIV or malaria.

Section 2 - Methods of blood collection and biochemical analyses in the individual studies

Study 1 [UKPresc][1]: National Diet and Nutrition Survey (NDNS) of Great Britain: children aged 1½ - 4½ years (Gregory *et al* 1995).

Subjects

A nationally representative sample of 2101 children aged 1½ - 4½ years was selected using a multi-stage random probability design, the Postcode Address File was used as the sampling frame, with postal sectors as first stage units. A total of 100 postal sectors were selected. Suitable households were identified by a sift form. Only children living in private households were eligible and only one child per household. To allow for seasonality, the fieldwork was distributed over 4 waves: July to September, October to December, January to March and April to June.

The first stage of the survey protocol was an interview which 1859 children completed. The sample of children was found to be representative of the population in terms of social and demographic characteristics as assessed by the 1992 General Household Survey and mid-year population estimates.

Blood sampling

Blood samples were taken in the child's home from 1003 children, 48% of the eligible sample. Parents were offered the choice of a finger-prick or venepuncture procedure. Where venepuncture was used 4 ml of blood was put in a 5 ml-lithium heparin container and subsequently delivered to a local laboratory within 4 hours of being taken. Once delivered to the local laboratory, the blood was centrifuged for 15 minutes at 3,000 rpm and the plasma removed and stored at - 40° C or below. Samples were transferred to the Dunn Nutrition Unit, Cambridge on dry ice, then stored at - 80° C. Only 7% of samples were taken using a finger-prick and these provided insufficient blood for the acute phase protein measurements. Approximately the same number of children was sampled in each wave.

Analysis

Retinol – Retinol and the carotenoids were assayed by high-pressure liquid chromatography (HPLC) based on the procedure described by Thurnham *et al* (1988). Plasma proteins were precipitated using ethyl alcohol to release the nutrients into the aqueous alcoholic solution from which they were extracted into heptane. A portion of the heptane was evaporated to dryness and reconstituted with mobile phase prior to injection on to a 100 x 4.6 mm, 3 µm Spherisorb ODS-2 column for separation and quantification. A guard column was not used and the column was protected by a 0.5 µm pore size stainless steel frit. Flow rate was approximately 0.8 ml/minute and the run time approximately 10 minutes.

The retinol and carotenoids were measured in 0.1 ml aliquots of plasma. The mobile phase was methanol, acetonitrile and dichloromethane (47:47:6 v/v/v) in which was dissolved 25 mg/L butylated hydroxytoluene.

Extinction coefficients were used to calculate the concentration of individual standards. Response factors were obtained from the areas of the standards after adjusting for purity. External quality assurance (EQA) was obtained by participating in the National Institute of Standards Scheme organised in Washington DC. In-house quality assurance (IQA) was obtained by using pooled serum samples, one at the beginning and one at the end, of each run. Retinol gave good agreement in both EQA and IQA throughout the survey.

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[1] Abbreviation refers to the identity of the study in the meta-analysis dat files.

Acute phase protein

α-1-antichymotrypsin (ACT) – the assay used was as described by DAKO which was based on the procedure of Calvin and Price (1986). Plasma and controls were diluted 1:41 times with dilution buffer. Standards were prepared from human serum standard calibrator (DAKO X908) to cover the range 0.2 – 2.39 g/L. Antisera (DAKO Q326) was diluted 1:5.5 with dilution buffer and left at room temperature for at least 10 minutes. Prior to use the diluted antibody was mixed with reaction buffer (1:5) and filtered through a 0.22 µm membrane filter (Millipore, Millex GV, SLGV 025 BS). The reaction mixture contained pre-treated sample (80 µL), diluted antiserum (50 µL) and diluent (10 µL water). Formation of the immunoprecipitate was monitored at 340 nm over 10 minutes at 37° C in a centrifugal spectrophotometer (Cobas Fara, Hoffmann La-Roche). The change in absorbance was plotted against the standard concentrations to construct a standard curve from which the unknowns were calculated. The assay was recalibrated with each new preparation of antibody. The reaction buffer consisted of 7 % (w/v) polyethylene glycol (PEG) 6000 in 0.1 M-phosphate buffer (pH 7.4) containing sodium azide (1.0 g/L sodium phosphate buffer). The dilution buffer is the same as the reaction buffer but without the PEG. IQA was obtained by using high (DAKO X940) and low (DAKO X 939) serum protein quality controls with each batch analysed.

Study 2 [Pakistan]: Survey of apparently healthy children in NorthWest Frontier Province, Pakistan (Paracha *et al* 2000).

Subjects
A two stage cluster sampling procedure was followed to select 3074 apparently-healthy children from 2193 households in both the urban and rural communities of North West Frontier Province (NWFP), Pakistan. The households in the selected clusters were identified and visited to identify children aged 6 – 60 months. Confirmation of the child's age was made with the mother with the help of a local-events calendar.

Blood sampling

A 5 mL blood sample was collected from each child using a disposable EDTA vacutainer (Becton & Dickenson, USA) with disposable monojet needle (Sherwood & Co, UK). The blood was centrifuged to separate the plasma. All plasma samples were immediately transported in a refrigerated van to the central storage room of the Agricultural University, Peshawar where samples were stored at –20° C until analysis. Those samples to be used for retinol analysis were wrapped in aluminium foil and transported as above.

Analysis
Retinol – Plasma retinol was determined by liquid chromatography using the method of DeRuyter and DeLeenheer (1978) at the Institute of Nutrition at Mahidol University, Bangkok, Thailand. Plasma proteins were denatured by adding 100 µL plasma and 15 µL of internal standard solution (retinyl acetate) to 100 µL methanol, the mixture was vortexed vigorously for 10 - 20 seconds. The retinol was extracted by adding 200 µL of extraction solvent (petroleum ether: dichloromethane:isopopranol, 80:19.3:0.7 v/v/v). The mixture was mixed by interrupted vortexing for 60 seconds, then centrifuged at 3000 rpm for 2 minutes. An aliquot (100 µL) of the supernatant was injected onto 15 x 0.2 cm MicroPak Si-10 column for separation. The mobile phase was the same as the extraction solvent (see above). The flow rate was 0.5 mL/min and the run time was up to 6 minutes. Retinol was detected using a sensitive UV detector set at 328 nm. The retinol concentration was calculated using peak height ratio of retinol relative to that of the internal standard. Accuracy and precision of the analytical method was checked by analysing pooled plasma samples (n = 150) with each batch and by using a lyophilised human serum standard reference material (SRM 968b) from the National Institute of Standards and Technology (NIST, Gaithersberg, USA). The mean (SD) for 5 determinations of low medium and high reference materials were: 1.03 (0.04), 1.79 (0.05) and 3.12 (0.16) compared with the certified values of 1.04 (0.05), 1.80 (0.06) and 3.12 (0.31) µmol/L, respectively.

Acute Phase Proteins
The plasma samples were transported on dry ice to the Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Northern Ireland

ACT – ACT was determined on a Cobas Fara centrifugal spectrophotometer using the immuno-turbidimetric technique described previously (see study 1). IQA was as described and the inter-batch precision was less than 8%.

α-1-Acid Glycoprotein (AGP) (also called orosomucoid) – the assay used was an in-house modification of the Dakopatts immunoturbidimetric method (DAKO, Ltd, Denmark). Plasma and controls were diluted 1:41 with dilution buffer (DAKO S2005). Standards were prepared from human serum standard calibrator (DAKO X908) to cover the range 0.14 – 3.28 g/L. Antiserum (DAKO Q326) was diluted 1:13.2 with dilution buffer and left at room temperature

for at least 10 minutes. Prior to use the diluted antiserum was mixed with reaction buffer (1:5)(DAKO S2008) and filtered through a 0.22 µm membrane filter (Millipore, Millex GV, SLGV 025 BS). The reaction buffer consisted of 7 % (w/v) PEG 6000 in 0.1 M-phosphate buffer (pH 7.4) containing sodium azide (1.0 g/L sodium phosphate buffer). The dilution buffer is the same as the reaction buffer but without the PEG.
The reaction mixture contained pre-diluted sample, calibrator or standard (20 µL), diluted antiserum (250 µL) and diluent (10 µL water). Formation of the immunoprecipitate was monitored at 340 nm over 10 minutes at 37° C in a centrifugal spectrophotometer (Cobas Fara, Hoffmann La-Roche). The change in absorbance was plotted against the standard concentrations to construct a standard curve from which the unknowns were calculated. The assay was recalibrated with each new preparation of antiserum. Between batch variation was 3 – 8 %, while within batch coefficients of variation (CVs) were 6 %.

Study 3 [PNG]: Effect of vitamin A supplementation on morbidity due to *Plasmodium falciparum* in young children in Papua New Guinea: a randomised trial (Shankar *et al* 1999).

Subjects

The study was done between July 1995 and August 1996 in the North Wosera District of East Sepik Province, in north-west Papua New Guinea (PNG). The study was a randomised double-blind placebo-controlled trial to assess the benefits of giving high-dose vitamin A supplements at 3-monthly intervals to children aged 6 – 60 months. Nine villages participated in the trial, forming a contiguous geographic area. The total population of the villages was about 4000, 520 of which were children aged 6 –60 months at the start of the study. Five hundred and eighteen children were screened at baseline and 90 plasma samples from these children were available for retinol and acute phase protein analysis.

Blood sampling

A venous sample (5 mL) was taken from each child. The blood was kept in a dark box at ambient temperature for no more than 6 hours before centrifugation and cryopreservation at – 70° C. Samples were placed in liquid nitrogen and transported to Baltimore, MD, USA. Retinol concentrations were measured by HPLC first hence the serum samples had been thawed-frozen 2 or 3 times before serum proteins were determined.

Analysis

Retinol – Retinol concentrations were measured by HPLC using a modification of the method described by Craft (1996). Briefly, after thawing, 150 µL aliquots of serum were diluted with 150 µL of water and deproteinated by vortexing with 300 µL of ethanol containing ß-apo-8' carotenal ethyl ester as an internal standard and butylated hydroxy-toluene as an anti-oxidant. The samples were extracted twice with 2 mL of hexane; the combined supernatant was evaporated under nitrogen. The residue was dissolved with vortexing in 35 µL of ethyl acetate, diluted with 100 µL of mobile phase and ultrasonically agitated for 15 seconds prior to placement in the autosampler. A 15 µL sample was injected.

The HPLC system consisted of a computer data system, an autosampler maintaining samples at 15° C, a Spherisorb ODS2 column (3 µm, 4.0 x 250 mm with titanium frits), a guard column containing some stationary phase, a column heater at 30° C, a UV detector to measure retinol at 325 nm. The separation was performed isocratically, using a mobile phase of acetonitrile, tetrahydrofuran and methanol (82:15:3) with 100 mM ammonium acetate and 0.1 % triethylamine as modifiers, at a flow rate of 1.5 mL/min.

Linear calibration curves were prepared. Quantitation was performed by internal standard calibration using peak area ratios. Samples from the National Institute of Standards and Technology were analysed each day at the beginning, end and at 2 sample intervals. The coefficient of variation of analytes in the quality control samples ranged from 3 to 10 %.

CRP, ACT and AGP – were measured using radial immunodiffusion assays based on the original method by Mancini et al (1965). The antiserum for each protein was purchased from DAKO (Carpinteria, CA). Briefly, glass plates, 10 by 8 cm (Hoefer Scientific Inst., San Francisco, CA) were cleaned and place on a level horizontal surface. Twenty four ml of 1% agarose solution (25 mmol/L tris-buffer saline solution, pH 7.4) at 45 °C, containing 1.5% polyethylene glycol (PEG 8000) and the respective antiserum was poured onto the glass plate. The samples were diluted in 7% bovine serum albumin (BSA) (Sigma, St Louis, Mo) and tris-buffer. Calibrators, external standards and samples were applied to wells in 5 µL volumes. After 48 to 72 hours of incubation in a humidified chamber at room temperature, the gel plates

were dried overnight and then stained with Coomassie Brilliant Blue R250 (BioRAD labs, Richmond, CA). After destaining the plates with a methanol/acetic acid solution, two perpendicular measurements of the diameter of each precipitin ring were taken with a calibrated magnifier, precision of 0.1 mm, and the square of the average was calculated. For the calibrators, plotting the diameter squared on the y-axis and the concentration of the antigen on the x-axis gave a linear function. Based on this linear function, the sample concentration was determined.

Calibrators from DAKO were titrated for each serum protein and four different concentrations were selected for each assay. During this procedure, it was determined that none of the human anti sera cross-reacted with bovine serum albumin (BSA). The College of American Pathologist reference standard for serum proteins (Northfield, IL) was used as an external standard for CRP and AGP, but the Binding site reference standard was used for ACT. The accuracy of each RID assay was checked against two different dilutions of external standards. Quality controls were conducted for each plate and the intra- and inter-assay CV were determined. The intra- and inter-assay CV's for CRP were 12 % and 2 % and for ACT 11% and 3% respectively. The limit of detection for CRP was 1.0 µg/mL, for ACT 29.0 µg/mL and for AGP 13.0 µg/mL.

Study 4 [Nepal]: Nepal Nutrition Intervention Project Sarlahi-2 (NNIPS-2) (Christian *et al* 1998).

Subjects

A population-based case-control study was conducted in Nepal between 1994 and 1995 to examine the risk factors of night-blindness during pregnancy. The case-control study was nested within a randomised, double-masked, maternal vitamin A and ß-carotene supplementation trial being conducted in the Sarlahi district of Nepal. Plasma was taken from 234 women for retinol and AGP estimations.

Blood sampling

Blood was collected in microtainer collection tubes (Becton-Dickinson, USA) and stored on ice in the field and centrifuged at 1530 x *g* for 10 minutes at room temperature within 4-5 hours of collection. The plasma was stored and transported in liquid nitrogen to the Center for Human Nutrition Laboratory of the John Hopkins University in Baltimore, USA. The samples were stored at –70° C until analysis.

Analysis

Retinol – retinol was analysed by reversed-phase HPLC with a Beckman System gold column (Beckman, USA), a 110 pump system, a Beckman 167 detector and a 710 Waters autosampler (Waters, USA). The mobile phase was acetonitrile, dioxane and methanol (83:13:4 %) with ammonium acetate (0.15 mol/L) added to the methanol and 0.1 % triethylamine. The detector wavelengths used were 450 nm for ß-apo-8'-carotenal (internal standard) and 325 nm for retinol.

Retinol was extracted using ethanol and hexane solvents and the residue, after evaporation, allowed to dissolve in 100 µL of the mobile phase. A portion (20 µL) of the sample was injected into the HPLC system. The concentration of retinol was calculated by the ratio of internal standard to that of the sample by using the areas under the peaks. Known concentrations of the pooled serum samples were run with the unknown standards simultaneously. A standard reference material (SRM 968b) from the Standard Reference Material Program of the National Institute of Standards and Technology (USA) was used for standardising the assay.

AGP - Plasma AGP was analysed with a competitive, time-resolved, fluorescent immunoassay method based on the enzyme linked immunosorbent assay (ELISA) method reported by Fiteau *et al* (1994). A 1:8000 dilution of anti-AGP (Dako Corp, USA) in bicarbonate buffer, pH 9.6 was bound to micro-wells. Standards (25 µL) (Behring Diagnostics Inc, USA) and samples, diluted 1:1000 in tris-HCL buffer pH 7.8, was added to each micro-well with an equal volume of biotinylated AGP diluted 1:8000 in tris-HCL buffer, pH 7.8. After a 2-hour incubation, europium labelled streptavidin (Wallac Inc, USA) was added to each micro-well. The fluorescence of each well was determined in an Arcus fluorometer (Wallac) and standard curves were developed for each micro-plate. Biotinylated AGP was prepared by incubating purified AGP (Sigma, USA) in a sodium acetate-sodium periodate solution, pH 4.5, in a ratio of 2 g/L. Excess periodate was removed with a PD-10 column, and biotin hydrazide was added and the mixture incubated at 37° C for 2 hours. Excess biotin was removed by dialysing the solution overnight in tris-buffered saline.

C-Reactive Protein (CRP) – plasma CRP was determined using a commercial ELISA kit (Hemagen Diagnostics, USA). Standards, controls and plasma were diluted (1:101) and pipetted into micro-wells bound with goat anti-human-CRP. Rabbit anti-CRP conjugated horseradish peroxidase was used as the second antibody. On activation with 3,3′, 5,5'-tetramethylbenzidine, plates were read at 450 nm.

Study 5 [GhanaM]: Influence of morbidity on serum retinol of children in a community-based study in Northern Ghana (Filteau *et al* 1993).

Subjects

The study took place in the guinea savannah area of Ghana in the Kassena-Nankana District, on the border with Burkina Faso. The Health Study included 1455 children aged 6 – 59 months who were monitored weekly for a year between June 1990 and August 1991. At baseline a subsample of 183 children was selected on the basis of morbidity as recorded by field-workers during the 2 weeks before the blood sample was taken. The samples were taken at four different times (October and December 1990, February and April 1991) and were geographically representative of the whole study area. Four subgroups of children were sampled: those with an observed raised temperature (> 37° C), those with reported vomiting but no observed fever, those with uncomplicated diarrhoea (> 3 motions/but no blood, fever or vomiting) and those with no reported symptoms. Each of the sub-groups was selected to include approximately equal numbers of children from the vitamin A-supplemented and placebo groups with the exception of the vomiting group where numbers were insufficient. The vitamin A treatment was 200,000 IU retinyl palmitate every 4 months (see study 6).

Blood sampling

A finger-prick blood sample was collected into an amber microtainer containing serum separator gel (Becton Dickinson, UK). The blood was kept on ice and centrifuged at 1500 x *g* for 10 minutes, at room temperature in the base laboratory, within 12 hours of collection. Serum samples were wrapped in foil, to protect them from the light, and frozen at -40° C until they were transported to London on dry ice, where they were then stored at –70° C until analysis.

Analysis

Retinol – Serum retinol was measured by HPLC (Filteau *et al*, 1993). Samples (100 µL) were vortexed with 100 µL methanol containing retinyl acetate as an internal standard. Hexane (500 µL) was added to the samples and they were vortexed and briefly centrifuged at 16,000 x *g* for 2 minutes at room temperature. The supernatant (250 µL) was removed and dried under a stream of nitrogen, 100 µL methanol was added and the samples were run on a HPLC (Spectraphysics, UK) equipped with a 10 x 0.4 cm ODS2 column (Pharmacia, UK) and an ultra-violet detector (Phillips, UK). The inter-assay and intra-assay coefficient of variations for a quality-control serum (2.39 µmol retinol/L) included in duplicate with each run were 12.6 % (n = 33 pairs) and 5.8 % (n =24 pairs) respectively. Sample values were normalised by comparison with the mean result for the quality control serum run in the same assay.

Serum Amyloid A (SAA) – SAA was measured by a competitive ELISA technique developed by Raynes (1988). The anti-SAA coating antibody, the SAA standard, and an alkaline phosphatase conjugate of the standard were made in-house. ELISA plates were coated in 1/200 anti-SAA diluted in 0.1 M bicarbonate buffer, pH 9.6 and left overnight. The following morning the plates were washed with PBS + 0.05% Tween 20, then blocked with 1% BSA in PBS and left for 1 hour at 37° C. The plates were washed again. Diluted standards (1.5 - 50 µg/L) or samples, plus an equal volume of diluted SAA-alkaline phosphatase were added and the plates were left at 37° C for 1.5 hours. The plates were washed. Alkaline phosphatase substrate (1 mg/ml) (Sigma N2507) in original coating buffer was added, then the reaction

started by adding 20 µL 1M magnesium chloridc just before adding thc substrate to the plate. The plates were left at 37° C for 1 hour, then read at 405 nm.

AGP – AGP was measured by turbidimetry with a COBAS Fara autoanalyser (Roche Products, UK). Samples, a laboratory standard calibrated against a control serum for nephelometry and turbidimetry (Behring Diagnostics UK) and rabbit anti-AGP (Dako, UK) were diluted in phosphate-buffered saline containing 6 % (w/v) PEG 6000. After 30 minutes at 4° C samples, standards and anti-AGP were centrifuged at 10,000 x *g* for 5 minutes at 4° C and run according to the manufacturers standard turbidimetry procedures for a Cobas Fara. The intra- and inter-assay CV's of a quality-control serum (mean 0.47 g/L) run in duplicate with each assay were 2.6 % and 10.7 % (n = 17), respectively.

Study 6 [GhanaR]: Vitamin A supplementation, morbidity and serum acute phase proteins in young Ghanaian children (Filteau *et al* 1995).

Subjects
The study took place in the guinea savannah area of Ghana in the Kassena-Nankana District, on the border with Burkina Faso. The Health Study included 1455 children aged 6 – 59 months who were monitored weekly for a year between June 1990 and August 1991. Every 4 months each child received either vitamin A (200,000 IU or 60 mg retinyl palmitate (30 mg for children aged 6 – 11 months) + 40 IU vitamin E) or placebo, then a blood sample was taken. A subsample of blood samples was randomly selected without stratification from the blood samples of the main study population, taken at any of the four monthly time points after baseline. Children less than 6 months at the time of blood sampling were not included. The sample size of 180 children in each treatment group was based on previous work, to give an 80 % chance of detecting differences between the groups. Plasma was successfully analysed from 329 children (166 vitamin A supplemented, 163 placebo).

Blood Sampling
Every 4 months a blood sample was taken from a different randomly chosen, one-third of children under surveillance. At later clinical examinations slightly larger numbers of children were sampled due to the continuous enrolment system. Finger-prick samples of blood were collected into amber micro-tubes containing serum separator gel (Becton Dickinson, UK). The tubes were kept on ice until the blood was centrifuged at 1500 x g for 10 minutes at room temperature and the serum was separated within 12 hours of blood collection in the base laboratory. Serum samples were wrapped in foil for protection from light and frozen at - 40º C until transport to London on dry ice where they were stored at -70º C until analysis.

Analysis
Retinol - was analysed as described previously (see study 5).

Acute phase protein

AGP – AGP was measured by competitive ELISA. Microtitre plates were coated overnight at 4 °C with anti-AGP (DAKO A011) diluted 1/4000 in 0.12 mol/L carbonate/bicarbonate buffer, pH 9.6. Plates were washed in phosphate buffered saline (PBS) containing 500 µL/L Tween 20 (Sigma) and then blocked by incubation at 37 °C for 2 hours with 10 g/L gelatin.

Biotinylated AGP was prepared by incubating 2 mg purified AGP (Sigma) in 1 mL of 0.1 mol/L sodium acetate and 5 mmol/L sodium periodate ($NaIO_4$) (pH 4.5) for 1 hour at 4 °C in the dark. Excess $NaIO_4$ was removed using a Sephadex G25 column, 15 mg biotin hydrazide (Sigma) was added and the mixture was incubated at 37 °C for 2 hours in the dark. The biotin-AGP was filtered and dialysed overnight against PBS. Standards (protein control serum, Behring OSKE07) and samples were incubated with the biotinylated AGP diluted $1/10^4$ with 0.1 mol/L carbonate/bicarbonate buffer, pH 9.6 and left overnight. The enzyme conjugate used was alkaline phosphatase-conjugated avidin (AP-avidin 1/1000 Sigma A7294) and the substrate p-nitrophenyl phosphate (Sigma), in 0.1 mol/L carbonate/bicarbonate buffer, pH 9.6, containing 2 mmol/L MgCl2. Absorbances were read at 405 nm and AGP concentrations determined from the standard curve. Samples and standards were run in duplicate on at least two separate plates. The inter-assay CV for a commercial serum (AGP = 0.71 g/L, Behring) was 13 %. When duplicates differed by more than 10% the plates were redone.

The correlation between this ELISA method and the turbidimetric method described for study 5 was good but the ELISA method tended to give lower results:

regression line ELISA = 0.135 + 0.615 x tubidimetric ($P < 0.001$, $n = 72$).

AGP results for this study were adjusted to equivalent turbidimetric values for the meta-analysis using this equation.

CRP - serum CRP was analysed by competitive ELISA similar to the one described for SAA (see study 5). The plates were coated with an affinity-purified rabbit anti-human CRP diluted in 0.1M bicarbonate buffer, pH9.2 and left overnight at 4 °C. The following morning, the plates were washed with PBS containing 0.05 % Tween 20 (Sigma), then blocked with 1% BSA in PBS and left for 1 hour at 37°C. The plates were washed again. Diluted standards or samples were then added with an alkaline phosphatase-conjugated CRP previously purified on phosphoryl choline-Sepharose. The substrate was p-nitrophenyl phosphate (Sigma). Plates were read at 405 nm. No QC data was available.

Study 7 **[SafricaS]: South African primary schools micronutrient and vitamin A supplementation and deworming intervention: effects on health, plasma retinol and gut integrity** (Noddy *et al*, unpublished).

Subjects

This study was part of a larger school children's vitamin A-micronutrient supplement and deworming treatment intervention. The study was carried out in the rural Vulamehlo Magisterial District in Kwa Zulu Natal (KZN) province of South Africa approximately 150 km south of Durban. The study population was drawn from school children aged 8 - 10 years attending 11 primary schools in the area. The study was a double-blind placebo controlled design.

A total of 587 children were enrolled into the study. Exclusion criteria for the study included: parental refusal for the child to participate; any physical or mental handicap; poor class attendance; participation in any other feeding programme; children without accurate age records or not formally registered with the school and children who were receiving any of the food supplements or treatments outside the context of the study. Blood samples were collected at baseline (June 1995) and a sub-sample of 201 plasma samples were taken for analysis of acute phase proteins and retinol.

Blood sampling

At baseline a 20 mL sample of blood was collected using KEDTA vacutainer tubes (Becton Dickinson, France). The blood samples were kept in a cooler box at 10º C before transportation to the University of Natal, Durban. Samples for retinol analysis were wrapped in foil to protect them from the light. The samples were centrifuged at 300 x g for 10 minutes and the plasma supernatant removed and stored in coloured tubes at - 20º C prior to analysis.

Analysis

Retinol - plasma retinol concentrations were determined by reverse phase HPLC using the method described by Catignan and Bieri (1983). A stock standard of retinyl acetate (100 mg/L) was prepared in ethanol. A working standard was prepared by diluting the stock standard 100-fold with ethanol. Briefly, 250 µL of sample and 50 µL of the internal standard (retinyl acetate) made up in ethanol were vortexed to mix and 500 µL of hexane was added. The mixture was vortexed for 1 minute and then centrifuged for 10 minutes. The supernatant was removed, then the hexane extract step was repeated. The hexane layers were combined and evaporated under a stream of nitrogen, the residue was reconstituted with 50 µL of methanol and 50µL was injected onto the column. The instrument was a Hewlett Packard 1090 with a dioderay detector. The column used was a Spherisorb S 5 ODS2 4.6mm X 250 mm (Waters) with a 5 cm guard column containing C18 packing. Retinol was detected at 325 nm. The flow rate was 1 mL per minute and the mobile phase was 98% methanol and 2 % water. The oven temperature was 40° C. All organic solvents were of HPLC grade.

The CVs for inter- and intra-assay variability were 1 % and 1.2 % respectively.

Acute phase proteins

ACT - was analysed according to an in-house modification of the Dako immuno-turbimetric method (Dako Ltd, Denmark) described earlier (see study 1).

AGP - plasma AGP was analysed according to an in-house modification of the Dako immuno-turbimetric method (Dako Ltd, Denmark) described earlier (see study 2).

Study 8 [SAFRmumH]: Vitamin A supplementation of women infected with HIV type-1: the relationship between breast-milk vitamin A levels and infant serum vitamin A levels. 1. Mothers (Mburu *et al*, submitted).

Subjects

Subjects were recruited from pregnant women attending the antenatal clinic at King Edward Hospital, University of Natal, Durban, South Africa. After giving their consent, women who tested positive for HIV-1 at 24 - 26 weeks gestation were recruited to the study. A total of 728 women were enrolled and randomly assigned to receive either placebo or vitamin A. The median age of the mothers in the placebo group was 24 years (range 15 - 38 years) and in the supplemented group was 26 years (range 17 - 38 years). At baseline, blood was taken from a sub-sample of women (n = 103) for a more intensive biochemical study. Data from women assigned to the placebo group (n = 54) was used for the meta-analysis.

Blood sampling

Baseline blood samples (20 mL) were taken by venipuncture of the antecubital vein and collected into KEDTA coated vacutainer tubes (Becton Dickinson, France). The samples were stored upright and kept cool in a covered polystyrene box to exclude light until centrifugation. The plasma was centrifuged at 3000 x *g* for 10 minutes. Samples were stored in 500 µL aliquots, at -20º C, for up to 4 weeks before being transferred to -70ºC until analysis. The retinol and acute phase proteins were analysed at NICHE, University of Ulster, Coleraine, Northern Ireland following transportation on dry ice. The plasma samples were analysed between 12 - 18 months after collection and were stored at -70ºC during that period.

Analysis

Retinol - plasma retinol was determined by reverse phase HPLC, using tocopherol acetate as the internal standard according to the method of Thurnham *et al* (1988). The method is described earlier (see study 1). Within-assay variation was 5 % and between-assay variation was 8 %.

Acute phase proteins

ACT - was analysed according to an in-house modification of the Dako immuno-turbimetric method (Dako Ltd, Denmark) described earlier (see study 1).

AGP - plasma AGP was analysed according to an in-house modification of the Dako immuno-turbimetric method (Dako Ltd, Denmark) described earlier (see study 2).

The inter- and intra-assay variation for both ACT and AGP were 3 - 8% and 6 % respectively.

Study 9 [SAFRinfH]: Vitamin A supplementation of women infected with HIV type-1: the relationship between breast-milk vitamin A levels and infant serum vitamin A levels. 2. Infants (Mburu *et al*, submitted).

Subjects

Subjects were the infants born to women who had been recruited as pregnant women attending the antenatal clinic at King Edward Hospital, University of Natal, Durban, South Africa. After giving their consent, women who tested positive for HIV-1 at 24 - 26 weeks gestation were recruited to the study. A total of 728 women were enrolled. The women were given a blister pack containing 14 capsules, each capsule contained 5000 IU (150 µg) retinyl palmitate and 30 µg of ß-carotene (Roche, Johannesburg, South Africa) or placebo, and were told to take one capsule every day after meals. The women were encouraged to attend the clinics every 14 days and the supplementation continued for 12 weeks or until the delivery of the infant. Blood samples were taken from those infants born to the sub-sample of HIV+ mothers used in study 8 (n = 54). Data used in the meta-analysis were obtained from the infants at 6 months of age.

Blood sampling

At delivery a 5 mL blood sample was taken from a vein near the groin of the infants and collected into a KEDTA-coated vacutainer tubes (Becton Dickinson, France). The samples were stored upright and kept cool in a covered polystyrene box to exclude light until centrifugation. The plasma was centrifuged at 3000 x *g* for 10 minutes. Samples were stored in 500 µL aliquots at -20º C for up to 4 weeks before being transferred to -70ºC until analysis. The retinol and acute phase proteins were analysed at NICHE, University of Ulster, Coleraine, Northern Ireland following transportation on dry ice. The plasma samples were analysed between 12 - 18 months after collection and were stored at -70ºC during that period.

Analysis

Retinol - plasma retinol was determined by reverse phase HPLC, using tocopherol acetate as the internal standard according to the method of Thurnham *et al* (1988). The method is described earlier (see study 1). Within assay variation was 5 % and between assay variation was 7 %.

Acute phase proteins

ACT - was analysed according to an in-house modification of the Dako immuno-turbimetric method (Dako Ltd, Denmark) described earlier (see study 1).

AGP - plasma AGP was analysed according to an in-house modification of the Dako immuno-turbimetric method (Dako Ltd, Denmark) described earlier (see study 2).

The inter- and intra-assay variation for both ACT and AGP were 3 - 8% and 6 % respectively.

Study 10 [SafricaP]: Anthropometric, vitamin A, iron and immunisation coverage status in children aged 6 - 71 months in South Africa, 1994 (Labadarios *et al,* unpublished).

Subjects

The study population consisted of all children aged 6 - 71 months in South Africa. A total of 18,219 households were selected on the basis of a national probability sample with disproportionate stratification by province. Most fieldwork was conducted between July and October 1994. A total of 360 clusters were studied of which 358 were available for analysis. Of these 163 were rural and 195 were urban. The age distribution of the children sampled (n = 11,430) was fairly consistent across all provinces and age groups. A total of 4,788 blood samples was drawn for retinol and of these 579 were randomly selected for analysis of acute phase proteins. Values from the latter group were the ones used in the meta-analysis.

Blood Sampling

Children were eligible to have their blood drawn if the following conditions were met: the parent or legal guardian gave consent, the child did not have a high temperature (< 38°C), and the child had not received high dose vitamin A supplementation in the preceding 6 months. Within each household, all children within the age group 6-71 months were included in the study. From the first 16 children for whom permission was obtained, blood was drawn for biochemical analysis. If the sixteenth child had siblings in the target age group, then blood was drawn from them as well.

Samples were stored at - 80° C at all times without any recorded power or instrument failures. No sample was more than 14 months old at analysis.

Analysis

Retinol – Plasma retinol concentration was determined using reversed phase HPLC by a modification of the method of Catignan and Bieri (1983). Retinol standards (calibrated at 324 nm), plasma controls (NIST reference material 968b) and plasma samples (250 µL) were mixed gently with 500 µL ethyl alcohol containing two internal standards, retinyl acetate and tocopherol acetate. Hexane (2.3 mL) was then mixed vortexed for 45 seconds interruptively and the tubes centrifuged for 15 minutes at 3000 rpm. The upper hexane layer was removed and the hexane extraction repeated. The combined extracts were evaporated under nitrogen and the residue reconstituted in 200 µL. Twenty-five µL was injected onto a 250 x 4.6 mm, 5 µm Supelco LC-18 HPLC column and peaks eluting were quantified using a fixed-wavelength detector at 292 nm. The flow rate was 1.8 mL per minute and the run time on the gradient system was 5 minutes using a mobile phase of methanol:water 9:1v/v). Concentrations of retinol were calculated using peak areas. Both inter- and intra-laboratory precision using this method were < 4 % respectively.

CRP – The principle of the assay is that samples are treated with a CRP reagent consisting of polystyrene particles coated with antibodies to CRP which agglutinate when mixed with samples containing CRP. The agglutination is monitored and the intensity of the resulting scattered light is dependent upon the CRP content of the sample so that, by comparison to standards of known concentration, the CRP content of the sample can be determined in a nephelometer (Behring N Latex CRP mono). The samples were initially diluted 1:400 with diluent (Behring OUMT). The dilutions of the standard 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280 and 1:2560 (Behring N CRP standard SY OQIK) were prepared automatically by the Behring nephelometer (Model BN100) and were used to make a reference curve. All subsequent steps were performed automatically by the BN 100: 40 µL of CRP reagent, 50 µL control serum or 50 µL sample, plus 60 µL reaction buffer were pipetted into cuvettes followed by 60 µL reaction buffer and 10 µL CRP accelerator, the mixture was stirred. The light scattering signal was recorded and stored in the computer for subsequent data processing.

Accuracy and precision was carried out using CRP control serum (Behring OUKU). The CV of the assay (4.1 %) was based on one control run with each batch of 155 samples, which gave a mean value of 14.0 mg/L, compared with the assigned value of 13.2 mg/L. The results of the measurements were calculated automatically using a logit-log function.

Study 11 [Equador]: National Nutrition and Health Survey of the Ecuadorian Population (Freire *et al,* 1988).

Subjects

The survey population was drawn from 8,100 children under the age of five years, from 10,000 households. There were five teams collecting data about the socio-economic factors that affect the nutrition, food and health situation of the Ecuadorian population. Six surveyors were split into 2 teams to gather data from 1,600 children to quantify and assess the dietary intake of the families and under fives in the chosen sectors, so as to determine the macro- and micro-nutrient intake of each family and their children.

The survey was carried out in 13 different provinces, and was conducted between March and November 1986. Of the 360 coded sectors, 144 sectors were used to collect socio-economic, dietary intake data and biochemical samples.

Blood sampling

The samples collected were of venous blood, except where venous sampling was not possible or failed, then capillary-prick samples were taken. Blood samples were taken from the children, at the family home, when each coded sector was visited.

a) Capillary prick samples - Capillary prick samples were obtained from either heel or finger. Blood collection tubes were either EDTA-coated or uncoated Microtainer tubes. All sample tubes were labelled with the child's survey identity number. Samples for haematological analysis were prioritised, therefore for each sample collection, the first 200 µL of blood were collected into EDTA coated tubes. After which up to 600 µL was collected into uncoated Microtainer tubes.

b)Venous blood samples - Blood was taken in EDTA coated and uncoated Eppendorf tubes. The EDTA coated tubes were inverted gently 10 times to ensure proper mixing of the sample with the anticoagulant. Approximately 750 µL of blood was collected.

Capillary prick and venous blood samples

Samples were transferred from the field to the field-laboratory and stored at 4 – 6° C, where all identification information was recorded. Blood samples in the uncoated Microtainer tubes were left to clot for at least 1 hour in the dark and then centrifuged at 4000 rpm for 10 minutes (Labofuge 1) at 4 – 10° C. Samples were considered properly centrifuged when the separation plate was between the serum and the clotted blood and proteins. Samples for retinol (250 µL) and acute phase (2 x 250 µL) analysis were stored in 0.5 mL Eppendorf tubes. Venous blood samples were handled similarly to capillary prick samples.

Sample storage and distribution.

Aliquoted samples were frozen vertically in the dark on dry ice, before being sent to the main laboratory at Quito. Samples were sent either daily or twice a week. In Quito, samples were divided up into batches for analysis either at Quito or at the Nestlé laboratories in Switzerland. Samples bound for the Swiss labs were packed and transported on dry ice, on arrival they were stored at - 80 °C.

As there were up to 50,000 analyses to be done, logistical constraints in the Quito laboratories meant that the acute phase proteins, CRP and AGP were done in Nestlé's Laboratory. CRP and AGP were determined either manually by radial immunodiffusion techniques or immunoturbidimetrically using the automated COBAS Fara centrifugal analyser.

Analysis

All samples were analysed within 6 months of collection.

Retinol

Serum retinol was assayed using HPLC with fluorometric detection according to the method of Mansourian *et al* (1982). Standardisation was carried out using a commercial retinol standard

(Hoffman La-Roche). The quality control CV was 5 % (n = 46) and the pooled sera CV was 5.5 % (n = 68).

Acute Phase Proteins

Manual determination - Individual proteins were determined using the radial immunodiffusion technique according to Mancini *et al*, (1965) using Nor Partigen (AGP), and LC Partigen plates (CRP), all plates and reagents were from Behring Werke (Behring Diagnostics, San Diego, CA.).

Automated methods: CRP and AGP were determined by immunoturbidimetry using antibodies and controls from Behring Werke (Behring Diagnostics, San Diego, CA.).

CRP - Samples and controls (ATAB, Atlantic Antibodies; Scarborough ME) were diluted in 1:5 dilution with isotonic saline. Diluted sample or control sera (20 µL) was used for the reaction on the COBAS Fara centrifugal analyser. Antisera (ORCS) was diluted 1:10 with reaction buffer containing PEG. The final volume of the reaction buffer containing the antibody was 130 µL. Calibration standards for the analysis were diluted in the range ¼, $^1/_8$, $^1/_{16}$, $^1/_{32}$, $^1/_{64}$. The inter-assay CV was 5.3 % (Atlantic Antibodies, n = 121).

AGP - Samples and control sera (OTCO, Behring) were diluted in 1:41 with dilution buffer and 10 µl of diluted sample or control sera was used for the reaction on the COBAS Fara. Antibody antisera (ORCW) was diluted 1:11 with the reaction buffer containing PEG. The final volume of the reaction buffer-antibody solution was 200 µL. Calibration standards (ORDT) were diluted in the series $^1/_{10}$, $^1/_{20}$, $^1/_{40}$, $^1/_{80}$, $^1/_{160}$. Control sera were generated with each rotor. Quality control was monitored using pooled sera (CV 4.4%, n = 68) and commercial control sera (CV 5.1%, n = 120).

Study 12 [Guatemal]: National survey in Guatemala (Dary *et al*, unpublished)
Subjects – A sample of 759 children aged 2 months to 5 years were recruited.
Blood sampling – A venous sample of blood (5 mL) was taken from each child into a heparinised tube. Samples were protected from the light.
Analysis
Retinol – Plasma retinol was first determined by spectrophotometry and the samples with retinol levels lower than 0.9 µmol/L were analyzed again using a HPLC assay. **Spectrometric method** - The method was as described by Dary *et al.* (1996), and it was based on the methods by Bessey *et al.* (1946) with modifications by Araujo and Flores (1978). In summary, 750 µL plasma were deproteinised with 750 µL of an alcoholic potassium hydroxide solution. After centrifugation, retinol was extracted with 1000 µL of a kerosene-xylene mixture (1:1v/v). Retinol in the extract was destroyed by irradiation of ultraviolet light. The difference between absorbance 325 nm before and after irradiation was attributable and proportional to total retinol. Readings were made in a spectrophotometer (Perkin Elmer lambda 3B UV/VIS) spectrophotometer, using 1,570 g^{-1} cm^{-1} dL as the retinol absorption coefficient at 325 nm. Performance of the method was checked by including in each run two control samples (a large pool stored in small vials). Variation of results of each sample and the control should have been within two times the intra-assay and the inter-assay variation of the method, respectively; otherwise, the complete run was repeated. Intra- and inter-assay variations were 10.0%. Quantitation limit of the method was 0.7 µmol/L, and its recovery was 93%. With some frequency, the reliability of the method was checked by analyzing a standard reference material (968b, NIST, USA).

HPLC - The HPLC method was described by Dary et al. (1996), and it was based on methods by Bankson et al. (1986), Bieri et al. (1979), DeRuyter and Leenheer (1976), and Packer (1990). In summary, 250 µL of plasma were diluted with a retinyl acetate (internal standard as 1.5 µg/mL) solution in ethanol, and mixed in Vortex for 1 minute. Extraction was carried out adding 500 µL hexane and mixed vigorously in a vortex for 1 minute. After centrifugation at 2,500 rpm for 2 minutes, the organic phase was separated and evaporated under nitrogen. The residue was resuspended with 250 µL HPLC-grade methanol and mixed in a vortex for 1 minute. After centrifugation at 2,000 rpm for 5 minutes, 250 µL of the upper solution were injected onto a 100 µL loop, using a reverse phase C-18 column (Supelcosil LC-18 15 cm x 4.6, 5 µm) as the solid phase and a 95% methanol:5% water as the mobile phase, at a flow rate of 1.6 mL/min. The run time was approximately 6 minutes. Both retinol and retinyl acetate were detected and measured in a UV Detector (Varian UV-200 uv/vis wavelength detector) at 325 nm in a HPLC apparatus (Varian, Vista 5500). Retinol was determined using peak height ratio of retinol relative to that of the internal standard. Daily, a standard curve of retinol (0.35 to 3.50 µmol/L) was calculated analyzing different levels of pure retinol with the internal standard (retinyl acetate). Retinol level of the original retinol solution in ethanol was determined spectrophotometrically at 325 nm using a Varian Techtron UV/VIS Model 635 spectrophotometer. As the absorption coefficient of retinol in ethanol at 325 nm was used the value of 0.1845 mg^{-1} cm^{-1} mL. Performance of the method was checked by including in each run one-control sample (a large pool stored in small vials). Variation in results of each sample and the control should have been within two times the intra-assay and the inter-assay variation of the method, respectively; otherwise, the complete run was repeated. Intra- and inter-assay variations were 5.0 and 7.0%, respectively. Quantitation limit of the method was 0.35 µmol/L. With some frequency, the reliability of the method was checked by analyzing a standard reference material (Standard reference material 968b Fat-Soluble Vitamins and Cholesterol in Human Serum) from the National Institute of Standards and Technology (NIST) USA.

Acute Phase Proteins

The plasma samples were transported on dry ice to the Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Northern Ireland

ACT – ACT was determined on a Cobas Fara centrifugal spectrophotometer using the immuno-turbidimetric technique described previously (see study 1).

AGP - plasma AGP was analysed according to an in-house modification of the Dako immuno-turbimetric method (Dako Ltd, Denmark) described earlier (see study 2).

Study 13 [Honduras]: The First National Micronutrient Survey of Children aged 12 –71 months in Honduras (Nestel *et al,* unpublished).

Subjects

The 1988 Master Sampling Frame (MSF) developed by the Direccion General de Estadisticas and Census, which was updated in Tegucigalpa and San Pedro Sula in 1991-2 to include the new peri-urban areas, was used to select the subjects. The MSF was divided into six strata or dominions: 3 urban and 3 rural areas. The survey covered the whole country except the Departments of Las Islas De La Bahia and Gracias A Dios, which are isolated and have a very low population density.

A two-stage sampling technique was used, in which clusters were first selected, after which houses within the selected clusters were chosen. For logistical reasons, 22 clusters were selected in each domain using systematic selection from a random start with probability proportional to number of households based on the 1988 census data. Within each cluster, 22 households were randomly selected in Tegucigalpa, 19 in San Pedro Sula and other medium sized cities and 16 in the remaining urban areas. In the rural areas 17 were in the south, 14 in the west and 18 in the north.

An eligible household was one in which there was at least one child between the age of 12 and 71 months. A total of 2421 households were visited of which 1726 were eligible. Plasma was obtained from 1584 children.

Blood sampling

Blood (2 ml) from the antecubital vein was collected for plasma retinol and AGP. Duplicate blood samples were taken from every tenth child for quality control. Blood was collected into a Sardsted plasma-gel tube using a butterfly needle and immediately placed in a cool box containing ice packs. Before collecting the blood, labelled tubes were covered in aluminium foil, except for a small window which allowed the phlebotomist to see when the tube was full. At the end of the each day, the blood samples were centrifuged and the plasma transferred to labelled vials covered in foil, then stored in a freezer until they were shipped on dry ice to the Institute of Nutrition for Central America and Panama (INCAP), Guatemala.

Analysis

Retinol – Plasma retinol was determined spectrophotometrically using the method of Dary and Arroyave (1996) which was based on the methods by Bessey et al. (1946) and modifications by Araujo and Flores (1978). The samples were analysed at INCAP, (see details in section on Guatemala, study 11). However, the quantitation limit of the method was 0.7 µmol/L, hence results below 0.7 µmol/L were reported as below that level and were not analysed using the HPLC method as described in study 11.

Acute Phase Proteins

AGP - AGP was determined using the Behring Turbitime system, an immunoturbidimetric assay, in which human AGP forms a precipitin with a specific antiserum. The serum sample was diluted 1 + 20 with isotonic saline. Standards were prepared from an international reference plasma protein preparation (US Reference Preparation for Proteins in Human Serum (RPPHS) lot 91/0619) to cover the range 0.4 – 5.5 g/L. The pre-diluted sample (50 µL) was pipetted into a cuvette, then the cuvette was put into the Behring Turbitimer. When prompted by the Turbitimer, the anti-rabbit anti-serum (500 µL) was also pipetted into the cuvette. The result of the protein determination was calculated automatically and printed out. No quality control data were given.

Study 14 **[Columbia]: Colombia National Demographic and Health Survey** (Navarro & Nicholls, 1996).

Subjects

The sample was a sub-sample of the National Demographic and Health survey based on the 1985 census and using projections for 1995. The population was divided into 5 large regions: Bogota, Atlantic, Pacific, Central and Orient, each subsample comprised 1023 segments of which 700 were in each municipality and 323 were in rural areas. The sample population was made up of 2187 children aged 12 – 59 months, which were probabilistically selected from all the municipalities in all the departments in the country. The sample was adjusted for non-compliance and weighted by a factor equivalent to the reciprocal of the final probability.

Blood Sampling

The study had a principal investigator, a co-investigator, 2 co-ordinators and 12 bacteriologists. The bacteriologists were trained and standardised in techniques for collection, handling, processing and storing of blood samples. The blood collection was done following the established procedures of the Instituto Nacional de Salud (INS) in the Procedures Manual. Each day the bacteriologists transported the blood samples to the health organisations laboratory to centrifuge, then freeze the blood. Frozen plasma was transported in dry ice, hermetically sealed to the INS for retinol analysis. Samples for AGP were then transported on dry ice to INCAP.

Analysis

Retinol – The assay was based on a method described by Bessey *et al* (1946). Briefly, the plasma was mixed with alcoholic potassium hydroxide to precipitate the proteins and partially saponify the retinol esters. The extraction of retinol and its esters was done with a mixture of kerosene and xylene (1:1), after which the free and esterified retinol was destroyed by ultra-violet light (UV). The difference in absorption at 323 nm before and after irradiation corresponds to total retinol (see study 11 for further details).

Retinol levels less than 0.77 µmol/L (22 µg/dl) were confirmed using HPLC which is more specific and sensitive. Plasma was diluted with a solution of retinol acetate in ethanol, the acetate serves as an internal standard and the ethanol precipitated the proteins. Retinol was extracted with hexane, evaporated under nitrogen, the residue was resuspended in methanol and injected on to the column. The retinol was separated on a C18 fixed phase column using methanol:water as the mobile phase and detected using a UV detector set a 328 nm.

AGP – AGP was determined using the Behring Turbitime system at INCAP (see method described earlier in Honduras section, study 12).

Study 15 [Nigeria]: Plasma retinol and acute phase proteins in Nigerian neonates (Adelekan *et al*, submitted).

Subjects: The subjects were 205 neonates delivered at the Wesley Guild Hospital, Ilesa, Nigeria, between June 1996 and July 1997. The hospital is part of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, SW Nigeria. The neonates were aged between 1 and 20 days and were apparently well at the time of the study. The socio-economic status of the neonates' mothers ranged from professional to unskilled workers. Most lived in urban areas.

Blood Sampling: 1-2 ml of blood was drawn from the heel of each neonate into heparinised tubes. Blood samples were mixed gently on a roller mixer and then centrifuged at 1200 x g for 5 minutes to separate plasma and red cells. Plasma was stored frozen at -50 °C up to 9 months before shipment on dry ice for analyses of fat-soluble vitamins, carotenoids and APP at NICHE, University of Ulster, Coleraine, Northern Ireland.

Analysis

Retinol - plasma retinol was determined by reverse phase HPLC, using tocopherol acetate as the internal standard according to the method of Thurnham *et al* (1988). The method is described earlier (see study 1). Within assay variation was 5 % and between assay variation was 15 %.

Acute phase proteins

ACT and AGP - were analysed according to an in-house modification of the Dako immuno-turbimetric method (Dako Ltd, Denmark) described earlier (see studies 1 and 2).
The inter- and intra-assay variation for both ACT and AGP were 3 - 8% and 6 % respectively.

Table 2.1 - Methods used to analyse plasma samples for retinol

Study	Method used	
	HPLC	Spectrophotometry
UKPresc	Thurnham *et al* 1988	
Pakistan	DeRuyter & DeLeenheer 1978	
PNG	Craft 1996	
Nepal	Christian *et al* 1998	
GhanaM	Filteau *et al* 1993	
GhanaR	Filteau *et al* 1993	
SafricaS	Catignan & Bieri 1983	
SAFRmumH	Thurnham *et al* 1988	
SAFRinfH	Thurnham *et al* 1988	
SafricaP	Catignan & Bieri 1983	
Equador	Mansourian *et al* 1982	
Guatemal	Dary *et al* 1996	Dary *et al* 1996
Honduras		Dary & Arroyave 1996
Columbia	Bessey *et al* 1946	
Nigeria	Thurnham et al 1988	

Table 2.2 - Methods used for ACT, AGP and CRP analysis

Study Code	ACT		AGP			CRP		
	Immuno-turbidimetric	Radial immuno-diffusion	Immuno-turbid-imetric	Radial immuno-diffusion	ELISA	Radial immuno-diffusion	ELISA	Immuno-turbid-imetric
UKPresc	DAKO							
Pakistan	DAKO		DAKO					
PNG		Mancini *et al* 1965		Mancini et al 1965		Mancini et al 1965		
Nepal					Filteau et al 1994		Hemagen Diagnostics	
GhanaM			DAKO					
GhanaR					Filteau et al 1994		Filteau et al 1994	
SafricaS	DAKO		DAKO					
SAFRmumH	DAKO		DAKO					
SAFRinfH	DAKO		DAKO					
SafricaP								Behring
Equador			Behring					Behring
Guatemal	DAKO		DAKO					
Honduras			Behring Turbitime					
Columbia			Behring Turbitime					
Nigeria	DAKO		DAKO					

Section 3 - Statistical methods

3.1 Correction to data sets

3.1.1 Corrections to AGP values in the GhanaR study (study 6)

The measurement of AGP in studies 5 and 6 (GhanaM and GhanaR respectively) were done in the same laboratory and on different subsets of samples obtained from the same study, but there were methodological differences in the analysis of AGP in the two subsets. The subset for GhanaM was analysed by immunoprecipitation techniques using the COBAS-Bio while those analysed for GhanaR, were done using an ELISA method developed in house.

To ensure comparability, 72 samples were analysed by both methods and the following regression equation was obtained (Filteau et al 1994).

$$\text{ELISA} = 0.135 + 0.615 \text{ X COBAS}$$

Hence the GhanaR results obtained by ELISA were corrected using the following formula before they were used in the meta-analysis:

$$\text{corrected AGP} = (\text{old AGP} - 0.135)/0.615$$

Nepalese data: An ELISA technique was also used to analyse AGP in the samples obtained in study 4 (Christian et al 1998). Details of the ELISA methodology were discussed by Dr Filteau with Dr Christian but there were no attempts to compare methods by the two groups of workers. In view of the lack of any comparative data between the two studies, it was felt that there was no justification for making corrections to the AGP concentrations in the Nepalese study

3.1.2 Corrections to acute phase proteins in Guatemala data (study 12)

Preliminary analysis indicated that ACT and AGP values in Guatemala appeared to be particularly high relative to the other studies, in particular relative to the neighbouring countries of Colombia and Honduras. In contrast mean retinol values in Guatemala were slightly higher (1.17 µmol/L, Table 4.1) than those in both Colombia and Honduras (1.10 µmol/L, Table 4.1). Subsequent investigation (Omar Dary, personal communication) revealed a high likelihood that the samples were somewhat evaporated when the measurements of ACT and AGP were made, thereby increasing the measured concentrations.

Plasma retinol in all three countries was measured in a similar fashion and at a similar time relative to the collection of the samples (Study details). In contrast, acute phase proteins in Guatemala were done two to three years later and in a different laboratory (Study details and Table 2.2). Samples were stored in 5 ml vials but sample volumes were small ranging from 0.1 ml to rarely greater than 0.4 ml. The samples were stored at -20C throughout and shipped in dry ice to the Northern Ireland for analysis of the acute phase proteins. There is no evidence that acute phase proteins deteriorate on storage.

The ACT and AGP values for Guatemala were corrected by multiplying each observation by the ratio of the geometric mean AGP for Colombia to the geometric mean AGP for Guatemala. A consequence of this correction is that the geometric AGP means for Colombia and Guatemala are

now equal. The correction factor used was 0.71/1.46=0.48630. Without correction, 67.11% (610 out of 760 measurements) were above the cutoff of 1.0 defining high AGP values; after correction 9.34% were high. Corresponding percents for ACT, with a cutoff of 0.6, were 49.74% and 1.71%.

3.1.3 Identification and re-coding of missing values in specific data sets

In several data sets missing values were represented by numerical values. These were noted and re-coded as ".", the SAS value for missing data. For some APPs, values below a threshold were given a specific code. These were re-coded to the lowest detectable value. Since our analyses used APP values only to classify individuals as normal or high, the particular choice of a low re-code value had no effect on our results.

3.1.3.1 Colombia

Retinol values greater than 990 were defined as missing.
AGP values of 0.04 (n=79), 0.07 (n=1) and 0.09 (n=1) were re-coded as 0.04.
An age of 4 years was re-coded to missing.
Retinol values were divided by 286.46 to convert to µmol/L.
AGP values were divided by 100 to convert to g/L.

3.1.3.2 Equador

Age given in months was divided by 12 to give age in years/
Retinol values were divided by 286.46 to convert to µmol/L.
RBP values were divided by 21 to convert to µmol/L.
CRP values equal to 1 were re-coded to 3.

3.1.3.3 GhanaR and GhanaM

Age given in months was divided by 12 to give age in years.
Retinol values less than or equal to zero were declared missing.

3.1.3.4 Honduras

Age given in months was divided by 12 to give age in years.
Retinol values were divided by 286.46 to convert to µmol/L.
Gender was defined to be male if q18 was equal to 1.
AGP values of 9 were re-coded as missing, 8 as 5.5, and 7 as 0.04.
Only individual with retinol greater than zero and AGP greater than zero were included in the analysis.

3.1.3.5 Nepal

Age = 24.55 years, average reported.
AGP and CRP were exponentiated to convert to common units.
Retinol values were divided by 286.46 to convert to µmol/L.

3.1.3.6 Nigeria

Age given in days was divided by 365.25 to give age in years.

3.1.3.7 Pakistan

Age givein in months was divided by 12 to give age in years.

3.1.3.8 Papua New Guinea

Age given in months was divided by 12 to give age in years.

3.1.3.9 South Africa HIV infants
Selected 6 months (time =7) data from group 1 (placebo)

3.1.3.10 South Africa HIV Mums
Selected baseline data (time = 1) data from group 1 (placebo)

3.1.3.11 South African Pre-School Children
Retinol values were divided by 28.646 to convert to µmol/L.
CRP values zero or less were re-coded as missing.

3.1.3.12 South African Schoolchildren
Age given in months was divided by 12 to give age in years.

3.1.3.13 United Kingdom Pre-School
Age given in months (age 8) was divided by 12 to give age in years.
ACT values less than zero were declared missing.
Retinol values less than zero were declared missing.

3.2 Outline of statistical process

The basic idea of a cross-project analysis is to combine information from different studies to draw conclusions based on all of the data available. The data used in this project are summarized in table 4.1. This is the kind of analysis that has typically been done qualitatively when reviewing the literature in a particular area. With the advent of meta-analysis and related approaches, we now combine the information quantitatively. The method starts with a summary statistic, sometimes called an effect size, that is calculated with an assessment of its variability, for each study. In our case the summary is a difference between two means.

The variability associated with the summaries typically varies from study to study and is related to the sample sizes; studies with large numbers of observations will generally have smaller variability associated with summaries than studies with small numbers of observations. We use the term, *within-study variance* to describe this variation. When we combine the individual study to obtain an overall summary, each study is given a weight. The traditional weights, that are actually optimal in a theoretical sense, are proportional to the inverse of the within-study variance. In this way, studies with large variance (and therefore, a relatively imprecise estimate of the study summary) receive less weight and studies with small variance (and therefore, more precise estimate of the study summary) receive more weight. If we were to combine two studies with variances 10 and 5, for example, we would first compute the inverses of the variances, 0.1 and 0.2, respectively and then rescale these so that the sum is one. Thus, the weights would be 0.1/(0.1+0.2)=1/3 and 0.2/(0.1+0.2)=2/3. The study with the variance equal to 5 would receive twice as much weight as the study with variance equal to 10. In the outputs, this weighting scheme is labeled *Invar*.

To examine the sensitivity of the overall summary to the weights, we have used two additional weighting methods. We have generated weights that are inversely proportional to the sample sizes (*Nsub*) and we have given the same weight to each study (*Equal*). With the weights and the summaries for each study, it is straightforward to compute the overall summary. In the example above, if the study summaries were 6 and 9, respectively, and we use the Invar weights, then the overall summary would be (1/3)(6)+(2/3)(9)=8.

Computing an estimate of the variability of the overall summary is a bit more complicated and there are two different approaches. These involve different assumptions about the study to study variation. In the first case, called the *fixed effects* model, we assume that all studies are estimating the same true parameter and any differences in the individual study results are due to statistical sampling error. In other words, if sufficiently large numbers of observations were available, all studies would obtain the same estimate. The second approach is based on the *random effects* model. Here we allow the possibility that there are small but non-zero differences among studies. These may be due to different populations being sampled, different laboratories performing the analyses, and other factors. In this model, we produce an estimate of the study to study variation, expressed as a standard deviation and labeled *tau*. Given the model assumptions, derivation of the appropriate formulas for the variability of the overall summary involves some relatively straightforward technical details that will not be discussed here. Output includes the overall summary for each weighting scheme and each choice of assumption (fixed or random) with 95% confidence intervals. In general, an overall summary will have the smallest variability when the Invar weighting scheme is used and, because we would not be surprised to see real study-to-study differences here, we prefer the *random effects* model. For these reasons, when drawing conclusions from these data, we focus on results for the *random effects* model with the Invar weights. Results for the *fixed effects* model and the other choices of weights are included to provide an assessment of the sensitivity of the results to this choice.

The questions of interest involve comparison of serum retinol values for different subsets of individuals. Because the distribution of these values is typically skewed toward larger values, we have performed all analyses on the (natural) logs of these values. The results are then transformed back into the original scale to facilitate interpretation. A consequence of this approach is that the confidence intervals are not necessarily symmetric about the overall summary estimate. The choice of the log transformation is closely related to using the geometric mean as a summary; the back transformed mean of the logs is the geometric mean of the original data.

First we examine the relationship between each APP and serum retinol as follows. To facilitate the explanation, consider first ACT. For each study where ACT was measured, subjects were classified into two groups: *normal* if ACT is less than or equal to 0.6, *high* if ACT is greater than 0.6. The mean of the log retinol is calculated for each group and the study summary is the difference in the mean log retinol for the normal group minus the mean log retinol for the high group. This summary and the associated standard error are then used in the procedures described above to produce overall summary estimates in the original (not the log scale) with confidence intervals. Similar calculations are performed for AGP, CRP and SAA, with cutoffs 1.0, 5.0, and 5.0. We use the term *two-group analyses* to describe these summaries because the subjects are classified into two groups: normal and high (Table 4.2).

Using a similar approach, a *four-group analysis* is based on classifying subjects into the following groups based on values of a pair of APPs (Table 4.3). For Guatemala, Nigeria. Pakistan, SAFRinfH, SAFRmumH, and SafricS, these are: *healthy* with AGP less that 1.0 and ACT less than 0.6; *recently ill* with AGP less than 1.0 and ACT greater than or equal to 0.6; *chronically ill* with AGP greater than or equal to 1.0 and ACT less than 0.6; and *currently ill* with AGP greater than or equal to 1.0 and ACT greater than or equal to 0.6. The following table summarizes these definitions.

	Normal AGP	**High AGP**
Normal ACT	Healthy	Chronically ill
High ACT	Recently Ill	Currently ill

For Equador, GhanaR, Nepal, and PNG, CRP with a cutoff of 5.0 replaces ACT. Note that Equador and GhanaR do not have measurements on ACT and for PNG, ACT is available but use of CRP is preferred in this study.

With four groups, there are six pairs of mean log retinol results that can be compared. Each of these is summarized by study and then analyzed by the methods described above. To avoid the difficulty in interpretation that would occur if different weights were computed for the comparison of each pair, weights are computed basis on the total sample size for the four groups (Nsub) and the sum of the variances for the four groups (Ivar).

Examination of the results of the four-group analyses reveals that it is difficult to see the differences between some groups because of relatively small sample sizes (see Table 4.3 and Figure 5.1). Although we still believe that the four-group approach is the most sound from a theoretical perspective, practical considerations have led us to consider a *three-group analysis* in which the recently ill and chronically ill groups are combined. Details of the analysis are similar to that for the four-group analysis (Table 4.4).

We have carefully considered how these results could be used to better interpret estimates of mean (or geometric mean) retinol values and prevalence of low retinol in populations with elevated APP concentrations. Our results clearly indicate that high APP concentrations are associated with depressed serum retinol. To what extent does serum retinol depressed in this way influence the observed distribution? One way to address this question is as follows. It is easiest to see the basic idea with the two-group analysis but the same concept is easily applied with three or four groups. Consider ACT. Subjects are classified as high or normal depending on whether of not the ACT is greater than 0.6. We can calculate the prevalence of low retinol concentrations (less than 0.7) for each of these subgroups. For example, the Colombia data have the prevalence of low retinol concentrations is 10.3% in the normal ACT group and 24.4% in the high ACT group. To standardize the Columbia prevalence to a reference population with no high ACT concentrations we would simply use the 10.3% rate for the normal ACT group; to standardize to a reference population with all high ACT concentrations, we would use the 24.4% rate for the high ACT group. For any desired mix of normal and high ACT concentrations that could be viewed as a standardized population, we can compute the appropriate rate by combining the two observed prevalences. For example, for a reference population with 88% normal ACT values (and therefore, 12% high ACT values), we compute the rate as 0.88(10.3%)+0.12(24.4%)=12.0%. Additional computations give the standard error for this estimate as 0.8%. The same idea can be applied to the three and four group analyses and to the geometric mean of the serum retinol concentrations.

Section 4 - TABLES

Tables 4.1 - Study Summaries

Study site = **Colombia**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	1756	0.47	0.50	0.00	0.00	0.00	1.00	1.00
Age (years)	1756	1.51	1.13	0.00	0.50	1.00	3.00	3.00
Retinol (umol/L)	1754	1.10	0.40	0.06	0.83	1.06	1.32	2.87
ACT	0	.	.	.	.	.	.	.
AGP	1641	0.80	0.36	0.40	0.55	0.71	0.94	4.34
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Study site = **Equador**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	1277	0.50	0.50	0.00	0.00	1.00	1.00	1.00
Age (years)	1277	2.64	1.40	0.00	1.50	2.75	3.92	4.92
Retinol (umol/L)	1277	0.99	0.28	0.24	0.80	0.97	1.16	2.08
ACT	0	.	.	.	.	.	.	.
AGP	919	0.85	0.32	0.28	0.63	0.77	1.00	2.66
CRP	916	6.30	12.07	3.00	3.00	3.00	3.00	263.00
SAA	0	.	.	.	.	.	.	.

Study site = **GhanaM**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	0	.	.	.	.	.	.	.
Age (years)	175	1.80	1.16	0.08	0.75	1.67	2.67	4.33
Retinol (umol/L)	178	0.61	0.34	0.15	0.39	0.54	0.73	2.05
ACT	0	.	.	.	.	.	.	.
AGP	172	1.26	0.50	0.41	0.92	1.18	1.54	2.84
CRP	0	.	.	.	.	.	.	.
SAA	178	8.58	18.67	0.05	0.45	1.91	6.72	188.94

Study site = **GhanaR**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	0	.	.	.	.	.	.	.
Age (years)	207	1.65	1.14	0.08	0.67	1.50	2.42	4.25
Retinol (umol/L)	303	0.72	0.35	0.13	0.47	0.66	0.94	2.05
ACT	0	.	.	.	.	.	.	.
AGP	303	1.21	0.74	0.24	0.74	1.03	1.54	5.27
CRP	302	15.76	24.92	0.22	1.68	7.35	19.60	216.50
SAA	303	8.03	15.88	0.10	0.53	2.33	6.99	134.10

Study site = **Guatemal**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	0	.	.	.	.	.	.	.
Age (years)	737	3.14	1.04	0.18	2.30	3.18	3.98	5.69
Retinol (umol/L)	759	1.17	0.38	0.26	0.89	1.11	1.37	2.67
ACT	760	0.34	0.14	0.06	0.23	0.31	0.43	1.01
AGP	760	0.80	0.32	0.13	0.57	0.76	0.98	2.26
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Study site = **Honduras**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	1523	0.52	0.50	0.00	0.00	1.00	1.00	1.00
Age (years)	1518	2.61	0.87	1.00	2.00	2.42	3.00	7.25
Retinol (umol/L)	1516	1.10	0.39	0.03	0.86	1.08	1.33	2.83
ACT	0	.	.	.	.	.	.	.
AGP	1523	0.80	0.42	0.04	0.56	0.71	0.96	5.50
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Study site = **Nepal**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	224	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Age (years)	224	24.55	0.00	24.55	24.55	24.55	24.55	24.55
Retinol (umol/L)	224	0.87	0.43	0.11	0.55	0.82	1.18	2.33
ACT	0	.	.	.	.	.	.	.
AGP	214	0.64	0.41	0.19	0.37	0.51	0.77	3.06
CRP	212	4.89	9.93	0.67	1.46	2.07	4.16	100.00
SAA	0	.	.	.	.	.	.	.

Study site = **Nigeria**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	194	0.59	0.49	0.00	0.00	1.00	1.00	1.00
Age (years)	194	0.01	0.01	0.00	0.01	0.01	0.02	0.05
Retinol (umol/L)	193	0.59	0.28	0.09	0.39	0.55	0.73	1.86
ACT	194	0.43	0.24	0.05	0.28	0.37	0.51	1.40
AGP	194	0.77	0.57	0.13	0.41	0.60	0.96	3.03
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Study site = **PNG**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	0	.	.	.	.	.	.	.
Age (years)	90	3.17	1.17	0.75	2.17	3.33	4.17	5.08
Retinol (umol/L)	90	0.68	0.35	0.23	0.37	0.65	0.97	1.90
ACT	90	0.33	0.25	0.07	0.16	0.31	0.41	1.98
AGP	89	0.80	0.28	0.30	0.61	0.76	0.96	1.82
CRP	90	17.49	30.10	3.00	3.00	7.49	20.78	223.20
SAA	0	.	.	.	.	.	.	.

Study site = **Pakistan**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	2755	0.53	0.50	0.00	0.00	1.00	1.00	1.00
Age (years)	2755	2.73	1.45	0.50	1.34	2.52	4.16	5.11
Retinol (umol/L)	2751	0.90	0.37	0.07	0.64	0.85	1.09	2.90
ACT	2528	0.41	0.16	0.06	0.31	0.39	0.49	1.38
AGP	2530	1.30	0.64	0.04	0.85	1.14	1.60	3.29
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Study site = **SAFRinfH**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	0	.	.	.	.	.	.	.
Age (years)	28	0.50	0.00	0.50	0.50	0.50	0.50	0.50
Retinol (umol/L)	28	1.19	0.49	0.53	0.87	1.13	1.34	2.33
ACT	27	0.30	0.13	0.12	0.21	0.27	0.33	0.65
AGP	27	0.87	0.46	0.29	0.55	0.79	1.12	2.24
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Study site = **SAFRmumH**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	54	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Age (years)	53	25.96	5.93	15.00	21.00	25.00	31.00	38.00
Retinol (umol/L)	54	1.12	0.57	0.09	0.73	0.99	1.45	2.58
ACT	54	0.24	0.07	0.13	0.19	0.25	0.27	0.50
AGP	54	0.47	0.16	0.15	0.37	0.46	0.52	1.08
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Study site = **SAfricS**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	205	0.44	0.50	0.00	0.00	0.00	1.00	1.00
Age (years)	187	9.21	0.75	7.92	8.58	9.17	9.75	10.92
Retinol (umol/L)	205	0.82	0.33	0.07	0.59	0.80	1.01	2.02
ACT	205	0.34	0.11	0.15	0.28	0.34	0.38	1.00
AGP	205	0.99	0.50	0.30	0.70	0.86	1.11	3.08
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Study site = **SAfricaP**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	4197	0.50	0.50	0.00	0.00	0.00	1.00	1.00
Age (years)	4197	3.41	1.45	0.50	2.25	3.50	4.67	5.92
Retinol (umol/L)	4283	0.87	0.31	0.07	0.66	0.85	1.06	2.51
ACT	0	.	.	.	.	.	.	.
AGP	0	.	.	.	.	.	.	.
CRP	579	13.02	17.14	2.40	3.90	6.50	13.10	124.00
SAA	0	.	.	.	.	.	.	.

Study site = **UKPresc**

Variable	N	Mean	SD	Min	Q1	Median	Q3	Max
Sexmale (0=f,1=m)	816	0.50	0.50	0.00	0.00	1:00	1.00	1.00
Age (years)	816	3.00	0.83	1.42	2.33	3.08	3.75	4.50
Retinol (umol/L)	815	1.01	0.25	0.26	0.84	1.00	1.16	2.07
ACT	753	0.48	0.15	0.13	0.38	0.45	0.53	1.18
AGP	0	.	.	.	.	.	.	.
CRP	0	.	.	.	.	.	.	.
SAA	0	.	.	.	.	.	.	.

Tables 4.2 - Two group analyses including Guatemala

Meta Analysis with two groups 1
Apparently normal Studies - log retinol analysis
ACT Meta Analysis

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	NHI	MHI	SEHI
1	Guatemal	App_Norm	759	1	0.56431	0.23514	32	4.73180	0.069631
2	Pakistan	App_Norm	2524	1	1.87658	1.45523	276	4.25507	0.026972
3	UKPresc	App_Norm	752	1	0.55911	1.30963	129	4.46661	0.028458

OBS	NLO	MLO	SELO	RATIO	LCLR	UCLR	Z	P
1	727	4.70452	0.012038	0.97309	0.84723	1.11764	-0.38606	0.69945
2	2248	4.43147	0.008908	1.19292	1.12832	1.26121	6.21023	0.00000
3	623	4.61474	0.009309	1.15967	1.09357	1.22976	4.94735	0.00000

Meta Analysis with two groups 2
Apparently normal Studies - log retinol analysis
ACT Meta Analysis

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4035	3	Equal	1.10416	0.97455	1.25101	1.55531
2	4035	3	Nsub	1.14204	0.99735	1.30773	1.92159
3	4035	3	Invar	1.15963	1.06407	1.26377	3.37525

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.11987	0.099718	1.04668	1.16480	3.63244	.00028076
2	0.05466	0.096020	1.09196	1.19443	5.80490	.00000001
3	0.00074	0.059586	1.11552	1.20547	7.48597	.00000000

Meta Analysis with two groups 3
Apparently normal Studies - log retinol analysis
AGP Meta Analysis

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	NHI	MHI	SEHI
1	Colombia	App_Norm	1639	1	1.28348	0.82914	344	4.46292	0.026372
2	Equador	App_Norm	919	1	0.71966	1.14737	224	4.40893	0.022036
3	GhanaR	App_Norm	303	1	0.23727	0.16806	158	4.06579	0.044265
4	Guatemal	App_Norm	759	1	0.59436	0.76426	177	4.63801	0.026270
5	Honduras	App_Norm	1516	1	1.18716	0.81562	328	4.39969	0.026130
6	Pakistan	App_Norm	2526	1	1.97807	2.27555	1555	4.37843	0.011246

OBS	NLO	MLO	SELO	RATIO	LCLR	UCLR	Z	P
1	1295	4.68489	0.010041	1.24854	1.18136	1.31954	7.8661	.0000000
2	695	4.63149	0.009478	1.24927	1.19189	1.30941	9.2777	.0000000
3	145	4.23487	0.044376	1.18421	1.04731	1.33901	2.6976	.0069848
4	582	4.72625	0.013183	1.09225	1.03110	1.15702	3.0020	.0026818
5	1188	4.69364	0.011257	1.34171	1.26894	1.41866	10.3314	.0000000
6	971	4.47026	0.012793	1.09618	1.06018	1.13339	5.3909	.0000001

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	7662	6	Equal	1.19874	1.12345	1.27908	5.47703
2	7662	6	Nsub	1.19487	1.09969	1.29829	4.20362
3	7662	6	Invar	1.17821	1.08365	1.28101	3.84212

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	.00000004	0.073222	1.16582	1.23258	12.7612	0
2	.00002627	0.087285	1.16922	1.22108	16.0817	0
3	.00012198	0.085349	1.15423	1.20268	15.6333	0

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	NHI	MHI	SEHI
1	Equador	App_Norm	916	1	1.52922	1.65826	161	4.37874	0.025044
2	GhanaR	App_Norm	302	1	0.50417	0.31803	173	4.02903	0.042897
3	SAfricaP	App_Norm	579	1	0.96661	1.02371	341	4.14983	0.022054

OBS	NLO	MLO	SELO	RATIO	LCLR	UCLR	Z	P
1	755	4.61800	0.009588	1.27032	1.20527	1.33887	8.92220	.0000000000
2	129	4.30245	0.043699	1.31446	1.16579	1.48208	4.46509	.0000080034
3	238	4.33087	0.026049	1.19846	1.12091	1.28138	5.30432	.0000001131

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	1797	3	Equal	1.26017	1.19529	1.32857	8.57462
2	1797	3	Nsub	1.25388	1.19922	1.31104	9.94911
3	1797	3	Invar	1.24984	1.19286	1.30955	9.36724

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0	0.017433	1.19986	1.32350	9.2425	0
2	0	0.016245	1.20488	1.30488	11.1234	0
3	0	0.019770	1.20194	1.29965	11.1858	0

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	NHI	MHI	SEHI	NLO	MLO
1	GhanaR	App_Norm	303	1	1	1	99	3.96067	0.056721	204	4.23698

OBS	SELO	RATIO	LCLR	UCLR	Z	P
1	0.036635	1.31826	1.15485	1.50480	4.09210	.000042749

Apparently normal Studies - log retinol analysis

SAA Meta Analysis

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	303	1	Equal	1.31826	1.15485	1.50480	4.09210
2	303	1	Nsub	1.31826	1.15485	1.50480	4.09210
3	303	1	Invar	1.31826	1.15485	1.50480	4.09210

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	.000042749	0	1.15485	1.50480	4.09210	.000042749
2	.000042749	0	1.15485	1.50480	4.09210	.000042749
3	.000042749	0	1.15485	1.50480	4.09210	.000042749

All Studies - log retinol analysis

ACT Meta Analysis

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	NHI	MHI	SEHI
1	Guatemal	App_Norm	759	1	1.16769	0.50357	32	4.73180	0.06963
2	Pakistan	App_Norm	2524	1	3.88308	3.11651	276	4.25507	0.02697
3	UKPresc	App_Norm	752	1	1.15692	2.80471	129	4.46661	0.02846
4	PNG	Sick	90	1	0.13846	0.05857	8	3.81835	0.19945
5	SAfricS	Sick	205	1	0.31538	0.10647	6	3.84482	0.14996
6	Nigeria	Infants	193	1	0.29692	0.29327	33	4.02592	0.08391
7	SAFRinfH	Infants	27	1	0.04154	0.11689	2	4.56144	0.12239

OBS	NLO	MLO	SELO	RATIO	LCLR	UCLR	Z	P
1	727	4.70452	0.012038	0.97309	0.84723	1.11764	-0.38606	0.69945
2	2248	4.43147	0.008908	1.19292	1.12832	1.26121	6.21023	0.00000
3	623	4.61474	0.009309	1.15967	1.09357	1.22976	4.94735	0.00000
4	82	4.11943	0.056131	1.35132	0.90031	2.02828	1.45313	0.14619
5	199	4.32034	0.033599	1.60886	1.19043	2.17436	3.09425	0.00197
6	160	3.96039	0.039151	0.93657	0.78113	1.12295	-0.70770	0.47913
7	25	4.71508	0.080825	1.16606	0.87474	1.55442	1.04750	0.29487

All Studies - log retinol analysis

ACT Meta Analysis

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4550	7	Equal	1.18057	1.02928	1.35409	2.37254
2	4550	7	Nsub	1.15407	0.99190	1.34276	1.85479
3	4550	7	Invar	1.15662	1.04353	1.28195	2.77178

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.017666	0.13966	1.07895	1.29175	3.61477	.00030062
2	0.063627	0.12213	1.10494	1.20539	6.45619	.00000000
3	0.005575	0.08089	1.11444	1.20039	7.67685	.00000000

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	NHI	MHI	SEHI
1	Colombia	App_Norm	1639	1	2.47296	1.67073	344	4.46292	0.02637
2	Equador	App_Norm	919	1	1.38661	2.31195	224	4.40893	0.02204
3	GhanaR	App_Norm	303	1	0.45717	0.33864	158	4.06579	0.04427
4	Guatemal	App_Norm	759	1	1.14519	1.53999	177	4.63801	0.02627
5	Honduras	App_Norm	1516	1	2.28737	1.64349	328	4.39969	0.02613
6	Pakistan	App_Norm	2526	1	3.81128	4.58524	1555	4.37843	0.01125
7	GhanaM	Sick	172	1	0.25952	0.19565	119	3.87856	0.04830
8	PNG	Sick	89	1	0.13429	0.08244	17	3.79854	0.11203
9	SAfricS	Sick	205	1	0.30931	0.26857	65	4.24837	0.05731
10	Nigeria	Infants	193	1	0.29120	0.20647	46	4.01358	0.06882
11	SAFRinfH	Infants	27	1	0.04074	0.08242	7	4.55617	0.08331
12	SAFRmumH	Women	54	1	0.08148	.	1	4.28562	.
13	Nepal	Women	214	1	0.32289	0.07441	30	4.00835	0.12683

OBS	NLO	MLO	SELO	RATIO	LCLR	UCLR	Z	P
1	1295	4.68489	0.010041	1.24854	1.18136	1.31954	7.8661	0.00000
2	695	4.63149	0.009478	1.24927	1.19189	1.30941	9.2777	0.00000
3	145	4.23487	0.044376	1.18421	1.04731	1.33901	2.6976	0.00698
4	582	4.72625	0.013183	1.09225	1.03110	1.15702	3.0020	0.00268
5	1188	4.69364	0.011257	1.34171	1.26894	1.41866	10.3314	0.00000
6	971	4.47026	0.012793	1.09618	1.06018	1.13339	5.3909	0.00000
7	53	4.14822	0.066837	1.30952	1.11409	1.53924	3.2701	0.00107
8	72	4.16808	0.059892	1.44706	1.12811	1.85619	2.9089	0.00363
9	140	4.33338	0.040861	1.08874	0.94844	1.24978	1.2079	0.22707
10	147	3.95846	0.041316	0.94637	0.80860	1.10761	-0.6867	0.49226
11	20	4.75533	0.095919	1.22037	0.95137	1.56544	1.5676	0.11698
12	53	4.57953	0.081280	1.34166	.	.	.	.
13	184	4.36723	0.042332	1.43173	1.10166	1.86071	2.6841	0.00727

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	8616	13	Equal	1.22222	1.14330	1.30658	5.89227
2	8616	13	Nsub	1.19685	1.10571	1.29550	4.44667
3	8616	13	Invar	1.19328	1.10010	1.29436	4.25968

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	.000000004	0.096232	1.17258	1.27395	9.4865	0
2	.000008721	0.092838	1.17157	1.22267	16.5015	0
3	.000020472	0.089016	1.16985	1.21718	17.4676	0

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	NHI	MHI	SEHI
1	Equador	App_Norm	916	1	2.18199	2.60941	161	4.37874	0.02504
2	GhanaR	App_Norm	302	1	0.71939	0.50044	173	4.02903	0.04290
3	SAfricaP	App_Norm	579	1	1.37923	1.61090	341	4.14983	0.02205
4	PNG	Sick	90	1	0.21439	0.15889	55	3.99528	0.06877
5	Nepal	Women	212	1	0.50500	0.12035	38	3.95405	0.11802

OBS	NLO	MLO	SELO	RATIO	LCLR	UCLR	Z	P
1	755	4.61800	0.009588	1.27032	1.20527	1.33887	8.92220	0.000000
2	129	4.30245	0.043699	1.31446	1.16579	1.48208	4.46509	0.000008
3	238	4.33087	0.026049	1.19846	1.12091	1.28138	5.30432	0.000000
4	35	4.24571	0.084147	1.28458	1.03814	1.58953	2.30439	0.021201
5	174	4.39877	0.040795	1.56006	1.22138	1.99265	3.56153	0.000369

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN	P_RAN
1	2099	5	Equal	1.32019	1.21009	1.44031	6.25216	.00000000040481
2	2099	5	Nsub	1.28319	1.18787	1.38616	6.33146	.00000000024285
3	2099	5	Invar	1.25763	1.19095	1.32804	8.24763	.00000000000000

OBS	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.057117	1.22940	1.41769	7.6413	2.1538E-14
2	0.059710	1.22904	1.33973	11.3342	0
3	0.032003	1.21077	1.30630	11.8323	0

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	NHI	MHI	SEHI
1	GhanaR	App_Norm	303	1	1.25988	1.20341	99	3.96067	0.056721
2	GhanaM	Sick	178	1	0.74012	0.79659	54	3.79689	0.067552

OBS	NLO	MLO	SELO	RATIO	LCLR	UCLR	Z	P
1	204	4.23698	0.036635	1.31826	1.15485	1.50480	4.09210	.0000427
2	124	4.03921	0.048216	1.27420	1.08290	1.49928	2.91967	.0035040

Meta Analysis with two groups
All Studies - log retinol analysis
SAA Meta Analysis

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	481	2	Equal	1.29604	1.16703	1.43931	4.84733
2	481	2	Nsub	1.30178	1.17457	1.44277	5.02682
3	481	2	Invar	1.30053	1.17364	1.44114	5.01685

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	.0000012514	0	1.16703	1.43931	4.84733	.0000012514
2	.0000004987	0	1.17457	1.44277	5.02682	.0000004987
3	.0000005253	0	1.17364	1.44114	5.01685	.0000005253

Table 4.3 - Four group analyses including Guatemala

Meta Analysis with four groups 1
00=healthy, 01=recent illness, 10=chronic illness, 11=current illness
Apparently normal Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	N00	M00	SE00
1	Equador	App_Norm	911	1	0.81230	2.84665	646	4.64327	0.009692
2	GhanaR	App_Norm	302	1	0.26928	0.65668	85	4.28072	0.059099
3	Guatemal	App_Norm	759	1	0.67677	0.38645	575	4.72466	0.013250
4	Pakistan	App_Norm	2514	1	2.24164	0.11022	959	4.47133	0.012770

OBS	N01	M01	SE01	N10	M10	SE10	N11	M11	SE11
1	45	4.46909	0.03704	104	4.47847	0.029396	116	4.34368	0.031134
2	60	4.16991	0.06670	44	4.34442	0.058506	113	3.95422	0.054205
3	7	4.85630	0.12945	152	4.62832	0.027557	25	4.69694	0.081312
4	5	4.06852	0.29010	1281	4.40331	0.012253	269	4.25879	0.027197

Meta Analysis with four groups 2
00=healthy, 01=recent illness, 10=chronic illness, 11=current illness
Apparently normal Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	D00_01	SED00_01	LCL00_01	UCL00_01	Z00_01	P00_01
1	Equador	App_Norm	0.17418	0.03829	0.09914	0.24922	4.54930	0.00001
2	GhanaR	App_Norm	0.11081	0.08911	-0.06385	0.28547	1.24349	0.21369
3	Guatemal	App_Norm	-0.13163	0.13012	-0.38668	0.12341	-1.01159	0.31173
4	Pakistan	App_Norm	0.40281	0.29038	-0.16634	0.97195	1.38717	0.16539

Meta Analysis with four groups 3
00=healthy, 01=recent illness, 10=chronic illness, 11=current illness
Apparently normal Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	D00_10	SED00_10	LCL00_10	UCL00_10	Z00_10	P00_10
1	Equador	App_Norm	0.16480	0.030953	0.10413	0.22547	5.32432	0.00000
2	GhanaR	App_Norm	-0.06370	0.083160	-0.22670	0.09929	-0.76604	0.44365
3	Guatemal	App_Norm	0.09635	0.030577	0.03641	0.15628	3.15088	0.00163
4	Pakistan	App_Norm	0.06802	0.017698	0.03333	0.10270	3.84308	0.00012

Meta Analysis with four groups 4
00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

Apparently normal Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	D00_11	SED00_11	LCL00_11	UCL00_11	Z00_11	P00_11
1	Equador	App_Norm	0.29959	0.032608	0.23567	0.36350	9.18754	0.00000
2	GhanaR	App_Norm	0.32650	0.080193	0.16932	0.48368	4.07143	0.00005
3	Guatemal	App_Norm	0.02772	0.082384	-0.13375	0.18919	0.33650	0.73650
4	Pakistan	App_Norm	0.21254	0.030046	0.15365	0.27143	7.07383	0.00000

OBS	STUDY	TYPE	D01_10	SED01_10	LCL01_10	UCL01_10	Z01_10	P01_10
1	Equador	App_Norm	-0.00937	0.04729	-0.10206	0.08331	-0.19825	0.84285
2	GhanaR	App_Norm	-0.17452	0.08872	-0.34841	-0.00062	-1.96701	0.04918
3	Guatemal	App_Norm	0.22798	0.13235	-0.03143	0.48738	1.72255	0.08497
4	Pakistan	App_Norm	-0.33479	0.29036	-0.90389	0.23431	-1.15302	0.24890

OBS	STUDY	TYPE	D01_11	SED01_11	LCL01_11	UCL01_11	Z01_11	P01_11
1	Equador	App_Norm	0.12541	0.04839	0.03057	0.22025	2.59182	0.00955
2	GhanaR	App_Norm	0.21569	0.08595	0.04724	0.38414	2.50960	0.01209
3	Guatemal	App_Norm	0.15935	0.15287	-0.14027	0.45898	1.04244	0.29721
4	Pakistan	App_Norm	-0.19027	0.29137	-0.76135	0.38082	-0.65300	0.51375

OBS	STUDY	TYPE	D10_11	SED10_11	LCL10_11	UCL10_11	Z10_11	P10_11
1	Equador	App_Norm	0.13478	0.042819	0.05086	0.21871	3.14776	0.00165
2	GhanaR	App_Norm	0.39020	0.079757	0.23388	0.54653	4.89242	0.00000
3	Guatemal	App_Norm	-0.06862	0.085855	-0.23690	0.09965	-0.79930	0.42412
4	Pakistan	App_Norm	0.14452	0.029830	0.08606	0.20299	4.84494	0.00000

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	1.14917	0.92653	1.42531	1.26545
2	4486	4	Nsub	1.27928	0.87562	1.86902	1.27331
3	4486	4	Invar	1.15088	0.97702	1.35569	1.68176

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.20571	0.14361	0.97632	1.35262	1.67188	0.09455
2	0.20291	0:16324	0.92669	1.76602	1.49717	0.13435
3	0.09262	0.10334	1.07599	1.23098	4.09356	0.00004

00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

Apparently normal Studies - log retinol analysis

Chronic illness (10) versus Healthy (00)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	1.06862	0.97289	1.17376	1.38608
2	4486	4	Nsub	1.08719	1.00591	1.17503	2.10874
3	4486	4	Invar	1.12525	0.94793	1.33575	1.34877

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.16572	0.08300	1.01976	1.11982	2.77942	.0054456
2	0.03497	0.05951	1.05776	1.11743	5.97133	.0000000
3	0.17741	0.11328	1.06916	1.18429	4.52344	.0000061

00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

Apparently normal Studies - log retinol analysis

Current illness (11) versus Healthy (00)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	1.24183	1.08797	1.41746	3.20925
2	4486	4	Nsub	1.22951	1.07617	1.40471	3.04007
3	4486	4	Invar	1.31698	1.11880	1.55026	3.30911

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	.0013308	0.12009	1.16907	1.31912	7.03068	2.0552E-12
2	.0023652	0.10234	1.17424	1.28739	8.80380	0
3	.0009359	0.10635	1.24701	1.39088	9.88499	0

00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

Apparently normal Studies - log retinol analysis

Chronic illness (10) versus Recent illness (01)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	0.92990	0.73467	1.17702	-0.60445
2	4486	4	Nsub	0.84985	0.56928	1.26868	-0.79588
3	4486	4	Invar	0.97773	0.80129	1.19302	-0.22181

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.54555	0.17275	0.78930	1.09555	-0.86890	0.38490
2	0.42610	0.19442	0.61545	1.17351	-0.98820	0.32305
3	0.82446	0.12679	0.90462	1.05674	-0.56801	0.57003

00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

Apparently normal Studies - log retinol analysis

Current illness (11) versus Recent illness (01)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	1.08063	0.90376	1.29212	0.85037
2	4486	4	Nsub	0.96110	0.68371	1.35104	-0.22836
3	4486	4	Invar	1.14432	1.05630	1.23967	3.30144

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.39512	0.061360	0.91323	1.27872	0.90301	0.36652
2	0.81937	0.083790	0.69456	1.32993	-0.23943	0.81077
3	0.00096	0.000000	1.05630	1.23967	3.30144	0.00096

00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

Apparently normal Studies - log retinol analysis

Current illness (11) versus Chronic illness (10)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	1.16209	0.96679	1.39685	1.60019
2	4486	4	Nsub	1.13091	0.96517	1.32511	1.52150
3	4486	4	Invar	1.17039	0.91484	1.49732	1.25180

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.10956	0.17646	1.09130	1.23748	4.68423	.0000028102
2	0.12814	0.12369	1.07812	1.18629	5.04382	.0000004563
3	0.21064	0.16405	1.09451	1.25152	4.60064	.0000042119

00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

All Studies - log retinol analysis

Study Summaries

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	N00	M00	SE00
1	Equador	App_Norm	911	1	1.28181	4.52202	646	4.64327	0.009692
2	GhanaR	App_Norm	302	1	0.42492	1.04316	85	4.28072	0.059099
3	Guatemal	App_Norm	759	1	1.06794	0.61389	575	4.72466	0.013250
4	Pakistan	App_Norm	2514	1	3.53729	0.17509	959	4.47133	0.012770
5	PNG	Sick	89	1	0.12523	0.11075	33	4.26788	0.086665
6	Nigeria	Infants	193	1	0.27156	0.37792	138	3.94382	0.042908
7	Nepal	Women	207	1	0.29126	0.15716	156	4.42116	0.042279

OBS	N01	M01	SE01	N10	M10	SE10	N11	M11	SE11
1	45	4.46909	0.03704	104	4.47847	0.02940	116	4.34368	0.03113
2	60	4.16991	0.06670	44	4.34442	0.05851	113	3.95422	0.05420
3	7	4.85630	0.12945	152	4.62832	0.02756	25	4.69694	0.08131
4	5	4.06852	0.29010	1281	4.40331	0.01225	269	4.25879	0.02720
5	39	4.08363	0.08131	2	3.88002	0.32467	15	3.78768	0.12321
6	9	4.18287	0.13672	22	4.06432	0.09200	24	3.96706	0.10237
7	22	3.99706	0.15962	14	4.15824	0.18079	15	3.85571	0.18703

00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

All Studies - log retinol analysis

Study Summaries

OBS	STUDY	TYPE	D00_01	SED00_01	LCL00_01	UCL00_01	Z00_01	P00_01
1	Equador	App_Norm	0.17418	0.03829	0.09914	0.24922	4.54930	0.00001
2	GhanaR	App_Norm	0.11081	0.08911	-0.06385	0.28547	1.24349	0.21369
3	Guatemal	App_Norm	-0.13163	0.13012	-0.38668	0.12341	-1.01159	0.31173
4	Pakistan	App_Norm	0.40281	0.29038	-0.16634	0.97195	1.38717	0.16539
5	PNG	Sick	0.18424	0.11884	-0.04868	0.41717	1.55036	0.12105
6	Nigeria	Infants	-0.23905	0.14329	-0.51990	0.04181	-1.66823	0.09527
7	Nepal	Women	0.42411	0.16513	0.10046	0.74775	2.56839	0.01022

00=healthy, 01=recent illness, 10=chronic illness, 11=current illness

All Studies - log retinol analysis

Study Summaries

OBS	STUDY	TYPE	D00_10	SED00_10	LCL00_10	UCL00_10	Z00_10	P00_10
1	Equador	App_Norm	0.16480	0.03095	0.10413	0.22547	5.32432	0.00000
2	GhanaR	App_Norm	-0.06370	0.08316	-0.22670	0.09929	-0.76604	0.44365
3	Guatemal	App_Norm	0.09635	0.03058	0.03641	0.15628	3.15088	0.00163
4	Pakistan	App_Norm	0.06802	0.01770	0.03333	0.10270	3.84308	0.00012
5	PNG	Sick	0.38786	0.33604	-0.27078	1.04650	1.15420	0.24842
6	Nigeria	Infants	-0.12050	0.10152	-0.31948	0.07847	-1.18700	0.23523
7	Nepal	Women	0.26292	0.18567	-0.10099	0.62683	1.41606	0.15676

All Studies - log retinol analysis

Study Summaries

OBS	STUDY	TYPE	D00_11	SED00_11	LCL00_11	UCL00_11	Z00_11	P00_11
1	Equador	App_Norm	0.29959	0.03261	0.23567	0.36350	9.18754	0.00000
2	GhanaR	App_Norm	0.32650	0.08019	0.16932	0.48368	4.07143	0.00005
3	Guatemal	App_Norm	0.02772	0.08238	-0.13375	0.18919	0.33650	0.73650
4	Pakistan	App_Norm	0.21254	0.03005	0.15365	0.27143	7.07383	0.00000
5	PNG	Sick	0.48020	0.15064	0.18495	0.77545	3.18778	0.00143
6	Nigeria	Infants	-0.02324	0.11100	-0.24080	0.19432	-0.20940	0.83413
7	Nepal	Women	0.56545	0.19175	0.18962	0.94128	2.94888	0.00319

All Studies - log retinol analysis

Study Summaries

OBS	STUDY	TYPE	D01_10	SED01_10	LCL01_10	UCL01_10	Z01_10	P01_10
1	Equador	App_Norm	-0.00937	0.04729	-0.10206	0.08331	-0.19825	0.84285
2	GhanaR	App_Norm	-0.17452	0.08872	-0.34841	-0.00062	-1.96701	0.04918
3	Guatemal	App_Norm	0.22798	0.13235	-0.03143	0.48738	1.72255	0.08497
4	Pakistan	App_Norm	-0.33479	0.29036	-0.90389	0.23431	-1.15302	0.24890
5	PNG	Sick	0.20361	0.33470	-0.45240	0.85962	0.60834	0.54296
6	Nigeria	Infants	0.11854	0.16479	-0.20445	0.44154	0.71935	0.47193
7	Nepal	Women	-0.16119	0.24117	-0.63389	0.31151	-0.66835	0.50391

All Studies - log retinol analysis

Study Summaries

OBS	STUDY	TYPE	D01_11	SED01_11	LCL01_11	UCL01_11	Z01_11	P01_11
1	Equador	App_Norm	0.12541	0.04839	0.03057	0.22025	2.59182	0.00955
2	GhanaR	App_Norm	0.21569	0.08595	0.04724	0.38414	2.50960	0.01209
3	Guatemal	App_Norm	0.15935	0.15287	-0.14027	0.45898	1.04244	0.29721
4	Pakistan	App_Norm	-0.19027	0.29137	-0.76135	0.38082	-0.65300	0.51375
5	PNG	Sick	0.29595	0.14762	0.00661	0.58530	2.00479	0.04499
6	Nigeria	Infants	0.21580	0.17080	-0.11896	0.55057	1.26350	0.20641
7	Nepal	Women	0.14134	0.24589	-0.34059	0.62328	0.57483	0.56541

All Studies - log retinol analysis

Study Summaries

OBS	STUDY	TYPE	D10_11	SED10_11	LCL10_11	UCL10_11	Z10_11	P10_11
1	Equador	App_Norm	0.13478	0.04282	0.05086	0.21871	3.14776	0.00165
2	GhanaR	App_Norm	0.39020	0.07976	0.23388	0.54653	4.89242	0.00000
3	Guatemal	App_Norm	-0.06862	0.08585	-0.23690	0.09965	-0.79930	0.42412
4	Pakistan	App_Norm	0.14452	0.02983	0.08606	0.20299	4.84494	0.00000
5	PNG	Sick	0.09234	0.34726	-0.58830	0.77298	0.26591	0.79031
6	Nigeria	Infants	0.09726	0.13764	-0.17252	0.36703	0.70661	0.47981
7	Nepal	Women	0.30253	0.26013	-0.20732	0.81238	1.16301	0.24483

Meta Analysis with four groups
00=healthy, 01=recent illness, 10=chronic illness, 11=current illness
All Studies - log retinol analysis
Recent illness (01) versus Healthy (00)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4975	7	Equal	1.14135	0.94937	1.37214	1.40708
2	4975	7	Nsub	1.26334	0.89100	1.79126	1.31216
3	4975	7	Invar	1.13552	0.92593	1.39256	1.22079

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.15940	0.19273	1.01602	1.28214	2.22773	0.02590
2	0.18947	0.17381	0.94409	1.69053	1.57288	0.11575
3	0.22217	0.14725	1.06571	1.20992	3.92559	0.00009

Meta Analysis with four groups
00=healthy, 01=recent illness, 10=chronic illness, 11=current illness
All Studies - log retinol analysis
Chronic illness (10) versus Healthy (00)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4975	7	Equal	1.12039	0.98244	1.27771	1.69570
2	4975	7	Nsub	1.09262	1.01088	1.18098	2.23265
3	4975	7	Invar	1.11924	0.95794	1.30771	1.41880

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.08994	0.08733	0.99931	1.25613	1.94822	0.051389
2	0.02557	0.06388	1.05795	1.12843	5.38416	0.000000
3	0.15596	0.11204	1.06525	1.17598	4.46533	0.000008

Meta Analysis with four groups
00=healthy, 01=recent illness, 10=chronic illness, 11=current illness
All Studies - log retinol analysis
Current illness (11) versus Healthy (00)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN	P_RAN
1	4975	7	Equal	1.30973	1.11492	1.53858	3.28397	.0010236
2	4975	7	Nsub	1.24300	1.06546	1.45011	2.76645	.0056671
3	4975	7	Invar	1.30862	1.08588	1.57705	2.82552	.0047204

OBS	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.18663	1.20593	1.42247	6.4048	.00000000015061
2	0.13295	1.18779	1.30077	9.3842	.00000000000000
3	0.13609	1.24252	1.37824	10.1704	.00000000000000

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4975	7	Equal	0.98164	0.83799	1.14991	-0.22959
2	4975	7	Nsub	0.86487	0.61312	1.21999	-0.82710
3	4975	7	Invar	0.98566	0.85774	1.13267	-0.20361

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.81841	0.03785	0.84009	1.14704	-0.23328	0.81554
2	0.40818	0.16399	0.64576	1.15833	-0.97395	0.33008
3	0.83866	0.08927	0.91520	1.06155	-0.38163	0.70274

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4975	7	Equal	1.14753	1.00351	1.31223	2.01114
2	4975	7	Nsub	0.98390	0.72692	1.33173	-0.10509
3	4975	7	Invar	1.15244	1.06833	1.24317	3.66957

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.04431	0.000000	1.00351	1.31223	2.01114	0.04431
2	0.91631	0.065465	0.73335	1.32004	-0.10824	0.91380
3	0.00024	0.000000	1.06833	1.24317	3.66957	0.00024

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4975	7	Equal	1.16900	1.02401	1.33451	2.31118
2	4975	7	Nsub	1.13762	1.02858	1.25823	2.50821
3	4975	7	Invar	1.16920	0.98980	1.38111	1.83942

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.020823	0.00000	1.02401	1.33451	2.31118	0.020823
2	0.012134	0.07863	1.08143	1.19674	4.98912	0.000001
3	0.065854	0.11660	1.09616	1.24711	4.74973	0.000002

Tables 4.4 - Three group analyses including Guatemala

Meta Analysis with three groups 1
0=healthy, 1=recent or chronic illness, 2=current illness
Apparently normal Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	N0	M0
1	Equador	App_Norm	911	1	0.81230	1.36698	646	4.64327
2	GhanaR	App_Norm	302	1	0.26928	0.25599	85	4.28072
3	Guatemal	App_Norm	759	1	0.67677	0.29189	575	4.72466
4	Pakistan	App_Norm	2514	1	2.24164	2.08514	959	4.47133

OBS	SE0	N1	M1	SE1	N2	M2	SE2
1	0.009692	149	4.47564	0.023304	116	4.34368	0.031134
2	0.059099	104	4.24374	0.046334	113	3.95422	0.054205
3	0.013250	159	4.63836	0.027123	25	4.69694	0.081312
4	0.012770	1286	4.40201	0.012261	269	4.25879	0.027197

Meta Analysis with three groups 2
0=healthy, 1=recent or chronic illness, 2=current illness
Apparently normal Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	D0_1	SED0_1	LCL0_1	UCL0_1	Z0_1	P0_1
1	Equador	App_Norm	0.16763	0.025239	0.11816	0.21710	6.64184	0.00000
2	GhanaR	App_Norm	0.03698	0.075097	-0.11021	0.18417	0.49240	0.62244
3	Guatemal	App_Norm	0.08631	0.030187	0.02714	0.14547	2.85917	0.00425
4	Pakistan	App_Norm	0.06932	0.017704	0.03462	0.10402	3.91544	0.00009

Meta Analysis with three groups 3
0=healthy, 1=recent or chronic illness, 2=current illness
Apparently normal Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	D0_2	SED0_2	LCL0_2	UCL0_2	Z0_2	P0_2
1	Equador	App_Norm	0.29959	0.032608	0.23567	0.36350	9.18754	0.00000
2	GhanaR	App_Norm	0.32650	0.080193	0.16932	0.48368	4.07143	0.00005
3	Guatemal	App_Norm	0.02772	0.082384	-0.13375	0.18919	0.33650	0.73650
4	Pakistan	App_Norm	0.21254	0.030046	0.15365	0.27143	7.07383	0.00000

Meta Analysis with three groups 4
0=healthy, 1=recent or chronic illness, 2=current illness
Apparently normal Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	D1_2	SED1_2	LCL1_2	UCL1_2	Z1_2	P1_2
1	Equador	App_Norm	0.13195	0.038890	0.05573	0.20818	3.39300	0.00069
2	GhanaR	App_Norm	0.28952	0.071309	0.14976	0.42929	4.06009	0.00005
3	Guatemal	App_Norm	-0.05859	0.085716	-0.22659	0.10942	-0.68350	0.49429
4	Pakistan	App_Norm	0.14322	0.029833	0.08475	0.20169	4.80079	0.00000

Meta Analysis with three groups
0=healthy, 1=recent or chronic illness, 2=current illness
Apparently normal Studies - log retinol analysis
Recent or chronic illness (1) versus Healthy (0)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	1.09424	1.03619	1.15554	3.23856
2	4486	4	Nsub	1.09415	1.03490	1.15680	3.16758
3	4486	4	Invar	1.10748	1.03167	1.18886	2.82174

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	.0012013	0.034899	1.04877	1.14168	4.15933	.000031918
2	.0015371	0.040291	1.06604	1.12301	6.77590	.000000000
3	.0047764	0.053084	1.07815	1.13761	7.45508	.000000000

Meta Analysis with three groups
0=healthy, 1=recent or chronic illness, 2=current illness
Apparently normal Studies - log retinol analysis
Current illness (2) versus Healthy (0)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	1.24183	1.08797	1.41746	3.20925
2	4486	4	Nsub	1.22951	1.07617	1.40471	3.04007
3	4486	4	Invar	1.26629	1.13521	1.41252	4.23448

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	.0013308	0.12009	1.16907	1.31912	7.0307	2.0552E-12
2	.0023652	0.10234	1.17424	1.28739	8.8038	0
3	.0000229	0.08201	1.21574	1.31894	11.3594	0

Meta Analysis with three groups
0=healthy, 1=recent or chronic illness, 2=current illness
Apparently normal Studies - log retinol analysis
Current illness (2) versus Recent or chronic illness (1)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	4486	4	Equal	1.13488	0.98671	1.30530	1.77257
2	4486	4	Nsub	1.12371	0.98846	1.27748	1.78256
3	4486	4	Invar	1.14340	1.03832	1.25912	2.72450

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.076300	0.12912	1.06913	1.20467	4.15532	.000032483
2	0.074658	0.09768	1.07211	1.17780	4.86304	.000001156
3	0.006440	0.06984	1.09542	1.19349	6.12657	.000000001

Meta Analysis with three groups
0=healthy, 1=recent or chronic illness, 2=current illness
All Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	NSUB	WTE	WTN	WTI	N0	M0
1	Equador	App_Norm	911	1	1.57461	2.82337	646	4.64327
2	GhanaR	App_Norm	302	1	0.52199	0.52873	85	4.28072
3	Guatemal	App_Norm	759	1	1.31189	0.60287	575	4.72466
4	Pakistan	App_Norm	2514	1	4.34530	4.30667	959	4.47133
5	PNG	Sick	89	1	0.15383	0.15724	33	4.26788
6	SAfricS	Sick	205	1	0.35433	0.11947	139	4.33608
7	Nigeria	Infants	193	1	0.33359	0.25099	138	3.94382
8	SAFRinfH	Infants	27	1	0.04667	0.12175	20	4.75533
9	Nepal	Women	207	1	0.35779	0.08891	156	4.42116

OBS	SE0	N1	M1	SE1	N2	M2	SE2
1	0.009692	149	4.47564	0.02330	116	4.34368	0.03113
2	0.059099	104	4.24374	0.04633	113	3.95422	0.05420
3	0.013250	159	4.63836	0.02712	25	4.69694	0.08131
4	0.012770	1286	4.40201	0.01226	269	4.25879	0.02720
5	0.086665	41	4.07370	0.07843	15	3.78768	0.12321
6	0.041066	61	4.27854	0.05755	5	3.82217	0.18156
7	0.042908	31	4.09874	0.07582	24	3.96706	0.10237
8	0.095919	5	4.55406	0.11434	2	4.56144	0.12239
9	0.042279	36	4.05974	0.11933	15	3.85571	0.18703

Meta Analysis with three groups
0=healthy, 1=recent or chronic illness, 2=current illness
All Studies - log retinol analysis
Study Summaries

OBS	STUDY	TYPE	D0_1	SED0_1	LCL0_1	UCL0_1	Z0_1	P0_1
1	Equador	App_Norm	0.16763	0.02524	0.11816	0.21710	6.64184	0.00000
2	GhanaR	App_Norm	0.03698	0.07510	-0.11021	0.18417	0.49240	0.62244
3	Guatemal	App_Norm	0.08631	0.03019	0.02714	0.14547	2.85917	0.00425
4	Pakistan	App_Norm	0.06932	0.01770	0.03462	0.10402	3.91544	0.00009
5	PNG	Sick	0.19418	0.11689	-0.03492	0.42327	1.66124	0.09667
6	SAfricS	Sick	0.05754	0.07070	-0.08103	0.19612	0.81388	0.41572
7	Nigeria	Infants	-0.15492	0.08712	-0.32567	0.01584	-1.77823	0.07537
8	SAFRinfH	Infants	0.20127	0.14924	-0.09125	0.49378	1.34857	0.17747
9	Nepal	Women	0.36142	0.12660	0.11329	0.60956	2.85485	0.00431

Study Summaries

OBS	STUDY	TYPE	D0_2	SED0_2	LCL0_2	UCL0_2	Z0_2	P0_2
1	Equador	App_Norm	0.29959	0.03261	0.23567	0.36350	9.18754	0.00000
2	GhanaR	App_Norm	0.32650	0.08019	0.16932	0.48368	4.07143	0.00005
3	Guatemal	App_Norm	0.02772	0.08238	-0.13375	0.18919	0.33650	0.73650
4	Pakistan	App_Norm	0.21254	0.03005	0.15365	0.27143	7.07383	0.00000
5	PNG	Sick	0.48020	0.15064	0.18495	0.77545	3.18778	0.00143
6	SAfricS	Sick	0.51391	0.18614	0.14907	0.87875	2.76082	0.00577
7	Nigeria	Infants	-0.02324	0.11100	-0.24080	0.19432	-0.20940	0.83413
8	SAFRinfH	Infants	0.19389	0.15550	-0.11088	0.49866	1.24690	0.21243
9	Nepal	Women	0.56545	0.19175	0.18962	0.94128	2.94888	0.00319

Study Summaries

OBS	STUDY	TYPE	D1_2	SED1_2	LCL1_2	UCL1_2	Z1_2	P1_2
1	Equador	App_Norm	0.13195	0.03889	0.05573	0.20818	3.39300	0.00069
2	GhanaR	App_Norm	0.28952	0.07131	0.14976	0.42929	4.06009	0.00005
3	Guatemal	App_Norm	-0.05859	0.08572	-0.22659	0.10942	-0.68350	0.49429
4	Pakistan	App_Norm	0.14322	0.02983	0.08475	0.20169	4.80079	0.00000
5	PNG	Sick	0.28602	0.14606	-0.00025	0.57229	1.95831	0.05019
6	SAfricS	Sick	0.45637	0.19046	0.08307	0.82967	2.39613	0.01657
7	Nigeria	Infants	0.13167	0.12739	-0.11801	0.38136	1.03362	0.30131
8	SAFRinfH	Infants	-0.00738	0.16749	-0.33565	0.32090	-0.04405	0.96486
9	Nepal	Women	0.20403	0.22186	-0.23081	0.63887	0.91963	0.35777

Recent or chronic illness (1) versus Healthy (0)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	5207	9	Equal	1.11997	1.02044	1.22921	2.38617
2	5207	9	Nsub	1.09722	1.00101	1.20266	1.98162
3	5207	9	Invar	1.10499	1.01456	1.20349	2.29173

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.017025	0.11084	1.05638	1.18739	3.79889	.00014535
2	0.047522	0.08286	1.06882	1.12637	6.93462	.00000000
3	0.021921	0.07152	1.07674	1.13399	7.55583	.00000000

Meta Analysis with three groups
0=healthy, 1=recent or chronic illness, 2=current illness
All Studies - log retinol analysis
Current illness (2) versus Healthy (0)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	5207	9	Equal	1.33443	1.16496	1.52856	4.16339
2	5207	9	Nsub	1.25743	1.07913	1.46520	2.93608
3	5207	9	Invar	1.27061	1.12467	1.43548	3.84754

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	.0000314	0.16437	1.22796	1.45014	6.8003	1.0441E-11
2	.0033239	0.13750	1.20120	1.31630	9.8137	0
3	.0001193	0.10164	1.22209	1.32106	12.0561	0

Meta Analysis with three groups
0=healthy, 1=recent or chronic illness, 2=current illness
All Studies - log retinol analysis
Current illness (2) versus Recent or chronic illness (1)

OBS	SUMNSUB	NSTUD	WTS	RATIO	L95RR	U95RR	ZRAN
1	5207	9	Equal	1.19149	1.07510	1.32047	3.34084
2	5207	9	Nsub	1.14602	1.03049	1.27451	2.51389
3	5207	9	Invar	1.14988	1.05922	1.24830	3.33308

OBS	P_RAN	TAU	L95FR	U95FR	ZFIX	P_FIX
1	0.000835	0.079460	1.09034	1.30202	3.87082	.00010847
2	0.011941	0.089520	1.09273	1.20191	5.60993	.00000002
3	0.000859	0.062526	1.10363	1.19806	6.66837	.00000000

Section 5: Results and discussion

5.1 The two-group meta-analysis

The results for the two-group analysis where subjects are classified according to whether the different acute phase proteins (APP) are normal or high, are shown in table 4.2. Because the distributions of retinol are skewed, geometric means are compared. The summary statistics for comparing retinol between the normal and high groups is the *ratio* of the geometric mean for the normal group to the geometric mean for the high group. Analyses are presented for each APP and separate analyses were done for those studies where the subjects were classified at 'apparently-healthy pre-school children' and for 'all studies' available for analysis. In each analysis three methods of 'weighting' are shown and the variability in the overall summaries is computed assuming both *fixed* and *random effects* models. As outlined in section 3.2, we prefer the '*Invar*' method of weighting and the '*random effects*' model. These are extracted and shown table 5.1.

Table 5.1 Summary results from two-group meta-analysis

APP*	Status	No. of studies	No. of subjects	ratio	Z-ran	P-ran <
ACT	App healthy#	3	4035	1.16 (1.17)**	3.38	0.001
ACT	All groups	7	4550	1.16 (1.17)	2.77	0.01
AGP	App healthy	6	7662	1.18 (1.19)	3.84	0.001
AGP	All groups	13	8616	1.19 (1.21)	4.26	0.001
CRP	App healthy	3	1797	1.25 (1.20)	9.37	0.001
CRP	All groups	5	2099	1.26 (1.26)	8.25	0.001
SAA	App healthy	1	303	1.32 (1.32)	4.09	0.001
SAA	All groups	2	481	1.30 (1.30)	5.01	0.001

* APP means acute phase proteins and the ones listed are α1-antichymotrypsin (ACT), α1-acid glycoprotein (AGP), C-reactive protein (CRP) and serum amyloid A (SAA)

** Values in parentheses are ratios from two-group analyses excluding Guatemala data.

App Healthy represents the apparently healthy pre-school children.

Other details in text.

Results obtained using 4 acute phase proteins: AGP, CRP, ACT and SAA are shown. The main objective in all this work was to determine to what extent acute phase proteins indicated alterations to serum retinol in apparently-healthy pre-school children. Data for such children are therefore shown separately and together with the other groups of subjects. The other groups included apparently-healthy subjects (Nigerian neonates - study 15, South African schoolchildren - study 7 'SAfricaS', Nepalese women - study 4), HIV+ pregnant women (study 8 'SAFRmumH') and their infants (study 9 'SAFRinfH') and two groups in whom malaria positivity was high (GhanaM, PNG). None of the subjects in any of the groups were clinically sick.

For all four acute phase proteins, retinol values are significantly higher in the subjects with the normal acute phase proteins. For example the ratio summary statistic given in Table 5.1 for ACT in apparently healthy children is 1.16; serum retinols are 16% higher in the normal group. The P-value of 0.001 indicates that this ratio is significantly different from one. The thresholds used to distinguish normal subjects and those with sub-clinically infections were 0.6 g/L for ACT, 1.0 g/L AGP, 5 mg/L CRP and 5 mg/L SAA. The results suggesta metabolic effect on retinol

accompanying the elevation of a specific APP. It is possible that the ratios represent the specific environmental conditions relating to the specific studies but this is unlikely in view of the procedures in the meta-analysis (Section 3.2) and the fact that the results obtained for the separate analyses of the 'apparently-healthy' and 'all groups', were essentially the same (Table 5.1). The difference in retinol between the subjects with normal and elevated acute phase proteins is smallest in the case of ACT and gets progressively larger in the following order AGP, CRP and SAA. These differences are entirely compatible with the known behaviour of these specific APP. That is ACT and AGP are generally considered to be markers of chronic disease and plasma retinol is only depressed by 16-18% in these circumstances, while CRP and SAA are more strongly associated with acute disease when the depression in plasma retinol is greater (25 to 32%).

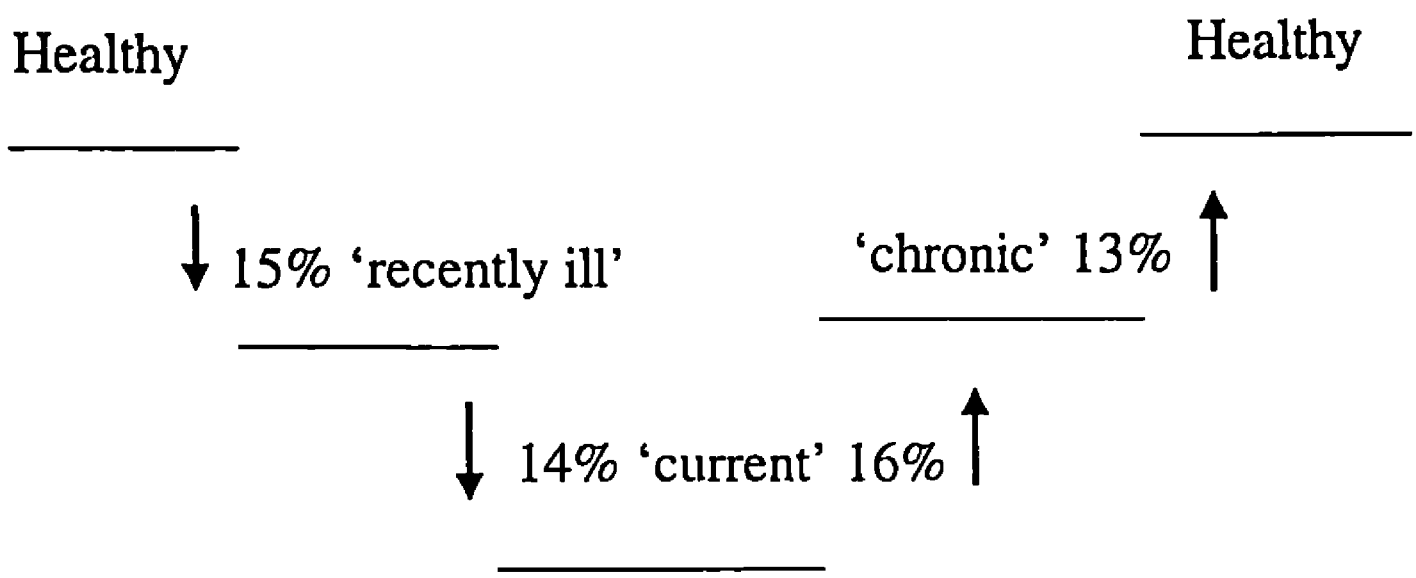
As indicated in section 3.1, the results obtained for the acute phase proteins ACT and AGP for the Guatemalan samples were corrected to allow for sample dehydration. Because various assumptions were made regarding the extent of dehydration, the two group analyses were also done without the Guatemalan samples (data not shown). That is the analysis of retinol and ACT for the apparently-normal children included only the Pakistan and UK studies (1 & 2) and there were only 6 groups available for the 'all group' analysis. In the case of AGP, the corresponding number of studies analysed were 5 and 12. The retinol ratios obtained were as follows: 1.18 & 1.17 for ACT and 1.19 & 1.21 for AGP with P-ran <0.001 in all cases. So in all four situations, when Guatemala was excluded from the analysis, the retinol ratios were essentially the same.

The results from the two-group analysis suggest that where large numbers of subjects have elevated acute phase proteins, the traditional thresholds to assess vitamin A status on the basis of plasma retinol may lead to overestimates of vitamin A deficiency. The ratios are potentially useful in this situation. For example in a a group of subjects without obvious evidence of clinical disease but having a CRP values greater than 5 mg/L, plasma retinol values could be increased by 25% to compensate for the effects of mild inflammation.

5.2 Four group meta-analysis

The two-group analyses looked at the effects of each APP on retinol concentrations but did not take into account the fact that different APPs reapond differently to infection. As outlined in section 3.2, using different combinations of the normal and elevated APP we can discern the stage and therefore the severity of infection in more detail. ACT and CRP increase rapidly on infection whereas AGP is much slower to rise. Therefore an individual with elevated values of either ACT or CRP but normal values of AGP has recently become ill. . Where either ACT or CRP and AGP are elevated, the individual is currently ill. Where only AGP is increased, we have chronical illness. The information outlined in table 4.3 enables us to construct figure 5.1 to show the depression in serum retinol associated with different disease states.

Figure 5.1 Influence of Four-group analysis on plasma retinol (including Guatemala)



Lines represent arbitrary retinol concentrations and nos indicate depression in retinol (%) between the adjoining health/disease states

Figure 5.1 is based on the analyses using the '*Invar*' method of weighting and the '*random effects*' model. It shows that the reduction in retinol in apparently-healthy pre-school children with a recent infection was 15% (NS), those with a chronic infection 12% (NS) and in those with a current illness, the overall depression is 32% ($P<0.001$; sub-tables 8, 9 &10 resp., Table 4.3). The four-group method of analysis reduced the potential number of studies which could be examined when 'all studies' were combined from 13 to 9. Comparable results for 'all studies' with those presented above were 13% (NS), 12% (NS) and 31% ($P=0.004$) (sub-tables 9-11 resp, Table 4.3).

Irrespective of whether only the pre-school children or all studies were analysed, the depression in plasma retinol in those recently infected and those chronically infected was approximately the same 12-15%. Furthermore, the degree of depression was not significant. Therefore it was decided to combine these two groups to increase the numbers of both subjects and studies.

5.3 Three group meta-analysis

The three group meta-analysis was done for two reasons. First, studies that did not have at least two observations (so a standard deviation could be calculated) in each of the four groups were excluded. Second, the numbers of subjects who were 'recently ill' (ie raised ACT or CRP but normal AGP) was relatively small and the depression in plasma retinol was not significant in this group. However, quantitatively, the depression in retinol in the recently-ill group (15%) was very similar to

that in the chronic group (13%). Therefore, these two groups were combined primarily to increase the number of studies which could be analysed in this way.

Table 5.2 Depression of plasma retinol within the different health-status groups by the three-group meta-analysis

	Subjects in meta-analyses			Depression of plasma retinol		
Groups included	**No. of studies**	**Inclusion of Guatemala**	**Recent or chronic illness (%)**	**P =**	**Current illness (%)**	**P <**
App Healthy#	4	yes	11	0.005	27	0.001
All groups	9	yes	11	0.02	29	0.001
App. Healthy	3	no	11	0.015	27	= 0.047
All groups	8	no	11	0.04	29	0.001

Apparently healthy pre-school children

Table 5.2 shows the summary results of the three-group analysis when the same method of calculation is used as in the previous two analyses described above. The *RATIO* provides a measure of the depression in plasma retinol associated with the different combinations of APP. The data were analysed to show the effects on retinol of sub-clinical infection in four apparently-healthy studies and when all nine studies with suitable data were combined. Table 5.2 also shows the effects of excluding the Guatemalan data from these. The results are remarkable similar; the effect on retinol of recent or chronic illness (a single elevated APP) was a reduction of 10% while current illness (elevated AGP and ACT or CRP) reduced retinol by 27-29%.

Section 6 - Conclusions

The two-group analyses of the effects of the different APP on plasma retinol suggest that ACT and AGP are associated with approximately a 16-18% depression in plasma retinol while CRP and SAA have greater effects of approximately 25 and 30% respectively. A comparison of these results with those obtained by the four and three group analyses suggest that the effect of CRP alone is very similar in its effect on retinol, to that of ACT or CRP plus AGP. That is a single measurement of CRP is sufficient to estimate the effect of current sub-clinical infection on plasma retinol.

In contrast, by themselves the two APPs, ACT and AGP, which are both believed to be good indicators of chronic sub-clinical infections, in fact overestimate the effects of chronic infection on plasma retinol when results of the two-group analyses are compared with the four- or three-group analyses. The reason for this of course is that both ACT and AGP are increased both in the acute (i.e. current) as well as the chronic phase of an infection and plasma retinol will be depressed more in the acute than the chronic phase. Therefore, in order to correctly assess the number of subjects in a study whose sub-clinical infection is genuinely chronic, it is important to measure two APP, and the best ones are probably CRP and AGP. The measurement of CRP is needed to differentiate those subjects currently ill from those in a chronic phase of a sub-clinical infection. ACT is probably not as useful as CRP because of the tendency for ACT to remain elevated for longer than CRP and therefore potentially decreasing the apparent effect of a current infection (or increasing the effect of a chronic infection) on plasma retinol.

The results of the three and four-group meta-analyses show that in chronic, sub-clinical infection (elevated AGP only) retinol is depressed between 10-15% while during a current infection (both AGP and CRP or AGP and ACT elevated) the depression in plasma retinol is approximately 27-31%.

Section 7 - References

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