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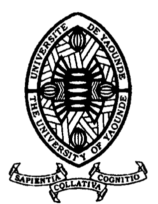

FACULTY OF SCIENCE

FACULTÉ DES SCIENCES

DEPARTMENT OF BIOCHEMISTRY

DEPARTEMENT DE BIOCHIMIE

LABORATORY OF NUTRITION AND NUTRITIONAL BIOCHEMISTRY

LABORATOIRE DE NUTRITION ET BIOCHIMIE NUTRITIONNELLE

Prevalence and determinants of Micronutrient deficiency amongst Children aged 12-59 months in the North and Far North Regions of Cameroon

THESIS

Submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy/Ph.D. in Biochemistry

Specialty: **Nutrition and Nutritional Biochemistry**

By:

NGWA AKONWI FUH

Matricule: 02Y173

DEA in Biochemistry

Supervised by :

JULIUS OBEN ENYONG
Professor

Academic Year 2015 - 2016

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P.O.BOX 812, Yaoundé, Cameroon

DEPARTEMET DE BIOCHIMIE
DEPARTMENT OF BIOCHEMISTRY

ATTESTATION DE CORRECTION DU MEMOIRE DE THESE DE DOCTORAT PhD

Nous soussignés : **Pr. NDJOUENKEU Robert** Président du Jury, **Pr. FOKOU Elie, Pr. KANSCI Germain, Pr. TANYA Agatha**, Membres du Jury et P**r. OBEN Julius ENYONG,** Rapporteur, attestons que **NGWA AKONWI FUH** (Matricule **02Y173**) a effectué les corrections conformément aux exigences du jury de soutenance de son mémoire de **DOCTORAT PhD** en **Biochimie** Option **Sciences des Aliments et Nutrition** intitulé **« Prevalence and determinants of Micronutrient deficiency amongst Children aged 12-59 months in the North and Far North Regions of Cameroon»**.

En foi de quoi la présente attestation lui est délivrée pour servir et valoir ce que de droit.

Rapporteur

Pr. OBEN Julius

Président du jury

Pr. NDJOUENKEU Robert.

Membres du Jury

Pr. FOKOU Elie, ________________

Pr. KANSCI Germain, ________________

Pr. TANYA Agatha, ________________

Chef de Département

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ANNEE ACADEMIQUE 2016/2017

(Par Département et par Grade)

DATE D'ACTUALISATION : 15 Novembre 2017

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7	NJIOKOU Flobert	Professeur	En Poste
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34	NJUA Clarisse YAFI	Chargé de Cours	En poste
35	TADU Zéphirin	Chargé de Cours	En poste
36	TOMBI Jeannette	Chargé de Cours	En poste
37	YEDE	Chargé de Cours	En poste
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39	KANDEDA KAVAYE Antoine	Assistant	En poste
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9	ZAPFACK Louis	Maître de Conférences	En poste
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11	BIYE Elvire Hortense	Chargé de Cours	En poste

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16	NGODO MELINGUI Jean Baptiste	Chargé de Cours	En poste
17	NGONKEU MAGAPTCHE Eddy Léonard	Chargé de Cours	En poste
18	NGOUO Lucas Vincent	Chargé de Cours	En poste
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22	TSOATA Esaïe	Chargé de Cours	En poste
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24	GONMADGE Christelle	Assistant	En poste
25	MAFFO MAFFO Nicole Liliane	Assistant	En poste
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20	GWET Simon – Pierre	Chargé de Cours	En poste
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26	NDOSIRI Bridget NDOYE	Chargé de Cours	En poste
27	NJIOMOU Chantale épse DJANGANG	Chargé de Cours	En poste
28	NJOYA Dayirou	Chargé de Cours	En poste
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31	TCHAKOUTE KOUAMO Hervé	Chargé de Cours	En poste
32	BELIBI BELIBI Placide Désiré	Assistant	En poste
33	KOUOTOU DAOUDA	Assistant	En poste
34	MAKON Thomas Beauregar	Assistant	En poste
35	NCHIMI NONO Katia	Assistant	En poste
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25	NGO MBING Joséphine	Chargé de Cours	En poste
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25	EDONGUE HERVAIS	Chargé de Cours	En poste
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30	NDOP Joseph	Chargé de Cours	En poste
31	OBOUNOU Marcel	Chargé de Cours	*Dir.acad/Univ.Int.Etat Cam-Congo*
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33	TABI Conrad Bertrand	Chargé de Cours	En poste
34	TCHOFFO Fidèle	Chargé de Cours	En poste
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39	CHAMANI Roméo	Assistant	En Poste

40	MELI’I Jorelle Larissa	Assistant	En Poste
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4	FOUATEU Rose épouse YONGUE	Maître de Conférences	En poste
5	KAMGANG Pierre	Maître de Conférences	En poste
6	MEDJO EKO Robert	Maître de Conférences	Conseiller Technique/UYII
7	MOUNDI Amidou	Maître de Conférences	*CT/MINMIDT*
8	NDAM NGOUPAYOU Jules-Remy	Maître de Conférences	En poste
9	NGOS III Simon	Maître de Conférences	*D.A.A.C./UM*
10	NJILAH Isaac KONFOR	Maître de Conférences	En poste
11	NKOUMBOU Charles	Maître de Conférences	En poste
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Répartition chiffrée des enseignants permanents par Département

(15 Novembre 2017)

Département	**Nombre d'enseignants**				
	Pr	**MC**	**CC**	**ASS**	**Total**
BC	05 (1)	9 (3)	18 (9)	08 (4)	**40 (18)**
BPA	10 (0)	10 (1)	18 (6)	6 (4)	**44 (11)**
BPV	3 (0)	6 (4)	13 (3)	5 (5)	**33 (9)**
C.I.	9 (1)	7 (0)	15 (3)	4 (1)	**35 (5)**
C.O.	9 (0)	10 (3)	12 (3)	2 (0)	**33 (6)**
IN	2 (0)	1 (0)	8 (0)	15 (2)	**26 (2)**
MA	3 (0)	4 (0)	18 (1)	5 (0)	**30 (1)**
MB	1 (0)	6 (1)	4 (2)	2 (0)	**13 (3)**

PH	8 (0)	14 (1)	15 (2)	4 (1)	**41 (5)**
ST	3 (0)	10 (1)	22 (4)	7 (0)	**44 (5)**
Total	**59 (2)**	**80 (14)**	**139 (33)**	**67 (18)**	**345 (67)**

Soit un total de : 345 (67) dont :

- Professeurs 59 (2)
- Maîtres de Conférences 80 (14)
- Chargés de Cours 139 (33)
- Assistants 67 (18)
- ( ) = Nombre de femmes.

The Dean of the Faculty of Science

DEDICATION

This Dissertation is dedicated to my wife (Mme Ngwa Hervee) and my beautiful daughters.

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This dissertation is the product of the hard work of many people and the support of many others; both are worth much more than these printed lines can convey.

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Table of Contents

LISTE PROTOCOLAIRE DES ENSEIGNANTS DE LA FACULTE DES SCIENCES I

LISTE DES ENSEIGNANTS PERMANENTS i

DEDICATION XV

ACKNOWLEDGMENTS XVI

TABLE OF CONTENTS XVII

ABSTRACT XXI

RESUME XXIII

LIST OF ABBREVIATIONS XXV

LIST OF TABLES XXVII

LIST OF FIGURES XXIX

INTRODUCTION - 1 -
Rationale - 3 -

OBJECTIVE - 5 -

CHAPTER 1: LITERATURE REVIEW - 6 -

1.1. MALNUTRITION - 6 -
1.1.1. DEFINITION - 6 -
1.1.2. CLASSIFICATION OF MALNUTRITION - 7 -
1.1.2.1. Acute Malnutrition - 10 -
1.1.2.1.1. Diagnosing severe acute malnutrition (severe wasting or kwashiorkor or marasmic kwashiorkor) - 11 -
1.1.2.1.2. Risk of death and severe acute malnutrition - 11 -
1.1.2.1.3. Specificity of recommended cut-offs for diagnosing severe acute malnutrition - 11 -
1.1.2.2. Chronic Malnutrition - 12 -

1.2. MICRONUTRIENTS AND MICRONUTRIENT DEFICIENCIES - 12 -
1.2.1. VITAMIN A - 12 -
1.2.2. IRON - 21 -
1.2.2.1. Overview of Iron metabolism and absorption - 22 -
1.2.2.2. Iron deficiency - 27 -
1.2.2.3. Zinc - 33 -
1.2.2.4. Overview of Zinc metabolism and absorption - 33 -
1.2.3. Vitamin B12 - 38 -
1.2.3.1. Role of vitamin B12 in human metabolic processes - 38 -
1.2.3.2. Dietary sources and availability - 38 -
1.2.3.3. Absorption - 39 -
1.2.3.4. Populations at risk for, and consequences of, vitamin B12 deficiency - 39 -
1.2.3.5. Vitamin B12 interaction with folate or folic acid - 41 -
1.2.3.6. Criteria for assessing vitamin B12 status - 42 -

1.2.4. Folate and Folic Acid - 42 -
1.2.4.1. Role of folate and folic acid in human metabolic processes - 42 -
1.2.4.2. Populations at risk for folate deficiency - 47 -
1.2.4.3. Dietary sources of folate - 47 -
1.2.5. Trace Elements - 48 -
1.2.5.1. Calcium - 48 -
1.2.5.2. Magnesium - 52 -
1.2.5.3. Copper - 55 -

1.3. BACKGROUND ON THE NORTH AND EXTREME NORTH REGIONS - 63 -
1.3.1. THE NORTH REGION - 63 -
1.3.2. THE FAR NORTH REGION - 64 -
1.3.3. HEALTH STATUS OF TWO REGIONS - 65 -

CHAPTER II: MATERIAL AND METHODS 66

2.1. SURVEY METHODOLOGY 66
2.1.1. GENERAL 66
2.1.2. SAMPLING 66
2.1.3. Target groups 69

2.2. DIETARY AND SOCIOECONOMIC STATUS DATA COLLECTION METHODS 70
2.2.1. Collection of Socio-Economic Status (SES) data 70
2.2.2. 24 hour recall for Food Frequency consumption 71

2.3. EVALUATION OF NUTRITION STATUS 72
2.3.1. EQUIPMENT 73
2.3.2. MEASURMENTS 73

2.4. EVALUATION OF PLASMA LEVELS OF MICRONUTRIENTS AND TRACE ELEMENTS 75
2.4.1. COLLECTION OF BIOLOGICAL SAMPLES 75

2.5. LABORATORY ANALYSIS OF BIOLOGICAL SAMPLES 76
2.5.1. EVALUATION OF PLASMA PROTEINS BY SANDWICH ELISA: 76
2.5.2. EVALUATION OF RETINOL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) 79
2.5.3. MEASURMENT OF PLASMA CONTENT OF METAL ELEMENTS BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY 83
2.5.4. MEASURMENT OF PLASMA FOLATE AND VITAMIN B12 BY RADIO LIGAND BINDING ASSAY 84

2.6. STATISTICAL ANALYSIS 85
2.6.1. Analysis 85
2.6.2. Calculation of Anthropometric indices 87
2.6.3. Measuring Food Consumption and Dietary Diversity Scores 88
2.6.4. Constructing the Socioeconomic status (SES) Index with Principal Correspondence Analysis (PCA) 89

CHAPTER III: RESULTS AND DISCUSSION 92

RESULTS 92

3.1. General Characteristics of the study population 92

3.2. Food Consumption 95

3.2.1. Food Frequency consumption 95

3.2.2. Food Consumption Score (FCS) and Dietary Diversity Score (DDS) 97

3.3. Nutritional status 98

3.3.1. Nutritional status as measured by stunting, wasting and underweight criteria 98

3.4. Markers of Inflamation 101

3.4.1. Distribution of Infection category by region 101

3.4.2. Distribution of Infection category by age group 102

3.4.3. Distribution of Infection category by SES quintile 102

3.4.4. Distribution of Infection category by Stunting, Wasting and Underweight categories 103

3.4.5. Distribution of Infection category by Level of education of Caregivers and HH heads. 105

3.5. Vitamin A status 106

3.5.1. ROH and RBP Plasma concentrations 106

3.5.2. Effect of sampling process ROH and RBP Plasma concentrations 107

3.5.3. Effect of infection on Plasma ROH and Plasma RBP concentrations 107

3.5.4. Adjusting for Elevated Acute phase proteins. 107

3.5.5. Vitamin A Deficiency (VAD) 109

3.6. Iron deficiency (ID) and Anemia 110

3.6.1. Plasma concentrations of biomarkers of Iron and anemia 110

3.6.2. Effect of infection on Biomarkers of Iron status and Anemia 112

3.6.3. Adjusted Plasma concentrations of biomarkers of Iron and anemia 114

3.6.4. Anemia and Iron deficiency (ID) and Iron Deficiency Anemia (IDA) 114

3.6.5. Comparison of ID among potential risk groups 115

3.7. ZINC Status 121

3.7.1. Plasma Zinc Concentration 121

3.7.2. Prevalence of Low Plasma Zinc Concentration 122

3.8. Folate and Vitamin B12 123

3.8.1. Plasma Concentration of Folate and vitamin B12 123

3.8.2. Prevalence of low Folate and vitamin B12 deficiency 124

3.9. Trace Elements (Copper, Calcium, Magnesium, and Zinc) 126

3.9.1. Plasma Concentration of Trace Elements 126

3.10. Multiple Micronutrient deficiency 128

3.10.1. Prevalence of all micronutrient deficiencies 129

3.10.2. Prevalence of multiple micronutrient deficiencies 129

3.10.3. Characterization of multiple micronutrient deficiencies 129

3.11. Determinants of Micronutrient deficiency 130

3.11.1. Determinants for vitamin A deficiency 131

3.11.2. Risk factors for iron deficiency 132

3.11.3. Risk factors for Low PZC 134

3.11.4. Risk factors for Low Folate 135

3.11.5. Risk factors for vitamin B12 deficiency 136

DISCUSSION 139

3.12. Vitamin A 139

3.12.1. Vitamin A Deficiency 139

3.12.2. Determinants of Vitamin A Deficiency 142

3.13. Anemia and Iron deficiency 145

3.13.1. Anemia 145

3.13.2. Distribution of Iron Deficiency 146

3.13.3. Iron deficiency and nutritional status 149

3.13.4. Determinants of Iron Deficiency 150

3.14. Low plasma Zinc Concentration (PZC) 151

3.14.1. Prevalence of Low PZC deficiency 151

3.14.2. Determinants of Low PZC deficiency 154

3.15. Plasma Folate and Vitamin B 12 155

CONCLUSION AND PERSPECTIVES 157

4.1. Conclusion 157

4.2. Perspectives 160

REFERENCES 161

ANNEXES I

4.3. Data collection Tools I

Socio Economic Status Questionnaire I

Food Frequency consumption questionnaire VIII

Picture Chart 24hour recall 10

Ethical Clearance 15

LIST OF PUBLICATIONS 16

ABSTRACT

The present study was designed to evaluate the relationship between multiple micronutrient levels and nutritional status among preschool school children. In this cross-sectional study, anthropometric data and blood samples were collected from 331 children in the North and Far North Regions of Cameroon. Serum concentration of magnesium, calcium, copper, and zinc were measured by inductively coupled plasma mass spectrometer. Ferritin, sTfR, RBP, CRP, AGP were measured by Sandwich ELISA. Body iron store was estimated using the ratio of sTfR: Ferritin. Anthropometric indices of weight-for-age, height-for-age and weight-for-height were used to estimate the children's nutritional status.

Descriptive statistics were calculated for all variables. Continuous variables were examined for adherence to a normal distribution and all micronutrients values in serum were normally distributed and hence no transformation was done. Using linear regression analysis, plasma concentrations were adjusted for CRP, AGP, and sampling process indicators. Principal correspondence analysis was used to create the Socio economic status scale.

Results showed that the three main food groups consumed in the North and Far North regions are Cereals (91.2%), vegetables (51.7%), and meat (38.1%). The prevalence of stunting, wasting and underweight were respectively 42.5%, 6.6% and 26.0%. The prevalence of stunting, wasting and underweight were all significantly higher for the Far North Region compared to the North region ($P<0.001$).
The most prevalent micro nutrient deficiency was low PZC (85.8%) followed by anemia (63.75), then vitamin A deficiency (40.8%), low vitamin B12 (39.4%), Iron deficiency (29.3%) and low folate (9.8%). In the multivariate models developed, VAD was positively associated with food consumption score, age, stunting, wasting, and underweight and care giver level of education. The model explained 81.1% of the variance in VAD. Univariate and multiple regression analysis showed that seven key factors were associated with ID which explain 68.1% of the overall variance in the ID. A very significant relationship between stunting ($n=303$, $r=0.134$, $p<0.01$) and wasting ($n=303$ $r=0.147$, $p<0.01$) and Plasma zinc concentration in the North and Far North regions of Cameroon was found. Eight determinants are strongly associated with plasma zinc concentration, explaining 78.6% of the total variance observed.

The overall prevalence of Low plasma folate was higher in the Far North Region (16.7%) as compared to the North Region (3.0%) and the reverse was true for Vitamin B12 (39.4% vs 43.9%). Seven determinants were strongly associated with low folate concentration, explaining 53.7% of the total variance observed while five others were positively associated with vitamin B12 explaining 74.1% of the variance.

The mean serum level of Calcium, Magnesium, copper and zinc was significantly lower in severely stunted compared to moderately stunted children ($p<0.05$). Wasting was significantly correlated with zinc ($r=0.094$, $p<0.05$), Cu/Zn ratio ($r=-0.085$, $p<0.05$), Body iron stores ($r=0.140$, $p<0.01$) and serum ferritin ($r=0.168$, $p<0.01$). Stunting was correlated to same micronutrients like wasting while underweight was only correlated to serum magnesium ($r=-0.101$, $p<0.01$), body iron stores ($r=0.106$, $p<0.01$) and serum ferritin ($r=0.166$, $p<0.01$).

The prevalence of multi-micronutrient deficiency was high with up to 70.4% of the children presenting two or more forms of micronutrient deficiency. The proportion of children presenting two, three, four, five and six micronutrient deficiencies was respectively 20.5%, 26.3%, 29.3%, 14.8%, 3.0% and 0.3%.

The determinants of micronutrient deficiency varied from one micronutrient to the other. The most recurrent determinants were: nutritional status, food consumption status and the deficiency in other micronutrients.

Key words: Preschool children, Nutritional status, Micronutrients deficiency, Cameroon

RESUME

La présente étude a été conçue pour évaluer la relation entre plusieurs niveaux de micronutriments et l'état nutritionnel des enfants d'âges préscolaire. Dans cette étude transversale, les données anthropométriques et des échantillons de sang ont été recueillies auprès de 331 enfants dans les régions du Nord et de l'Extrême-Nord du Cameroun. La concentration sérique de magnésium, le calcium, le cuivre et le zinc ont été mesurées par couplage inductif spectromètre de masse à plasma. Ferritine, sTfR, RBP, CRP, AGP ont été mesurés par sandwich ELISA. La réserve corporelle en fer a été estimée en utilisant le ratio de sTfR: ferritine. Indices anthropométriques de poids-pour-âge, taille-pour-âge et le poids-pour-taille ont été utilisés pour estimer l'état nutritionnel des enfants.

Les statistiques descriptives ont été calculées pour toutes les variables. Les variables continues ont été examinées pour l'adhésion à une distribution normale et toutes les valeurs de micronutriments dans le sérum ont été distribuées normalement et donc aucune transformation n'a été faite. En utilisant une analyse de régression linéaire, les concentrations plasmatiques ont été ajustées pour les indicateurs CRP, AGP, et le processus d'échantillonnage. Analyse des correspondances principal a été utilisé pour créer l'échelle de statut socio-économique.

Les trois principaux groupes d'aliments consommés dans les régions du Nord et de l'Extrême-Nord sont les céréales (91,2%), les légumes (51,7%) et la viande (38,1%). La prévalence du retard de croissance, d'émaciation et d'insuffisance pondérale était respectivement de 42,5%, 6,6% et 26,0%. La prévalence du retard de croissance, d'émaciation et d'insuffisance pondérale étaient tous significativement plus élevé pour la région de l'Extrême Nord par rapport à la région du Nord (P <0,001).

La carence la plus répandue des éléments nutritifs est faible PZC (85,8%), suivie par l'anémie (63,75), puis carence en vitamine A (40,8%), faible taux de vitamine B12 (39,4%), la carence en fer (29,3%) et une faible folate (9,8%). Dans les modèles multivariés développés, la carence en vitamine A a été positivement associée avec le score de la consommation alimentaire, l'âge, le retard de croissance, émaciation, et le niveau d'insuffisance pondérale et le niveau d'éducation de la mère. Le modèle explique 81,1% de la variance. Analyse de régression univariée et multiple montrent que sept facteurs clés ont été associés à la carence en fer, expliquant 68,1% de la variance globale. Des relations très importantes entre retard de croissance (n = 303, r = 0,134, p <0,01) et l'émaciation (n = 303 r = 0,147, p <0,01) et la concentration plasmatique de zinc dans le Nord et les régions de l'Extrême Nord du Cameroun a été observée. Huit déterminants sont fortement associés à la concentration de zinc plasmatique, expliquant 78,6% de la variance totale observée.

La prévalence globale de faible folate plasmatique était plus élevée dans la région de l'Extrême-Nord (16,7%) par rapport à la région du Nord (3,0%) et l'inverse était vrai pour la vitamine B12 (39,4% vs 43,9%). Sept déterminants étaient fortement associés à la faible concentration de folates, expliquant 53,7% de la variance totale observée, tandis que cinq autres ont été positivement associés à la vitamine B12 expliquant 74,1% de la variance.

Le niveau moyen de calcium, le magnésium, le cuivre et le zinc sérique était significativement plus faible pour les enfants ayant un retard de croissance grave par rapport modérément retardées (p <0,05). L'émaciation était significativement corrélée avec le zinc (r = 0,094, p <0,05), rapport Cu / Zn (r = -0,085, p <0,05), les réserves en fer du corporelle (r = 0,140, p <0,01) et la ferritine sérique (r = 0,168, p <0,01). Le retard de croissance a été corrélée aux mêmes micronutriments comme l'émaciation tandis que l'insuffisance pondérale était seulement corrélée au magnésium (r = -0,101, p <0,01), les réserves en fer du corporelle (r = 0,106, p <0,01) et la ferritine sérique (r = 0,166, p <0,01).

La prévalence de la carence en multiples micronutriments été élevé avec 70,4% des enfants présentant deux ou plusieurs formes de carences en micronutriments. La proportion d'enfants présentant deux, trois, quatre, cinq et six carences en micronutriments été respectivement de 20,5%, 26,3%, 29,3%, 14,8%, 3,0% et 0,3%.

Les déterminants de carence en micronutriments varient d'un oligo-élément à l'autre. Les déterminants les plus récurrents sont: l'état nutritionnel, état de la consommation alimentaire et l'insuffisance dans d'autres micronutriments.

Mots clés: Enfants D'âge Préscolaire, état nutritionnel, déficience en micronutriments, Cameroun

LIST OF ABBREVIATIONS

ACD	Anemia Of Chronic Disease
AGP	alpha (1)-acid glycoprotein
Apo-RBP	Apo Retinol Binding Protein
APP	Acute Phase proteins
BIS	Body Iron Stores
BMI	Body Mass Index
BUCREP	Bureau Central de Recensement et d'Etude de la population au Cameroun
CDC	Centre for Disease Control
CRP	C-Reactive Protein
DDS	Dietary diversity scores
DHS	Demographic Health Survey
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid,
DTT	Dithiothreitol
EA	Enumeration Area
EAR	Estimated Average Requirement
ECF	Extracellular Fluid
EDS	Enquete Demographique de Santé
ENA	Essential Nutrition Actions
EPI	Expanded Program on Immunization
FAO	Food and Agricultural Organization
FCS	Food Consumption Score
Fer	Ferritin
HAZ	Height for Age z-Score
Hb	Hemoglobin
HDL	High Density lipoprotein
HH	House Holds
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Atomic Emission Spectrometry
ID	Iron deficiency
IDA	Iron deficiency Anemia
IVACG	International Vitamin A Consultative Group
IZiNCG	International Zinc Nutrition Consultative Group
LDL	Low density Lipoprotein
MAO	Monoamine oxidase
MD	Micro nutrient Deficiency
MMA	Methylmalonic acid
MPO	Myeloperoxidase
MSP	Minsitère de Santé publique
NTDs	neural tube defects
PA	pernicious anemia
PCA	Principal Correspondence Analysis
PEM	protein–energy malnutrition
PZC	Plasma Zinc Concentration
QC	Quality control
RBP	Retinol binding Protein
RDA	Recommended Dietary Allowance
RNA	Ribonucleic Acid
RNIs	Recommended Nutrient Intakes
ROH	Retinol
SD	Standard Deviation
SEM	Standard Error on Mean

SES	Socio Economic Status
SOD	Superoxide dismutase
sTfR	Soluble Transferrin Receptor
THF	Tetrahydrofolate
TMB	Tetramethyl-benzidine dihydrochloride
UNICEF	United Nations children Fund
VA	Vitamin A
VAD	Vitamin A Deficiency
WAZ	Weight-For Age z-Score
WHO	World Health Organization
WHZ	Weight-For Height z-Score

LIST OF TABLES

Table 1: Indicators of subclinical VAD in mothers and children aged 6-71 months .... - 20 -
Table 2: Iron intakes required for growth under the age of 18 years, median basal iron losses, menstrual losses in women and total absolute iron requirement .... - 23 -
Table 3: Daily protein and calcium intakes in different regions of the world, 1987-1989 (*et al*., 1990) . - 50 -
Table 4: Typical daily intakes of magnesium by infants (6kg) and adults (65kg), in selected countries (*et al*., 1994) .... - 54 -
Table 5 Parameters for the calculation of sample size 66
Table 6 Number of house holds to be surveyed 67
Table 7: Dilution of Liquicheck Bio-Rad control sample for the preparation of calibration curves..... 78
Table 8: Extinction coefficient of retinol standards 81
Table 9: WHO cut-offs for nutritional status classification 87
Table 10: Detail of Food group weights... 88
Table 11: General Characteristics of the study population.. 93
Table 12: Frequency of households/individuals consuming each food group by region 95
Table 13: Frequency of households/individuals consuming each food group by SES Quintile........... 95
Table 14: Frequency of households/individuals consuming each food group by Age Group.............. 96
Table 15: Frequency of house and individuals consuming food groups that are sources of vitamin A , Iron and Zinc by Region... 96
Table 16: Frequency of house and individuals consuming food groups that are sources of vitamin A , Iron and Zinc by SES Quintile 96
Table 17: Frequency of house and individuals consuming food groups that are sources of vitamin A , Iron and Zinc by Age group 97
Table 18: Regional Distribution of FCS and DDS 97
Table 19: Distribution of FCS and DDS by SES Quintile 98
Table 20: Distribution of FCS and DDS by Age Group 98
Table 21: Distribution of Nutritional status by region 99
Table 22: Distribution of Nutritional status by region 99
Table 23: Distribution of Nutritional status by sex 100
Table 24: Distribution of Nutritional status by Age group.. 100
Table 25: Distribution of Nutritional status by SES quintile... 100
Table 26: Distribution of Infection category by region 101
Table 27: Distribution of Infection category by age group 102
Table 28: Distribution of Infection category by SES quintile 102
Table 29: Stunting Category and infection... 103
Table 30: Underweight Category and infection 104
Table 31: Wasting Category and infection.. 104
Table 32: Distribution of Infection category by Level of education of Caregivers............................ 105
Table 33: Distribution of Infection category by Level of education of HH heads 105
Table 34: Mean ROH and RBP plasma concentration by age group 106
Table 35: Mean ROH and RBP plasma concentration by SES Quintile 106
Table 36: Mean ROH and RBP plasma concentration by Region 106
Table 37: Spearman correlations between Plasma RBP, ROH and processing factors....................... 107
Table 38: Spearman correlation between AGP, CRP, RBP and ROH 107
Table 39: Adjusted Mean ROH and RBP plasma concentration by age group................................... 108
Table 40: Adjusted Mean ROH and RBP plasma concentration by region 108
Table 41: Adjusted Mean ROH and RBP plasma concentration by SES Quintile............................... 108

Table 42: Vitamin A deficiency by Region 109
Table 43: Vitamin A deficiency by Age group 109
Table 44: Vitamin A deficiency by Age SES quintile 110
Table 45: Spearman correlation between RBP and ROH per age group........ 110
Table 46: Mean Hemoglobin, sTfR, Ferritin and BIS by Region 111
Table 47: Mean Hemoglobin, sTfR, Ferritin and BIS by Agegroup 111
Table 48: Mean Hemoglobin, sTfR, Ferritin and BIS by SES Quintile 111
Table 49: spearman correlation between acute phase proteins and Iron Markers 112
Table 50: Adjusted mean Hemoglobin, sTfR, Ferritin and BIS by Region 114
Table 51: Adjusted mean Hemoglobin, sTfR, Ferritin and BIS by SES Quintile 114
Table 52: Adjusted mean Hemoglobin, sTfR, Ferritin and BIS by Age group 114
Table 53: Prevalence of ID, IDA and the proportion of anemia associated with ID by iron indicator 115
Table 54: Effect infection and sampling process on plasma Zinc Concentration 121
Table 55: Distribution of mean PZC by Region 121
Table 56: Distribution of mean PZC by SES Quintile 122
Table 57: Distribution of mean PZC by Age group 122
Table 58: Prevalence of Low Plasma Zinc Concentration 123
Table 59: Mean plas ma concentrations of Folate and Vitamin B12 by Region 124
Table 60: Mean plas ma concentrations of Folate and Vitamin B12 by SES Quintile 124
Table 61: Mean plas ma concentrations of Folate and Vitamine B12 by Agegroup 124
Table 62: Prevalence of low Folate and Folate and vitamin B12 deficiency 125
Table 63: Mean Values of Trace Elements by region, SES quintile and Agegroup 126
Table 64: Correlations between Trace Elements, FCS, and DDS 127
Table 65: Levels of Serum Micro-nutrients (Mean±SEM) in relation to Nutritional status of children of the North and Far North regions of Cameroon 127
Table 66: Correlation between Micro-nutrients and Nutritional in children of the North and Far North regions of Cameroon 128
Table 67: Prevalence of Major Micronutrient deficiencies 129
Table 68: Prevalence of Multiple micronutreint deficiencies 129
Table 69: Characterization of Multiple micronutrient deficiencies........ 129
Table 70: Distribution of Vitamin A Deficiency by Nutritional Status 131
Table 71: Determinants of Vitamin A Deficiency 132
Table 72: Distribution of iron deficiency by Nutritional status........ 133
Table 73: Determinants of Iron Deficiency 133
Table 74: Distribution of Low Plasma Zinc concentration by nutritional status........ 134
Table 75: Determinants of Low plasma Zinc Concentration 134
Table 76: distribution of Low folate by Nutritional Status 135
Table 77: Determinants of low folate 135
Table 78: Distribution Vitamin B12 Category by Nutritional status........ 136
Table 79: Determinants of vitamin B12 Deficiency........ 136
Table 80: Determinants of both folate and Vitamin B 12 137

LIST OF FIGURES

Figure 1: Effect of different amounts of Calcium on iron absorption (Baynes *et al.,* 1990)............- 26 -
Figure 2: the chemical formula of Folic Acid and the most important natural folates (*et al*., 1994) - 43 -
Figure 3: the role of the Folate cofactors in the DNA cycle and the methylation cycle (*et al*., 1997) - 45 -
Figure 4: Major calcium Movements in the Body (*et al*., 1981)...- 49 -
Figure 5 Schematic representation of sampling Results.. 70
Figure 9: The arrangement of the samples on a 96-well plate (01– 40: study samples; BL, L1–L5: standards, QC1 and QC2 quality controls).. 77
Figure 10: Prevalence of ID by age group among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals............................. 116
Figure 11: Prevalence of ID by Vit A status Category among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals. 116
Figure 12: Prevalence of ID by caregiver educational level among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals. .. 117
Figure 13: Prevalence of ID by Socio-economic status quintile among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals 117
Figure 14: Prevalence of ID by Urban and rural milieu among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals. 118
Figure 15: Prevalence of ID by child stunting among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals. 118
Figure 16: Prevalence of ID by child wasting among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals 119
Figure 17: Prevalence of ID by child Underweight among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals. 119
Figure 18: Prevalence of ID by study region among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals............................. 120
Figure 19: Prevalence of ID by presence of inflammation among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals. .. 120
Figure 20: Characterization of Multiple micronutrient deficiencies 130
Figure 21: Patterns of both Folate and Vitamin B12 deficiency with Nutritional status 138

INTRODUCTION

Micronutrient deficiencies are caused by inadequate dietary intake, increased losses from the body, and/or increased requirements (Harrador *et al*., 2014). It has become recognized by the nutrition community that micronutrient malnutrition is very widespread, probably one of the main nutritional problems in the world (Allen *et al*., 2003) and a major contributor to childhood morbidity and mortality (WHO, 2009). Micronutrients of known public health importance include the following: zinc, iodine, iron, selenium, copper, vitamins A, E, C, D, B2, B6, B12 and folate (UNICEF 2014). Besides, micronutrient deficiencies (MD) and especially iron deficiency, is believed to be one of the main underlying causes of anemia (Van den Broek 2003). Zinc is an essential micronutrient, which is required for normal growth, immune function, neuro-behavioral development, and pregnancy outcomes (Hess *et al*., 2009). More than 2 billion people in the world today are estimated to be deficient in key vitamins and minerals, mainly vitamin A, iodine and zinc. Particularly in Africa, MD affect millions of people, especially the most vulnerable groups, which are children and pregnant women (WHO, 2014).

The World Health Organization estimates that 190 million preschool-age children and 19.1 million pregnant women worldwide are vitamin A (VA) deficient (WHO, 2009). VA is important for epithelial differentiation and function (i.e., skin, gut, etc.), vision, hemoglobin synthesis, and adequate immune function. As a result, VA deficiency (VAD) leads to a diverse set of consequences, including increased severity of infection and increased risk of mortality, night blindness and xerophthalmis, and anemia. Supplementation of night-blind (i.e., vitamin A-deficient) women in Nepal was associated with a 40% reduction in maternal mortality. A recent meta-analysis reported that high dose supplementation of children less than 5 years resulted in a 24% reduction in all-cause mortality (Sommer, 1982).

Iron deficiency (ID) and its more severe form, iron-deficiency anemia (IDA), are public health problems worldwide (WHO, 2005). Although there are no global estimates of the prevalence of ID, an estimated 2 billion people are anemic, with a non-negligible proportion of these cases likely due to iron deficiency. Iron-deficiency anemia is related to reduce physical capacity, adverse pregnancy outcomes, increased risk of maternal mortality, and impaired cognitive development in children (McGregor et al., 2001). Increased iron intake, through both iron supplements and fortified products, increases hemoglobin and markers of iron status among individuals with iron-deficiency anemia, although the provision of prophylactic iron

supplements to young children in malaria endemic areas is a subject of recent concern. A recent meta-analysis estimated a 73% reduction of ID and 67% reduction of IDA among pregnant women who took oral iron supplements (Gibson, 2005).
An estimated 20% of the world's population is at risk of inadequate zinc intake, with a higher prevalence in low-income countries (FAO, 2009). Zinc deficiency can lead to stunting (low height-for age), which is associated with lower cognitive performance and reduced economic productivity later in life (Rosado *et al*., 1997). Supplementation of zinc-deficient children increases serum zinc, improves linear growth, and decreases the incidence of diarrhea and pneumonia. Zinc supplementation during diarrhea treatment reduces the severity and duration of diarrheal episodes (Behrens *et al*., 1990).

Folate and vitamin B-12 are both key components of one-carbon metabolism (Borjel *et al*., 2005). Deficiency of folate or B-12 inhibits production of thymidylate, which is critical for DNA synthesis. As a result, deficiency of either nutrient can progress to megaloblastic anemia and increase the risk of neural tube defects (Srikantia et a., 1967).

Micronutrient deficiencies are likely to be common in Cameroon, although population micronutrient status has not been thoroughly and consistently monitored. A national survey conducted in 2000 found that 39% of children 1-5 years of age had serum retinol concentrations less than 0.70 μmol/L. However, the study did not measure any indicators of infection or inflammation, which can temporarily lower serum retinol, independent of VA status. Thus, the "true" prevalence of VA deficiency (VAD) is likely somewhat lower than this estimate, but still above a level that would be considered indicative of a public health problem. Assuming a 'true prevalence' of 40% VAD, an estimated 12,000 deaths of children 12-59 months of age per year would be attributable to VAD in the absence of appropriate VAD control strategies (EDS-MICS, 2011).

The 2000 survey also reported that 27.3% of women and 57.9% of children were anemic, although indicators of iron, folate, and vitamin B-12 status were not measured (MSP 2001). In the Demographic and Health Survey in 2004, the prevalence of anemia among women of reproductive age (hemoglobin < 110 g/L for pregnant women and < 120 g/L for nonpregnant women) and children 6-59 months of age (hemoglobin < 110 g/L) was 45% and 70%, respectively (EDS, 2004).

Although zinc deficiency has not been specifically measured in Cameroon, the high prevalence of stunting suggests that zinc deficiency may also be a public health problem (Hotz *et al*., 2004).

The national prevalence of stunting among children 0-59 months of age as measured by the Cameroon Demographics and Health Surveys (27% in 1991 and 32% in 2004) was greater than the cutoff of > 20%, which is considered indicative of a public health problem. Additional suggestive evidence of zinc deficiency can be derived from the FAO Food Balance Sheets, which contain national estimates of food availability. Using this method, the estimated proportion of the population at risk of inadequate zinc intake in Cameroon was greater than the cutoff of > 25% considered to indicate elevated risk of zinc deficiency (FAO, 2009).

Rationale

Studies in Cameroon have shown that MD are prevalent and likely contribute to increased morbidity and mortality (Aguayo 2005). A national survey conducted in 2001 found that 39% of children 1–5 years of age had serum retinol concentrations < 0.70 µmol/L (without adjustment for elevated acute phase proteins) (MSP 2001). The prevalence of anemia among children 6–59 mo (hemoglobin <110 g/L) was 70%, in 2004.

Although the prevalence of zinc deficiency has not been specifically measured, the high prevalence of stunting among children 0–59 mo old (32%) suggests that zinc deficiency may also be a public health problem. More specifically, a study found that the prevalence of stunting was 33% (32% South; 46% North; 13% Yaoundé/Douala). Among women, 82% had low adjusted PZC (<50 mg/dL for pregnant women; <66 mg/dL for others; 79% South, 89% North, 76% Yaoundé Douala). Among children, 83% had low adjusted PZC (<65 mg/dL; 80% South, 92% North, 74% Yaoundé/Douala). Though this study identified micronutrient deficiency in the North zone (Admawa, North and Far North Regions), this study did not assess what the risk factors for these specific regions were. And given that the sample was nationally representative, region specific cofactors were not assessed. Thus given that the risk of Zinc deficiency is highest in the Northern part of the country, in-depth analysis of the risk factors associated to low PZC is essential to better characterize the phenomenon (Engle-Stone *et al*., 2014).

In 2009, a national survey in Cameroon found that the prevalence of ID in children 12–59 mo of age ranged from 14 to 68%, depending on the indicator and adjustment for inflammation applied (Engle-Stone *et al*., 2012). This broad range of estimated prevalence complicates policy decisions, which are generally related to specific levels of disease prevalence. Another study published in 2013 demonstrated that different markers of irons status identify similar risk factors for Iron deficiency (Engle-Stone *et al*., 2013). There is no awareness of any study that has assessed the risk factors for iron deficiency in the North and Far North regions, and specifically the influence for food consumption indicators as predictors of ID.

The same national survey in Cameroon found out the prevalence of Vitamin A was 35% in the national Sample and 43% in the Northern Zone. Another published report (Engle-Stone *et al*., 2011) showed that in Cameroon, plasma retinol-binding protein predicts plasma retinol concentration and could be a good indicator for VAD in population studies. These studies did not identify specific risk factors for Vitamin A deficiency.

Another study published in 2012 (Shabah-Ferdows *et al*., 2012) revealed that the prevalence (%) of B12 deficiency (B12<150 pM) in mothers and children was 25% and 24% respectiveyly in urban setting vs. 33% and 34% respectively in rural areas; and folate deficiency (folate<10 nM) 17 and 6 urban vs. 25 and 24 rural. Living in the North, low SES and pregnancy predicted low B12, while folate was highest in the south and negatively associated with SES. This study however did not asses the possible interference of the other micronutrient deficiencies on the risk or low folate or low B12. Also, there is little or no data on the prevalence of low B12 or low folate in Cameroon.

Finally, though many studies (EDS-MICS, 2011; EDS, 2004,) have documented that malnutrition as assessed by many indices are a major public health problem in Cameroon and especially in the North and Far North regions, there is no wareness of any study that has assessed the risk factors for malnutrition in the North and Far North regions.

Though recent studies that have discussed on insufficiencies of some micronutrients in Cameroonian children, there is no awareness of any recent publication regarding a comprehensive study that includes multiple micronutrients, hemoglobin, as well as Nutritional status among children in the North and Far North Regions of Cameroon.

Therefore, the present study aimed at contributing to describing the distribution of selected micronutrients and the presence of anemia among children aged 12-59 months living in rural and urban areas of the North and Far North regions of Cameroon and their relationship with Nutritional status.

OBJECTIVE

The main objective of this study is to describe the distribution of selected micronutrients and the presence of anemia among children aged 12-59 months living in rural and urban areas of the North and Far North regions of Cameroon and their relationship with Nutritional status.

Specific Objectives

1. **To determine the consumption patterns foods among children 12-59 months of age.**
2. **Assess the nutritional Status of children 12-59 months in the North and Far North Regions of Cameroon**
3. **To determine the prevalence of micronutrient deficiencies (iron, zinc, folate, and vitamins A and B12, Calcium, Magnesium and Copper) among children 12-59 months of age.**
4. **To determine the predictors of micronutrient deficiencies (iron, zinc, folate, and vitamins A and B12) and Nutritional status among children 12-59 months of age.**

CHAPTER 1: LITERATURE REVIEW

1.1. MALNUTRITION

Malnutrition has been recognized for millennia as being due to a shortage of food. Ancel Keys lists many of the famines recorded in history (keys *et al*., 1945) which disappeared when the normal diet of the population returned. Kwashiorkor as a separate entity was recognized early in Latin America and called “multi-deficiency syndrome”(Autrete *et al*., 1954) and in Europe as “flour dystrophy” (Reitschel *et al*., 1908). Later it was described from Africa by Cecile Williams in the English literature (Williams 1933) given the name kwashiorkor, and recorded as responding to milk. After a brief argument whether this was a form of pellagra(Gillman *et al*., 1951) most experts accepted that the cause was protein deficiency(Dean *et al*., 1954) a view that has persisted(Waterlow ,1984) particularly among those who invested their life’s work investigating protein metabolism and deficiency on the basis that it would illuminate kwashiorkor. Marasmus was thought to be due to energy deficiency as failure to give sufficient energy always leads to weight loss and dietary surveys showed a low energy intake in marasmic children. Starvation was studied extensively during and after the Second World War and the etiology assumed to apply to marasmic children. This led to the treatment of all types of malnutrition with high-protein, high-energy diets and the naming of these forms of malnutrition as first protein-calorie malnutrition and then protein-energy malnutrition (which is still the Index Medicus and International Classification of Diseases nomenclature). There are many technical terms and concepts used in any discussion of malnutrition. It is important to understand what these mean and to use the terms correctly.

1.1.1. DEFINITION

The term malnutrition generally refers both to under nutrition and over nutrition. Many factors can cause malnutrition, most of which relate to poor diet or severe and repeated infections, particularly in underprivileged populations. Inadequate diet and disease, in turn, are closely linked to the general standard of living, the environmental conditions, and whether a population is able to meet its basic needs such as food, housing and health care. Malnutrition is thus a health outcome as well as a risk factor for disease and exacerbated malnutrition and it can increase the risk both of morbidity and mortality.

The World Health Organization defines malnutrition as "***the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions.***"(WHO, 2006)- Malnutrition generally implies under nutrition and refers to all deviations from adequate and optimal nutritional status in infants, children and in adults. In children, under nutrition manifests as underweight and stunting (short stature), while severely undernourished children present with the symptoms and signs that characterize conditions known as kwashiorkor, marasmus or marasmic-kwashiorkor

Changes in body dimensions reflect the overall health and welfare of individuals and populations. Anthropometry is used to assess and predict performance, health and survival of individuals and reflect the economic and social wellbeing of populations. Anthropometry is a widely used, inexpensive and non-invasive measure of the general nutritional status of an individual or a population group. Recent studies have demonstrated the applications of anthropometry to include the prediction of who will benefit from interventions, identifying social and economic inequity and evaluating responses to interventions.

1.1.2. CLASSIFICATION OF MALNUTRITION

The first real attack on the protein deficiency theory of kwashiorkor came from Gopalan (Gopalan *et al*., 1968) where he found that the antecedent diets of children with kwashiorkor and marasmus were not different in terms of protein, a finding that has since been confirmed(Lin *et al*., 2007)·. Shrikantia then ascribed the edema of kwashiorkor to the antidiuretic effects of ferritin, which he found elevated in edematous malnutrition (Srikantia *et al*., 1958)·. This seminal work was discounted internationally because the paper was not in a peer reviewed journal and the electrolyte pattern did not usually accord with an antidiuretic effect. Importantly, there was no alternative paradigm at that time so that the protein hypothesis was not abandoned. The next advance was to show that children could lose all their edema without a change in plasma albumin level (Golden *et al*., 1980) and that protein intake was not associated with rates of recovery (Golden *et al*., 1982). Furthermore, the high ferritin values found by Sri kantia were also confirmed (Ramdath *et al*., 1989). These children have liver dysfunction with reduced levels of amino acid metabolizing enzymes and abnormal urinary metabolites (Whitehead,1964) . One would not give high levels of protein to a child with an inborn error of amino-acid metabolism. It is unfortunate that the studies of the livers of malnourished children, showing similar defects, albeit acquired, should not have been translated into clinical practice and led to the restriction of protein in the diet of malnourished children. In Somalia mortality fell when protein was restricted in the diets of severely malnourished adult patients (Collins et al., 1998) (mortality on high and moderate protein diets – edematous 51% *vs* 25%, $P<0.05$, marasmic 22% *vs* 13%, $P = 0.08$). There then developed the concept of kwashiorkor being due to a lack of antioxidant nutrients (Golden *et al*., 1991)·, a hypothesis which has not been confirmed by intervention trials(Ciliberto et al., 2005) despite evidence of oxidative damage in the same population (Manary *et al*., 2000) It is incontrovertible that if sufficient food is not taken, for whatever reason, the child will lose weight and become marasmic. This was interpreted as energy deficiency and the treatment response was to give additional energy in the diet. Furthermore, metabolic studies showed that wasted children's rates of weight gain were closely related to their energy intake. For this reason, the energy density of the diets was increased by the addition of lipid (Ashworth *et al*., 1973) to limits where

water deficiency and hypernatraemic dehydration were real possibilities. The reason for the decreased energy intake could of course be starvation, and it is notable that those that get marasmus are almost always dependent upon others for food: infants and children, prisoners, the elderly, infirm, mentally ill and indigent.

As a measure of metabolic wellbeing, appetite is particularly disturbed with liver dysfunction, during the metabolic response to infection (Brown et al., 1990) and with deficiency of certain essential nutrients. During these conditions, loss of appetite is the main reason for weight loss (Miall et al., 1970); with infection, during convalescence with a good diet there is an increased appetite and regain of lost weight. The studies that showed a relationship between infection and malnutrition are cross-sectional statistical analyses – this effect is not seen with longitudinal studies where under normal circumstances acute infection does not result in wasting (Briend et al., 1989) after convalescence.

One of the most potent causes of loss of appetite in all experimental animals is a dietary imbalance of certain nutrients, and malnourished children given traditional weaning foods normally have a reduced appetite (Brown et al., 1995). If diminution of appetite is due to deficiency of a specific nutrient in the habitual diet of the children, then dietary surveys will indeed showed that they have a reduced energy intake, but this will not be due to energy deficiency and not be corrected by giving additional energy in the form of carbohydrate or lipid. It will only be cured by giving the specific nutrients that are missing in the habitual diet that cause the loss of appetite. The appetites of children given zinc supplements improved and they started to catch up in height (Krebs et al., 1984). From these realizations, the theory of type I (functional nutrients) and type II nutrients was generated (Golden *et al*., 1988). The implication is that if protein deficiency is involved in malnutrition the clinical expression will be marasmus and not kwashiorkor, however other type II nutrients, such as zinc, phosphorus or magnesium are likely to become limiting in the diet before protein (Golden *et al*., 1981). If the diet is poor then when weight is lost from an infection, there will be insufficient type II nutrient density to allow for catch-up growth during convalescence. Zinc is frequently the limiting type II nutrient, although not always (Maraziegos *et al*., 2010); the effect of zinc on convalescence from diarrhea, now a world-wide WHO promoted intervention, is simply a specific example of a general phenomenon. There has to be sufficient and the right balance of type II nutrients in the diet to promote convalescence. If the diets were adequate there would be no requirement for zinc supplements for the recovering child. Perhaps the supplement should contain all the type II nutrients in balance and be given to all children after an acute weight loss. If kwashiorkor is not due to protein deficiency and marasmus is not usually due to energy deficiency, then the name "protein-energy malnutrition" gives quite the wrong message and leads to inappropriate treatment of the malnourished individual. This nomenclature has not only led to quite

inappropriate policy decisions but also probably led to deaths. Based upon the protein-energy deficiency theories, diets were devised and tested in large scale trials. They either failed to prevent malnutrition of all sorts, or had a marginal effect. An influential review of these programs(Beaton *et al*., 1982) led to disillusionment with “food” shortage as a cause of malnutrition, and the focus switched to infection as the primary cause, a view which persists to this day(Black *et al*., 2008). Although improved sanitation prevents mortality and morbidity from water-borne infection, it does not prevent stunting, wasting or edematous malnutrition any more than the trials of improving diets in protein and energy.

Malnutrition can be of the acute, chronic or mixed type (Joosten *et al*., 2008). Acute malnutrition is the type that usually occurs in with illness, but children with underlying chronic diseases who are admitted to the hospital because of an acute illness can also present with chronic malnutrition.

Anthropometric variables are used to define nutritional status worldwide but various classification systems and cutoff points are used to define malnutrition. One such classification method includes kwashiorkor and marasmus. These terms were originally established to describe syndromes of protein-energy malnutrition in children in developing countries. The most used classification system was that described by Waterlow(Waterlow ,1972), in which acute and chronic malnutrition were divided into four stages, on the basis of the actual weight to the 50th percentile of weight for height (WFH) for acute malnutrition and the actual height to the 50th percentile for height for chronic malnutrition.

In 2006, WHO published child growth standards for attained weight and height to replace the previously recommended 1977 NCHS/WHO child growth reference. These new standards are based on breastfed infants and appropriately fed children of different ethnic origins raised in optimal conditions and measured in a standardized way(WHO, 2006). The same cohort was used to produce standards of mid-upper arm circumference (MUAC) in relation to age. The new WHO growth standards confirm earlier observations that the effect of ethnic differences on the growth of infants and young children in populations is small compared with the effects of the environment. Studies have shown that there may be some ethnic differences among groups, just as there are genetic differences among individuals, but for practical purposes they are not considered large enough to invalidate the general use of the WHO growth standards population as a standard in all populations. These new standards have been endorsed by international bodies such as the United Nations Standing Committee on Nutrition, the International Union of Nutritional Sciences and International Pediatric Association and adopted in more than 90 countries (WHO, 2006).

1.1.2.1. Acute Malnutrition

The main characteristic of acute malnutrition is wasting. Wasting occurs as a result of recent rapid weight loss, malnutrition or a failure to gain weight within a relatively short period of time. Wasting occurs more commonly in infants and younger children, often during the stage when complementary foods are being introduced and children are more susceptible to infectious diseases. Recovery from wasting is relatively quick once optimal feeding, health and care are restored. Wasting occurs as a result of deficiencies in both macronutrients (fat, carbohydrate and protein) and some micronutrients (vitamins and minerals). **Weight-for-height** (WFH) is a widely used nutritional or anthropometric index, and is the best indicator of wasting. WFH is recommended for assessments of recent nutrition, and is especially important for assessments of nutrition-related humanitarian emergencies. Depending on the anthropometric parameters, the WHO has classified acute malnutrition in to two types (WHO 2009):

Global acute Malnutrition (GAM): this encomprises both Severe and Moderate acute malnutrition.

- Weight-for-hieght < - 2 SD
- Mid Upper Arm Circumference < 125mm

Moderate Acute Malnutrition (MAM)

- Weight-for-hieght ≥ - 3 SD and < - 2 SD
- Mid Upper Arm Circumference ≥ 115mm and < 125mm
- **NO** Billateral oedema

Severe Acute Malnutrition (SAM)

For children 6-60 months, severe acute malnutrition is defined[1] as:

- Weight-for-hieght < - 3 SD
- Mid Upper Arm Circumference <115mm
- Billateral oedema (*Bilateral oedema is an essential indicator for determining the presence of Severe Acute Malnutrition or kwashiorkor. It presents first in feet, then in ankles and lower limbs. Oedema results from the excessive accumulation of extracellular fluid as a result of severe nutritional deficiencies, and is a serious cause for concern. All children with nutritional oedema are automatically classified as severely malnourished. Oedema may be detected by the production of a definite pit as a result of moderate pressure for three seconds with the thumb just above the ankle*.)

The following terms are used to describe the clinical manifestations of SAM: Marasmus (severe wasting) Kwashiorkor (bilateral pitting oedema) and Marasmic kwashiorkor (mixed form of bilateral pitting oedema and severe wasting). The following paragraphs will look closely at marasmus and kwashiorkor (WHO, 2006).

[1] Report of a meeting to harmonize the criteria for monitoring and evaluation of the treatment of acute malnutrition in west and central africa, 2010

1.1.2.1.1. *Diagnosing severe acute malnutrition (severe wasting or kwashiorkor or marasmic kwashiorkor)*

WHO defined severe malnutrition in children as a weight-for-height[2] below -3 SD[3] (based on NCHS reference) and/or the presence of oedema(WHO,1999). Experts in a meeting in 2005, recommended to add Mid Upper Arm Circumference (MUAC) less than 110 mm (in 6 to 60 month old children) as an independent diagnostic criterion. Since the 2005 meeting, the WHO standards have been published and there is therefore a need to reassess diagnostic criteria including MUAC. The rationale for keeping the same cut-off for weight-for-height when defining severe acute malnutrition and for adjusting the MUAC cut-off up to 115 mm, based on the WHO standards is given below (WHO,2007).

1.1.2.1.2. *Risk of death and severe acute malnutrition*

Following the release of the WHO child growth standards, the relationship between weight-for-height and the risk of dying was reassessed in existing epidemiological studies. This analysis showed that children with a weight-for-height below -3 SD based on the WHO standards have a high risk of death exceeding 9-fold that of children with a weight-for-height above -1 SD (Black *et al*., 2008). Similar studies using MUAC as diagnostic criteria showed that the risk of dying is increased below 115 mm (Myatt *et al*., 2007).

1.1.2.1.3. *Specificity of recommended cut-offs for diagnosing severe acute malnutrition*

Weight-for-height below -3 SD is a highly specific criterion to identify severely acutely malnourished infants and children. Statistical theory shows that in a well-nourished population, only 0.13% of children will have a weight-for-height less than -3 SD, giving a specificity of more than 99%[4] for this cut-off. With the release of the WHO standards for MUAC-for-age, the revision of the earlier recommended MUAC cut-off of 110 mm as an independent diagnostic criterion for severe acute malnutrition was necessary. A higher cut-off of 115 mm is recommended as it will identify more infants and children as having severe acute malnutrition and still have a high specificity of more than 99% over the age range 6–60 months (WHO,2007).

The child's nutritional status is expressed either as a percentage of the reference median, or alternatively as a Zscore. The calculation of the percentage of the median is relatively easy. Z-scores are the equivalent of standard deviations in a normal bell-shaped distribution curve. The normal range for growth is assumed to lie between –2 and +2 standard deviations, which include 95% of the reference population. This means that, even within the reference population,

---

[2] When assessing weight-for-height, infants and children under 24 months of age should have their lengths measured lying down (supine). hildren over 24 months of age should have their heights measured while standing. For simplicity, however, infants and children under 87 cm can be measured lying down (or supine) and those above 87 cm standing.

[3] A z-score is the number of standard deviations (SD) below or above the reference median value.

[4] Specificity is defined as the percentage of healthy individuals correctly diagnosed as healthy by the diagnostic test

5% of children lie outside of the normal range. Z-scores are expressed in multiples of the standard deviation, so that a Z-score of 0 is equivalent to the median (100% percentage WFH), while a Z-score of –2 lies two standard deviations below the median (WHO,2007) .

1.1.2.2. Chronic Malnutrition

Chronic malnutrition is most often reflected by stunting i.e. inadequate height or length[5] relative to age. **Stunting** is a failure to grow in stature, and occurs as a result of inadequate nutrition over a longer time period, which is why it is also referred to as chronic malnutrition. It is a slow, cumulative process, the effects of which are not usually apparent until the age of two years, although to prevent stunting action is needed before a child reaches the age of two. Stunting is not a good indicator of growth failure in emergencies as it does not reflect recent changes and requires a long-term response. The effects of stunting are not completely reversible, and children who suffer from chronic malnutrition and become stunted will grow up to become small adults. The nutritional index Height – for – Age (HFA) reflects skeletal growth (stature), and is the best indicator of stunting. The longer time-scale over which height-for-age is affected makes it more useful for long-term planning and policy development, rather than emergencies (WHO,2007) . Using the WHO 2006 height for age cutoffs, Chronic malnutrition can be subdivided into two types:

Severe Chronic Malnutrition

- Height – for - Age < - 3 SD

Moderate Chronic Malnutrition

- Height – for - Age ≥ - 3 SD and < -2 SD

1.2. MICRONUTRIENTS AND MICRONUTRIENT DEFICIENCIES

1.2.1. VITAMIN A

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction. These dietary needs for vitamin A are normally provided for as preformed retinol (mainly as retinyl ester) and provitamin A carotenoids.

Overview of vitamin A metabolism

Preformed vitamin A in animal foods occurs as retinyl esters of fatty acids in association with membrane-bound cellular lipid and fat-containing storage cells. Provitamin A carotenoids in foods of vegetable origin are also associated with cellular lipids but are embedded in complex cellular structures such as the cellulose-containing matrix of chloroplasts or the pigment-containing portion of chromoplasts. Normal digestive processes free vitamin A and carotenoids

[5] Children under 2 years are measured lying down (length) and children 2–5 years are measured standing up (height).

from food matrices, which is a more efficient process from animal than from vegetable tissues. Retinyl esters are hydrolysed and the retinol and freed carotenoids are incorporated into lipid-containing, water-miscible micellar solutions. Products of fat digestion (e.g. fatty acids, monoglycerides, cholesterol, and phospholipids) and secretions in bile (e.g. bile salts and hydrolytic enzymes) are essential for the efficient solubilization of retinol and especially for solubilization of the very lipophilic carotenoids (e.g. a- and bcarotene, b-cryptoxanthin, and lycopene) in the aqueous intestinal milieu (Jayarajan *et al*., 1980).

Micellar solubilization is a prerequisite to their efficient passage into the lipidrich membrane of intestinal mucosal cells (i.e. enterocytes) (Blomhoff *et al*., 1991 ,). Diets critically low in dietary fat (under about 5–10 g daily) (Jayarajan *et al*., 1980) or disease conditions that interfere with normal digestion and absorption leading to steatorrhoea (e.g. pancreatic and liver diseases and frequent gastroenteritis) can therefore impede the efficient absorption of retinol and carotenoids. Retinol and some carotenoids enter the intestinal mucosal brush border by diffusion in accord with the concentration gradient between the micelle and plasma membrane of enterocytes. Some carotenoids pass into the enterocyte and are solubilized into chylomicrons without further change whereas some of the provitamin A carotenoids are converted to retinol by a cleavage enzyme in the brush border (Parker *et al*., 1996). Retinol is trapped intracellularly by re-esterification or binding to specific intracellular binding proteins. Retinyl esters and unconverted carotenoids together with other lipids are incorporated into chylomicrons, excreted into intestinal lymphatic channels, and delivered to the blood through the thoracic duct (Ong *et al*., 1994).

Tissues extract most lipids and some carotenoids from circulating chylomicrons, but most retinyl esters are stripped from the chylomicron remnant, hydrolysed, and taken up primarily by parenchymal liver cells. If not immediately needed, retinol is re-esterified and retained in the fat-storing cells of the liver (variously called adipocytes, stellate cells, or Ito cells). The liver parenchymal cells also take in substantial amounts of carotenoids. Whereas most of the body's vitamin A reserve remains in the liver, carotenoids are also deposited elsewhere in fatty tissues throughout the body (Bloomhoff *et al*., 1991). Usually, turnover of carotenoids in tissues is relatively slow, but in times of low dietary carotenoid intake, stored carotenoids are mobilized. A recent study in one subject using stable isotopes suggests that retinol can be derived not only from conversion of dietary provitamin carotenoids in enterocytes—the major site of bioconversion—but also from hepatic conversion of circulating provitamin carotenoids (Novotny *et al*., 1995). The quantitative contribution to vitamin A requirements of carotenoid converted to retinoids beyond the enterocyte is unknown. Following hydrolysis of stored retinyl esters, retinol combines with a plasma-specific transport protein, retinol-binding protein (RBP). This process, including synthesis of the unoccupied RBP (apo-RBP), occurs to the greatest

extent within liver cells but it may also occur in some peripheral tissues. The RBP-retinol complex (holo-RBP) is secreted into the blood where it associates with another hepatically synthesized and excreted larger protein, transthyretin. The transthyretin-RBP-retinol complex circulates in the blood, delivering the lipophilic retinol to tissues; its large size prevents its loss through kidney filtration. Dietary restriction in energy, proteins, and some micronutrients can limit hepatic synthesis of proteins specific to mobilization and transport of vitamin A. Altered kidney functions or fever associated with infections (e.g. respiratory infections (Stephensen *et al*., 1994) or diarrhoea (Avarez *et al*., 1995) can increase urinary vitamin A loss.

Holo-RBP transiently associates with target tissue membranes, and specific intracellular binding proteins then extract the retinol. Some of the transiently sequestered retinol is released into the blood unchanged and is recycled (i.e. conserved) (Green *et al*., 1994). A limited reserve of intracellular retinyl esters is formed that subsequently can provide functionally active retinol and its oxidation products (i.e. isomers of retinoic acid) as needed intracellularly. These biologically active forms of vitamin A are associated with specific cellular proteins which bind with retinoids within cells during metabolism and with nuclear receptors that mediate retinoid action on the genome (Ross *et al*., 1994). Retinoids modulate the transcription of several hundreds of genes (Chambon *et al*., 1996). In addition to the latter role of retinoic acid, retinol is the form required for functions in the visual (Rando *et al*., 1994) and reproductive systems (Eskild *et al*., 1994) and during embryonic development.

Holo-RBP is filtered into the glomerulus but recovered from the kidney tubule and recycled. Normally vitamin A leaves the body in urine only as inactive metabolites resulting from tissue utilization and in bile secretions as potentially recyclable active glucuronide conjugates of retinol. No single urinary metabolite has been identified which accurately reflects tissue levels of vitamin A or its rate of utilization. Hence, at this time urine is not a useful biological fluid for assessment of vitamin A nutriture (Morris-kay *et al*., 1996).

Biochemical mechanisms for vitamin A functions

Vitamin A functions at two levels in the body: the first is in the visual cycle in the retina of the eye; the second is in all body tissues where it systemically maintains the growth and soundness of cells. In the visual system, carrier bound retinol is transported to ocular tissue and to the retina by intracellular binding and transport proteins. Rhodopsin, the visual pigment critical to dim-light vision, is formed in rod cells after conversion of all-*trans*-retinol to retinaldehyde, isomerization to the 11-*cis*-form, and binding to opsin (Eskild *et al*., 1994). Alteration of rhodopsin through a cascade of photochemical reactions results in the ability to see objects in dim light. The speed at which rhodopsin is regenerated is related to the availability of retinol. Night blindness is usually an indicator of inadequate available retinol, but it can also be due to a deficit of other nutrients that are critical to the regeneration of rhodopsin, such as protein and

zinc, and to some inherited diseases, such as *retinitis pigmentosa*. The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency (VAD). In addition, goblet cell numbers are reduced in epithelial tissues and as a consequence, mucous secretions (with their antimicrobial components) diminish. Cells lining protective tissue surfaces fail to regenerate and differentiate, hence they flatten and accumulate keratin. Both factors—the decline in mucous secretions and loss of cellular integrity—reduce the body's ability to resist invasion from potentially pathogenic organisms. Pathogens can also compromise the immune system by directly interfering with the production of some types of protective secretions and cells. Classical symptoms of xerosis (drying or non-wetability) and desquamation of dead surface cells as seen in ocular tissue (i.e. xerophthalmia) are the external evidence of the changes also occurring to various degrees in internal epithelial tissues (Morris-kay *et al*., 1996).

Current understanding of the mechanism of vitamin A action within cells outside the visual cycle is that cellular functions are mediated through specific nuclear receptors. Binding with specific isomers of retinoic acid (i.e. all*trans*-and 9-*cis*-retinoic acid) activates these receptors. Activated receptors bind to DNA response elements located upstream of specific genes to regulate the level of expression of those genes. These retinoid-activated genes regulate the synthesis of a large number of proteins vital to maintaining normal physiologic functions. There may, however, be other mechanisms of action that are as yet undiscovered (Eskild *et al*., 1994).

Populations at risk for, and consequences of, vitamin A deficiency

Definition of vitamin A deficiency (VAD)

VAD is not easily defined. WHO defines it as tissue concentrations of vitamin A low enough to have adverse health consequences even if there is no evidence of clinical xerophthalmia (WHO,2004). In addition to the specific signs and symptoms of xerophthalmia and the risk of irreversible blindness, nonspecific symptoms include increased morbidity and mortality, poor reproductive health, increased risk of anaemia, and contributions to slowed growth and development. However, these nonspecific adverse effects may be caused by other nutrient deficits as well, making it difficult to attribute non-ocular symptoms specifically to VAD in the absence of biochemical measurements reflective of vitamin A status.

Units of expression

In blood, tissues, and human milk, vitamin A levels are conventionally expressed in µg/dl or µmol/l of all-trans-retinol. Except for postprandial conditions, most of the circulating vitamin A is retinol whereas in most tissues (such as the liver), secretions (such as human milk), and

other animal food sources, it exists mainly as retinyl esters, which are frequently hydrolysed before analytical detection.

Age and sex

VAD can occur in individuals of any age. However, it is a disabling and potentially fatal public health problem for children under 6 years of age. VAD related blindness is most prevalent in children under 3 years of age (Sommer *et al*., 1994). This period of life is characterized by high requirements for vitamin A to support rapid growth, and the transition from breastfeeding to dependence on other dietary sources of the vitamin. In addition, adequate intake of vitamin A reduces the risk of catching respiratory and gastrointestinal infections. The increased mortality risk from concurrent infections extends at least to 6 years of age and is associated with both clinical and subclinical VAD (Beaton *et al*., 1993). There is little information regarding the health consequences of VAD in school-age children. The prevalence of Bitot's spots (i.e. white foamy patches on the conjunctiva) may be highest in this age group but their occurrence may reflect past more than current history of VAD (sommer *et al*., 1980). Women of reproductive age are also thought to be vulnerable to VAD during pregnancy and lactation because they often report night blindness (Bloem *et al*., 1994,) and because their breast milk is frequently low in vitamin A (Wallingford *et al*., 1996,). Not all night blindness in pregnant women, however, responds to vitamin A treatment (Christian *et al*., 1998).

There is no consistent, clear indication in humans of a sex differential in vitamin A requirements during childhood. Growth rates, and presumably the need for vitamin A, from birth to 10 years for boys are consistently higher than those for girls (WHO 1995). In the context of varied cultural and community settings, however, variations in gender-specific child-feeding and care practices are likely to subsume a small sex differential in requirements to account for reported sex differences in the prevalence of xerophthalmia. Pregnant and lactating women require additional vitamin A to support maternal and fetal tissue growth and lactation losses, additional vitamin A which is not needed by other post-adolescent adults (Committee on Nutritional status 1990).

Risk factors

VAD is most common in populations consuming most of their vitamin A needs from provitamin carotenoid sources and where minimal dietary fat is available (Mele *et al*., 1991). About 90% of ingested preformed vitamin A is absorbed, whereas the absorption efficiency of provitamin A carotenoids varies widely, depending on the type of plant source and the fat content of the accompanying meal (Erdman *et al*., 1988). Where possible, an increased intake of dietary fat is likely to improve the absorption of vitamin A in the body. In areas with endemic VAD, fluctuations in the incidence of VAD throughout the year reflect the balance between intake and need. Periods of general food shortage (and specific shortages in vitamin A-rich foods)

coincide with peak incidence of VAD and common childhood infectious diseases (e.g. diarrhoea, respiratory infections, and measles). Seasonal food availability influences VAD prevalence directly by influencing access to provitamin A sources; for example, the scarcity of mangoes in hot arid months followed by the glutting of the market with mangoes during harvest seasons (Marsh *et al.*, 1995). Seasonal growth spurts in children, which frequently follow seasonal post-harvest increases in energy and macronutrient intakes, can also affect the balance. These increases are usually obtained from staple grains (e.g. rice) and tubers (e.g. light coloured yams) that are not, however, good sources of some micronutrients (e.g. vitamin A) to support the growth spurt (Sinha *et al.*, 1973). Food habits and taboos often restrict consumption of potentially good food sources of vitamin A (e.g. mangoes and green leafy vegetables). Culture specific factors for feeding children, adolescents, and pregnant and lactating women are common (Johns *et al.*, 1992, ,). Illness- and childbirth-related proscriptions of the use of specific foods pervade many traditional cultures (Mahadevan *et al.*, 1961). Such influences alter short- and long-term food distribution within families. However, some cultural practices can be protective of vitamin A status and they need to be identified and reinforced.

Morbidity and mortality

The consequences of VAD are manifested differently in different tissues. In the eye, the symptoms and signs, together referred to as xerophthalmia, have a long, well-recognized history and have until recently been the basis for estimating the global burden from the disease (Sommer *et al.*, 1994). Although ocular symptoms and signs are the most specific indicators of VAD, they occur only after other tissues have impaired functions that are less specific and less easily assessed.

The prevalence of ocular manifestations (i.e. xerophthalmia or clinical VAD) is now recognized to far underestimate the magnitude of the problem of functionally significant VAD. Many more preschool-age children, and perhaps older children and women who are pregnant or lactating, have their health compromised when they are subclinically deficient. In young children, subclinical deficiency, like clinical deficiency, increases the severity of some infections, particularly diarrhoea and measles, and increases the risk of death (Beaton *et al*, 1993,). Moreover, the incidence (Barreto *et al.*, 1994) and prevalence (Bhandari *et al.*, 1994) of diarrhoea may also increase with subclinical VAD. Meta-analyses conducted by three independent groups using data from several randomized trials provide convincing evidence that community-based improvement of the vitamin A status of deficient children aged 6 months to 6 years reduces their risk of dying by 20–30% on average (Fawzi *et al.*, 1993 ,). Mortality in children who are blind from keratomalacia or who have corneal disease is reported to be from 50% to 90% (Menon *et al.*, 1979), and measles mortality associated with VAD is increased by

up to 50% (Hussey *et al*., 1990). Limited data are available from controlled studies of the possible link between morbidity history and vitamin A status of pregnant and lactating women (West *et al*., 1997).

There are discrepancies in the link between incidence and severity of infectious morbidity of various etiologies and vitamin A status. A great deal of evidence supports an association of VAD with severity of an infection once acquired, except for respiratory diseases, which are non-responsive to treatment (The vitamin A and Pneumonia working Group 1995). The severity of pneumonia associated with measles, however, is an exception because it decreases with the treatment of vitamin A supplementation (Coutsoudis *et al*., 1991).

Infectious diseases depress circulating retinol and contribute to vitamin A depletion. Enteric infections may alter the absorptive surface area, compete for absorption-binding sites, and increase urinary loss (Solomons *et al*., 1991,). Febrile systemic infections also increase urinary loss (Thurnham *et al*., 1991) and metabolic utilization rates and may reduce apparent retinol stores if fever occurs frequently (Campos *et al*., 1987). In the presence of latent deficiency, disease occurrence is often associated with precipitating ocular signs (Curtail *et al*., 1995,). Measles virus infection is especially devastating to vitamin A metabolism, adversely interfering with both efficiencies of utilization and conservation (Foster *et al*., 1992). Severe protein–energy malnutrition affects many aspects of vitamin A metabolism, and even when some retinyl ester stores are still present, malnutrition—often coupled with infection— can prevent transport-protein synthesis, resulting in immobilization of existing vitamin A stores (Arrogave *et al*., 1991).

The compromised integrity of the epithelium, together with the possible alteration in hormonal balance at severe levels of deficiency, impairs normal reproductive functions in animals (Bates *et al*., 1983,). Controlled human studies are, of course, lacking. In animals and humans, congenital anomalies can result if the fetus is exposed to severe deficiency or large excesses of vitamin A at critical periods early in gestation (first trimester) when fetal organs are being formed (Takahashi *et al*., 1975). Reproductive performance, as measured by infant outcomes, in one community-based clinical intervention trial, however, was not influenced by vitamin A status (West *et al*., 1997).

The growth of children may be impaired by VAD. Interventions with vitamin A only have not consistently demonstrated improved growth in community studies because VAD seldom occurs in isolation from other nutrient deficiencies that also affect growth and may be more limiting

(Underwood *et al*., 1994). A lack of vitamin A can affect iron metabolism when deficiencies of both nutrients coexist and particularly in environments that favour frequent infections (IVACG 1998). Maximum haemoglobin response occurs when iron and vitamin A deficiencies are corrected together (Suharno *et al*., 1993). VAD appears to influence the availability of storage iron for use by haematopoietic tissue (sijtsma *et al*., 1993). However, additional research is needed to clarify the mechanisms of the apparent interaction.

Dietary sources of Vitamin A

Preformed vitamin A is found almost exclusively in animal products, such as human milk, glandular meats, liver and fish liver oils (especially), egg yolk, whole milk, and other dairy products. Preformed vitamin A is also used to fortify processed foods, which may include sugar, cereals, condiments, fats, and oils (Rodriguez, 1997). Provitamin A carotenoids are found in green leafy vegetables (e.g. spinach, amaranth, and young leaves from various sources), yellow vegetables (e.g. pumpkins, squash, and carrots), and yellow and orange non-citrus fruits (e.g. mangoes, apricots, and papayas). Red palm oil produced in several countries worldwide is especially rich in provitamin A (Booth *et al*., 1992). Some other indigenous plants also may be unusually rich sources of provitamin A. Such examples are the palm fruit known in Brazil as burití, found in areas along the Amazon River (as well as elsewhere in Latin America), and the fruit known as gac in Viet Nam, which is used to colour rice, particularly on ceremonial occasions (Vuong *et al*., 1997). Foods containing provitamin A carotenoids tend to have less biologically available vitamin A but are more affordable than animal products. It is mainly for this reason that carotenoids provide most of the vitamin A activity in the diets of economically deprived populations.

Indicators of vitamin A deficiency

Clinical indicators of vitamin A deficiency

Ocular signs of VAD are assessed by clinical examination and history, and are quite specific in preschool-age children. However, these are rare occurrences that require examination of large populations in order to obtain incidence and prevalence data. Subclinical VAD being the more prevalent requires smaller sample sizes for valid prevalence estimates (WHO,2004). A full description of clinical indicators of VAD, with coloured illustrations for each, can be found in the WHO field guide (Sommer, 1994). The most frequently occurring is night-blindness, which is the earliest manifestation of xerophthalmia. In its mild form it is generally noticeable after stress from a bright light that bleaches the rhodopsin (visual purple) found in the retina. VAD prolongs the time to regenerate rhodopsin, and thus delays adaptation time in dark environments. Night-blind young children tend to stumble when going from bright to dimly-lit

areas and they, as well as night-blind mothers, tend to remain inactive at dusk and at night (Christian *et al*., 1998).

No field-applicable objective tool is currently available for measuring night blindness in children under about 3 years of age. However, it can be measured by history in certain cultures (Underwood *et al*., 1993). In areas where night-blindness is prevalent, many cultures coin a word descriptive of the characteristic symptom that they can reliably recall on questioning, making this a useful tool for assessing the prevalence of VAD (Sommer *et al*., 1980). It must be noted that questioning for night-blindness is not always a reliable assessment measure where a local term is absent. In addition, there is no clearly defined blood retinol level that is directly associated with occurrence of the symptom, such that could be used in conjunction with questioning. Vitamin A-related night-blindness, however, responds rapidly (usually within 1–2 days) to administration of vitamin A (Eskild *et al*., 1994).

Subclinical indicators of vitamin A deficiency

Direct measurement of concentrations of vitamin A in the liver (where it is stored) or in the total body pool relative to known specific vitamin A-related conditions (e.g. night-blindness) would be the indicator of choice for determining requirements. This cannot be done with the methodology currently available for population use. There are several more practical biochemical methods for estimating subclinical vitamin A status but all have limitations (Underwood , 1990,). Each method is useful for identifying deficient populations, but not one of these indicators is definitive or directly related quantitatively to disease occurrence. The indicators of choice are listed in Table 1.

Table 1: Indicators of subclinical VAD in mothers and children aged 6-71 months

Indicator	Cut-off to indicate deficiency
Night-blindness (24-71 months)	≥1% report a history of night-blindness
Biochemical	
Breask-milk retinot	≤1.05µmol/l (≤8µg/g milk fat)
Serum retinol	≤0.70µmol/l
Relative dose response	≥20%`
Modified relative dose response	Ratio ≥0.06
Source: Adapted from reference []	

These indicators are less specific to VAD than clinical signs of the eye and less sensitive than direct measurements for evaluating subclinical vitamin A status. WHO recommends that where feasible at least two subclinical biochemical indicators, or one biochemical and a composite of non-biochemical risk factors, should be measured and that both types of indicators should point to deficiency in order to identify populations at high risk of VAD (WHO, 2004). Cut-off points given in Table 1 represent the consensus gained from practical experience in comparing

populations with some evidence of VAD with those without VAD. There are no field studies that quantitatively relate the prevalence of adverse health symptoms (e.g. incidence or prevalence of severe diarrhoeal disease) and relative levels of biologic indicator cut-off values. Furthermore, each of the biochemical indicators listed is subject to confounding factors which may be unrelated to vitamin A status (e.g. infections) (Eskild *et al.*, 1994). Although all biochemical indicators currently available have limitations, the preferred biochemical indicator for population assessment is the distribution of serum levels of vitamin A (serum retinol). Only at very low blood levels (<0.35µmol/l) is there an association with corneal disease prevalence (sommer *et al.*, 1982). Blood levels between 0.35 and 0.70µmol/l are likely to characterize subclinical deficiency (Watchmiester *et al.*, 1988), but subclinical deficiency may still be present at levels between 0.70 and 1.05µmol/l and occasionally above 1.05µmol/l (Flores *et al.*, 1984). The prevalence of values below 0.70mmol/l is a generally accepted population cutoff for preschool-age children to indicate risk of inadequate vitamin A status and above 1.05mmol/l to indicate an adequate status (Flores *et al.*, 1991). As noted elsewhere, clinical and subclinical infections can lower serum levels of vitamin A on average by as much as 25%, independently of vitamin A intake (Christian *et al.*, 1998,). Therefore, at levels between about 0.5 and 1.05mmol/l, the relative dose response or the modified relative dose response test on a subsample of the population can be useful for identifying the prevalence of critically depleted body stores when interpreting the left portion of serum retinol distribution curves (sommer *et al.*, 1982).

1.2.2. IRON

Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues. The physiology of iron has been extensively reviewed (Bothwell , 1979).

Most of the iron in the body is present in the erythrocytes as haemoglobin, a molecule composed of four units, each containing one haem group and one protein chain. The structure of haemoglobin allows it to be fully loaded with oxygen in the lungs and partially unloaded in the tissues (e.g. in the muscles). The iron-containing oxygen storage protein in the muscles, myoglobin, is similar in structure to haemoglobin but has only one haem unit and one globin chain. Several iron-containing enzymes, the cytochromes, also have one haem group and one globin protein chain. These enzymes act as electron carriers within the cell and their structures do not permit reversible loading and unloading of oxygen. Their role in the oxidative metabolism is to transfer energy within the cell and specifically in the mitochondria. Other key functions for the iron-containing enzymes (e.g. cytochrome P450) include the synthesis of

steroid hormones and bile acids; detoxification of foreign substances in the liver; and signal controlling in some neurotransmitters, such as the dopamine and serotonin systems in the brain. Iron is reversibly stored within the liver as ferritin and haemosiderin whereas it is transported between different compartments in the body by the protein transferrin (Bothwell, 1979).

1.2.2.1. Overview of Iron metabolism and absorption

Basal iron losses

Iron is not actively excreted from the body in urine or in the intestines. Iron is only lost with cells from the skin and the interior surfaces of the body—intestines, urinary tract, and airways. The total amount lost is estimated at 14µg/kg body weight/day (Green *et al.*, 1968). In children, it is probably more correct to relate these losses to body surface. A non-menstruating 55-kg woman loses about 0.8 mg Fe/day and a 70-kg man loses about 1mg/day. The range of individual variation has been estimated to be ±15% (FAO, 1988). Earlier studies suggested that sweat iron losses could be considerable, especially in a hot, humid climate. However, new studies which took extensive precautions to avoid the interference of contamination of iron from the skin during the collection of total body sweat have shown that sweat iron losses are negligible (Brune *et al.*, 1986).

Requirements for growth

The newborn term infant has an iron content of about 250–300mg (75mg/kg body weight). During the first 2 months of life, haemoglobin concentration falls because of the improved oxygen situation in the newborn infant compared with the intrauterine fetus. This leads to a considerable redistribution of iron from catabolized erythrocytes to iron stores. This iron will cover the needs of the term infant during the first 4–6 months of life and is why iron requirements during this period can be provided by human milk, which contains very little iron. Because of the marked supply of iron to the fetus during the last trimester of pregnancy, the iron situation is much less favourable in the premature and low-birth-weight infant than in the healthy term infant. An extra supply of iron is therefore needed in these infants during the first 6 months of life (Bothwell, 1979).

In the term infant, iron requirements rise markedly after age 4–6 months and amount to about 0.7–0.9 mg/day during the remaining part of the first year. These requirements are very high, especially in relation to body size and energy intake[6]. In the first year of life, the term infant almost doubles its total iron stores and triples its body weight. The increase in body iron during this period occurs mainly during the latter 6 months. Between 1 and 6 years of age, the body iron content is again doubled. The requirements for absorbed iron in infants and children are

[6] Nutrient and energy intakes for the European Community: a report of the Scientific Committee for Food. Brussels, Commission of the European Communities, 1993.

very high in relation to their energy requirements. For example, in infants 6–12 months of age, about 1.5 mg of iron need to be absorbed per 4.184 MJ and about half of this amount is required up to age 4 years.

In the weaning period, the iron requirements in relation to energy intake are at the highest level of the lifespan except for the last trimester of pregnancy, when iron requirements to a large extent have to be covered from the iron stores of the mother. Infants have no iron stores and have to rely on dietary iron alone. It is possible to meet these high requirements if the diet has a consistently high content of meat and foods rich in ascorbic acid. In most developed countries today, infant cereal products are the staple foods for that period of life. Commercial products are regularly fortified with iron and ascorbic acid, and they are usually given together with fruit juices and solid foods containing meat, fish, and vegetables. The fortification of cereal products with iron and ascorbic acid is important in meeting the high dietary needs, especially considering the importance of an optimal iron nutrititure during this phase of brain development. Iron requirements are also very high in adolescents, particularly during the period of rapid growth (Rossander-Hulthén *et al.*, 1996). There is a marked individual variation in growth rate, and the requirements of adolescents may be considerably higher than the calculated mean values given in Table 2. Girls usually have their growth spurt before menarche, but growth is not finished at that time. Their total iron requirements are therefore considerable. In boys during puberty there is a marked increase in haemoglobin mass and concentration, further increasing iron requirements to a level above the average iron requirements in menstruating women (Brune *et al.*, 1986).

Table 2: Iron intakes required for growth under the age of 18 years, median basal iron losses, menstrual losses in women and total absolute iron requirement (Rossander-Hulthén et al., 1996)

Group	Age (years)	Mean body Weight (kg)	Required iron intakes for growth (mg/day)	Median basal iron losses (mg/day	Menstrual losses		Total absolute requirements [a]	
					Median (mg/day	95th Percentil (mg/day)	Median (mg/day	95th Percentil (mg/day)
Infants and children	0.5-1	9	0.55	0.17			0.72	0.93
	1-3	13	0.27	0.19			0.46	0.58
	4-6	19	0.23	0.27			0.50	0.63
	7-10	28	0.32	0.39			0.71	0.89
Males	11-14	45	0.55	0.62			1.17	1.46
	15-17	64	0.60	0.90			1.50	1.88
	18+	46		1.05			1.05	1.37
Females	11-14[b]	46	0.55	0.65			1.20	1.40
	11-14	56	0.55	0.65	0.48[c]	1.90[c]	1.68	3.27
	15-17	62	0.35	0.79	0.48[c]	1.90[c]	1.62	3.10
	18+	62		0.87	0.48[c]	1.90[c]	1.46	2.94
Post menaupausal		62		0.87			0.87	1.13
lactating		62		1.15			1.15	1.50

[a] *Total absolute requirements = Requirements for Growth +Basal losses + Menstrual losses*
[b] *Premenarch*

[c] *Effect of the normal variation in heamoglobin concentration not included in this figure*
Source: adapted in part from (Requirements of vitamin A, iron, folate and vitamin B12. Report of a Joint FAO/WHO 1988)

Iron absorption

With respect to the mechanism of absorption, there are two kinds of dietary iron: haem iron and non-haem iron (Hallberg L 1981). In the human diet, the primary sources of haem iron are the haemoglobin and myoglobin from consumption of meat, poultry, and fish whereas non-haem iron is obtained from cereals, pulses, legumes, fruits, and vegetables. The average absorption of haem iron from meat-containing meals is about 25% (Hallberg *et al* ., 1979). The absorption of haem iron can vary from about 40% during iron deficiency to about 10% during iron repletion (Hallberg *et al*.,1997). Haem iron can be degraded and converted to non-haem iron if foods are cooked at a high temperature for too long. Calcium (discussed below) is the only dietary factor that negatively influences the absorption of haem iron and does so to the same extent that it influences non-haem iron (Hallberg *et al* ., 1993).

Non-haem iron is the main form of dietary iron. The absorption of non-haem iron is influenced by individual iron status and by several factors in the diet. Iron compounds used for the fortification of foods will only be partially available for absorption. Once dissolved, however, the absorption of iron from fortificants (and food contaminants) is influenced by the same factors as the iron native to the food substance (Hallberg *et al*., 1974,). Iron from the soil (e.g. from various forms of clay) is sometimes present on the surface of foods as a contaminant, having originated from dust on air-dried foods or from the residue of the water used in irrigation. Even if the fraction of iron that is available is often small, contamination iron may still be nutritionally significant because of its addition to the overall dietary intake of iron (Hallberg *et al* ., 1981). Reducing substances (i.e. substances that keep iron in the ferrous form) must be present for iron to be absorbed (Wollenberg *et al*., 1987). The presence of meat, poultry, and fish in the diet enhance iron absorption. Other foods contain chemical entities (ligands) that strongly bind ferrous ions, and thus inhibit absorption. Examples are phytates and certain iron-binding polyphenols (Wollenberg *et al*., 1987).

Inhibition of iron absorption

Phytates are found in all kinds of grains, seeds, nuts, vegetables, roots (e.g. potatoes), and fruits. Chemically, phytates are inositol hexaphosphate salts and are a storage form of phosphates and minerals. Other phosphates have not been shown to inhibit non-haem iron absorption. In North American and European diets, about 90% of phytates originate from cereals. Phytates strongly inhibit iron absorption in a dose-dependent fashion and even small amounts of phytates have a marked effect (Gillooly *et al* ., 1983,). Bran has a high content of phytate and strongly inhibits iron absorption. Wholewheat flour, therefore, has a much higher phytate content than does white-wheat flour (Hallberg *et al*., 1978). In bread, some of the phytates in bran are degraded

during the fermentation of the dough. Fermentation for a couple of days (sourdough fermentation) can almost completely degrade the phytate and increase the bioavailability of iron in bread made from wholewheat flour (Brune *et al* 1992). Oats strongly inhibit iron absorption because of their high phytate content that results from native phytase in oats being destroyed by the normal heat process used to avoid rancidity (Rossander-Hulthén *et al*., 1990). Sufficient amounts of ascorbic acid can counteract this inhibition (Siegenberg *et al* ., 1991). In contrast, non-phytate-containing dietary fibre components have almost no influence on iron absorption. Almost all plants contain phenolic compounds as part of their defence system against insects and animals. Only some of the phenolic compounds (mainly those containing galloyl groups) seem to be responsible for the inhibition of iron absorption (Brune *et al*., 1989). Tea, coffee, and cocoa are common plant products that contain iron-binding polyphenols (Disler *et al*. 1982). Many vegetables, especially green leafy vegetables (e.g. spinach), and herbs and spices (e.g. oregano) contain appreciable amounts of galloyl groups, which strongly inhibit iron absorption as well. Consumption of betel leaves, common in areas of Asia, also has a marked negative effect on iron absorption. Calcium, consumed as a salt or in dairy products interferes significantly with the absorption of both haem and non-haem iron (Hallberg *et al*., 1991). However, because calcium is an essential nutrient, it cannot be considered to be an inhibitor of iron absorption in the same way as phytates or phenolic compounds. In order to lessen this interference, practical solutions include increasing iron intake, increasing its bioavailability, or avoiding the intake of foods rich in calcium and foods rich in iron at the same meal (Gleerup *et al*.1995).

The mechanism of action for absorption inhibition is unknown, but the balance of evidence strongly suggests that the inhibitory effect takes place within the mucosal cell itself at the common final transfer step for haem and non-haem iron. Recent analyses of the dose–effect relationship showed that the first 40 mg of calcium in a meal does not inhibit absorption of haem and nonhaem iron. Above this level of calcium intake, a sigmoid relationship develops, and at levels of 300–600 mg calcium, reaches a 60% maximal inhibition of iron absorption. The form of this curve suggests a one-site competitive binding of iron and calcium (Figure 13.3). This relationship explains some of the seemingly conflicting results obtained in studies on the interaction between calcium and iron (Hallberg *et al*., 1998).

For unknown reasons, the addition of soya to a meal reduces the fraction of iron absorbed (Cook *et al*., 1981). This inhibition is not solely explained by the high phytate content of soya. However, because of the high iron content of soya, the net effect on iron absorption with an addition of soya products to a meal is usually positive. In infant foods containing soya, the

inhibiting effect can be overcome by the addition of sufficient amounts of ascorbic acid. Conversely, some fermented soy sauces have been found to enhance iron absorption (Baynes *et al.*, 1990).

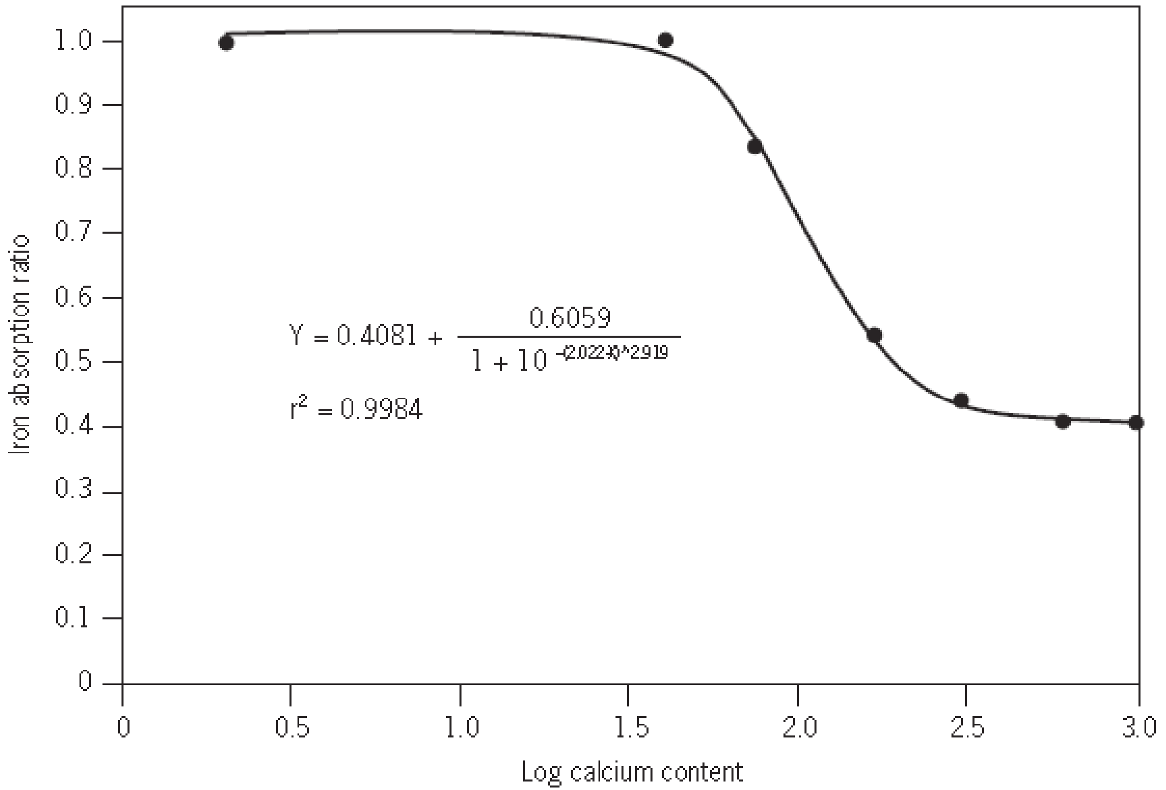

Figure 1: Effect of different amounts of Calcium on iron absorption (Baynes *et al.,* 1990)

Iron balance and regulation of iron absorption

The body has three unique mechanisms for maintaining iron balance. The first is the continuous reutilization of iron from catabolized erythrocytes in the body. When an erythrocyte dies after about 120 days, it is usually degraded by the macrophages of the reticular endothelium. The iron is released and delivered to transferrin in the plasma, which brings the iron back to red blood cell precursors in the bone marrow or to other cells in different tissues. Uptake and distribution of iron in the body is regulated by the synthesis of transferrin receptors on the cell surface. This system for internal iron transport not only controls the rate of flow of iron to different tissues according to their needs, but also effectively prevents the appearance of free iron and the formation of free radicals in the circulation (Wollenberg *et al.*, 1987).

The second mechanism involves access to the specific storage protein, ferritin. This protein stores iron in periods of relatively low need and releases it to meet excessive iron demands. This iron reservoir is especially important in the third trimester of pregnancy.

The third mechanism involves the regulation of absorption of iron from the intestines; decreasing body iron stores trigger increased iron absorption and increasing iron stores trigger decreased iron absorption. Iron absorption decreases until equilibrium is established between

absorption and requirement. For a given diet this regulation of iron absorption, however, can only balance losses up to a certain critical point beyond which iron deficiency will develop (Hallberg *et al* 1995). About half of the basal iron losses are from blood and occur primarily in the gastrointestinal tract. Both these losses and the menstrual iron losses are influenced by the haemoglobin level; during the development of an iron deficiency, menstrual and basal iron losses will successively decrease when the haemoglobin level decreases. In a state of more severe iron deficiency, skin iron losses may also decrease. Iron balance (absorption equals losses) may be present not only in normal subjects but also during iron deficiency and iron overload. The three main factors that affect iron balance are absorption (intake and bioavailability of iron), losses, and stored amount. The interrelationship among these factors has recently been described in mathematical terms, making it possible to predict, for example, the amount of stored iron when iron losses and bioavailability of dietary iron are known (Hallberg *et al* 1998). In states of increased iron requirement or decreased bioavailability, the regulatory capacity to prevent iron deficiency is limited. However, the regulatory capacity seems to be extremely good in preventing iron overload in a state of increased dietary iron intake or bioavailability.

1.2.2.2. Iron deficiency

Populations at risk for iron deficiency

Populations most at risk for iron deficiency are infants, children, adolescents, and women of childbearing age, especially pregnant women. The weaning period in infants is especially critical because of the very high iron requirement needed in relation to energy. Thanks to better information about iron deficiency and the addition of fortified cereals to the diets of infants and children, the iron situation has markedly improved in these groups in most developed countries, such that the groups currently considered to be most at risk are menstruating and pregnant women, and adolescents of both sexes. In developing countries, however, the iron situation is still very critical in many groups—especially in infants in the weaning period. During this period, iron nutrition is of great importance for the adequate development of the brain and other tissues such as muscles, which are differentiated early in life (Hallberg *et al*. 1993).

Iron deficiency and iron deficiency anaemia are often incorrectly used as synonyms. A definition of these terms may clarify some of the confusion about different prevalence figures given in the literature (Hallberg *et al*. 1993).

Iron deficiency is defined as a haemoglobin concentration below the optimum value in an individual, whereas ***iron deficiency anaemia*** implies that the haemoglobin concentration is below the 95th percentile of the distribution of haemoglobin concentration in a population

(disregarding effects of altitude, age and sex, etc. on haemoglobin concentration). The confusion arises due to the very wide distribution of the haemoglobin concentration in healthy, fully iron-replete subjects (in women, 120–160 g/l; in men, 140–180 g/l). During the development of a negative iron balance in subjects with no mobilizable iron from iron stores (i.e. no visible iron in technically perfect bone marrow smears or a serum ferritin concentration <15mg/l), there will be an immediate impairment in the production of haemoglobin with a resulting decrease in haemoglobin and different erythrocyte indexes (e.g. mean corpuscular haemoglobin and mean corpuscular volume). In turn, this will lead to an overlap in the distributions of haemoglobin in iron-deficient and iron-replete women. The extent of overlap depends on the prevalence and severity of iron deficiency. In populations with more severe iron deficiency, for example, the overlap is much less marked (Wintrobe,1981).

In women, anaemia is defined as a haemoglobin level <120g/l. For a woman who has her normal homeostatic value set at 150 g/l, her haemoglobin level must decrease by 26% to 119g/l before she is considered to be anaemic, whereas for a woman who has her normal haemoglobin set at 121g/l, her haemoglobin level must only decrease by 1.5% to 119 g/l. Iron deficiency anaemia is a rather imprecise concept for evaluating the single subject and has no immediate physiological meaning. By definition, this implies that the prevalence of iron deficiency anaemia is less frequent than iron deficiency and that the presence of anaemia in a subject is a statistical rather than a functional concept. The main use of the cut-off value in defining anaemia is in comparisons between population groups. In practical work, iron deficiency anaemia should be replaced by the functional concept of iron deficiency. Anaemia per se is mainly important when it becomes so severe that oxygen delivery to tissues is impaired. An iron deficiency anaemia which develops slowly in otherwise healthy subjects with moderately heavy work output will not give any symptoms until the haemoglobin level is about 80g/l or lower. The reason for the continued use of the concept of iron deficiency anaemia is the ease of determining haemoglobin. Therefore, in clinical practice, knowledge of previous haemoglobin values in a subject is of great importance for evaluating the diagnosis (Yip *et al*., 1996).

Iron deficiency being defined as an absence of iron stores combined with signs of an iron-deficient erythropoiesis implies that in a state of iron deficiency there is an insufficient supply of iron to various tissues. This occurs at a serum ferritin level <15mg/l. At this point, insufficient amounts of iron will be delivered to transferrin, the circulating transport protein for iron, and the binding sites for iron on transferrin will therefore contain less and less iron. This is usually described as a reduction in transferrin saturation. When transferrin saturation drops to a certain critical level, erythrocyte precursors, which continuously need iron for the formation of

haemoglobin, will get an insufficient supply of iron. At the same time, the supply of iron by transferrin to other tissues will also be impaired. Liver cells will get less iron, more transferrin will be synthesized, and the concentration of transferrin in plasma will then suddenly increase. Cells with a high turnover rate are the first ones to be affected (e.g. intestinal mucosal cells with a short lifespan). The iron–transferrin complex binds to transferrin receptors on certain cell surfaces and is then taken up by invagination of the whole complex on the cell wall. The uptake of iron seems to be related both to transferrin saturation and the number of transferrin receptors on the cell surface (Harford *et al*., 1994). There is a marked diurnal variation in the saturation of transferrin because the turnover rate of iron in plasma is very high. This fact makes it difficult to evaluate the iron status from single determinations of transferrin saturation.

Indicators of iron deficiency

The absence of iron stores (iron deficiency) can be diagnosed by showing that there is no stainable iron in the reticuloendothelial cells in bone marrow smears or, more easily, by a low concentration of ferritin in serum (<15μg/l). Even if an absence of iron stores per se may not necessarily be associated with any immediate adverse effects, it is a reliable and good indirect indicator of iron-deficient erythropoiesis and of an increased risk of a compromised supply of iron to different tissues (Yip *et al*., 1996)..

Even before iron stores are completely exhausted, the supply of iron to the erythrocyte precursors in the bone marrow is compromised, leading to iron deficient erythropoiesis. A possible explanation is that the rate of release of iron from stores is influenced by the amount of iron remaining. As mentioned above, it can then be assumed that the supply of iron to other tissues needing iron is also insufficient because the identical transport system is used. During the development of iron deficiency haemoglobin concentration, transferrin concentration, transferrin saturation, transferrin receptors in plasma, erythrocyte protoporphyrin, and erythrocyte indexes are changed. All these indicators, however, showed a marked overlap between normal and iron-deficient subjects, which makes it impossible to identify the single subject with mild iron deficiency by looking at any single one of these indicators. Therefore, these tests are generally used in combination (e.g. for interpreting results from the second National Health and Nutrition Examination Survey in the United States (Pilch *et al*., 1985). By increasing the number of tests used, the diagnostic specificity then increases but the sensitivity decreases, and thus the true prevalence of iron deficiency is markedly underestimated if multiple diagnostic criteria are used. Fortunately, a low serum ferritin (<15μg/l) is always associated with an iron-deficient erythropoiesis. The use of serum ferritin alone as a measure will also underestimate the true prevalence of iron deficiency but to a lesser degree than when the combined criteria are used. A diagnosis of iron deficiency anaemia can be suspected if

anaemia is present in subjects who are iron-deficient as described above. Preferably, to fully establish the diagnosis, the subjects should respond adequately to iron treatment. The pitfalls with this method are the random variation in haemoglobin concentrations over time and the effect of the regression towards the mean when a new measurement is made. The use of serum ferritin has improved the diagnostic accuracy of iron deficiency. It is the only simple method available to detect early iron deficiency. Its practical value is somewhat reduced, however, by the fact that serum ferritin is a very sensitive acute-phase reactant and may be increased for weeks after a simple infection with fever for a day or two (Hulthén *et al*., 1998). Several other conditions, such as use of alcohol (Osler *et al*., 1990), liver disease, and collagen diseases, may also increase serum ferritin concentrations. Determination of transferrin receptors in plasma has also been recommended in the diagnosis of iron deficiency. The advantage of this procedure is that it is not influenced by infections. Its main use is in subjects who are already anaemic and it is not sensitive enough for the early diagnosis of iron deficiency. The use of a combination of determinations of serum ferritin and serum transferrin receptors has also been suggested (Cook *et al*., 1996).

Causes of iron deficiency

Nutritional iron deficiency implies that the diet cannot supply enough iron to cover the body's physiological requirements for this mineral. Worldwide this is the most common cause of iron deficiency. In many tropical countries, infestations with hookworms lead to intestinal blood losses that in some individuals can be considerable. The average blood loss can be reliably estimated by egg counts in stools. Usually the diet in these populations is also limited with respect to iron content and availability. The severity of the infestations varies markedly between subjects and regions. In clinical practice, a diagnosis of iron deficiency must always lead to a search for pathologic causes of blood loss (e.g. tumours in the gastrointestinal tract or uterus, especially if uterine bleedings have increased or changed in regularity). Patients with achlorhydria absorb dietary iron less well (a reduction of about 50%) than healthy individuals, and patients who have undergone gastric surgery, especially if the surgery was extensive, may eventually develop iron deficiency because of impaired iron absorption. Gluten enteropathy is another possibility to consider, especially in young patients (Yip *et al*., 1996)..

Prevalence of iron deficiency

Iron deficiency is probably the most common nutritional deficiency disorder in the world. A recent estimate based on WHO criteria indicated that around 600–700 million people worldwide have marked iron deficiency anaemia (DeMaeyer *et al*., 1985), and the bulk of these people live in developing countries. In developed countries, the prevalence of iron deficiency anaemia is much lower and usually varies between 2% and 8%. However, the prevalence of iron deficiency, including both anaemic and non-anaemic subjects (see definitions above), is

much higher. In developed countries, for example, an absence of iron stores or subnormal serum ferritin values is found in about 20–30% of women of fertile age. In adolescent girls, the prevalence is even higher. It is difficult to determine the prevalence of iron deficiency more exactly because representative populations for clinical investigation are hard to obtain. Laboratory methods and techniques for blood sampling need careful standardization. One often neglected source of error (e.g. when samples from different regions, or samples taken at different times, are compared) comes from the use of reagent kits for determining serum ferritin that are not adequately calibrated to international WHO standards. In addition, seasonal variations in infection rates influence the sensitivity and specificity of most methods used (Yip *et al*., 1996).

Worldwide, the highest prevalence figures for iron deficiency are found in infants, children, adolescents, and women of childbearing age. Both better information about iron deficiency prevention and increased consumption of fortified cereals by infants and children have markedly improved the iron situation in these groups in most developed countries, such that, the highest prevalence of iron deficiency today is observed in menstruating and pregnant women, and adolescents of both sexes (Osler *et al*., 1990).

In developing countries, where the prevalence of iron deficiency is very high and the severity of anaemia is marked, studies on the distribution of haemoglobin in different population groups can provide important information that can then be used as a basis for action programmes (Yip R *et al*., 1996). A more detailed analysis of subsamples may then give excellent information for the planning of more extensive programmes.

Effects of iron deficiency

Studies in animals have clearly shown that iron deficiency has several negative effects on important functions in the body (Dallman *et al*., 1986). The physical working capacity of rats is significantly reduced in states of iron deficiency, especially during endurance activities (Edgerton *et al*., 1972). This negative effect seems to be less related to the degree of anaemia than to impaired oxidative metabolism in the muscles with an increased formation of lactic acid. Thus, the effect witnessed seems to be due to a lack of iron-containing enzymes which are rate limiting for oxidative metabolism. Further to this, several groups have observed a reduction in physical working capacity in human populations with longstanding iron deficiency, and demonstrated an improvement in working capacity in these populations after iron administration (Scrimshaw *et al*., 1984).

The relationship between iron deficiency and brain function and development is very important to consider when choosing a strategy to combat iron deficiency (Lozoff *et al*., 1991). Several structures in the brain have a high iron content; levels are of the same order of magnitude as those observed in the liver. The observation that the lower iron content of the brain in iron-deficient growing rats cannot be increased by giving iron at a later date strongly suggests that the supply of iron to brain cells takes place during an early phase of brain development and that, as such, early iron deficiency may lead to irreparable damage to brain cells. In humans about 10% of brain-iron is present at birth; at the age of 10 years the brain has only reached half its normal iron content, and optimal amounts are first reached between the ages of 20 and 30 years (Scrimshaw *et al*., 1984).

Iron deficiency also negatively influences the normal defence systems against infections. In animal studies, the cell-mediated immunologic response by the action of T-lymphocytes is impaired as a result of a reduced formation of these cells. This in turn is due to a reduced DNA synthesis dependent on the function of ribonucleotide reductase, which requires a continuous supply of iron for its function. In addition, the phagocytosis and killing of bacteria by the neutrophil leukocytes is an important component of the defence mechanism against infections. These functions are impaired in iron deficiency as well. The killing function is based on the formation of free hydroxyl radicals within the leukocytes, the respiratory burst, and results from the activation of the iron-sulfur enzyme NADPH oxidase and probably also cytochrome b (a haem enzyme) (Brock *et al*., 1994).

The impairment of the immunologic defence against infections that was found in animals is also regularly found in humans. Administration of iron normalizes these changes within 4–7 days. It has been difficult to demonstrate, however, that the prevalence of infections is higher or that their severity is more marked in iron-deficient subjects than in control subjects. This may well be ascribed to the difficulty in studying this problem with an adequate experimental design (Scrimshaw *et al*., 1984).

Several groups have demonstrated a relationship between iron deficiency and attention, memory, and learning in infants and small children. In the most recent well-controlled studies, no effect was noted from the administration of iron. This finding is consistent with the observations in animals. Therapy resistant behavioural impairment and the fact that there is an accumulation of iron during the whole period of brain growth should be considered strong arguments for the early detection and treatment of iron deficiency. This is valid for women, especially during pregnancy, and for infants and children, up through the period of adolescence

to adulthood. In a recent well controlled study, administration of iron to non-anaemic but iron-deficient adolescent girls improved verbal learning and memory (Bruner *et al*., 1996). Well-controlled studies in adolescent girls showed that iron-deficiency without anaemia is associated with reduced physical endurance (Rowland *et al*., 1988) and changes in mood and ability to concentrate (Ballin *et al*., 1992). Another recent study showed that there was a reduction in maximum oxygen consumption in non-anaemic women with iron deficiency that was unrelated to a decreased oxygen transport capacity of the blood (Zhu *et al*., 1997).

1.2.2.3. Zinc

1.2.2.4. Overview of Zinc metabolism and absorption

Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30mmol (2g). Skeletal muscle accounts for approximately 60% of the total body content and bone mass, with a zinc concentration of 1.5–3mmol/g (100–200mg/g), for approximately 30%. The concentration of zinc in lean body mass is approximately 0.46mmol/g (30mg/g) (Shankar *et al*., 1998).

Plasma zinc has a rapid turnover rate and it represents only about 0.1% of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye (4.2mmol/g or 274mg/g) and in prostatic fluids (4.6–7.7mmol/l or 300–500mg/l) (Hambridge *et al*., 1987). Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components and membranes and in this way contributes to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms.

Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shankar *et al*., 1998). Shankar and Prasad have reviewed the role of zinc in immunity extensively.

The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes. The effects of marginal or mild zinc deficiency are less clear. A reduced growth rate and impairments of immune defence are so far the only clearly demonstrated signs

of mild zinc deficiency in humans. Other effects, such as impaired taste and wound healing, which have been claimed to result from a low zinc intake, are less consistently observed (Lukaski *et al*., 1984).

Zinc metabolism and homeostasis

Zinc absorption is concentration dependent and occurs throughout the small intestine. Under normal physiological conditions, transport processes of uptake are not saturated. Zinc administered in aqueous solutions to fasting subjects is absorbed efficiently (60–70%), whereas absorption from solid diets is less efficient and varies depending on zinc content and diet composition (Sandström *et al*., 1997). The major losses of zinc from the body are through the intestine and urine, by desquamation of epithelial cells, and in sweat. Endogenous intestinal losses can vary from 7mmol/day (0.5 mg/day) to more than 45mmol/day (3mg/day), depending on zinc intake—the higher the intake, the greater the losses (King, 1998). Urinary and integumental losses are of the order of 7–10mmol/day (0.5– 0.7 mg/day) each and depend less on normal variations in zinc intake . Starvation and muscle catabolism increase zinc losses in urine. Strenuous exercise and elevated ambient temperatures can lead to high losses through perspiration.

The body has no zinc stores in the conventional sense. In conditions of bone resorption and tissue catabolism, zinc is released and may be reutilized to some extent. Human experimental studies with low zinc diets containing 2.6–3.6mg/day (40–55mmol/day) have shown that circulating zinc levels and activities of zinc-containing enzymes can be maintained within a normal range over several months (Lukaski *et al*., 1984), a finding which highlights the efficiency of the zinc homeostasis mechanism. Controlled depletion–repletion studies in humans have shown that changes in the endogenous excretion of intestinal, urinary, and integumental zinc as well as changes in absorptive efficiency are how body zinc content is maintained (Baer *et al*., 1984,, ,). However, the underlying mechanisms are poorly understood. Sensitive indexes for assessing zinc status are unknown at present. Static indexes, such as zinc concentration in plasma, blood cells, and hair, and urinary zinc excretion are decreased in severe zinc deficiency. A number of conditions that are unrelated to zinc status can affect all these indexes, especially zinc plasma levels. Food intake, stress situations such as fever, infection, and pregnancy lower plasma zinc concentrations whereas, for example, long-term fasting increases it (Agett *et al*., 1993). However, on a population basis, reduced plasma zinc concentrations seem to be a marker for zinc-responsive growth reductions (Goldenberg *et al*., 1995,). Experimental zinc depletion studies suggest that changes in immune response occur before reductions in plasma zinc concentrations are apparent (Beck *et al*., 1997). To date, it has

not been possible to identify zinc dependent enzymes which could serve as early markers for zinc status.

A number of functional indexes of zinc status have been suggested, for example, wound healing, taste acuity, and visual adaptation to the dark. Changes in these functions are, however, not specific to zinc and these indexes have not been proven useful for identifying marginal zinc deficiency in humans thus far. The introduction of stable isotope techniques in zinc research (Sandström *et al*., 1993) has created possibilities for evaluating the relationship between diet and zinc status and is likely to lead to a better understanding of the mechanisms underlying the homeostatic regulation of zinc. Estimations of the turnover rates of administered isotopes in plasma or urine have revealed the existence of a relatively small but rapidly exchangeable body pool of zinc of about 1.5–3.0mmol (100–200mg) (Wastney *et al*., 1991,,,). The size of the pool seems to be correlated to habitual dietary intake and it is reduced in controlled depletion studies. The zinc pool was also found to be correlated to endogenous intestinal excretion of zinc and to total daily absorption of zinc. These data suggest that the size of the pool depends on recently absorbed zinc and that a larger exchangeable pool results in larger endogenous excretion. Changes in endogenous intestinal excretion of zinc seem to be more important than changes in absorptive efficiency for maintenance of zinc homeostasis (Lukaski *et al*., 1984).

Dietary sources and bioavailability of zinc

Lean red meat, whole-grain cereals, pulses, and legumes provide the highest concentrations of zinc: concentrations in such foods are generally in the range of 25–50mg/kg (380–760mmol/kg) raw weight. Processed cereals with low extraction rates, polished rice, and chicken, pork or meat with high fat content have a moderate zinc content, typically between 10 and 25mg/kg (150–380 mmol/kg). Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc, having concentrations <10mg/kg (<150mmol/kg) (Sandström *et al*., 1989). Saturated fats and oils, sugar, and alcohol have very low zinc contents. The utilization of zinc depends on the overall composition of the diet. Experimental studies have identified a number of dietary factors as potential promoters or antagonists of zinc absorption (Sandström *et al*., 1989). Soluble organic substances of low relative molecular mass, such as amino and hydroxy acids, facilitate zinc absorption. In contrast, organic compounds forming stable and poorly soluble complexes with zinc can impair absorption. In addition, competitive interactions between zinc and other ions with similar physicochemical properties can affect the uptake and intestinal absorption of zinc. The risk of competitive interactions with zinc seems to be mainly related to the consumption of high doses of these other ions, in the form of supplements or in aqueous solutions. However, at levels present in food and at realistic fortification levels, zinc absorption appears not to be affected, for example, by iron or copper (Sandström *et al*., 1989).

Isotope studies with human subjects have identified two factors that, together with the total zinc content of the diet, are major determinants of absorption and utilization of dietary zinc. The first is the content of inositol hexaphosphate (phytate) in the diet and the second is the level and source of dietary protein. Phytates are present in whole-grain cereals and legumes and in smaller amounts in other vegetables. They have a strong potential for binding divalent cations and their depressive effect on zinc absorption has been demonstrated in humans. The molar ratio between phytates and zinc in meals or diets is a useful indicator of the effect of phytates in depressing zinc absorption. At molar ratios above the range of 6–10, zinc absorption starts to decline; at ratios above 15, absorption is typically less than 15%. The effect of phytate is, however, modified by the source and amount of dietary proteins consumed. Animal proteins improve zinc absorption from a phytate-containing diet (Sandström *et al*., 1998). Zinc absorption from some legume-based diets (e.g. white beans and lupin protein) is comparable with that from animalprotein- based diets despite a higher phytate content in the former (Petterson *et al*., 1994). High dietary calcium potentiated the antagonistic effects of phytates on zinc absorption in experimental studies. The results from human studies are less consistent and any effects seem to depend on the source of calcium and the composition of the diet (Sandström *et al*., 1989).

Several recently published absorption studies illustrate the effect of zinc content and diet composition on fractional zinc absorption (Knudsen *et al*., 1994). The results from the total diet studies, where all main meals of a day's intake were extrinsically labelled, showed a remarkable consistency in fractional absorption despite relatively large variations in meal composition and zinc content. Thus, approximately twice as much zinc is absorbed from a nonvegetarian or high-meat diet (Hunt *et al*., 1995) than from a diet based on rice and wheat flour. Data are lacking on zinc absorption from typical diets of developing countries, which usually have high phytate contents. The availability of zinc from the diet can be improved by reducing the phytate content and including sources of animal protein. Lower extraction rates of cereal grains will result in lower phytate content but at the same time the zinc content is reduced, so that the net effect on zinc supply is limited. The phytate content can be reduced by activating the phytase present in most phytate-containing foods or through the addition of microbial or fungal phytases. Phytases hydrolyse the phytate to lower inositol phosphates, resulting in improved zinc absorption (Nävert *et al*., 1985,). The activity of phytases in tropical cereals such as maize and sorghum is lower than that in wheat and rye (Gibson *et al*., 1998). Germination of cereals and legumes increases phytase activity and addition of some germinated flour to ungerminated maize or sorghum followed by soaking at ambient temperature for 12–24 hours can reduce the phytate content substantially. Additional reduction can be achieved by the fermentation of porridge for

weaning foods or dough for bread making. Commercially available phytase preparations could also be used but may not be economically accessible in many populations (Sandström *et al.*, 1989).

Populations at risk for zinc deficiency

The central role of zinc in cell division, protein synthesis, and growth is especially important for infants, children, adolescents, and pregnant women; these groups suffer most from an inadequate zinc intake. Zinc-responsive stunting has been identified in several studies; for example, a more rapid body weight gain in malnourished children from Bangladash supplemented with zinc was reported (Simmer *et al.*, 1988). However, other studies have failed to showed a growth promoting effect of zinc supplementation. A recent meta-analysis of 25 intervention trials comprising 1834 children under 13 years of age, with a mean duration of approximately 7 months and a mean dose of zinc of 14mg/day (214mmol/day), showed a small but significant positive effect of zinc supplementation on height and weight increases. Zinc supplementation had a positive effect when stunting was initially present; a more pronounced effect on weight gain was associated with initial low plasma zinc concentrations. Results from zinc supplementation studies suggest that a low zinc status in children not only affects growth but is also associated with an increased risk of severe infectious diseases (Black *et al.*, 1998). Episodes of acute diarrhoea were characterized by shorter duration and less severity in zinc-supplemented groups; reductions in incidence of diarrhoea were also reported. Other studies indicate that the incidence of acute lower respiratory tract infections and malaria may also be reduced by zinc supplementation. Prevention of suboptimal zinc status and zinc deficiency in children by an increased intake and availability of zinc could consequently have a significant effect on child health in developing countries (Black *et al.*, 1998).

The role of maternal zinc status on pregnancy outcome is still unclear. Positive as well as negative associations between plasma zinc concentration and fetal growth or labour and delivery complications have been reported (Caulfield *et al.*, 1998). Results of zinc supplementation studies also remain inconclusive (WHO, 1996). Interpretation of plasma zinc concentrations in pregnancy is complicated by the effect of haemodilution, and the fact that low plasma zinc levels may reflect other metabolic disturbances. Zinc supplementation studies of pregnant women have been performed mainly in relatively well-nourished populations, which may be one of the reasons for the mixed results . A recent study among low-income American women with plasma zinc concentrations below the mean at enrolment in prenatal care showed that a zinc intake of 25mg/day resulted in greater infant birth weights and head circumferences as well as a reduced frequency of very low-birth-weight infants among non-obese women compared with the placebo group (Caulfield *et al.*, 1998).

1.2.3. Vitamin B12

1.2.3.1. Role of vitamin B12 in human metabolic processes

Although the nutritional literature still uses the term vitamin B12, a more specific name for vitamin B12 is cobalamin. Vitamin B12 is the largest of the B complex vitamins, with a relative molecular mass of over 1000. It consists of a corrin ring made up of four pyrroles with cobalt at the centre of the ring (Weir *et al*., 1998,).

There are several vitamin B12-dependent enzymes in bacteria and algae, but no species of plants have the enzymes necessary for vitamin B12 synthesis. This fact has significant implications for the dietary sources and availability of vitamin B12. In mammalian cells, there are only two vitamin B12-dependent enzymes (Scott *et al*., 1994). One of these enzymes, methionine synthase, uses the chemical form of the vitamin which has a methyl group attached to the cobalt and is called methylcobalamin. The other enzyme, methylmalonyl coenzyme (CoA) mutase, uses a form of vitamin B12 that has a 5¢-adeoxyadenosyl moiety attached to the cobalt and is called 5¢- deoxyadenosylcobalamin, or coenzyme B12. In nature, there are two other forms of vitamin B12: hydroxycobalamin and aquacobalamin, where hydroxyl and water groups, respectively, are attached to the cobalt. The synthetic form of vitamin B12 found in supplements and fortified foods is cyanocobalamin, which has cyanide attached to the cobalt. These three forms of vitamin B12 are enzymatically activated to the methyl- or deoxyadenosylcobalamins in all mammalian cells (Scott *et al*., 1994).

1.2.3.2. Dietary sources and availability

Most microorganisms, including bacteria and algae, synthesize vitamin B12, and they constitute the only source of the vitamin (Chanarin *et al*., 1979). The vitamin B12 synthesized in microorganisms enters the human food chain through incorporation into food of animal origin. In many animals, gastrointestinal fermentation supports the growth of these vitamin B12 synthesizing microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues. This is particularly true for the liver, where vitamin B12 is stored in large concentrations. Products from herbivorous animals, such as milk, meat, and eggs, thus constitute important dietary sources of the vitamin, unless the animal is subsisting in one of the many regions known to be geochemically deficient in cobalt (Smith *et al*., 1978). Milk from cows and humans contains binders with very high affinity for vitamin B12, though whether they hinder or promote intestinal absorption is not entirely clear. Omnivores and carnivores, including humans, derive dietary vitamin B12 almost exclusively from animal tissues or products (i.e. milk, butter, cheese, eggs, meat, poultry). It appears that the vitamin B12 required

by humans is not derived from microflora in any appreciable quantities, although vegetable fermentation preparations have been reported as being possible sources of vitamin B12 (van den Berg *et al*., 1988).

1.2.3.3. Absorption

The absorption of vitamin B12 in humans is complex (Weir *et al*., 1998, Weir *et al*., 1999). Vitamin B12 in food is bound to proteins and is only released by the action of a high concentration of hydrochloric acid present in the stomach. This process results in the free form of the vitamin, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands. These glycoproteins, called R-binders (or haptocorrins), protect vitamin B12 from chemical denaturation in the stomach. The stomach's parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor. Intrinsic factor binds vitamin B12 and ultimately enables its active absorption. Although the formation of the vitamin B12–intrinsic factor complex was initially thought to happen in the stomach, it is now clear that this is not the case. At an acidic pH, the affinity of the intrinsic factor for vitamin B12 is low whereas its affinity for the R-binders is high. When the contents of the stomach enter the duodenum, the R-binders become partly digested by the pancreatic proteases, which in turn causes them to release their vitamin B12. Because the pH in the duodenum is more neutral than that in the stomach, the intrinsic factor has a high binding affinity to vitamin B12, and it quickly binds the vitamin as it is released from the R-binders. The vitamin B12–intrinsic factor complex then proceeds to the lower end of the small intestine, where it is absorbed by phagocytosis by specific ileal receptors (Weir *et al*., 1998, Weir *et al*., 1999).

1.2.3.4. Populations at risk for, and consequences of, vitamin B12 deficiency

Vegetarians

Because plants do not synthesize vitamin B12, individuals who consume diets completely free of animal products (vegan diets) are at risk of vitamin B12 deficiency. This is not true of lacto-ovo vegetarians, who consume the vitamin in eggs, milk, and other dairy products (Scott *et al*., 1994).

Pernicious anaemia

Malabsorption of vitamin B12 can occur at several points during digestion (Chanarin *et al*., 1979). By far the most important condition resulting in vitamin B12 malabsorption is the autoimmune disease called pernicious anaemia (PA). In most cases of PA, antibodies are produced against the parietal cells causing them to atrophy, and lose their ability to produce intrinsic factor and secrete hydrochloric acid. In some forms of PA, the parietal cells remain intact but autoantibodies are produced against the intrinsic factor itself and attach to it, thus

preventing it from binding vitamin B12. In another less common form of PA, the antibodies allow vitamin B12 to bind to the intrinsic factor but prevent the absorption of the intrinsic factor–vitamin B12 complex by the ileal receptors. As is the case with most autoimmune diseases, the incidence of PA increases markedly with age. In most ethnic groups, it is virtually unknown to occur before the age of 50, with a progressive rise in incidence thereafter (Chanarin *et al*., 1979). However, African American populations are known to have an earlier age of presentation (Chanarin *et al*., 1979). In addition to causing malabsorption of dietary vitamin B12, PA also results in an inability to reabsorb the vitamin B12 which is secreted in the bile. Biliary secretion of vitamin B12 is estimated to be between 0.3 and 0.5mg/day. Interruption of this so-called enterohepatic circulation of vitamin B12 causes the body to go into a significant negative balance for the vitamin. Although the body typically has sufficient vitamin B12 stores to last 3–5 years, once PA has been established, the lack of absorption of new vitamin B12 is compounded by the loss of the vitamin because of negative balance. When the stores have been depleted, the final stages of deficiency are often quite rapid, resulting in death in a period of months if left untreated (Scott *et al*., 1994).

Atrophic gastritis

Historically, PA was considered to be the major cause of vitamin B12 deficiency, but it was a fairly rare condition, perhaps affecting between one and a few per cent of elderly populations. More recently, it has been suggested that a far more common problem is that of hypochlorhydria associated with atrophic gastritis, where there is a progressive reduction with age of the ability of the parietal cells to secrete hydrochloric acid (Carmel *et al*., 1996). It is claimed that perhaps up to one quarter of elderly subjects could have various degrees of hypochlorhydria as a result of atrophic gastritis. It has also been suggested that bacterial overgrowth in the stomach and intestine in individuals suffering from atrophic gastritis may also reduce vitamin B12 absorption. The absence of acid in the stomach is postulated to prevent the release of proteinbound vitamin B12 contained in food but not to interfere with the absorption of the free vitamin B12 found in fortified foods or supplements. Atrophic gastritis does not prevent the reabsorption of biliary vitamin B12 and therefore does not result in the negative balance seen in individuals with PA. Nonetheless, it is agreed that with time, a reduction in the amount of vitamin B12 absorbed from the diet will eventually deplete vitamin B12 stores, resulting in overt deficiency (Carmel *et al*., 1996).

When considering recommended nutrient intakes (RNIs) for vitamin B12 for the elderly, it is important to take into account the absorption of vitamin B12 from sources such as fortified foods or supplements as compared with dietary vitamin B12. In the latter instances, it is clear that absorption of intakes of less than 1.5–2.0mg/day is complete—that is, for daily intakes of

less than 1.5–2.0mg of free vitamin B12, the intrinsic factor-mediated system absorbs that entire amount. It is probable that this is also true of vitamin B12 in fortified foods, although this has not been specifically examined. However, absorption of food-bound vitamin B12 has been reported to vary from 9% to 60% depending on the study and the source of the vitamin, which is perhaps related to its incomplete release from food (Food and Nutrition Board 1998). This has led many to estimate absorption as being up to 50% to correct for the bioavailability of vitamin B12 from food (Carmel *et al*., 1996).

1.2.3.5. Vitamin B12 interaction with folate or folic acid

One of the vitamin B12-dependent enzymes, methionine synthase, functions in one of the two folate cycles, namely, the methylation cycle. This cycle is necessary to maintain availability of the methyl donor, S-adenosylmethionine. Interruption of the cycle reduces the level of S-adenosylmethionine. This occurs in PA and other causes of vitamin B12 deficiency, producing as a result demyelination of the peripheral nerves and the spinal column, giving rise to the clinical condition called subacute combined degeneration. This neuropathy is one of the main presenting conditions in PA. The other principal presenting condition in PA is a megaloblastic anaemia morphologically identical to that seen in folate deficiency. Disruption of the methylation cycle also causes a lack of DNA biosynthesis and anaemia. The methyl trap hypothesis is based on the fact that once the cofactor 5,10-methylenetetrahydrofolate is reduced by its reductase to form 5-methyltetrahydrofolate, the reverse reaction cannot occur. This suggests that the only way for the 5-methyltetrahydrofolate to be recycled to tetrahydrofolate, and thus to participate in DNA biosynthesis and cell division, is through the vitamin B12-dependent enzyme methionine synthase. When the activity of this synthase is compromised, as it would be in PA, the cellular folate will become progressively trapped as 5-methyltetrahydrofolate. This will result in a cellular pseudo-folate deficiency where, despite adequate amounts of folate, anaemia will develop, which is identical to that seen in true folate deficiency. Clinical symptoms of PA, therefore, include neuropathy, anaemia, or both. Treatment with vitamin B12, if given intramuscularly, will reactivate methionine synthase, allowing myelination to restart. The trapped folate will be released and DNA synthesis and generation of red cells will cure the anaemia. Treatment with high concentrations of folic acid will treat the anaemia but not the neuropathy of PA. It should be stressed that the so-called “masking” of the anaemia of PA is generally agreed not to occur at concentrations of folate found in food or at intakes of the synthetic form of folic acid at usual RNI levels of 200 or 400mg/day. However, there is some evidence that amounts less than 400mg may cause a haematologic response and thus potentially treat the anaemia (Savage *et al*., 1995). The masking of the anaemia definitely occurs at high concentrations of folic acid (>1000mg/day). This

becomes a concern when considering fortification with synthetic folic acid of a dietary staple such as flour (Carmel *et al*., 1996).

In humans, the vitamin B12-dependent enzyme methylmalonyl CoA mutase functions both in the metabolism of propionate and certain amino acids—converting them into succinyl CoA—and in the subsequent metabolism of these amino acids via the citric acid cycle. It is clear that in vitamin B12 deficiency the activity of the mutase is compromised, resulting in high plasma or urine concentrations of methylmalonic acid (MMA), a degradation product of methylmalonyl CoA mutase. In adults, this mutase does not appear to have any vital function, but it clearly has an important role during embryonic life and in early development. Children deficient in this enzyme, through rare genetic mutations, suffer from mental retardation and other developmental defects (Carmel *et al*., 1996).

1.2.3.6. Criteria for assessing vitamin B12 status

Traditionally it was thought that low vitamin B12 status was accompanied by a low serum or plasma vitamin B12 level . Recently, Lindenbaum challenged this assumption, by suggesting that a proportion of people with normal serum and plasma vitamin B12 levels are in fact vitamin B12 deficient. They also suggested that elevation of plasma homocysteine and plasma MMA are more sensitive indicators of vitamin B12 status. Although plasma homocysteine can also be elevated because of folate or vitamin B6 deficiency, elevation of MMA apparently always occurs with poor vitamin B12 status. However, there may be other reasons why MMA is elevated, such as renal insufficiency, so the elevation of MMA, in itself, is not diagnostic. Thus, low serum or plasma levels of vitamin B12 should be the first indication of poor status and this could be confirmed by an elevated MMA if this assay was available (Lindenbaum *et al*., 1990).

1.2.4. Folate and Folic Acid

1.2.4.1. Role of folate and folic acid in human metabolic processes

Folates accept one-carbon units from donor molecules and pass them on via various biosynthetic reactions (Scott *et al*., 1994). In their reduced form cellular folates function conjugated to a polyglutamate chain. These folates are a mixture of unsubstituted polyglutamyl tetrahydrofolates and various substituted onecarbon forms of tetrahydrofolate (e.g. 10-formyl-, 5,10-methylene-, and 5-methyl-tetrahydrofolate) (Figure 2).

Folic acid

Tetrahydrofolate

10-Formyltetrahydrofolate

5,10-Methylenetetrahydrofolate

5-Methyltetrahydrofolate

Figure 2: the chemical formula of Folic Acid and the most important natural folates (Scott *et al.*, 1994)

The reduced forms of the vitamin, particularly the unsubstituted dihydro and tetrahydro forms, are unstable chemically. They are easily split between the C-9 and N-10 bond to yield a substituted pteridine and p-aminobenzoylglutamate, which have no biologic activity (Blakley *et al.*, 1969). Substituting a carbon group at N-5 or N-10 decreases the tendency of the molecule to split; however, the substituted forms are also susceptible to oxidative chemical rearrangements and, consequently, loss of activity (Blakley *et al.*, 1969). The folates found in food consist of a mixture of reduced folate polyglutamates.

The chemical lability of all naturally-occurring folates results in a significant loss of biochemical activity during harvesting, storage, processing, and preparation. Half or even three quarters of initial folate activity may be lost during these processes. Although natural folates

rapidly lose activity in foods over periods of days or weeks, the synthetic form of this vitamin, folic acid, (e.g. in fortified foods) is almost completely stable for months or even years. In this form, the pteridine (2-amino-4-hydroxypteridine) ring is not reduced (Figure 2), rendering it very resistant to chemical oxidation. However, folic acid is reduced in cells by the enzyme dihydrofolate reductase to the dihydro and tetrahydro forms. This takes place within the intestinal mucosal cells, and 5-methyltetrahydrofolate is released into the plasma (Blakley *et al*., 1969).

Natural folates found in foods are all conjugated to a polyglutamyl chain containing different numbers of glutamic acids depending on the type of food. This polyglutamyl chain is removed in the brush border of the mucosal cells by the enzyme, folate conjugase, and folate monoglutamate is subsequently absorbed. The primary form of folate entering human circulation from the intestinal cells is 5-methyltetrahydrofolate monoglutamate. This process is, however, limited in capacity. If enough folic acid is given orally, unaltered folic acid appears in the circulation (Kelly *et al*., 1975), is taken up by cells, and is reduced by dihydrofolate reductase to tetrahydrofolate.

The bioavailability of natural folates is affected by the removal of the polyglutamate chain by the intestinal conjugase. This process is apparently not complete (Gregory *et al*., 1997), thereby reducing the bioavailability of natural folates by as much as 25–50%. In contrast, synthetic folic acid appears to be highly bioavailable—85% or greater (Cuskelly *et al*., 1996). The low bioavailability and, more importantly, the poor chemical stability of the natural folates have a profound influence on the development of nutrient recommendations. This is particularly true if some of the dietary intake is as the more stable and bioavailable synthetic form, folic acid. Fortification of foods such as breakfast cereals and flour can add significant amounts of folic acid to the diet. Functional folates have one-carbon groups derived from several metabolic precursors (e.g. serine, N-formino-l-glutamate, and folate). With 10-formyltetrahydrofolate, the formyl group is incorporated sequentially into C-2 and C-8 of the purine ring during its biosynthesis. Similarly, the conversion of deoxyuridylate (a precursor to RNA) into thymidylate (a precursor to DNA) is catalysed by thymidylate synthase, which requires 5,10-methylenetetrahydrofolate. Thus, folate in its reduced and polyglutamylated forms is essential for the DNA biosynthesis cycle shown in Figure 15.2. Alternatively, 5,10-methylenetetrahydrofolate can be channelled to the methylation cycle (Figure 3).

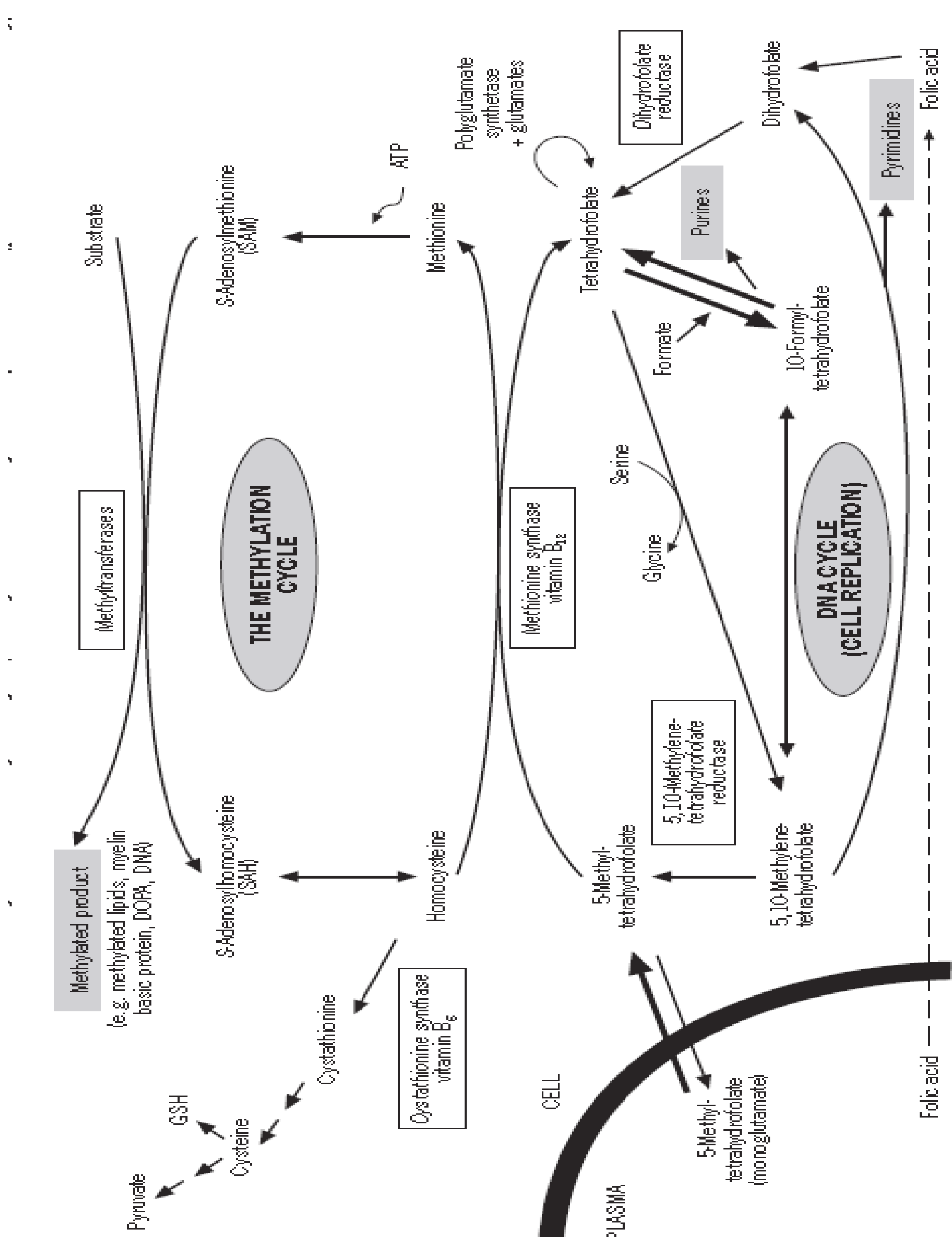

Figure 3: the role of the Folate cofactors in the DNA cycle and the methylation cycle (Gregory *et al*., 1997)

This cycle has two functions. It ensures that the cell always has an adequate supply of S-adenosylmethionine, an activated form of methionine which acts as a methyl donor to a wide range of methyltransferases. The methyltransferases methylate a wide range of substrates including lipids, hormones, DNA, and proteins. One particularly important methylation is that of myelin basic protein, which acts as insulation for nerve cells. When the methylation cycle is interrupted, as it is during vitamin B12 deficiency, one of the clinical consequences is the demyelination of nerve cells resulting in a neuropathy which leads to ataxia, paralysis, and, if

untreated, ultimately death. Other important methyltransferase enzymes down-regulate DNA and suppress cell division (Blakley *et al*., 1969).

In the liver, the methylation cycle also serves to degrade methionine. Methionine is an essential amino acid in humans and is present in the diet of people in developed countries at about 60% over that required for protein synthesis and other uses. The excess methionine is degraded via the methylation cycle to homocysteine, which can either be catabolized to sulfate and pyruvate (with the latter being used for energy) or remethylated to methionine. All cells including the liver metabolize methionine to homocysteine as part of the methylation cycle. This cycle results in converting methionine to S-adenosylmethionine, which is used as a methyl donor for the numerous methyltransferences that exist in all cells. This cycle effectively consumes methyl (-CH3) groups and these must be replenished if the cycle is to maintain an adequate concentration of S-adenosylmethionine, and thus the methylation reactions necessary for cell metabolism and survival. These methyl groups are added to the cycle as 5-methyltetrahydrofolate, which the enzyme methionine synthase uses to remethylate homocysteine back to methionine and thus to S-adenosylmethionine (Figure 3) (McPartlin *et al*., 1993).

The DNA and methylation cycles both regenerate tetrahydrofolate. However, there is a considerable amount of catabolism of folate (McPartlin *et al*., 1993) and a small loss of folate via excretion from the urine, skin, and bile. Thus, there is a need to replenish the body's folate content by uptake from the diet. If there is inadequate dietary folate, the activity of both the DNA and the methylation cycles will be reduced. A decrease in the former will reduce DNA biosynthesis and thereby reduce cell division. Although this will be seen in all dividing cells, the deficiency will be most obvious in cells that rapidly divide, including for example red blood cells, thereby producing anaemia; in cells derived from bone marrow, leading to leucopenia and thrombocytopenia; and in cells in the lining of the gastrointestinal tract. Taken together, the effects caused by the reduction in the DNA cycle result in an increased susceptibility to infection, a decrease in blood coagulation, and intestinal malabsorption. Folate deficiency will also decrease the flux through the methylation cycle but the DNA cycle may be the more sensitive. The most obvious expression of the decrease in the methylation cycle is an elevation in plasma homocysteine. This is due to a decreased availability of new methyl groups provided as 5-methyltetrahydrofolate, necessary for the remethylation of plasma homocysteine. Previously it was believed that a rise in plasma homocysteine was nothing more than a biochemical marker of possible folate deficiency. However, there is increasing evidence that elevations in plasma homocysteine are implicated in the etiology of cardiovascular disease

(Scott *et al.*, 1996). Moreover, this moderate elevation of plasma homocysteine occurs in subjects with a folate status previously considered adequate (Wald *et al.*, 1998).

Interruption of the methylation cycle resulting from impaired folate status or decreased vitamin B12 or vitamin B6 status may have serious long-term risks. Such interruption, as seen in vitamin B12 deficiency (e.g. pernicious anaemia), causes a very characteristic demyelination and neuropathy known as subacute combined degeneration of the spinal cord and peripheral nerves. If untreated, this leads to ataxia, paralysis, and ultimately death. Such neuropathy is not usually associated with folate deficiency but is seen if folate deficiency is very severe and prolonged (Manzoor *et al.*, 1976). The explanation for this observation may lie in the well-established ability of nerve tissue to concentrate folate to a level of about five times that in the plasma. This may ensure that nerve tissue has an adequate level of folate when folate being provided to the rapidly dividing cells of the marrow has been severely compromised for a prolonged period. The resultant anaemia will thus inevitably present clinically earlier than the neuropathy (Manzoor *et al.*, 1976).

1.2.4.2. Populations at risk for folate deficiency

Nutritional deficiency of folate is common in people consuming a limited diet (Chanarin *et al.*, 1979). This can be exacerbated by malabsorption conditions, including coeliac disease and tropical sprue. Pregnant women are at risk for folate deficiency because pregnancy significantly increases the folate requirement, especially during periods of rapid fetal growth (i.e. in the second and third trimester) (Mcpartlin *et al.*, 1993). During lactation, losses of folate in milk also increase the folate requirement.

During pregnancy, there is an increased risk of fetal neural tube defects (NTDs), with risk increasing 10-fold as folate status goes from adequate to poor (Daly *et al.*, 1995). Between days 21 and 27 post-conception, the neural plate closes to form what will eventually be the spinal cord and cranium. Spina bifida, anencephaly, and other similar conditions are collectively called NTDs. They result from improper closure of the spinal cord and cranium, respectively, and are the most common congenital abnormalities associated with folate deficiency (Scott *et al.*, 1994).

1.2.4.3. Dietary sources of folate

Although folate is found in a wide variety of foods, it is present in a relatively low density except in liver. Diets that contain adequate amounts of fresh green vegetables (i.e. in excess of three servings per day) will be good folate sources. Folate losses during harvesting, storage, distribution, and cooking can be considerable. Similarly, folate derived from animal products is subject to loss during cooking. Some staples, such as white rice and unfortified corn, are low

in folate. In view of the increased requirement for folate during pregnancy and lactation and by select population groups, and in view of its low bioavailability, it may be necessary to consider fortification of foods or selected supplementation of diets of women of childbearing years (Chanarin *et al*., 1979).

1.2.5. Trace Elements

1.2.5.1. Calcium

Calcium is a divalent cation with an atomic weight of 40. In the elementary composition of the human body, it ranks fifth after oxygen, carbon, hydrogen, and nitrogen, and it makes up 1.9% of the body by weight (Scott *et al*., 1976). Carcass analyses showed that calcium constitutes 0.1–0.2% of early fetal fat-free weight, rising to about 2% of adult fat-free weight. In absolute terms, this represents a rise from about 24 g (600 mmol) at birth to 1300 g (32.5 mol) at maturity, requiring an average daily positive calcium balance of 180mg (4.5mmol) during the first 20 years of growth.

Nearly all (99%) of total body calcium is located in the skeleton. The remaining 1% is equally distributed between the teeth and soft tissues, with only 0.1% in the extracellular fluid (ECF). In the skeleton it constitutes 25% of the dry weight and 40% of the ash weight. The ECF contains ionized calcium at concentrations of about 4.8mg/100ml (1.20 mmol/l) maintained by the parathyroid–vitamin D system as well as complexed calcium at concentrations of about 1.6mg/100ml (0.4 mmol/l). In the plasma there is also a protein-bound calcium fraction, which is present at a concentration of 3.2mg/100ml (0.8 mmol/l). In the cellular compartment, the total calcium concentration is comparable with that in the ECF, but the free calcium concentration is lower by several orders of magnitude (Robertson *et al*., 1981).

Biological role of calcium

Calcium salts provide rigidity to the skeleton and calcium ions play a role in many, if not most, metabolic processes. In the primitive exoskeleton and in shells, rigidity is generally provided by calcium carbonate, but in the vertebrate skeleton, it is provided by a form of calcium phosphate which approximates hydroxyapatite [Ca10(OH)2(PO4)6] and is embedded in collagen fibrils. Bone mineral serves as the ultimate reservoir for the calcium circulating in the ECF. Calcium enters the ECF from the gastrointestinal tract by absorption and from bone by resorption. Calcium leaves the ECF via the gastrointestinal tract, kidneys, and skin and enters into bone via bone formation (Figure 4) (Robertson et al., 1981).

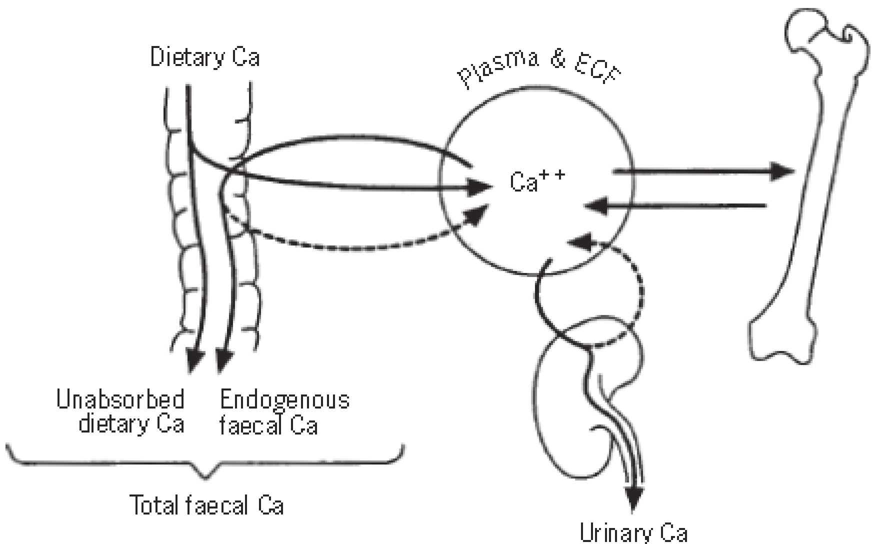

Figure 4: Major calcium Movements in the Body (Robertson *et al.*, 1981)

In addition, calcium fluxes occur across all cell membranes. Many neuromuscular and other cellular functions depend on the maintenance of the ionized calcium concentration in the ECF. Calcium fluxes are also important mediators of hormonal effects on target organs through several intracellular signaling pathways, such as the phosphoinositide and cyclic adenosine monophosphate systems. The cytoplasmic calcium concentration is regulated by a series of calcium pumps, which either concentrate calcium ions within the intracellular storage sites or extrude them from the cells (where they flow in by diffusion). The physiology of calcium metabolism is primarily directed towards the maintenance of the concentration of ionized calcium in the ECF. This concentration is protected and maintained by a feedback loop through calcium receptors in the parathyroid glands (Brown et a., 1997), which control the secretion of parathyroid hormone. This hormone increases the renal tubular reabsorption of calcium, promotes intestinal calcium absorption by stimulating the renal production of 1,25-dihydroxyvitamin D or calcitriol [1,25-(OH)2D], and, if necessary, resorbs bone. However, the integrity of the system depends critically on vitamin D status; if there is a deficiency of vitamin D, the loss of its calcaemic action (Jones *et al.*, 1998) leads to a decrease in the ionized calcium and secondary hyperparathyroidism and hypophosphataemia. This is why experimental vitamin D deficiency results in rickets and osteomalacia whereas calcium deficiency gives rise to osteoporosis (Wu *et al.*, 1990).

Determinants of calcium balance

Calcium intake

In a strictly operational sense, calcium balance is determined by the relationship between calcium intake and calcium absorption and excretion. A striking feature of the system is that relatively small changes in calcium absorption and excretion can neutralize a high intake or

compensate for a low one. There is a wide variation in calcium intake between countries, generally following the animal protein intake and depending largely on dairy product consumption. The lowest calcium intakes occur in developing countries, particularly in Asia, and the highest in developed countries, particularly in North America and Europe (Table 3).

Table 3: Daily protein and calcium intakes in different regions of the world, 1987-1989 (Wu *et al.*, 1990)

	Protein (g)			Calcium (mg)		
Region	Total	Animal	Vegetable	Total	Animal	Vegetable
North America	108.7	72.2	36.5	1031	717	314
Europe	102.0	59.6	42.4	896	684	212
Oceania	98.3	66.5	31.8	836	603	233
Other developed	91.1	47.3	43.8	565	314	251
USSR	106.2	56.1	50.1	751	567	184
All developed	103.0	60.1	42.9	850	617	233
Africa	54.1	10.6	43.5	368	108	260
Latin America	66.8	28.6	38.2	477	305	171
Near East	78.7	18.0	60.7	484	223	261
Far East	58.2	11.0	47.2	305	109	196
Other developing	55.8	22.7	33.1	432	140	292
All developing	59.9	13.3	46.6	344	138	206

Calcium absorption

Ingested calcium mixes with digestive juice calcium in the proximal small intestine from where it is absorbed by a process which has an active saturable component and a diffusion component (Ireland *et al*., 1973). When calcium intake is low, calcium is mainly absorbed by active (transcellular) transport, but at higher intakes, an increasing proportion of calcium is absorbed by simple (paracellular) diffusion. The unabsorbed component appears in the faeces together with the unabsorbed component of digestive juice calcium known as endoge- nous faecal calcium. Thus, the faeces contain unabsorbed dietary calcium and digestive juice calcium that was not reabsorbed.

True absorbed calcium is the total amount of calcium absorbed from the calcium pool in the intestines and therefore contains both dietary and digestive juice components. Net absorbed calcium is the difference between dietary calcium and faecal calcium and is numerically the same as true absorbed calcium minus endogenous faecal calcium. At zero calcium intake, all the faecal calcium is endogenous and represents the digestive juice calcium which has not been reabsorbed; net absorbed calcium at this intake is therefore negative to the extent of about 200mg (5mmol) (Nordin *et al*., 1976). When the intake reaches about 200mg (5mmol), dietary and faecal calcium become equal and net absorbed calcium is zero. As calcium intake increases, net absorbed calcium also increases, steeply at first but then, as the active transport becomes saturated, more slowly until the slope of absorbed on ingested calcium approaches linearity

with an ultimate gradient of about 5–10% (Nordin *et al*., 1975,,). The relationship between intestinal calcium absorption and calcium intake, derived from 210 balance experiments performed in 81 individuals collected from the literature. True absorption is an inverse function of calcium intake, falling from some 70% at very low intakes to about 35% at high intakes. Percentage net absorption is negative at low intake, becomes positive as intake increases, reaches a peak of about 35% at an intake of about 400 mg, and then falls off as intake increases further. True and net absorption converge as intake rises because the endogenous faecal component that separates them becomes proportionately smaller (Clarkson *et al*., 1970).

Many factors influence the availability of calcium for absorption and the absorptive mechanism itself. In the case of the former, factors include the presence of substances which form insoluble complexes with calcium, such as the phosphate ion. The relatively high calcium–phosphate ratio of 2.2 in human milk compared with 0.77 in cow milk (Nordin *et al*., 1976) may be a factor in the higher absorption of calcium from human milk than cow milk (see below). Intestinal calcium absorption is mainly controlled by the serum concentration of 1,25-(OH)2D. The activity of the 1-a-hydroxylase, which catalyses 1,25-(OH)2D production from 25-hydroxyvitamin D (25- OH-D) in the kidneys, is negatively related to plasma calcium and phosphate concentrations and positively related to plasma parathyroid hormone concentrations (Jones *et al*., 1998). Thus the inverse relationship between calcium intake and fractional absorption described above is enhanced by the inverse relationship between dietary calcium and serum 1,25-(OH)2D (Gallagher *et al*., 1979). Phytates, present in the husks of many cereals as well as in nuts, seeds, and legumes, can form insoluble calcium phytate salts in the gastrointestinal tract. Excess oxalates can precipitate calcium in the bowel but are not an important factor in most diets.

Urinary calcium

Urinary calcium is the fraction of the filtered plasma water calcium which is not reabsorbed in the renal tubules. At a normal glomerular filtration rate of 120ml/min and an ultrafiltrable calcium concentration of 6.4mg/100ml (1.60 mmol/l), the filtered load of calcium is about 8mg/min (0.20mmol/min) or 11.6 g/day (290 mmol/day). Because the average 24-hour calcium excretion in subjects from developed countries is about 160–200mg (4–5mmol), it follows that 98–99% of the filtered calcium is usually reabsorbed in the renal tubules. However, calcium excretion is extremely sensitive to changes in filtered load. A decrease in plasma water calcium of only 0.17mg/100ml (0.043 mmol/l), which is barely detectable, was sufficient to account for a decrease in urinary calcium of 63 mg (1.51mmol) when 27 subjects changed from a normal- to a low-calcium diet (MacFadyen *et al*., 1965). This very sensitive renal response to calcium deprivation combines with the inverse relationship between calcium intake and absorption to

stabilize the plasma ionized calcium concentration and to preserve the equilibrium between calcium entering and leaving the ECF over a wide range of calcium intakes. However, there is always a significant obligatory loss of calcium in the urine (as there is in the faeces), even on a low calcium intake, simply because maintenance of the plasma ionized calcium and, therefore, of the filtered load, prevents total elimination of calcium from the urine. The lower limit for urinary calcium in developed countries is about 140 mg (3.5mmol) but depends on protein and salt intakes. From this obligatory minimum, urinary calcium increases on intake with a slope of about 5–10%. In Figure 4.3, the relationship between urinary calcium excretion and calcium intake is represented by the line which intersects the absorbed calcium line at an intake of 520mg (Heaney *et al*., 1999).

Insensible losses

Urinary and endogenous faecal calcium are not the only forms of excreted calcium; losses through skin, hair, and nails also need to be taken into account. These are not easily measured, but a combined balance and isotope procedure has yielded estimates of daily insensible calcium losses in the range of 40–80mg (1–2mmol), which are unrelated to calcium intake (Charles *et al*., 1983,). Thus, the additional loss of a mean of 60mg (1.5 mmol) as a constant to urinary calcium loss raises the level of dietary calcium at which absorbed and excreted calcium reach equilibrium from 520 to 840 mg (13 to 21 mmol) (Heaney *et al*., 1999).

1.2.5.2. Magnesium

The human body contains about 760 mg of magnesium at birth, approximately 5g at age 4–5 months, and 25 g when adult (Widdowson *et al*., 1951). Of the body's magnesium, 30–40% is found in muscles and soft tissues, 1% is found in extracellular fluid, and the remainder is in the skeleton, where it accounts for up to 1% of bone ash (Heaton *et al*., 1976,). Soft tissue magnesium functions as a cofactor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes. Of particular importance with respect to the pathological effects of magnesium depletion is the role of this element in regulating potassium fluxes and its involvement in the metabolism of calcium (Waterlow, 1992,). Magnesium depletion depresses both cellular and extracellular potassium and exacerbates the effects of low-potassium diets on cellular potassium content. Muscle potassium becomes depleted as magnesium deficiency develops, and tissue repletion of potassium is virtually impossible unless magnesium status is restored to normal (Widdowson *et al*., 1951).

In addition, low plasma calcium often develops as magnesium status declines. It is not clear whether this occurs because parathyroid hormone release is inhibited or, more probably, because of a reduced sensitivity of bone to parathyroid hormone, thus restricting withdrawal of

calcium from the skeletal matrix. Between 50% and 60% of body magnesium is located within bone, where it is thought to form a surface constituent of the hydroxyapatite (calcium phosphate) mineral component. Initially much of this magnesium is readily exchangeable with serum and therefore represents a moderately accessible magnesium store which can be drawn on in times of deficiency. However, the proportion of bone magnesium in this exchangeable form declines significantly with increasing age (Breibart *et al*., 1960).

Significant increases in bone mineral density of the femur have been associatedpositively with rises in erythrocyte magnesium when the diets of subjects with gluten-sensitive enteropathy were fortified with magnesium (Rude *et al*., 1996). Little is known of other roles for magnesium in skeletal tissues.

Populations at risk for, and consequences of, magnesium deficiency

Pathological effects of primary nutritional deficiency of magnesium occur infrequently in infants (Lönnerdal *et al*., 1995) but are even less common in adults unless a relatively low magnesium intake is accompanied by prolonged diarrhoea or excessive urinary magnesium losses (Shils *et al*., 1988). Susceptibility to the effects of magnesium deficiency rises when demands for magnesium increase markedly with the resumption of tissue growth during rehabilitation from general malnutrition (Gibson *et al*., 1990). Studies have shown that a decline in urinary magnesium excretion during protein–energy malnutrition (PEM) is accompanied by a reduced intestinal absorption of magnesium. The catch-up growth associated with recovery from PEM is achieved only if magnesium supply is increased substantially (Nichols *et al*., 1978).

Most of the early pathological consequences of depletion are neurologic or neuromuscular defects (Shils *et al*., 1969), some of which probably reflect the influence of magnesium on potassium flux within tissues. Thus, a decline in magnesium status produces anorexia, nausea, muscular weakness, lethargy, staggering, and, if deficiency is prolonged, weight loss. Progressively increasing with the severity and duration of depletion are manifestations of hyperirritability, hyperexcitability, muscular spasms, and tetany, leading ultimately to convulsions. An increased susceptibility to audiogenic shock is common in experimental animals. Cardiac arrhythmia and pulmonary oedema frequently have fatal consequences. It has been suggested that a suboptimal magnesium status may be a factor in the etiology of coronary heart disease and hypertension but additional evidence is needed (Elwood *et al*., 1994).

Table 4: Typical daily intakes of magnesium by infants (6kg) and adults (65kg), in selected countries (Elwood *et al*., 1994)

Group and source of intake	Magnesium intake (mg/day)[a]	Reference(s)
Infants[b]		
Human-milk fed		
Finland	24 (23–25)	17
India	24 ± 0.9	20
United Kingdom	21 (20–23)	21,22
United States	23 (18–30)	11,23
Formula-fed		
United Kingdom (soya-based)	38–60	24
United Kingdom (whey-based)	30–52	24
United States	30–52	11,23
Adults: conventional diets		
China, Changle county	232 ± 62	25
China, Tuoli county	190 ± 59	25
China, females	333 ± 103	25
France, females	280 ± 84	26
France, males	369 ± 106	26
India	300–680	27
United Kingdom, females	237	28
United Kingdom, males	323	28
United States, females	207	29,30
United States, males	329	29,30

[a] Mean ± SD or mean (range).
[b] 750 ml liquid milk or formula as sole food source.

Dietary sources, absorption, and excretion of magnesium

Dietary deficiency of magnesium of a severity sufficient to provoke pathological changes is rare. Magnesium is widely distributed in plant and animal foods, and geochemical and other environmental variables rarely have a major influence on its content in foods. Most green vegetables, legume seeds, beans, and nuts are rich in magnesium, as are some shellfish, spices, and soya flour, all of which usually contain more than 500mg/kg fresh weight. Although most unrefined cereal grains are reasonable sources, many highly-refined flours, tubers, fruits, fungi, and most oils and fats contribute little dietary magnesium (<100mg/kg fresh weight) (Tan *et al*., 1985). Corn flour, cassava and sago flour, and polished rice flour have extremely low magnesium contents. Table 4 presents representative data for the dietary magnesium intakes of infants and adults.

Stable isotope studies with 25Mg and 26Mg indicate that between 50% and 90% of the labelled magnesium from maternal milk and infant formula can be absorbed by infants (Lönnerdal *et al*., 1997). Studies with adults consuming conventional diets showed that the efficiency of magnesium absorption can vary greatly depending on magnesium intake (Spencer *et al*., 1980). One study showed that 25% of magnesium was absorbed when magnesium intake was high compared with 75% when intake was low (Schwartz *et al*., 1984). During a 14-day balance study a net absorption of 52 ± 8% was recorded for 26 adolescent females consuming 176mg magnesium daily (Andon *et al*., 1996). Although this intake is far below the United States recommended dietary allowance (RDA) for this age group (280mg/day), magnesium balance was still positive and averaged 21mg/day. This study provided one of several sets of data that

illustrate the homeostatic capacity of the body to adapt to a wide range of magnesium intakes (Abrams *et al*., 1997). Magnesium absorption appears to be greatest within the duodenum and ileum and occurs by both passive and active processes (Greger *et al*., 1981). High intakes of dietary fibre (40–50 g/day) lower magnesium absorption. This is probably attributable to the magnesium-binding action of phytate phosphorus associated with the fibre (Kelsay *et al.,* 1979). However, consumption of phytate- and cellulose-rich products increases magnesium intake (as they usually contain high concentrations of magnesium) which often compensates for the decrease in absorption. The effects of dietary components such as phytate on magnesium absorption are probably critically important only when magnesium intake is low. There is no consistent evidence that modest increases in the intake of calcium (Kelsay *et al*., 1996), iron, or manganese affect magnesium balance. In contrast, high intakes of zinc (142 mg/day) decrease magnesium absorption and contribute to a shift towards negative balance in adult males (Quarme *et al*., 1986).

The kidney has a very significant role in magnesium homeostasis. Active reabsorption of magnesium takes place in the loop of Henle in the proximal convoluted tubule and is influenced by both the urinary concentration of sodium and probably by acid–base balance. The latter relationship may well account for the observation drawn from Chinese studies that dietary changes which result in increased urinary pH and decreased titratable acidity also reduce urinary magnesium output by 35% despite marked increases in magnesium input from vegetable protein diets. Several studies have now shown that dietary calcium intakes in excess of 2600mg/day (Greger *et al*., 1981), particularly if associated with high sodium intakes, contribute to a shift towards negative magnesium balance or enhance its urinary output (Kesteloot *et al*., 1990).

1.2.5.3. Copper

Copper (Cu) is an essential trace element for humans and animals. In the human organism, copper exists in two forms – the first and second oxidation form, as most of the copper in the human organism is in the second form. The ability of copper to easily attach and accept electrons explains its importance in oxidative reduction processes and in disposing and removing free radicals from the organism (Uauy *et al*., 1998). Although scientists identified copper compounds to treat diseases in 400 BC (during Hippocrates) (Turnlund *et al*., 2006), researchers still discover new information regarding the biochemistry, physiology, toxicology, many clinical, laboratory and other indicators of the impact of copper in the organism. In this respect, the exact composition and structure of copper compounds, generated in the organism, are not yet fully elucidated. The overall and interorgan distribution of copper is not quite

determined (Rosalind *et al*., 2005). The role and the participation of copper and copper enzymes in the metabolism, as well as the interaction of copper with other micronutrients are not clearly specified (Brunetto *et al*., 1999). Changes in copper concentrations in body fluids and tissues are observed in different diseases and conditions. There are indications of serious diseases, caused by disorders of the metabolism of copper in the organism, but the role of copper in most of them is not completely clarified (Ghayour-Mobarhan *et al*., 2009). Investigation of copper functions in human bodies requires accurate, affordable, informative, low-detection-limit methods for determination of trace copper in biological samples (Hendler S. S, 2001). Although a considerable number of methods for copper investigation exists, research continues in search of more sophisticated analytical approaches, for its determination in serum and urine, applicable in clinical laboratory diagnostics. These current developments justify the purpose of this research; to make a brief review of some issues related to studying the trace element copper.

Copper Status and Physiological Impact

The human organism contains about 70 ÷ 80 mg of copper (). There is evidence that its content varies over the year and depends on gender and age (4-9). With age significant differences are observed in the concentrations of ceruloplasmin (the main carrier of copper in the blood) (Ghayour-Mobarhan *et al*., 2005). Regarding gender, some studies have found no statistically significant differences in the copper content of blood serum in healthy children, but other studies discovered that the concentration of serum copper has significantly higher levels in women ($p < 0.05$) than in men. There cannot be distinguished clear trends in serum copper of subjects with drinking and smoking habits. Differences in serum copper are reported in people from different regions. According to some authors, the demographic differences are due to variations in copper content of the soil and / or food habits of the population. Increased physical activity is also found to reduce the concentration of serum copper ($p < 0.05$). A strong positive correlation is established between serum copper and body mass index (BMI) ($R = 0.85$, $p < 0.001$) in large-scale sample of 2233 subjects, aged 15-65 years. There is evidence for a link between copper levels in children and their parents. In a study of serum copper in 66 healthy children, aged 3- 14 years, a positive correlation is found between levels of trace elements in children and their parents. It is believed that this is due to identical dietary habits of children and their parents (Carlos *et al*., 2002).

Copper is a trace element which can be found in almost every cell of the human organism. The highest concentrations of copper are discovered in the brain and the liver; the central nervous system and the heart have high concentrations of copper as well. About 50% of copper content is stored in bones and muscles (in skeletal muscle it is about 25%), 15% in skin, 15% in bone marrow, 8 to 15% in the liver and 8% in the brain. Copper is a functional component of several

essential enzymes, known as copper enzymes – cytochrome c oxidase, lysyl oxidase, feroxidase, 2-furoate-CoA dehydrogenase, amine oxidase, catechol oxidase, tyrosinase, dopamine beta-monooxygenase, D-galaktozo oxidase, D-hexozo oxidoreductase, indole 2.3-dioxygenase, L-ascorbatoxidase, nitratreductase, peptidylglycine monooxygenase, flavonol 2,4-dioxygenase, superoxide dismutase, PHM (peptidylglycine monooxygenase hydroxylation) and others. (Malte *et al*., 2008)

Some physiological functions, dependent on the presence these enzymes in the organism, are described below. Cytochrome oxidase plays an essential role in cellular energy. As catalyzing the reduction of molecular oxygen (O_2) to water (H_2O), cytochrome c oxidase generates an electrical gradient, which is used by mitochondria to create vital energy for the organism and stored in molecules of ATP. Another copper enzyme, lysyl oxidase, participates in cross-linking of collagen and elastin, which form the connective tissue. The effects of lysyl oxidase helps maintain the integrity and elasticity of connective tissue in the heart and blood vessels, but also plays a role in bone formation. Two copper-containing enzymes, ceruloplasmin (feroxidase I) and (feroxidase II) have the ability to oxidize iron (Fe_2 +) to iron (Fe_3 +), which are connected to the protein transferrin for transportation to the red blood cells and blood formation. Although feroxidase activity of these two copper enzymes is still not thoroughly understood, the physiological significance and the involvement of copper in iron metabolism has been clearly demonstrated (Harris *et al*., 1997).

Many enzymatic reactions, which are essential for the proper functioning of the brain and the nervous system, are catalyzed by copper enzymes. Dopamine-beta-mono oxidase catalyses the conversion of the neurotransmitter dopamine into norepinephrine. Monoamine oxidase (MAO) plays a role in the metabolism of the neurotransmitters norepinephrine, epinephrine and dopamine. MAO functions in the breakdown of the neurotransmitter serotonin, which justifies the use of MAO inhibitors as antidepressants . Myelin sheath is made of phospholipids whose synthesis depends on the activity of the cytochrome c oxidase copper enzyme. Copper enzyme tyrosinase is required for the formation of melanin pigment. Melanin is produced in cells, called melanocytes, and plays a role in the pigmentation of hair, skin and eyes (Carlos *et al*., 2002).

Superoxide dismutase (SOD) functions as an antioxidant, which catalyses the conversion of superoxide radicals (free radicals) in hydrogen peroxide, that can subsequently be reduced to water by other antioxidant enzymes (Murthy *et al*., 1987). Two forms of SOD contain copper: 1) copper / zinc SOD is found in most cells of the organism, including red blood cells, and 2) extracellular SOD is a copper-containing enzyme, located in large quantities in the lungs and

in low levels – in plasma. Ceruloplasmin can function as an antioxidant in two different ways. Copper and iron ions are powerful catalysts in neutralizing of free radicals. Ceruloplasmin facilitates the catalyzation oxidative processes of the disposal of free radicals. In feroxidase, the activity of ceruloplasmin facilitates iron binding and the degradation of the transport protein transferrin, and may also prevent free ions (Fe_2+) from participating in the generation of harmful free radicals (Johnson *et al.*, 1992). Copper-dependent enzymes are involved in the regulation of gene expression. Cellular copper levels may affect the synthesis of proteins in the organism by enhancing or inhibiting the transcription of specific genes. Enzymes, which are involved in the regulation of gene expression, are copper / zinc superoxide dismutase (Cu/Zn SOD), catalase (another antioxidant enzyme); proteins, associated with cell storage of copper, can be included here as well (Murthy *et al.*, 1987).

There are two main forms of copper in serum, one form is tightly associated with the plasma protein ceruloplasmin, and the other is bound reversibly to serum albumin. Serum also contains copper enzymes – cytochrome c oxidase and monoamine oxidase. There is still no consensus on the best biomarker for determination of copper status. Serum copper or serum ceruloplasmin are most commonly used in order to investigate copper levels. According to some authors, these markers should be combined with more sensitive, specific and functional markers such as cytochrome c oxidase, platelets and superoxide dismutase in erythrocytes. In the future it is possible to use as functional indicators the antioxidant status, the changes in immune function, in combination with other biochemical markers.

Metabolism and Interactions with Micronutrients

Common daily intake of copper is 2 ÷ 5 mg, which exceeds the required amount. Copper is carried by the blood protein ceruloplasmin. Once absorbed from the digestive system, it is transported to the liver. Copper is absorbed in the duodenum and upper sections of the small intestine. It is believed that it is mainly absorbed in the small intestine, although there is evidence for its absorption in the stomach. In circulation, copper ions are connected to proteins: ceruloplasmin by 95% and the remainder – to albumin and amino acids. Small quantities of copper are excreted in the urine. The main part of the copper intake in the organism is excreted in bile juice. The proper amount of copper which an adult person requires per day is about 0.9 mg. There is no evidence that high dietary intake of copper is a problem for human health. In the USA, the recommended intake of copper is: for adult ≥ 19.10 mg/d.; for pregnant and lactating women and adolescents – 8 mg/d (Rosalind, 2001).

Copper is necessary in human nutrition for normal iron metabolism and the formation of red blood cells. Anemia is a clinical sign of deficiency of both iron and copper. Infants, who receive

food with high iron content, absorb less copper than babies who take food with low iron content. This suggests that high doses of iron can interfere with copper absorption in infants (Johnson *et al*., 1992). There are contradictive data on the copper content of blood serum in anemia. The results of some studies in children with IDA (Turgut *et al*., 2007) showed increased serum concentrations of copper. The authors concluded that high levels of copper reduced the absorption of iron and adversely affected haematological indices. High additional intake of zinc - 50 mg/d or more for an extended period of time can lead to copper deficiency. Zinc supplemented diet increases the intestinal synthesis of cellular proteins, called metallothioneins. They bind metals and do not allow their absorption by intestinal cells. Metallothioneins have a stronger affinity for copper than for zinc, so high levels of metallothioneins due to increased zinc can cause reduced absorption of copper. On the other hand, it is found that high doses of copper affect zinc nutritional status (Gurgoze *et al*., 2006).

The effect of supplements of vitamin C and copper on nutritional status of humans is not clear. Two studies in healthy men showed that the activity of ceruloplasmin oxidase may be impaired by relatively high doses of supplementary vitamin C (Jacob *et al*., 1987). Adverse effects of vitamin C supplements on copper nutritional status are not reported in any of these studies. In another study, the correlation between serum content of copper, zinc, iron and fat soluble vitamin A and E in healthy pre-school children is examined. A strong correlation between serum zinc and serum copper and iron is discovered. Serum levels of vitamin A are found to be significantly correlated to serum zinc and vitamin E as well. The authors believe that in order to investigate thoroughly the trace elements and fat-soluble vitamins, they should include in the study nutritional surveys, metabolic balance and correlations between micronutrient levels and anthropometric variables (stature, weight, body mass index) (Turgut *et al*., 2007).

Changes in Serum Copper in Various Diseases and Conditions

Deficiency or excess of copper in the organism is observed in metabolic disturbances and in various diseases and conditions. In several studies, the authors concluded that medical conditions in which low concentrations (abnormal) of copper are found are: Menkes syndrome, Parkinson's disease, impaired intestinal resorption, parenteral nutrition, protein loss (nephrosis syndrome, exudative enteropathy and others. Increased concentration of copper is observed in: pregnancy, cholestasis, increased ceruloplasmin – inflammation, tumors, lymphomas, liver cirrhosis, myeloid leukosis; Wilson’s disease. Hypercupremia is considered to be related to several acute and chronic infections and malignancies – leukemia, Hodgkin's disease, severe anemia hemohromatosys, myocardial infarction, hyperthyroidism, etc. Serum levels of copper are higher in patients who use contraceptives or estrogens. Two genetic diseases, which are caused by impaired metabolism of copper, are well studied (Menkes *et al*., 1962).

Menkes syndrome is an acquired condition and a recessive disease which leads to copper deficiency (de Bie *et al*., 2007). Intake and transport of copper is changed, whereby mineral substances in cells and organs are allocated abnormally. Symptoms include sparse and coarse hair, weak muscle tone (hypotonia), sagging facial features, seizures, mental retardation. Menkes disease is characterized as a recessive disorder with retardation of growth, brittle hair and focal cerebral and cerebellar degeneration (Menkes *et al*., 1962). The neurodegenerative processes change the grey matter of the brain – impaired twisted cerebral arteries (Barnes *et al*., 2005). This can lead to rupture or blockage of arteries. Weakened bones (osteoporosis) may result in fractures. Symptoms appear during infancy and are largely due to abnormal intestinal absorption of copper with an average deficit in mitochondrial copper-dependent enzymes. Reduced supply of copper decreases the activity of copper enzymes (e.g. lysine oxidase), which are necessary for structuring and functioning of bones, skin, hair, blood vessels and nervous system. (Voskoboinik *et al*., 2002,). In rare cases, symptoms appear later in childhood and are less severe. Early treatment with subcutaneous or intravenous injection of copper supplements (in the form of acetate salts) may be applied in the treatment of this disease. (Kaler *et al*., 2008) Wilson's disease is an autosomal recessive disorder, which causes copper overload and toxicity in the organism. This impairment of copper metabolism causes copper accumulation and toxic damage to cells primarily in the liver and the brain tissue, but also in the kidneys, eyes, joints and other organs. In some cases, large quantities of copper can destruct many red blood cells which results in severe anemia. (Attri *et al*., 2006) Symptoms of Wilson's disease usually appear at the age of 5-6 years. Kayser- Fleischer ring is a significant diagnostic indicator, because of the accumulation of copper in the eye. Neurological symptoms of the disease include behavioral abnormalities, tremor of the hands, unclear speech, and mask expression on the face. Once the symptoms emerge, they usually quickly progress. Disease diagnosis is confirmed by low levels of the protein ceruloplasmin, increased excretion of copper in the urine, high levels of copper in the liver or through confirmation of the genetic defect (Menkes *et al*., 1962).

A retrospective study was performed in Bulgaria, which analyzed the clinical and laboratory profile of 13 patients with Wilson's disease for a period of 12 years. The onset of clinical symptoms includes predominantly neurological manifestations (61.5%). In 54% of the patients there are clinical and laboratory evidence of liver cirrhosis. The ring of K-F is observed in 69% of the cases. Serum concentration of ceruloplasmin was reduced in 92% of the patients, urine excretion of copper in 24-hour urine was increased in 92% and copper in serum was increased in all patients (Tankova *et al*., 2007). It was discovered that the level of copper in the liver in Wilson's disease is about 25 times higher than in healthy subjects. A content of 89.8 μg/g copper

in the liver is reported in a 22-year-old woman who was in the subclinical stage of the disease and suddenly died. (Lech *et al*., 2007) Wilson's disease has a specific treatment. If the treatment is not implemented on time, it can lead to death after the age of 30. Cardiovascular diseases are widely spread and their diagnostics and treatment are essential for human life and health. Studies on atherosclerosis have shown contradictory results. Some scientists suggest that elevated levels of copper can increase the risk of atherosclerosis (Fox *et al*., 2000) and others that copper deficiency rather than copper excess holds an increasing risk of cardiovascular disease (Jones *et al*., 1997). Contradictory results were discovered in epidemiological studies on serum copper and copper enzymes in cardiovascular diseases – coronary heart disease (Malek *et al*., 2006), chronic heart failure and coronary artery disease, rheumatic heart disease (Kosar et a.., 2005), atherosclerosis, myocardial infarction (Wang *et al*., 1998). It is believed that these results are due to a lack of a reliable biomarker of copper status and it is not clear how copper is associated with cardiovascular diseases. Contradictory results were obtained in experimental studies on oxidative stress. When the dietary intake of copper was low, adverse changes had been observed in blood cholesterol, including increased total and LDL cholesterol and decreased HDL-cholesterol (Wang *et al*., 1998).

Results from other studies did not confirm these findings (Milne *et al*., 196). High copper supplements for four to six weeks did not lead to clinically significant changes in cholesterol levels, e.g. it was not proved that increased intake of copper lead to increase of oxidative stress. It is known that copper plays an important role in the development and maintenance of immune system function, whose exact mechanism is not revealed yet. Neutropenia (abnormally low number of white blood cells – neutrophils) is a clinical sign of copper deficiency in the human organism. Adverse effects of copper deficiency on immune function is most pronounced in infants. Infants with Menkes disease, which causes severe copper deficiency, suffer from frequent and severe infections (Failla *et al*., 1998).

In a study of 11 malnourished children with copper deficiency, the ability of white blood cells to absorb pathogens has increased significantly one month after administration of copper supplementations (Heresi *et al*., 1985). Osteoporosis is a socially significant disease of the bones. Copper-containing enzyme lysyl oxidase is required for the development (crosslinking) of collagen, which is a key element in the organic matrix of bone. Osteoporosis occurs in children and adults with severe copper deficiency, but it is not clear whether copper deficiency contributes to the manifestation and development of the disease. Serum levels of copper in elderly patients with hip fractures were found to be significantly lower than these of controls (Conlan *et al*., 1990). Studies in healthy adult men and women showed that copper supplements

significantly increased bone density (Baker *et al*., 1985). However, in another study, the addition of copper did not have effect on biochemical markers of bone resorption and bone formation in healthy adult men and women (Baker *et al*., 1999).

The role of copper in glucose homeostasis in diabetic patients has not been thoroughly determined. In a study of plasma selenium, zinc and copper in patients with diabetes Type 1and healthy controls, there were not found any significant differences in plasma copper concentrations of males and females – controls and patients with diabetes. The reduction in plasma copper concentration in patients with poor metabolic control is less strong in women than in men with diabetes. (Ruiz *et al*., 1998)

Concentrations of copper in blood serum in type 2 diabetes were significantly higher than those in controls. In recent years, many experiments with animals are performed in order to discover a treatment for impaired copper status in diabetes. A copper chelating agent was used in these experiments. As a result, serum copper was reduced to levels consistent with those of controls, treatment with copper-chelating agent decreased insulin resistance and improved glucose intolerance in diabetic patients. In addition, the treatment reduced triglyceride levels in blood serum. In conclusion, the results showed that copper was related to the manifestation of type 2 diabetes and should be applied in the treatment of diabetic patients. (Tanaka *et al*., 2009).

A research on patients with obesity and hypertension showed statistically significant higher serum levels of copper as compared to healthy subjects ($p < 0.001$). (11) Studies in children with chronic diarrhea investigated zinc and copper status (Rodriques *et al*., 1985). The level of both trace elements in serum was reduced. The authors have found deficit of serum copper in chronic diarrhea (Sachdev *et al*., 1990). Best K *et al*, 2004 () found moderate copper deficiency in cystic fibrosis patients. Other authors (Percival *et al*., 1995) found that patients with cystic fibrosis were at risk of nutritional deficiency due to the malabsorption syndrome, associated with endocrine pancreatic insufficiency. According to them, serum levels of copper and zinc demonstrate deficiency of these micronutrients in cystic fibrosis patients. Trace elements alone or in combination can be used as additives to the treatment of different diseases. Copper supplements are used in the form of copper oxide, copper gluconate, copper sulphate and copper amino acid chelates (Hendler *et al*.., 2001). Often they are combined with vitamins and other micronutrients. These supplements are produced by renowned pharmaceutical companies and could be found under different brand names – Suppravit, Doppelherz aktiv products, etc. In patients with Wilson's disease penicillamine is used to bind with copper and enhance its elimination from the organism. Since penicillamine dramatically increases urine excretion of

copper, the dosing should be very precise. It is known that antacids can interfere with copper absorption when applied in very large quantities.

Of the ten regions of the country, the two northernmost regions, the North and the Far North are part of the semi-arid Sahel. They are limited to the North by Lake Chad to the east by the Republic of Chad and Central African Republic, to the west by the Republic of Nigeria and South by the region of Adamawa.

1.3. BACKGROUND ON THE NORTH AND EXTREME NORTH REGIONS

Cameroon is a Central African state, located in the Gulf of Guinea between the latitude 2 and 13 degrees North and longitude 9 and 16 degrees has a rich human mosaic of over 200 ethnic groups

1.3.1. THE NORTH REGION

Location

The Northern Region covers an area of 66,090 square kilometers. Its terrain is diverse. It consists mainly of plains and basins, but also of plateaus and mountains. This region is subject to the Sudano-Sahelian climate with a rainy season of about five months which often makes roads access impassable, isolating certain populations for long periods. It is sometimes affected by large floods. The North is watered by a major water network of rivers: Mayo-Louti, Mayo-Kebi, Benue, Faro and Vina.

Demography

According to the results of the 2005 population census updated and published in 2010, the population of the Northern Region is estimated at 2,050,229 people i.e. 9.7% of the population of Cameroon, with a density of 25.5 inhab / km ^{2}. The average annual population growth of 5.1% is the highest of Cameroon, against a national average of 2.8% (DHS 2004). The position of the North Region supports a large mixed population. It has a high immigration population primarily from the Far North in search of fertile land. These migrants settled primarily in the Benue, the Mayo-Rey and the Faro, but also clog the towns of Garoua, Guider, Figuil, and Touboro, creating slums or over populated neighborhoods, which favors social problems.

Economic Situation

The dominant economic activities in the region are livestock (cattle and goat), cotton, groundnuts and corn. However, rice and millet also occupies an important place in the farming population. The North Region, by its geographical location, is the true economic powerhouse in the northern part of the country because it is crossed by a main highway (National road No 1) which allows the flow of agricultural products to the Far North, the south and neighboring countries. Business is thriving, thanks in particular to the major border markets. Touristic

activity also plays a significant role, despite continuing insecurity.In addition, major companies located there and are a very important economic asset offering employment opportunities (airline, cotton factory, breweries etc.).

Sociological situation

Many ethnic groups live together: Fulbe, Douroum, Mboum, Guidar, Fali, Bata and ethnic migrants from regions of the Far North, South Cameroon and neighboring countries (Chad, Central Africa and Nigeria in particular). This intersection of human and geographic factors gives the North region a potential high epidemiological risk. Islam and Christianity are the dominant religions but there are also many animist populations.

Health Map

The North Region is divided into 15 health districts, including 145 health areas. It counts 242 health facilities/support structures in all categories divided between the public and para-public and the private sector. Paramedic Training is provided by four structures.

1.3.2. THE FAR NORTH REGION

Location

The region covers an area of 34,263k2m. The terrain is mainly composed of plains and mountains to the West. The climate is of the Sudano-Sahelian type but predominantly Sahelian with a short rainy season from 3 to 4 months from June / July to September. In the rainy season, the lowland areas are inaccessible thereby slowing almost all activities including health. The Vele, Gure, Kar-Hay, Mada, Makary, Goulfey and the southern part of the Kousseri health districts are particularly difficult to access during the rainy season. The few rivers that water the area dry up during the dry season except the Logone. Also, it should be noted that there are three lakes: Maga, Guéré and Chad where fishing and agriculture are carried on in season.

Demography

In 2010, the region's population was estimated at over 3,480,414 inhabitants, with a density of 90.8 inhabitants/km ^{2}. It is the most populated region of Cameroon with 17.8% of the total population. The rural exodus of young people to the cities and especially to the rest of the country is important. This is partly due to the adversity of the nature of the area. Indeed, this region is experiencing droughts and floods that sometimes considerably weaken the household food security.

The average annual rate of population growth, steady over the past two decades, is 2.5%.The Far North Region shares long borders with neighboring countries, Chad and Nigeria, with a large porosity promoting populations mixing. These factors contribute to the occurrence of multiple outbreaks that affect the area and vary by season: Cholera in 2010 with some 9421 cases and 602 deaths, meningitis and measles, which usually appear and get worse in the dry

season. The capital of the region on the other hand is witnessing a substantial population growth with the creation and opening of the University of Maroua and two professional schools that are the Higher Institute Sahel and the Higher Teachers Training College.

Economic Situation

Agro-pastoral activities dominate the local economy with farming and selling of millet and dry season-millet in the words called "Mouskouari", rice in the Mayo-Danay, cattle and small ruminants. The main cash crop is cotton. In addition to the sale of agricultural products, trade is practiced by a small minority of people that imports items from Chad and Nigeria. Tourism activity is also important due to the presence of many touristic sites. In terms of business, the Far North has only two cotton plants (Maroua and Tchatibali) that offer only meager temporary jobs for young people.

Social situation

The Region of Far North is filled with several ethnic groups including:

- Toupouri straddling the two Divisions of the Mayo Danay and Mayo Kani;
- Mundangs and Guiwiga in Mayo Kani;
- The Fulani are spread throughout the region. Their language Fulani or "Fulfulde" is the common language between different ethnic groups;
- The Arabs and the Kotoko Choas that populate the Logone and Chari;
- The Mousgoum in Mayo Danay and part of the Logone and Chari such as Zina
- The Mandara, the Mada, the Zoulgo and Kanuri in the Mayo Sava;
- The Mafa, the Kapsiki the Bana and the Mayo Tsanaga Goude;
- The Moufou and Guiziga in Diamaré
- The dominant religions are Christianity and Islam, but there are also animist populations

1.3.3. HEALTH STATUS OF TWO REGIONS

It is in these two regions that malnutrition rates are highest in the country and many health indicators are the worst according to the Multiple Indicator Cluster Survey conducted in 2006 (MICS 2006). This survey showed that rates of global acute malnutrition among children 0-59 months were 15.1% and 14.2% respectively in the North and Far North and the chronic malnutrition rate was 50.8% and 41.6% (according to WHO 2006). The MICS was conducted during the dry season. In addition, the ten regions of the country both included 42.3% of deaths among children aged 0 to 59 months, 62.9% diarrhea, 53.8% of anemic children and 55.4% of pneumonia in two weeks preceding the survey (MICS 2006).

CHAPTER II: MATERIAL AND METHODS

2.1. SURVEY METHODOLOGY

2.1.1. GENERAL

The sampling method is random sampling in two-stage cluster, stratified for the two regions: North and Far North. Forty-three clusters were selected randomly in the Far North and 45 clusters in the North. A total of 1795 children under five years were interviewed, including 1343 from 12-59 months.

2.1.2. SAMPLING

Calculation of sample size for anthropometry and Food Frequency Questionnaire

To calculate the sample size (n) of children for anthropometric measurements, the following formula was used (ENA software):

$$n = \frac{k \times t^2 \times (1 - p) \times p}{\varepsilon^2}$$

Where:

n : required Sample size

k : cluster effect

t : for a confidence interval of 95 %, t = 1,96

p : estimated prevalence of global acute malnutrition in children 6-59 months

ε : Required relative precision

Table 5 Parameters for the calculation of sample size

Parameter	Anthropometry in children
t = for confidence interval at 95 %, t = 1,96	95%
p = prevalence of Global acute malnutrition	14%
ε : required relative precision	3%
k : cluster effect	1.5
n = sample size	770

Calculation of sample size for Micro nutrient Analysis

For the evaluation of the level of serum and plasma micronutrients, the sample size was calculated using on a method previously described by Pfeiffer *et al.*, 2007 which takes into account each biological indicator. According to the WHO(WHO, 2007), when considering resources and validity, the most appropriate assessment method for use in surveys is the 24-hour recall. Ideally, more than one day of intake data would be collected for each individual in

the sample, or for a subset of individuals, thus allowing for a better estimation of the distribution of micronutrient intakes in the population. It is recognized, however, that limitations in available resources may not always permit repeated recalls to be used. Thus the sample size for Plasma soluble Transferrin Receptor (sTfR), Plasma ferritin, Plasma folate, Plasma retinol and RBP and plasma vitamin A were calculated using the fisher formula (Araoye 2003) with a tolerable margin of error of 10%, and estimated effect size of 0.2 and a prevalence of 17.0 % from a previous study (MSP 2011) as previously described by Sin-Ho *et al.*, in 2014. The sample size formula for stratified Fisher's exact test was derived by specifying the values of the same input parameters as those for Mantel-Haenszel (Jung *et al.*, 2007). Following are input parameters specified for the sample size calculation. This gave a minimum sample size of 132 subjects for the study.

Calculation of the total number of households to be surveyed

The total number of households to be surveyed for an indicator is equal to the sample size divided by the number of individuals in target population in the household.

Table 6 Number of house holds to be surveyed

Parameter	Anthropometry	Micronutrient status	Total
n= Sample size	770	132	902
Number of targets in each household	1,5	1,5	1,5
Number of households to survey	642	198	840
Adding 10% for non-response	65	20	85
Adding 10% for ineligible house holds	65	20	85
Total	772	238	1010

Calculating the number of clusters and number of households to be surveyed

Considering that 24 households would be surveyed per day per cluster and dividing by the number of households to be surveyed by 24, the number of clusters required per region (42.8 clusters) was calculated. This figure was increased by 5% (2.14 clusters), for a total of 44.94 cluster, approximating to 45 clusters.

$$Numberof\ household = (total\ number\ of\ clusters\ \times 24\ households\ perday)$$

The **household** in the context of this study is defined as all people living under the authority of a single head of house and sharing the same meals on a regular basis, regardless of the relationship.

Choosing the type of sampling and selection of clusters

The choice of sampling depends primarily on the size of the sample, information and data available on the study population and the geographical layout of this population.

The sampling frame available and the one used in this study was the list of enumeration areas (EAs) of the North and the Far North regions from the last General Population and Housing Census, conducted by the Central Bureau of Study and Research on Population (BUCREP-2005). BUCREP defines an EA as a well-defined geographical area comprising generally between 120 to 220 households with a population of 700 to 1000 people. The size of each EA is known. Considering the size of the EA evolved consistently since 2005, the method of random sampling in clusters using probability proportional to size was chosen for the first stage of survey. The two regions are the two strata. The study area included 4474 EAS 1338 in the North and 3136 in the Far North. The second stage of the draw is random selection of households once in the EA.

The sampling method highly used is the Expanded program on Immunization (EPI) method also called pencil method. According to this method, the surveyor stands at the center of the site / village and turn a pencil (or bottle) on the ground, following the direction pointed to by the pencil to the edge of village. Once there, the same operation is repeated until the pencil point a direction towards the interior of the village. Walking in the direction indicated, households are numbered progressively. A draw is made from the numbered list of households to determine the first household to visit. The next households are selected by proximity until the number of children required for the cluster size attained. The disadvantage here is that the probability of a household being selected is unknown, since the total number of households is unknown, except weighted probabilities are calculated.

For this survey, households in the selected sites are listed exhaustively. After numbering these households, it is possible to randomly select households to be visited. With this method, households have the same probability of being selected. Also, selection by proximity was not used which reduces the cluster effect and thus allows for greater precision. Indeed, households close to each other may have more common characteristics (whether or not related to their situation) than households in the remote site.

The full census was conducted as follows: Initially, the EA is demarcated with the help of maps produced during the 2005 mapping census. An EA may contain one or more areas and / or one or more villages.

With the help of a guide and the village head/neighborhood, the EA is censured and the names of heads of households noted. A rough sketch is done in urban areas in order to find households. Each house is numbered with chalk; the household number assigned is noted on the map, and the name of the household head. Households whose inhabitants have traveled and who (according to the neighbors) would not return before the end of the day were not included.

Then, the draw of 10 households is carried out with the ENA software, from the list of all the households numbered in the EAs. The cluster consists of the selected households and respondents with the statistical unit being the households. This choice is due to the fact that some households, especially polygamous households, may have many children under five years. In such cases, the cluster size can be attained with very few households, which reduces the representativeness of the cluster.

Finally, no substitute clusters or households were previewed. This choice allows avoiding ending up with only accessible EAs that can replace EAs most at risk of malnutrition and so on.

The sampling methodology described here has limitations, since the maps date as far back as 2005. In fact in some EAs like that of Koza, one of the villages was not located in the EA as per the map, but after a migration, this village was no more within the limits of the EA. Such cases were therefore not included in the count.

2.1.3. Target groups

Children 1 to 5 years of age were selected as the target groups because they are especially vulnerable to malnutrition and micronutrient deficiency and could conveniently donate blood samples for the biological assessments. The North and Far North regions are zones were the level of malnutrition is a public health problem. This explains why in this study these two regions were targeted.

For this study, 1010 Households (HH) were targeted for this study, only 850 actually participated in the study. 5 Households were not eligible, 125 HH declined participating, and 28 HH did not participate. Among the 1795 children present in the 850 Households, 331 children consented to provide samples for micronutrient assessments and only 325 households successfully completed the Food Frequency questionnaire. (Figure 9 below).

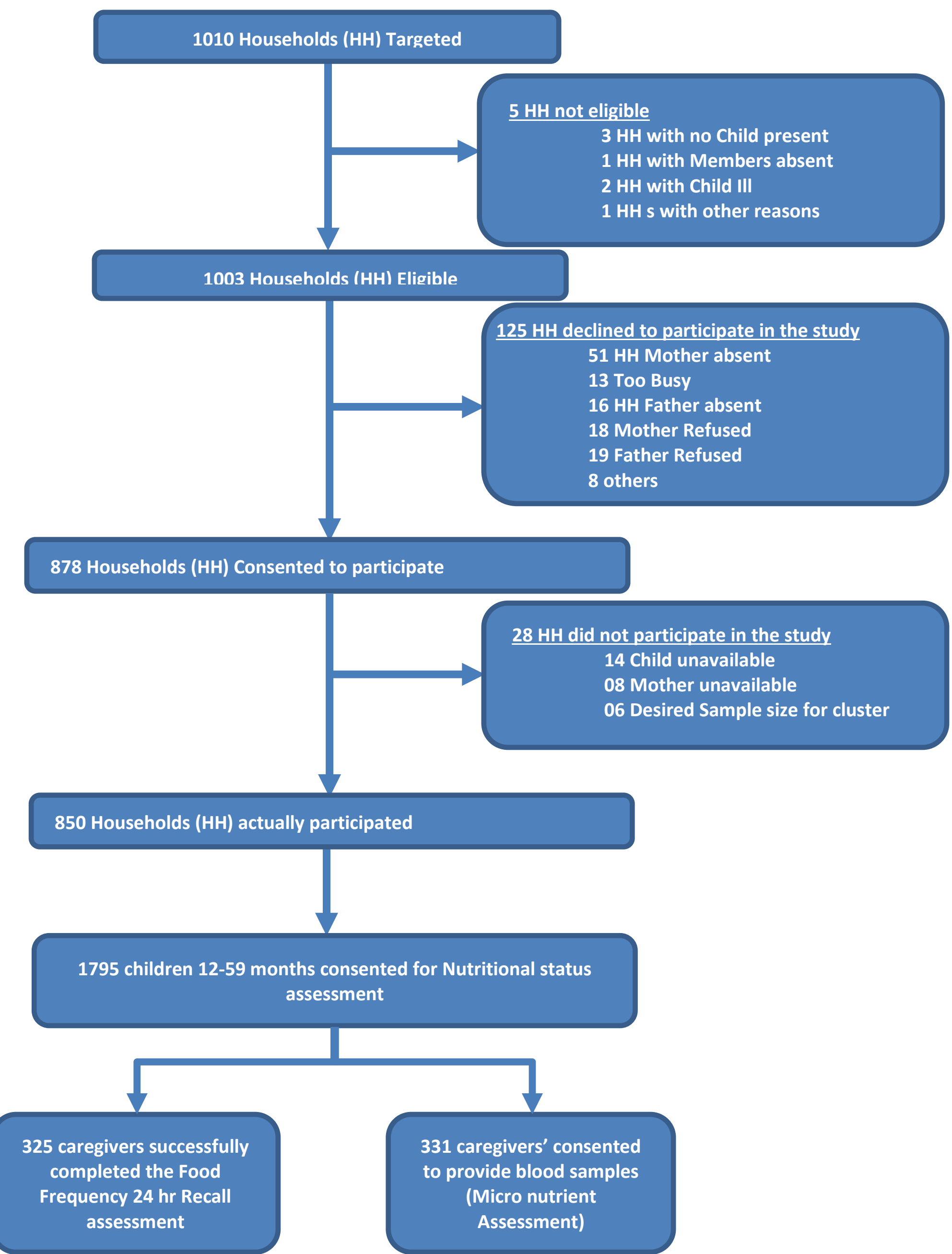

Figure 5 Schematic representation of sampling Results

2.2. DIETARY AND SOCIOECONOMIC STATUS DATA COLLECTION METHODS

2.2.1. Collection of Socio-Economic Status (SES) data

Data on the socio-economic status of participants was collected using a standardized data collection questionnaire with questions relating to the occupation/employment, and education level of the head of household and the caregiver interviewed, possession of certain assets including livestock, energy sources for cooking and domestic lighting, type of toilet, and access to water. The basic living conditions of the households were also considered by simply observing the housing materials (wall, floor, and roof) and household possessions.

2.2.2. 24 hour recall for Food Frequency consumption

On the day of the interview, the following standardized approach was used by trained interviewers to collects the frequency of the foods and Drinks consumed during the previous day. A data collection food frequency questionnaire that covered the previous week to the survey was used. Participants were asked how many times they had consumed each food over the past 7 days. For each food consumed, participants were then asked how many times per day the food was consumed on the last day on which they had consumed the food.

The success of the 24-hour recall depends on the subject's memory, how well the respondent estimates the portion sizes consumed, the respondent's degree of motivation, and the skill and persistence of the interviewer. Research indicates that a four-stage, multiple-pass interviewing technique yields the most accurate data.

To calculate food intake in this study, dietary data were collected using and adapted protocol of the for-stage multiple pass 24 hour recall method suitable for developing countries published by Gibson *et al*., 2008. This protocol consisted of carrying out only the first 3 pass of the four stage multiple pass recall approach. Two days prior to the interviews, participants were given a Picture charts (developed by a previous study – Engle-Stone *et al*., 2012) of commonly consumed foods. Participants were asked to place a check on the form next to each food they consumed during the 24-hour period preceding the dietary interview. An interviewer kit to help participants estimate the quantity of each food consumed was developed

Pass 1 – Recalling the foods and Drinks Consumed

For this first pass of the recall interview, a list of all the foods and drinks (including drinking water) consumed during the preceding 24-hour period was obtained. The interviewer started by reestablishing a rapport with the respondent.

1. The interviewer used the following simple and open questions to prompt the respondent: *"I would like you to tell me what you had to eat or drink after you woke up yesterday morning. Did you eat that food at home? What did you have next and at what time?"*
2. The interviewer then proceeded through the day, repeating these questions as necessary, and recording each food or drink (including drinking water) consumed in column 4 of the 24-hour recall food list. Probe for any snacks and drinks consumed between meals was done.
3. When the interviewer reached the end of the day, he checked the respondent's responses against the picture chart. If a food has been mentioned but it is not recorded on the chart, he probed for information: *Did the respondent forget to write it down? Was it eaten away from home? Was there a mistake made in recording it on the picture chart?* If a

food was on the chart but not mentioned, he probed to see whether the food was forgotten in the interview.

Pass 2 – Describing the foods and Drinks Consumed

In the second pass of the recall interview, the interviewer went over, in chronological order, each of the responses made by the respondent in pass 1, probing for more specific descriptions of all the foods and drinks consumed, including the cooking methods and (where possible or relevant) brand names. At this stage, the interviewer also asked if the respondent had remembered any additional items that were consumed but which were forgotten in the first pass.

For each food and drink item in the recall the following was done:

- Record the time and place of eating in the appropriate columns on the form.
- Use the appropriate probes to obtain further descriptive information.
- Record a detailed description of each food and drink item on the form. When commercial products are reported, information from the product label was also be recorded on the form in the appropriate columns.
- Amount of the mixed dish consumed by the respondent in the same units
- For homemade mixed dishes only, record on the recipe form and on the 24-hour recall form: the following additional details:
    - Name of mixed dish (Recipe name);
    - Descriptive list of all ingredients in descending order of quantity;
    - Amount of each raw ingredient (excluding water);
    - Method of preparation and cooking;
    - Total amount of cooked dish and the UNITS of the quantity and

Pass 4 – Reviewing the Recall Interview Data

In the final stage of the interview (i.e., pass four), the interviewer reviewed the recall to ensure that all the items have been recorded correctly. Finding and correcting errors at the time of the recall interview, when both the interviewer and the respondent are focused on the previous day's food, yielded more accurate information.

2.3. EVALUATION OF NUTRITION STATUS

Accurate anthropometric measurement is a skill requiring specific training for the proper assessment of the nutritional status of children. Step-by-step procedures are to be followed when taking measurements. Standardizing methods helps ensure that the measurements are correct and makes comparisons possible. Comparisons may be done between data collected from different areas of a country, between different surveys or between measurements and the reference standards. None of these comparisons will be possible without a standard method for

taking measurements. This section will cover the necessary field equipment and methods used in this study for these measurements.

2.3.1. EQUIPMENT

The equipment and materials used to measure anthropometric parameters of children were:

- UNICEF electronic UNISCALE (Weighing scale)
- Length measuring board (Seca 416, Infantometer; Seca Medical Scales and Measuring Systems)
- Portable standiometer (Seca Leicester Portable Height Measure; Seca Medical Scales and Measuring Systems).
- Clipboard

2.3.2. MEASURMENTS

Height for children 24 months and older

1. Measurer or assistant: Place the measuring board on a hard flat surface against a wall, table, tree, staircase, etc. Make sure the board is not moving.

2. Measurer or assistant: Ask the mother to remove the child's shoes and unbraid any hair that would interfere with the height measurement. Ask her to walk the child to the board and to kneel in front of the child. If a Microtoise measure is used, stand the child vertically in the middle of the platform.

3. Assistant: Place the questionnaire and pencil on the ground (Arrow 1). Kneel with both knees on the right side of the child (Arrow 2).

4. Measurer: Kneel on your right knee on the child's left side (Arrow 3). This will give you maximum mobility.

5. Assistant: Place the child's feet flat and together in the center of and against the back and base of the board/wall. Place your right hand just above the child's ankles on the shins (Arrow 4), your left hand on the child's knees (Arrow 5) and push against the board/wall. Make sure the child's legs are straight and the heels and calves are against the board/wall (Arrows 6 and 7). Tell the measurer when you have completed positioning the feet and legs.

6. Measurer: Tell the child to look straight ahead at the mother who should stand in front of the child. Make sure the child's line of sight is level with the ground (Arrow 8). Place your open left hand under the child's chin. Gradually close your hand (Arrow 9). Do not cover the child's mouth or ears. Make sure the shoulders are level (Arrow 10), the hands are at the child's side (Arrow 11), and the head, shoulder blades and buttocks are against the board/wall (Arrows 12,

13, and 14). With your right hand, lower the headpiece on top of the child's head. Make sure you push through the child's hair (Arrow 15).

7. Measurer and assistant: Check the child's position (Arrows 1-15). Repeat any steps as necessary.

8. Measurer: When the child's position is correct, read and call out the measurement to the nearest 0.1 cm. Remove the headpiece from the child's head and your left hand from the child's chin.

9. Assistant: Immediately record the measurement and show it to the measurer.

10. Measurer: Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to erase and correct any errors.

Length for infants and children 0-23 months

1. Measurer or assistant: Place the measuring board on a hard flat surface, i.e., ground, floor, or steady table.

2. Assistant: Place the questionnaire and pencil on the ground, floor, or table (Arrow 1). Kneel with both knees behind the base of the board if it is on the ground or floor (Arrow 2).

3. Measurer: Kneel on the right side of the child so that you can hold the foot piece with your right hand (Arrow 3).

4. Measurer and assistant: With the mother's help, lay the child on the board by supporting the back of the child's head with one hand and the trunk of the body with the other hand. Gradually lower the child onto the board.

5. Measurer or assistant: Ask the mother to kneel close on the opposite side of the board facing the measurer as this will help to keep the child calm.

6. Assistant: Cup your hands over the child's ears (Arrow 4). With your arms comfortably straight (Arrow 5), place the child's head against the base of the board so that the child is looking straight up. The child's line of sight should be perpendicular to the ground (Arrow 6). Your head should be straight over the child's head. Look directly into the child's eyes.

7. Measurer: Make sure the child is lying flat and in the center of the board (Arrows 7). Place your left hand on the child's shins (above the ankles) or on the knees (Arrow 8). Press them firmly against the board. With your right hand, place the foot piece firmly against the child's heels (Arrow 9).

8. Measurer and assistant: Check the child's position (Arrows 1-9). Repeat any steps as necessary.

9. Measurer: When the child's position is correct, read and call out the measurement to the nearest 0.1 cm. Remove the foot piece and release your left hand from the child's shins or knees.

10. Assistant: Immediately release the child's head, record the measurement, and show it to the measurer.

11. Measurer: Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to erase and correct any errors.

Child Weight Using UNICEF UNISCALE

The UNICEF mother/child electronic scale (Item No. 0141015) requires the mother and child to be weighed simultaneously. Minimize the clothing on the child. Ensure the scale is not over-heated in the sun and is on an even surface enabling the reading to be clear. Ask the mother to stand on the scale. Record the weight of just the mother and include the reading with one decimal point (e.g. 65.5 Kg). Tare the scale by passing your thumb over the blue section of the screen. Pass the child to the mother who is still no the scale. Record the second reading (e.g. 8.3 Kg). That is the weight of the child (UNICEF , 2000).

2.4. EVALUATION OF PLASMA LEVELS OF MICRONUTRIENTS AND TRACE ELEMENTS

2.4.1. COLLECTION OF BIOLOGICAL SAMPLES

Blood collection and processing

Previously trained biological sample collection agents (phlebotomists) collected 5-7ml of blood into tubes certified trace-element free and containing lithium heparin as an anticoagulant (Sarstedt, United Kingdom). The blood was taken from the antecubital vein in the majority of children. In the case of children for whom sample collection was difficult, the blood was drawn from the back of the wrist. The tourniquet was removed immediately after the needle penetrated the vein to minimize changes in blood pressure which may alter the concentrations of blood constituents.

Upon removing the syringe, a drop of venous blood was collected into a micro cuvette for immediate analysis of hemoglobin using a portable photometer (Hemocue Hb201 +). This model of photometer contains an internal quality control monitor and is appropriate for the analysis of venous, arterial or capillary blood. In cases where it was not possible to obtain venous blood, a drop of capillary blood was collected from a finger prick for the analysis of hemoglobin.

After collection of the blood into tubes that were previously wrapped in aluminum foil, blood samples were immediately placed into coolers containing cold packs. The samples were then centrifuged at 2500 rev / min (Hermle Z206A) for 10 minutes to separate plasma from the pellet. Samples were generally centrifuged 45 min to 1 hour after collection. Plasma was aliquoted into polypropylene cryotubes using adjustable pipettes with sterile polypropylene tips.

To avoid contamination by dust, the samples were aliquoted in a portable "hood", which consisted of a large plastic box with the opening 2/3 covered by a plastic film. In addition, to avoid degradation of vitamin A and folate due to exposure to light, the "hood" was covered with black plastic packaging and the cryotubes containing plasma to be analyzed for vitamin A and folic acid were covered with aluminum foil.

Plasma samples were transported in coolers directly to the central laboratory (Centre Pasteur of Cameroon – Annex Garoua) and frozen at -20 ° C. All samples collected were then packed in dry ice and shipped to specialized laboratories for analysis.

2.5. LABORATORY ANALYSIS OF BIOLOGICAL SAMPLES

Four main biochemical analysis techniques were used for the analysis of the different biomarkers:

2.5.1. EVALUATION OF PLASMA PROTEINS BY SANDWICH ELISA:

This method was used to analyze five plasma proteins: Ferritin, Soluble Transferrin Receptor (sTfR), Retinol Binding Protein (RBP), C-reactive protein (CRP), and alpha (1)-acid glycoprotein (AGP).The method was a follows:

Chemicals and other materials: The following chemicals were used as purchased: NaH_2PO_4, Na_2HPO_4, NaCl, Citric acid, phosphoric acid, 3,3 ,5,5 -tetramethyl-benzidine dihydrochloride (TMB), 30% H_2O_2 (Sigma).

Capture antibodies: Capture antibodies were as follows: ferritin (Code A0133, Dako), sTFR (Cat. No. 4Tr26; Clone 23D10, Hytest), RBP (Code A0040, Dako), CRP (Code A0073, Dako Denmark).

Detection antibodies: Detection antibodies were as follows: anti-ferritin-horseradish peroxidase (HRP) (Code P0145, Dako), anti-sTFR-HRP (Cat. No. 4Tr26-c; Clone 13E4, Hytest), anti-RBP-HRP (Code P0304, Dako), anti-CRP-HRP (Code P0227, Dako).

Serum control samples (Liquicheck, Bio-Rad) were used as standards for the calibration curves. Quality control (QC) samples were prepared from serum samples with a low and high content of analytes.

Coating 96-well plates with capture antibody. Antibodies were diluted in coating buffer (0.01 mol/L phosphate buffer, 0.15 mol/L NaCl, pH 7.2). To 96-well plates (Maxi sorb C-shape, Nunc) was added 100 L of appropriately diluted antibody. The plates were covered with parafilm and were incubated overnight in the refrigerator. The antibody concentrations for coating were anti-ferritin, 0.05 g/well (1:5000); anti-sTFR, 0.1 μg/well (1:10000); anti-RBP, 0.82 μg/well (1:1000); and anti-CRP, 0.05 μg/well (1:20,000). The following morning the plates were emptied by inversion over a sink and prewashed by pouring wash buffer (0.01 mol/L phosphate buffer, pH 7.2, 0.5 mol/L NaCl, 0.1% Tween 20) over the plate and then slinging the buffer out into a sink. This was repeated 3 times, each time leaving the wash buffer 3–5 min in the wells. After the last wash any remaining wash buffer was removed by tapping the inverted plate on a paper towel.

Application of serum and standards on the plate. After the coated plates were washed, 100 μL diluted serum and standard samples were added to the wells. The samples and calibration standards were placed on the plates in a way that minimizes biased readings to obtain reliable results. Therefore the measurement was done in duplicate and the 2 replicate samples were placed in different positions on the plate. The same procedure was done for the standards. An example for the arrangement of the samples on a plate is shown in Figure 8. A copy of this figure was also placed under the 96-well plate, which helps in finding the correct well during pipetting.

	1	2	3	4	5	6	7	8	9	10	11	12
A	01	02	03	04	05	06	07	08	09	10	11	12
B	BL	L1	L2	L3	L4	L5	BL	L1	L2	L3	L4	L5
C	13	14	15	16	17	18	19	20	21	22	23	24
D	25	26	27	28	29	30	31	32	33	34	35	36
E	37	38	39	40	QC1	QC2	01	02	03	04	05	06
F	07	08	09	10	11	12	13	14	15	16	17	18
G	19	20	21	22	23	24	25	26	27	28	29	30
H	31	32	33	34	35	36	37	38	39	40	QC1	QC2

Figure 6: The arrangement of the samples on a 96-well plate (01– 40: study samples; BL, L1–L5: standards, QC1 and QC2 quality controls).

Dilution scheme for serum. The serum dilution scheme was as follows: D1, 15 μL serum 150 μL wash buffer (1:11 dilution performed in 1.7-mL microtubes); D2, 10 μL D1 1500 μL wash buffer (1:1661 dilution performed in 1.7-mL microtubes or a 96-deep-well plate).

Volumes of diluted serum applied to the final reaction plate. The following volumes were applied to the final reaction plate: ferritin, 100 μL D1 (1:11 final dilution); sTfR, 100 μL D2 (1:1661 final dilution); RBP: 25 μL D2 (1:6644 final dilution); CRP: 50 μL D2 (1:3322 final dilution).

For sTfR, RBP, and CRP the diluted serum was applied using a 12-channel pipette from the 96-deep-well plate or a plate that was filled with the D2 dilution of the samples. Before addition of the diluted serum to the plate, the wells for RBP and CRP were filled with the necessary amount of wash buffer to obtain a final volume of 100μL.

Preparation of standards. A commercially available control sample from Bio-Rad (Liquichek Immunology Control, Level 3) was used to obtain a calibration curve on each plate. The manufacturer provides values for many analytes and methods of analysis. The mean value of all methods mentioned for each analyte of interest was calculated and used this value as the basis for further dilutions to obtain a calibration curve. The values were 296.9 μg/L for ferritin, 2.87 μmol/L for RBP, and 54.33 mg/L for CRP. Because no values were available for sTfR, commercially available kit (Ramco Laboratories) were used to measure the concentration of sTfR (8.93 mg/L). The dilution scheme as shown Table 1 was used to get calibration curves in the physiologically most interesting range.

Table 7: Dilution of Liquicheck Bio-Rad control sample for the preparation of calibration curves

	Ferritin		sTfR		RBP		CRP	
	SD1[1] μL	*μg/L[2]*	*SD2,[3] μL*	*mg/L[2]*	*SD3,[4] μL*	*μmol/L[2]*	*SD4,[5] μL*	*mg/L[2]*
Level 1	3	8.9	20	3.55	3	0.34	6	0.59
Level 2	6	17.8	40	7.1	6	0.69	12	1.18
Level 3	12	35.6	60	10.65	9	1.03	25	2.47
Level 4	25	74.2	80	14.19	12	1.38	50	4.94
Level 5	50	148.5	100	17.74	15	1.72	100	9.88

1 SD1: 15 L Liquichek Immunology Control (Level 3) 150 L wash buffer.
2 Resulting concentration after filling up the wells to 100 L.
3 SD2: 20 L SD1 1500 L wash buffer.
4 SD3: 10 L SD1 1500 L wash buffer.
5 SD4: 100 L SD3 1000 L wash buffer.

After 2-h incubation at room temperature, the plate washing procedure was repeated as described above at the coating step. Considering the large number of samples, timing was critical: the samples and the standards were incubated for same amounts of time.

Detection antibody binding. A total of 100 μL of diluted HRP coupled antibodies in coating buffer was added to the wells. The detection antibody concentrations were anti-ferritin-HRP, 0.015 μg/well (1:8000); anti-sTFR-HRP, 0.015 μg/well (1:15000); anti-RBP-HRP, 0.06 μg/well (1:2000); and anti-CRP-HRP, 0.016 μg/well (1:4000). The plates were again incubated for 1 h at room temperature and the standard washing step was repeated.

Color reagent and plate development. To prepare the color reagent, 1 mg TMB (pre-dissolved in Dimethyl sulfoxide - DMSO) was added to 12 mL 0.1mol/L citric acid phosphate buffer (pH 5.2). The TMB citric acid buffer solution were prepared in larger amounts and stored frozen in 12-mL portions. To prevent border effects, the plate and the color reagent were both at room temperature. Before the addition of 100 μL of this color reagent to each well 2 μL of 30% H_2O_2 was mixed in to the 12-mL TMB solution. After sufficient blue color development (5–10 min) the reaction was stopped by the addition of 100 μL/well of 1 mol/L phosphoric acid. Each well was measured at 450 nm with the reference wavelength set at 650 nm. Although it is possible to measure without a reference wavelength the use of this reference wavelength improved the quality of the measurement.

2.5.2. EVALUATION OF RETINOL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The recent application of high performance liquid chromatography (HPLC) to the analysis of biological samples has provided nutritional biochemists with a highly useful technique. In this study, HPLC was chosen over spectrophotometry, fluorometry or gas chromatography because of its rapidity, relative freedom from interfering impurities, nondestructive conditions and its simplified methodology that is applicable to a wide range of samples.

Retinol Binding Protein (RBP) as the primary transporter of retinol in blood is most often used as a biomarker for Vitamin A for children blow 5 years. However, the ratio of Retinol to RBP is not always 1:1 thus HPLC was used to measure Plasma Retinol in the population. The use of HPLC for the analysis of plasma retinol has been documented (Engle-Stone *et al*., 2011).

HPLC conditions: A Gilson liquid chromatography system was used, comprising a 305 piston pump connected to an 805S manometric module and a 234 auto-injector with a 500 µl interchangeable sample loop. The detector used was a 119 UV detector with dual wavelength detection at 325 nm at 0.002 absorbance unit full scale and 295 nm at 0.002 absorbance unit full scale. The pump and detector were controlled by the 715 system controller software which saved, viewed and enabled re-integration of all stored chromatographic data. All chromatograms and results analyzed were then recorded on a Hewlett Packard 5L laser printer.

A stainless steel 30 cm by 3.9 mm I.D. 10 µm µBondapak C_{18} column was used for the chromatographic separation. This was preceded by a Sentry guard column holder housing a disposable guard column insert which was packed with the same material as that in the analytical column.

Chemicals and reagents: Solvents used for preparation of mobile phase for liquid chromatography were of HPLC grade and ultra-pure water of resistivity of around 18 megohms centimetres (MΩ - cm) was used. Ethanol used for standard preparation and sample extraction was HPLC grade. Retinol and - tocopherol used as standards and retinol acetate and -tocopherol acetate as internal standards were from Sigma Chemicals (USA). Ascorbic acid used was analar grade. Petroleum ether (b.p. 40 - 60ºC) used for sample preparation was analytical grade as HPLC grade was not available. The solvent was redistilled to prevent interference with retinol peak during the chromatographic run.

Extraction procedure: A 10% solution of vitamin C in ultra-pure water was prepared and 200 µl added to a 10 ml flask containing 0.065 µg/ml of retinol acetate and 23.03 µg/ml of tocopherol acetate and made up to 10 ml with ethanol. The addition of ascorbic acid to ethanol has been reported to prevent deterioration of retinol and tocopherol during the extraction step (Driskell, Bashor & Neese, 1985; Tee & Khor, 1995). A volume of 20 µl of this solution was added to 20 µl of serum in a 8 mm x 75 mm extraction tube and mixed for 30 seconds to precipitate the protein. 200 µl of redistilled petroleum ether was then added to extract the retinol and tocopherol from the sample using an electronic shaker for at least 2 minutes. The extract was then centrifuged at 1300 rpm for 5 minutes to separate the petroleum ether layer which was then removed with either a pasteur pipette or micropipette into a 1.5 ml, eppendorf tube. The extract was completely dried using a vacuum concentrator (Hetovac HS-1-110) for 10 minutes after which 200 µl of ethanol was added and then mixed with a vortex mixer. After a period of 5 to 10 minutes, the extract was filtered with a 0.45 µm disposable membrane filter into a disposable vial for chromatography using the Gilson's 234 autoinjector. Not more than 100 µl of the extract was injected as excess solvent would result in ethanol interfering in the HPLC chromatogram.

Preparation of mobile phase: Two mobile phase systems were used in the study. The first was a mixture of acetonitrile, methanol and ethyl acetate (88:10:2, v/v) developed by Tee, Lim & Chong (1994) and Tee & Khor (1995). The second mobile phase comprises a mixture of methanol and water (95:5, v/v). All mobile phase solutions were filtered through a 47 mm, 0.45 µm nylon membrane filter and subsequently degassed for 15 minutes with an ultra-sonic bath. The analytical column was washed with at least 100 to 150 ml of mobile phase and then equilibrated at a flow rate of 2 ml per minute before injecting 100 µl of the standard mixture.

Preparation of standard solutions: Retinol, retinol acetate, -tocopherol and -tocopherol acetate of 100 µg/ml were prepared in HPLC grade ethanol. These standard solutions were kept refrigerated in amber containers and were found to be stable for 3-4 months. Absorbance readings of each standard was also taken before each assay and the appropriate absorptivities (extinction coefficients) of De Ritter & Purcell (1981), Schudel, Mayer & Isler (1972) and Sigma Chemicals were used to calculate the exact concentrations of each compounds as shown in Table 2 below. The preparation of all standard solution were carried out rapidly in a room with subdued light and with all windows tinted with a light-protective film. All sample extraction and analytical procedures were also carried out in this room.

Table 8: Extinction coefficient of retinol standards

Standards	Solvent (wavelength maxima, nm)	Extinction Coefficient	Reference
Retinol	Ethanol (325)	1570	Sigma Chemical Co.
Retinol acetate	Ethanol (325)	1550	Sober HA (1970)
α -tocopherol	Ethanol (292)	75.8	Schudel, Mayer & Isler (1972)
α -tocopherol acetate	Ethanol (298)	43.6	Sober HA (1970)

Quantitation using internal standards: Internal standards were added to the sample at the beginning of the extraction procedure to compensate for losses at each step of the sample preparation. After processing as described in the extraction procedure, the final extract was filtered with a 4 mm, 0.45µm disposable membrane filter and then chromatographed for the simultaneous determination of the above vitamins. Calibration curves for the two standards were prepared weekly and unknown samples quantitated by the software using the standard curves obtained. Quantitation was accomplished by comparing the peak area of the analyte in the unknown to the peak area of the internal standard in the standard solution i.e. retinol to retinol acetate and α-tocopherol to α-tocopherol acetate. With internal standard data analysis, the system controller software automatically adjusts the amount of the analyte in unknown samples, in relation to the amount of internal standard added at the beginning of the whole procedure.

Measuring Plasma Retinol Concentration By HPLC

All procedures were carried out in dim light to protect samples from light at all times.

1. Label two 7 mL glass, screw-cap vials for each plasma sample.
2. Pipet 100 µL plasma into a new, 7 mL glass vial, add 900 µL of water.
3. Add 1.0 mL ethanol containing ~100 ng/mL retinyl acetate, vortex for 15 seconds.
4. Add 3.0 mL hexane, vortex vigorously for ~45 s.

5. Centrifuge for 2 minutes (use bench-top centrifuge). Transfer hexane layer to clean 7mL glass vial.
6. Evaporate under N2 in fume hood. Be careful to protect samples from light (cover samples with aluminum foil). Clean needles on evaporator with alcohol swabs before using.
7. Redissolve the residue in 70 µL methanol, vortex vigorously for 15s.
8. Prepare autosampler vials: put 100 µL plastic inserts inside amber-colored 2 mL, glass autosampler vials. Replace red septums in blue caps. Label the autosampler vials (0, 1, 2,…etc.; start with 0 because the software on the HPLC starts with 0).
9. Make a list of samples to be run and the position in which they will be placed in the auto-sampler rack.

(example:	sample	vial#
	Pool	0
	1A	1
	1B	2, etc…)

10. Transfer samples to inserts in auto-sampler vials using a pipet (~70 µL; all of the sample), place vials in auto-sampler (according to list).
11. Prepare Sequence on HPLC, using the "retinol.met" method. Inject 60 µL onto HPLC. Mobile phase 95/5 (Methanol/water; run time is ~10minutes; column: C18, 3µm, 15cm column, with 5µm guard column (Alltech)); monitor absorbance at 325 nm.
12. Run 3 plasma pool samples that have been calibrated against the NIST control serum with each set of unknowns. Compare the ratios of the peak areas of retinol:retinyl acetate in the unknowns to the ratio of peak areas of retinol:retinyl acetate in the calibrated pool samples to calculate the retinol concentration of the unknowns. Use a second plasma pool sample (different concentration from the first plasma pool) that has also been calibrated against NIST control serum as a "control" sample. Run a single aliquot of the "control" plasma pool with each set of unknowns to assess the accuracy of the measurements. Precision of the measurements is estimated by calculating the CV of the ratio of peak areas of retinol:retinyl acetate in the three plasma pool samples.

Preparation of retinyl acetate as an internal standard for plasma retinol analyses

1. Prepare stock solution of ~1 mg/mL of retinyl acetate in ethanol. Wrap 7 mL glass vial in foil, tare the vial on the electronic balance (0.0000g precision), add few mgs of retinyl acetate (<5 mg). Add enough ethanol (d=0.76 g/mL) to make solution of ~1 mg/mL.

(example: if retinyl acetate weighs 3.5 mg, add 3.5 mL of ethanol which is 3.5 * 0.76 g/mL = 2.66 g). Vortex until retinyl acetate goes into solution.

2. Dilute stock solution to ~ 5 µg/mL to check the concentration by UV-Vis spectrophotometry. Assume the concentration is close to 1 mg/mL, dilute 1:200. Use a positive displacement pipet to make the dilution. Example: Wrap a 20 mL glass scint vial in foil, tare it on the electronic balance, add ~5 mL of ethanol. Carefully pipet 50 µL of the stock solution into the vial, rinse the tip several times in the ethanol inside the vial. Bring the total volume to 10 mL (7.76 g) with ethanol. Mix well, read the OD 325nm on the spectrophotometer in the Clifford Lab, calculate the concentration using the molar extinction for retinol in ethanol of ~52000 and molecular weight of 286 (RE) or 328 (retinyl acetate). The OD must be between 0.5 and 1 absorbance units to be accurate, if it's not, adjust the dilution until it's within that range.
3. Dilute the stock solution to make a working solution for the internal standard of 100 ng/mL ethanol. Make a total volume of 250 to 500 mL and store in an amber-colored glass bottle or wrap a clear glass bottle in foil. Label should include the concentration, solvent and date. Example: retinyl acetate in ethanol, *mm/dd/yyyy*.

The safety measures observed were as follows: Wear lab coat, close-toed shoes. Work with organic solvents in the chemical fume hood. Work with plasma in the Biological Safety Cabinet. Dispose of all glass pipet tips in the box labeled "glass only". Dispose of all pipet tips, gloves, etc. that come in contact with plasma in the waste container labeled "biologic hazard". Wear goggles when pouring organic solvent to make mobile phase.

2.5.3. MEASURMENT OF PLASMA CONTENT OF METAL ELEMENTS BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY

This technique was used in the analysis of Plasma Zinc, Iron, Copper, Calcium, Magnesium, and Potassium. An analytical portion of the sample (0.4 to 0.5 µg) was decomposed with Nitric acid - HNO3 (Wako Pure Chemicals, Japan) and hydrogen peroxide in a high-pressure (Teflon® lined digestion vessel) and heated on an aluminium heating block (IWAKI, Asahi Techno Glass, Japan) at 120°C for 5 hours. The sample was further heated almost to dryness at 200°C after removing the teflon ball. A 50 mL analytical solution was prepared from the digest. Analytical solutions were nebulized and aerosol was transported to a plasma where desolvation and excitation occured. A pneumatic nebulization sample introduction was used. Characteristic atomic emission spectra were produced by radio frequency inductively coupled plasma. Spectra were dispersed by a grating spectrometer, and line intensities were measured with a light sensitive detector (a photomultiplier tube). Photocurrents were processed by a computer system. A background correction technique was required to compensate for variable background emission contribution to analyte signal. Extensive quality control procedures were incorporated

for monitoring laboratory contamination. Finally, the residue was dissolved with 2 ml of 0.1 M HNO3 which contained 10 ng/ml internal standard elements (In, Re, and Tl). The diluted serum solution was used for analysis of the elements in ICP-MS. Commercially available single element standard solutions (1000 μg/ml) were purchased from Wako Pure Chemicals (Osaka, Japan) and used for standardization of calibration curves. The result was expressed in μg/dl.

2.5.4. MEASURMENT OF PLASMA FOLATE AND VITAMIN B12 BY RADIO LIGAND BINDING ASSAY

The principle behind this method is that in competitive protein binding, the binder should have an equal affinity for the standard and the substance which is present in the sample. The unlabeled vitamin B12 or folate competes with its labeled species for the limited number of available binding sites on its specific binder, thus reducing the amount of labeled vitamin B12 or folate bound. Therefore, the level of radioactivity bound is inversely related to the concentration in the specimen or standard.

In the ICN Pharmaceuticals SimulTRAC-SNB Radioassay Kit, levels of vitamin B12 and folate are determined simultaneously in a single tube. The vitamin B12 and folate tracers, binders and standards are supplied in combined form. The pteroylglutamic acid form of folate (PGA) is used as both standard and tracer in an incubation mixture at pH 9.5.

At this binding pH, both 5-methyltetrahydrofolic acid (MTFA) in the patient sample and PGA in the standards have equal affinity for the milk binder. The two tracers, [57Co] for vitamin B12 and [125I] for folate, produce energies at levels which can be easily separated by two-channel counters.

REAGENTS

SimulTRAC-SNB Vitamin B12/ Folate Tracer, Catalog No. 06B257133. A bottle contains <1.5 μCi (55.5 kBq) [^{57}Co] Vitamin B12 and <3 μCi (111 kBq) [^{125}I] folate in borate buffer with human serum albumin, dextran, potassium cyanide, endogenous binder blocker, dye and preservative. Volume: >10 mL/bottle. One bottle/100 tube kit, 2 bottles/200 tube kit. Storage: Refrigerate at 2-8°C; protect from strong light. Stability: Refer to expiration date on bottle.

REAGENT PREPARATION:

Equal amounts (100 µL each) of tracer and dithiothreitol (DTT) were added per assay tube. The tracer and dithiothreitol were mixed at a 1:1 ratio in a 200 µL/tube with the tracer pipetted first and followed by the dithiothreitol.

ASSAY PROCEDURE:

Sixteen (16) tubes were numbered for the standards and beginning with 17 two tubes per sample were numbered. The standards and samples were added as follows:

- 200 µL Working Tracer/ DTT Solution (Reagent B1) were added to all tubes including the Total Count tubes (1 and 2) and vortexed,
- Incubation for 15 minutes at room temperature. (18 - 25°C)
- Addition of 100 µL Extracting Reagent to tubes 3-16 and all sample tubes. Vortex.
- Incubation for 10 minutes at room temperature. (18 - 25°C)
- Thorough mixing of the bottle of SimulTRAC-SNB Blank Reagent and addition of 1000 µL blank reagent to tubes 3 and 4.
- Vigorous mixing of the bottle of SimulTRAC-SNB Binder and addition of 1000 µL binder to tubes 5-16 and all sample tubes. Vortex.
- Incubation of tubes 3-16 and all sample tubes at room temperature (18 - 25°C) for 60 minutes from the time of the last addition of the binder. The rack of tubes were covered with aluminum foil to exclude light,
- Centrifugation at of 12050 x g for 10 minutes in the cold.
- Gentle decantation and discard of supernatant. Removal of last drop by touching the tube to a paper towel or absorbent paper.
- Counting of the radioactivity in the pellets and in tubes 1 and 2 in sequence for one minute with a gamma counter. The total count per minute for tubes 1 and 2 for [^{57}Co] were between 10,000 and 25,000 and for [^{125}I] between 15,000 and 35,000.
- Calculations. Drawing of the standard curve and determination of sample assay values.

2.6. STATISTICAL ANALYSIS

2.6.1. Analysis

Data was analyzed using SPSS version 20 statistical package (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were calculated for all variables. Continuous variables were examined for adherence to a normal distribution and all micronutrients values in serum were normally distributed and hence no transformation was done

The variables related to socioeconomic status were used to construct a score representing socio-economic status (SES). The socio-economic score was created using principal components analysis of a number of variables (some dichotomized) representing the living conditions of households. The primary factors explaining 64.4% of the total variance. The SES scores were divided into five quintiles to indicate: the very poor, poor, average, rich, and very rich.

Plasma concentrations of RBP and ferritin were arithmetically adjusted for the presence of infection (CRP 0.5 mg/L and/or AGP 0.1 g/L) prior to analysis (Thurnham *et al*., 20011). Using linear regression analysis, plasma zinc concentrations were adjusted for CRP, AGP, time of day

of blood collection, time elapsed since previous meal, and time from blood collection to centrifugation.

Specifically, using biological indicators of risk of micronutrient deficiency, children with and without low plasma RBP (RBP<0.83 µmol/L for children-Engle-stone *et al*., 2011), low iron stores (ferritin <0,12 µg/L), low tissue iron (sTfR> 8.3 mg/L), low plasma zinc concentration (plasma zinc <650 µg/L), anemia (hemoglobin <110 g/L), low plasma vitamin B-12 (B-12 <210 pmol/L), and low plasma folate (folate <10 nmol/L) and children with and without stunting (height-for-age Z-score <-2) were assessed.

Due to the fact that the acute phase response to infection or inflammation results in a reduction in the plasma concentrations of RBP and zinc and increases the concentration of ferritin (Biesalski *et al*., 2007). To better estimate the prevalence of vitamin A, zinc, and iron deficiency, the prevalence of deficiency was calculated using values that were first adjusted mathematically to account for elevated CRP and / or AGP (Thurnham *et al*., 2010). To do this, all individuals are divided into four categories reflecting the status of infection:

- CRP > 5 mg / L and AGP <1g / L (incubation)
- CRP > 5 mg / L and AGP > 1 g / L (presence of symptoms / early convalescence)
- CRP < 5 mg / L and AGP > 1 g / L (late convalescence), and
- CRP < 5 mg / L and AGP < 1g / L (healthy / reference group).

For RBP and zinc, individual values in each of the three 'infection groups' were multiplied by the ratio of the geometric mean of the group of healthy individuals to the geometric mean of the 'infection group'. The procedure for adjustment of ferritin was the same, except that the median instead of the geometric mean was used. Adjusted body iron stores were calculated using sTfR and infection-adjusted ferritin values.

Cut off value for magnesium, calcium, and copper, was defined at their serum levels of 18 µg/dl, 84 µg/dl, and 7 µg/dl respectively (Sauberlich HE, 1999). The cutoffs used to define deficiency for each micronutrient indicator were as follows: Unadjusted and adjusted ferritin, <12 mg/L; unadjusted ferritin, <30 mg/L (WHO recommendation – WHO 2011); unadjusted and adjusted BIS, <0 mg/kg; sTfR, >8.3 mg/L; haemoglobin, <110 g/L; plasma zinc, <65 µg/dl.

Body iron Stores (BIS) were calculated using the ratio of sTfR and ferritin concentrations according to a formula derived by Cook *et al*. Plasma concentrations of ferritin were adjusted for the presence of inflammation using the method previously published by Thurnham *et al*. (Thurnham *et al*., 2010) and involves first stratifying individuals into categories based on elevated CRP and/or AGP: apparently healthy reference group (CRP ≤5 mg/L and AGP ≤1

g/L), incubation (CRP >5 mg/L and AGP ≤1 g/L), early convalescence (CRP >5 mg/L and AGP >1g/L), and late convalescence (CRP ≤5 mg/L and AGP >1 g/L). Individual values were then adjusted by multiplying by the ratio of the median of the apparently healthy group to the median of that individual's inflammation group.

Pearson's test was used to assess the correlation between two continuous variables. Statistical significance was assigned for p values less than 0.05. The z score values for height-, weight- and BMI-for-age relative to the WHO 2006 reference were calculated using Epi Info and WHO Anthro Plus softwares (WHO AnthroPlus, 2009).

2.6.2. Calculation of Anthropometric indices

The indicators calculated for this study include:

- The prevalence of Global Acute Malnutrition, Moderate Acute Malnutrition and Severe Acute Malnutrition (Weight for Hieght)
- The prevalence of Global, Moderate and Severe Wasting (Weight for Age)
- The prevalence of Global, Moderate and Severe Stunting (Height for Age)

The Anthropometric indices calculated for children 12 to 59 months compared to the WHO 2006 reference population. They include:

Prevalence of acute malnutrition – Wasting: It is characterized by a low weight for height index - WHZ (acute malnutrition or wasting). It is an expression of recent, progressive and severe situation causing rapid weight loss. It is generally the result of a serious illness increasing nutritional needs, reducing food consumption and/or a combination with a high dietary deficiency. The child has approximately the same size as those of his age but is thinner.

Prevalence of Chronic Malnutrition - Stunting: It is measured by a low height for age index - HAZ (chronic malnutrition) which reflects the cumulative effect of inadequate nutrition and/or with a weakened health status or a combination of both over a long period. The child has a smaller size than those of his age.

Prevalence of underweight: it is represented by a low weight for age index - WAZ (underweight). It is a composite of both wasting and stunting. It informs a change in nutritional status without specifying if it's recent or chronic. It is often biased by the low mastery of the ages.

Table 9: WHO cut-offs for nutritional status classification

Category of Malnutrition	Acute Malnutrition or wasting (WHZ)	Underweight (WAZ)	Chronic Malnutrition or stunting (HAZ)

Severe	<-3 z score and/or oedema	<-3 z score	<-3 z score
Moderate	≥ - 3 z score and < - 2 z score	≥ - 3 z score and < - 2 z score	≥ - 3 z score and < - 2 z score
Global	< - 2 z score and/or oedema	< - 2 z score	< - 2 z score

2.6.3. Measuring Food Consumption and Dietary Diversity Scores

Food consumption Score (FCS)

The Food Consumption Score (FCS), a tool developed by WFP, is commonly used as a proxy indicator for access to food. It is a weighted score based on dietary diversity, food frequency and the nutritional importance of food groups consumed.

Data is collected on the number of days in the last 7 days a household ate specific food items. A seven day recall period is used to make the FCS as precise as possible and reduce recall bias. The FCS of a household is calculated by multiplying the frequency of foods consumed in the last seven days with the weighting of each food group. The weighting of food groups has been determined by WFP according to the nutrition density of the food group. Table 10 shows the food group weights.

Table 10: Detail of Food group weights

Food Item	*Food Group*	*Weight*
Rice	Cereals and tubers	2
Wheat/other Cereals		
Potato (including sweet potato)		
Pulses/Beans/Nuts	Pulses	3
Milk/Milk products	Milk	4
Yoghurt		
Other dairy products		
Meat	Meat and Fish	4
Poultry		
Fish and seafood (fresh/dried)		
Eggs	Eggs	4
Dark green vegetable – leafy	Vegetables	1
Other vegetables		
Sugar/Honey	Sugar	0.5
Fruits	Fruits	1
Oil	Oil	0.5

The sum of the scores is then used to determine the FCS as indicated in the formula below:

$$FCS = \alpha_{cereals}\beta_{cereals} + \alpha_{pulses}\beta_{pulses} + \alpha_{milk}\beta_{milk} + \alpha_{meat\,\&fish}\beta_{meat\,\&\,fish} + \alpha_{vegetables}\beta_{vegetables} + \alpha_{sugar}\beta_{sugar} + \alpha_{fruits}\beta_{fruits} + \alpha_{oil}\beta_{oil} + \alpha_{eggs}\beta_{eggs}$$

Where:

$\alpha_i = Frequency\ of\ food\ consumption -$
$Number\ of\ days\ each\ food\ group\ was\ consumed\ in\ the\ pas\ 7\ days$

$\beta_i = weight\ of\ each\ food\ group$

The maximum FCS has a value of 112 which would be achieved if a household ate each food group every day during the last 7 days. The total scores were then compared to pre-established thresholds:

Poor food consumption: 0 to 21
Borderline food consumption: 21.5 to 35
Acceptable food consumption: > 35

Dietary Diversity Score (DDS)

Dietary diversity scores (DDS) were calculated by summing the number of food groups consumed in the household or by the individual respondent over the 24-hour recall period.

The following steps are included in creating either the HDDS or WDDS:

$$DDS = \gamma_{cereals} + \gamma_{pulses} + \gamma_{milk} + \gamma_{meat\,\&fish} + \gamma_{vegetables} + \gamma_{sugar} + \gamma_{fruits} + \gamma_{oil} + \gamma_{eggs}$$

Where:

$\gamma_i = 1,\ if\ Food\ group\ is\ consumed\ in\ the\ pas\ 7\ days$

The maximum DDS has a value of 9 which would be achieved if a household ate each food group during the last 7 days. The total scores were then compared to pre-established thresholds:

Acceptable dietary diversity: ≥ 4

2.6.4. Constructing the Socioeconomic status (SES) Index with Principal Correspondence Analysis (PCA)

Theoretically, measures of household wealth can be reflected by income, consumption or expenditure information. However, the collection of accurate income and consumption data requires extensive resources for household surveys. PCA has been validated as a method to describe SES differentiation within a population. Issues related to the underlying data will affect PCA and this was considered when generating and interpreting results.

PCA is a multivariate statistical technique used to reduce the number of variables in a data set into a smaller number of 'dimensions'. In mathematical terms, from an initial set of n correlated variables, PCA creates uncorrelated indices or components, where each component is a linear

weighted combination of the initial variables. For example, from a set of variables X1 through to Xn,

$$PC_1 = a_{11}X_1 + a_{12}X_2 + \cdots + a_{1n}X_n$$

$$\vdots$$

$$PC_m = a_{m1}X_1 + a_{m2}X_2 + \cdots + a_{mn}X_n$$

where amn represents the weight for the mth principal component and the nth variable.

There are four main steps in constructing a SES index: selection of asset variables; application of PCA; interpretation of results; and classification of households into socio-economic groups.

Selection of asset variables

As a first step, descriptive analyses were carried out for all the variables, looking at means, frequencies and standard deviations. Descriptive analysis informed decisions on which variables to include in the analysis, and highlight data management issues, such as coding of variables and missing values.

McKenzie in 2003 highlights that a major challenge for PCA-based asset indices is to ensure the range of asset variables included is broad enough to avoid problems of 'clumping' and 'truncation'. Clumping or clustering is described as households being grouped together in a small number of distinct clusters. Truncation implies a more even distribution of SES, but spread over a narrow range, making differentiating between socio-economic groups difficult (e.g. not being able to distinguish between the poor and the very poor). From the distribution of asset ownership, access to utilities and infrastructure, and housing characteristics in the analysis, clumping and truncation are likely to be issues for the data from the rural areas of the North and far North region. This is because many households do not own the durable items, have similar access to utilities and infrastructure, and similar housing characteristics, and so will be grouped together. Also, of the households that do own assets, they have the same ones, which will make differentiating among them difficult. To resolve the problem of clumping and truncation, additional variables were included that capture inequality between households most of which were continues variables.

In order to mitigate for the stability of household classification into SES groups, infrastructure variables were included in the index which increased the representation of households from urban areas into the richest groups, and subsequently increased inequality.

Application of PCA

Data in categorical form (such as religion) are not suitable for PCA, as the categories were converted into a quantitative scale which does not have any meaning. To avoid this, qualitative

categorical variables were re-coded into binary variables. Similar variables with relatively high frequencies were kept as separate variables. All binary variables created from a categorical variable, with those that had low frequencies but were not similar enough to another variable to combine were included in order to ensure all the data for each household were measured. Durable assets that were initially binary with very low counts were excluded.

Given that the analysis of data on household characteristics and asset ownership is complicated by the fact that there are potentially a large number of variables which could be collected, some of which may yield similar information, methods such as PCA to try and organize the data to reduce its dimensionality with as little loss of information as possible in the total variation these variables explain.

In the data analysis, when specifying PCA, the user is given the choice of deriving eigenvectors (weights) from either the correlation matrix or the co-variance matrix of the data. The number of principal components extracted can also be defined by the user, and a common method used is to select components where the associated eigenvalue is greater than one.

CHAPTER III: RESULTS AND DISCUSSION

RESULTS

3.1. General Characteristics of the study population

Of the 1795 children interviewed, 50.5% were from the North and 49.5% from the Far North regions. There was not statistical difference between these proportions ($p>0.05$). Even though more Males (53.2%) participated in the study compared to females (46.8%), the difference was only significant ($p=0.034$) for the North region. Most of the household heads were males (96.1%) and the average household size was 8.3 persons per household. The age distribution suggests that children of the lower ages (12-23 months) are slightly more represented compared to children of the higher ages.

A majority of the study population were Muslims (55.3%) though there were relatively high representations of Catholics (15.1%) and Protestants (19.6%). A great proportion of the caregivers were married (89.1%) and only 35% had attended school (24.2% Primary education and 10.3% secondary education). Comparatively, much more Heads of house hold had attended school (49.8%) with 24.2% attending Primary education and 22.7% attending secondary education. Only 3.05% of the household heads had attendant higher education.

Considering the environment of the households, 63.1% of the population use Pit latrine (improved with slab) while up to 29.0% have modern toilets. The proportion of modern toilets was significantly higher for the Far North region compared to the North Region ($p<0.05$). The major source of energy for cooking was Wood/Charcoal (91.2%). However, a comparatively high proportion of the households in the North region use Kerosene/Gas as source of energy for cooking. Three major sources of energy for lighting were identified: Electricity (36.9%), Flash light/tube light –battery (28.1%) and Oil/Gas (24.8%). Similarly, the three main sources of drinking water that made up 87.6% of all the sources of drinking water were: Dug well (36.6%), Tube well/Borehole (33.5%) and piped water (17.5%). The average distance from the source of drinking water was 2hrs 13 minutes' walk from the household. This distance was significantly longer for the Far North region compared to the North Region.

The two topmost principal economic activity of the caregivers were petit business (39.9%) and Famer (46.5%). Contrarily, for the principal economic activity of the head of Household (HH), 35.0% did business, 47.4% were unemployed and only 7.6% were farmers.

Principal correspondence analysis of the factors contributing to the socioeconomic status(the primary factors explaining 64.4% of the total variance) revealed that 42.6% of the households were classified in the "poorest" quintile, 26.3% in the "poor", 17.8% in the "average", 4.8% in

the Rich and 8.5% in the "Richest" quintile. Comparative analysis show that the households in the North are generally Richer compared to the households in the Far North Region. For instance, a significant higher number of households were classified as poorest in the Far North compared to the North ($p<0.05$) and a significant lower number of households were classified as richest in the Far North compared to the North region ($p<0.05$). Table 11 below presents the general characteristics of the study population.

Table 11: General Characteristics of the study population

Variable	*Far North Region*	*North Region*	*Total*
N	889	906	1795
SEX of Child			
Male	51.2%	55.1%	53.2%
Female	48.8%	44.9%	46.8%
Age Group			
12-23	29.3%	38.9%	34.1%
24-35	29.3%	25.7%	27.5%
36-47	27.4%	19.8%	23.6%
48-59	14.0%	15.6%	14.8%
Overall	49.5%	50.5%	100.0%
SEX of Head of Household			
Male	98.2%	94.0%	96.1%
Female	1.8%	6.0%	3.9%
Mean HH size			
Mean ± SEM	9.2 ± 0.4	7.5 ± 0.3	8.3 ± 0.2
SES Quintile			
Poorest	64.0%	21.6%	42.6%
Poor	23.2%	29.3%	26.3%
Average	9.1%	26.3%	17.8%
Rich	3.0%	6.6%	4.8%
Richest	0.6%	16.2%	8.5%
Primary House Hold Religion			
Catholic	9.1%	21.0%	15.1%
Protestant	15.9%	23.4%	19.6%
Muslim	59.8%	50.9%	55.3%
Others	15.2%	4.8%	10.0%
Marital Status of Caregiver			
Single	0.6%	5.4%	3.0%
Married	96.3%	82.0%	89.1%
Living with Partner	0.0%	7.2%	3.6%
Divorced/widowed	3.0%	5.4%	4.2%
Care giver Level of Education			
No schooling	72.0%	58.1%	65.0%
Primary	23.2%	25.1%	24.2%
Secondary	4.9%	15.6%	10.3%
Higher Education	0.0%	1.2%	0.6%
Head of House Hold Level of Education			
No schooling	54.3%	46.1%	50.2%
Primary	23.2%	25.1%	24.2%
Secondary	21.3%	24.0%	22.7%

Variable	*Far North Region*	*North Region*	*Total*
N	889	906	1795
Higher Education	1.2%	4.8%	3.0%
Marital Status of Head of House Hold			
single	0.6%	1.8%	1.2%
Married	98.2%	89.2%	93.7%
Living with Partner	0.0%	6.0%	3.0%
Divorced/widowed	1.2%	3.0%	2.1%
Type of toilet			
Modern toilet	33.5%	24.6%	29.0%
Pit Latrine (Improved with slab)	60.4%	65.9%	63.1%
Pit Latrine without slab/open pit	1.8%	8.4%	5.1%
Other	4.3%	1.2%	2.7%
Source of energy for cooking			
Wood/Charcoal	98.8%	83.8%	91.2%
kerosene/Gas	0.0%	15.6%	7.9%
Other	1.2%	0.6%	0.9%
Source of energy for Lighting			
Electricity	30.5%	43.1%	36.9%
Oil/Gas lantern	20.1%	29.3%	24.8%
Flashlight/tube light (Battery)	43.3%	13.2%	28.1%
no lights	1.8%	6.6%	4.2%
Other	4.3%	7.8%	6.0%
Source of drinking water			
Piped water inside/outside	11.0%	24.0%	17.5%
Tube well/Borehole	53.0%	14.4%	33.5%
Dug Well	32.3%	40.7%	36.6%
Spring	0.0%	4.8%	2.4%
Surface water	1.2%	8.4%	4.8%
Other	2.4%	7.8%	5.1%
Average distance from source of drinking Water			
Mean (minutes) ±SEM	165.2 ± 28.1	101.6 ± 22.1	133.0 ± 17.9
Principal Economic activity of Caregiver			
Business	44.5%	35.3%	39.9%
Unemployed	11.6%	12.0%	11.8%
Farmer	43.9%	49.1%	46.5%
Other	0.0%	3.6%	1.8%
Principal Economic activity of Head of Household			
Business	40.2%	29.9%	35.0%
Unemployed	45.1%	49.7%	47.4%
Farmer	4.9%	10.2%	7.6%
Other	9.8%	10.2%	10.0%
Employment status of Caregiver over the past year			
Worked	37.8%	33.5%	35.6%
Seasonal	37.8%	48.5%	43.2%
Occasional	23.8%	15.0%	19.3%
Other	0.6%	3.0%	1.8%

3.2. Food Consumption

3.2.1. Food Frequency consumption

The three main food groups consumed in this region are Cereals (91.2%), vegetables (51.7%), and meat (38.1%) Table 12. This high consumption of these three food groups can be attributed to the feeding habits of the North and Far North regions who have cereals as the base and Meat and vegetables as the complement.

Table 12: Frequency of households/individuals consuming each food group by region

	Region		
Food Group	***Far North***	***North***	***Total***
n	***154***	***155***	***309***
Cereals	93.9%	88.6%	91.2%
Condiments	0.0%	1.2%	0.6%
Eggs	0.0%	5.4%	2.7%
Fish	22.0%	22.2%	22.1%
Fruits	7.9%	21.6%	14.8%
Meat	40.9%	35.3%	38.1%
Milk	7.9%	21.6%	14.8%
Oil	40.9%	34.1%	37.5%
Pulses	29.3%	41.3%	35.3%
Sugar	25.6%	40.1%	32.9%
Tubers	14.0%	29.3%	21.8%
Vegetables	59.1%	55.1%	57.1%

As shown in table 13 below, the consumption of food groups seem not to vary significantly by socioeconomic status quintile. However apart from Pulses, Sugar, meat and Fish, the consumption other food groups tend to drop as SES Quintile moves from the poorest to the richest quintiles.

Table 13: Frequency of households/individuals consuming each food group by SES Quintile

	SES Quintile				
Food Group	***Poorest***	***Poor***	***Average***	***Rich***	***Richest***
n	***131***	***85***	***58***	***15***	***25***
Cereals	92.9%	95.4%	91.5%	81.3%	75.0%
Condiments	0.0%	1.1%	0.0%	6.3%	0.0%
Eggs	0.7%	0.0%	6.8%	0.0%	14.3%
Fish	19.9%	21.8%	16.9%	31.3%	39.3%
Fruits	8.5%	6.9%	23.7%	37.5%	39.3%
Meat	33.3%	46.0%	44.1%	31.3%	28.6%
Milk	8.5%	6.9%	23.7%	37.5%	39.3%
Oil	37.6%	39.1%	35.6%	43.8%	32.1%

Pulses	34.8%	37.9%	28.8%	50.0%	35.7%
Sugar	23.4%	39.1%	37.3%	31.3%	53.6%
Tubers	13.5%	21.8%	23.7%	31.3%	53.6%
Vegetables	58.9%	63.2%	67.8%	25.0%	25.0%

Looking at table 14 below, the consumption of food groups seem not to vary significantly by Age group. Nevertheless, cereals and vegetables are more consumed by other children while eggs are more consumed by younger children.

Table 14: Frequency of households/individuals consuming each food group by Age Group

	Age Group			
Food Group	***12-23***	***24-35***	***36-47***	***47-59***
n	***98***	***89***	***73***	***49***
Cereals	88.5%	91.2%	93.6%	93.9%
Condiments	0.9%	1.1%	0.0%	0.0%
Eggs	4.4%	0.0%	3.8%	2.0%
Fish	25.7%	17.6%	19.2%	26.5%
Fruits	14.2%	16.5%	11.5%	18.4%
Meat	25.7%	41.8%	50.0%	40.8%
Milk	14.2%	16.5%	11.5%	18.4%
Oil	32.7%	39.6%	35.9%	46.9%
Pulses	24.8%	36.3%	47.4%	38.8%
Sugar	29.2%	36.3%	32.1%	36.7%
Tubers	19.5%	24.2%	17.9%	28.6%
Vegetables	45.1%	65.9%	64.1%	57.1%

As described in table 15 below, the proportion of households consuming food sources of Vitamin A and Zinc is higher in the North region compared to the Far North region while the inverse is true for the proportion of households consuming food sources of Zinc.

Table 15: Frequency of house and individuals consuming food groups that are sources of vitamin A , Iron and Zinc by Region

	Region		
	Far North	***North***	***Total***
n	***154***	***155***	***309***
Food groups Sources of Vitamin A	82.9%	84.4%	83.7%
Food groups Sources of Iron	82.3%	81.4%	81.9%
Food groups Sources of Zinc	77.4%	82.6%	80.1%

The consumption of food sources of Vitamin A, Iron and Zinc decreases as SES quintile moves from Poorest to Richest. Due to the high intake of meat amongs the richest quintile, the proportion of households consuming food sources of iron spike between the rich and richest quintiles (Table 16 below)

Table 16: Frequency of house and individuals consuming food groups that are sources of vitamin A , Iron and Zinc by SES Quintile

	SES Quintile				
	Poorest	***Poor***	***Average***	***Rich***	***Richest***
n	***131***	***85***	***58***	***15***	***25***
Food groups Sources of Vitamin A	83.0%	86.2%	88.1%	68.8%	78.6%
Food groups Sources of Iron	82.3%	86.2%	86.4%	56.3%	71.4%
Food groups Sources of Zinc	80.1%	82.8%	83.1%	75.0%	67.9%

As shown in table 17 below, the consumption of food group sources of Vitamin A, Iron and Zinc decrease with increasing age group.

Table 17: Frequency of house and individuals consuming food groups that are sources of vitamin A , Iron and Zinc by Age group

	Age Group			
	12-23	***24-35***	***36-47***	***47-59***
n	***98***	***89***	***73***	***49***
Food groups Sources of Vitamin A	91.2%	91.2%	84.6%	85.7%
Food groups Sources of Iron	90.1%	90.1%	84.6%	83.7%
Food groups Sources of Zinc	87.9%	87.9%	87.2%	81.6%

3.2.2. Food Consumption Score (FCS) and Dietary Diversity Score (DDS)

Of the factors that affect child health outcomes, dietary diversity and nutrition have received attention in recent times. Dietary diversity is particularly important for infants and young children who need nutrient and energy-dense food for healthy growth as well as physical and mental development.

The observed high dietary diversity score from both North and Far North Regions is therefore an indication that the preschool children are meeting their micronutrient needs.

Results showed that event though up to 89.4% of the population have an Acceptable food consumption score only 59.8% had an acceptable dietary diversity. This is an indication that though the frequency of food is acceptable, the population is feeding on the same types of food. This is confirmed by findings that the three major classes of food consumed are Cereals, Meat and vegetables. The results of ***table 18*** below indicate that the participants from the North region present a relatively better food frequency and Diet diversity when compared to participants from the Far North Region.

Table 18: Regional Distribution of FCS and DDS

Region	***Far North***	***North***	***Total***
n	***154***	***155***	***309***
Food Consumption Score Class			
Poor food Consumption	3.0%	5.4%	4.2%
Borderline food Consumption	5.5%	7.2%	6.3%
Acceptable food consumption	91.5%	87.4%	89.4%

Mean Food Consumption Score ± SEM	9.5±0.6	10.2±0.5	9.9±0.4
Dietary Diversity Score Class			
Unacceptable dietary Diversity	48.8%	31.7%	40.2%
Acceptable dietary Diversity	51.2%	68.3%	59.8%
Mean Dietary Diversity Score ± SEM	3.8±0.1	4.4±0.1	4.0±0.1

A comparison of the evolution of Acceptable food consumption and Acceptable dietary diversity with SES Quintile showed a significant inverse relation (r=-0.643, P<0.01). This suggests that the poorest eat the same meals many more times compared to the Richest while the richest each very few but diverse meals (Table 19 below).

Table 19: Distribution of FCS and DDS by SES Quintile

SES Quintile	***Poorest***	***Poor***	***Average***	***Rich***	***Richest***
n	***131***	***85***	***58***	***15***	***25***
Food Consumption Score Class					
Poor food Consumption	4.3%	3.4%	1.7%	12.5%	7.1%
Borderline food Consumption	5.0%	5.7%	8.5%	12.5%	7.1%
Acceptable food consumption	90.8%	90.8%	89.8%	75.0%	85.7%
Mean Food Consumption Score ± SEM	8.8±0.1	10.1±0.7	10.6±0.8	11.9±1.9	11.8±1.2
Dietary Diversity Score Class					
Unacceptable dietary Diversity	52.5%	34.5%	30.5%	37.5%	17.9%
Acceptable dietary Diversity	47.5%	65.5%	69.5%	62.5%	82.1%
Mean Dietary Diversity Score ± SEM	3.7±0.1	4.1±0.2	4.3±0.2	4.9±0.5	4.9±0.3

With Respect to the age group of the children (table 20), the global tendency is that the food consumption and dietary diversity indicators of the children ameliorate with increasing age.

Table 20: Distribution of FCS and DDS by Age Group

Age Group	***12-23***	***24-35***	***36-47***	***47-59***
n	***98***	***89***	***73***	***49***
Food Consumption Score Class				
Poor food Consumption	3.5%	4.4%	6.4%	2.0%
Borderline food Consumption	1.8%	9.9%	6.4%	10.2%
Acceptable food consumption	94.7%	85.7%	87.2%	87.8%
Mean Food Consumption Score ± SEM	7.6±0.5	11.4±0.8	10.9±0.8	10.8±1.0
Dietary Diversity Score Class				
Unacceptable dietary Diversity	46.0%	37.4%	35.9%	38.8%
Acceptable dietary Diversity	54.0%	62.6%	64.1%	61.2%
Mean Dietary Diversity Score ± SEM	3.6±0.2	4.3±0.2	4.4±0.3	4.1±0.3

3.3. Nutritional status

3.3.1. Nutritional status as measured by stunting, wasting and underweight criteria

In this study, the mean age of the study population was 28.8 months with 42% of stunting observed. The mean HAZ was -1.66 indicating that stunting is a major issue but the mean WHZ

and WAZ are -0.32 confirming that stunting is more prevalent in the population compared to wasting and under weight.

There was no significantly difference in the prevalence of stunting, wasting and underweight with sex. Also the prevalence of the different indices of nutritional status decreased with age and increase with increasing SES quintile.

Tables 21 and 22 below shows the prevalence of acute malnutrition expressed as z-score for children 12 to 59 months. The prevalence of severe acute malnutrition is significantly (P <0.00001) higher in the North compared to the Far North. Boys seem more affected by this form of malnutrition than girls. Also it was noticed that the prevalence of Acute malnutrition amongst children 12-23 months was higher than the 24-59 months age group (p=0.00142)

Table 21: Distribution of Nutritional status by region

Region		*Stunting, % (95% CI)*	*Wasting, % (95% CI)*	*Underweight, % (95% CI)*
	N	1795	1795	1795
Far North	Normal	53.8 (46.3-61.9)	91.3 (86.9-95.0)	66.9 (58.8-74.4)
	Moderate	25.6 (18.8-32.5)	7.5 (3.8-11.9)	22.5 (15.6-29.4)
	Severe	20.6 (14.4-26.9)	1.3 (0.0-3.1)	10.6 (6.3-15.6)
North	Normal	62.3 (54.7-70.4)	95.6 (91.8-98.7)	81.1 (74.8-87.4)
	Moderate	23.3 (16.4-30.2)	3.8 (1.3-7.5)	17.6 (11.3-23.9)
	Severe	14.5 (9.4-20.1)	0.6 (0.0-1.9)	1.3 (0.0-3.1)
Total	Normal	58.0 (48.3-62.1)	93.4 (85.9 – 94.0)	74.0 (68.1-79.6)
	Moderate	24.5 (16.6-30.4)	5.6 (1.8-9.9)	20.1 (15.1-25.1)
	Severe	17.6(13.4-19.6)	0.9 (0.0-2.0)	6.0 (3.2-9.5)

Table 22: Distribution of Nutritional status by region

Variable	Far North Region	North Region	Total
n	889	906	1795
Age, months (Mean, 95% CI)	29.4 (27.7, 31.1)	28.2 (26.3, 30.1)	28.8 (27.5, 30.1)
HAZ (Mean, 95% CI)	-1.84 (-2.07, -1.59)	-1.49 (-1.73, -1.28)	-1.66 (-1.83, -1.50)
Stunted, HAZ<-2SD; % (CI)	46.3 (38.8, 53.8)	37.7 (30.2, 45.3)	42.0 (37.3 - 47.3)
WHZ (Mean, 95% CI)	-0.51 (-0.68, -0.35)	-0.12 (-0.29, 0.06)	-0.32 (-0.43, -0.19)
Wasted, WHZ<-2SD; % (CI)	8.8 (4.4, 13.1)	4.4 (1.9, 8.20	6.6 (4.1, 9.4)
WAZ (Mean, 95% CI)	-1.36 (-1.55, -1.15)	-0.91 (-1.10, -0.74)	-0.32 (-0.43, -0.20)
Underweight, WAZ<-2SD; % (CI)	33.1 (26.3, 41.3)	18.9 (12.6, 25.2)	26.0 (21.3, 31.0)

There was a significant differences in the variation of nutritional status with sex. As for stunting, the proportion of both moderate and severe stunting, wasting and underweight were higher for

males than females (Table 23). This can be associated to the socio-cultural norms of these regions were the male children tend to be regarded as more valuable compared to the female children.

Table 23: Distribution of Nutritional status by sex

Sex		*Stunting, % (95% CI)*	*Wasting, % (95% CI)*	*Underweight, % (95% CI)*
	N	1795	1795	1795
Female	Normal	62.4 (54.5-70.5)	92.6 (87.9-96.0)	77.9 (71.1 -83.9)
	Moderate	22.8 (16.8-29.5)	6.7 (3.4-11.4)	16.1 (10.7-22.1)
	Severe	14.8 (9.4-20.8)	0.7 (0.0 – 2.0)	6.0 (2.7-10.7)
Male	Normal	54.1 (45.9-61.2)	94.1 (90.6 -97.6)	70.6 (63.5-77.1)
	Moderate	25.9 (20.0-32.9)	4.7 (1.8-8.2)	23.5 (17.1-30.6)
	Severe	20.0 (14.1-26.5)	1.2 (0.0-2.9)	5.9 (2.4 - 9.4)

Age wise, stunting, wasting and underweight ten to degrease with age group. The highest proportion of severe stunting as in the 48-59 months age group, the highest proportion of severe underweight was in the 24-35 months age group. (Table 24).

Table 24: Distribution of Nutritional status by Age group

Age Group (months)		*Stunting, % (95% CI)*	*Wasting, % (95% CI)*	*Underweight, % (95% CI)*
	N	***1795***	***1795***	***1795***
12-23	Normal	66.0 (56.6-74.5)	87.7 (81.1-94.3)	69.8 (61.3-78.3)
	Moderate	19.8 (12.3-27.4)	9.4 (3.8-16.0)	23.6 (15.1-31.1)
	Severe	14.2 (8.5-21.7)	2.8 (0.0-6.6)	6.6 (1.9-11.3)
24-35	Normal	53.9 (42.7-64.0)	93.3 (87.6-97.8)	73.0 (62.9-82.0)
	Moderate	23.6 (15.7-32.6)	6.7 (2.2-12.4)	15.7 (9.0-23.6)
	Severe	22.5 (14.6-31.5)	-	11.2 (5.6-18.0)
36-47	Normal	46.1 (35.5-56.6)	97.4 (93.4-100.0)	72.4 (61.8-82.9)
	Moderate	36.8 (26.3-47.4)	2.6 (0.0-6.6)	25.0 (15.8-35.5)
	Severe	17.1 (9.2-26.3)	-	2.6 (0.0-6.6)
48-59	Normal	66.7 (52.1-79.2)	100.0	87.5 (77.1-95.8)
	Moderate	16.7 (6.3-29.2)	-	12.5 (4.2-22.9)
	Severe	16.7 (6.3-27.1)	-	-

The distribution of markers of nutritionnel status significantly decrease as SES quintile moves from the poorest quintile to the richest quintile (Table 25).

Table 25: Distribution of Nutritional status by SES quintile

SES Quintile		*Stunting, % (95% CI)*	*Wasting, % (95% CI)*	*Underweight, % (95% CI)*
	N	1795	1795	1795
Poorest	Normal	56.7 (47.8-64.9)	90.3 (85.1-94.8)	72.4 (64.2-79.9)
	Moderate	23.1 (16.4-30.6)	7.5 (3.7-11.9)	17.9 (11.2-24.6)

	Severe	20.1 (13.4-26.9)	2.2 (0.0-5.2)	9.7 (5.2-14.9)
Poor	Normal	49.4 (37.6-60.0)	94.1 (89.4-97.6)	69.4 (58.8-78.8)
	Moderate	29.4 (20.0-40.0)	5.9 (2.4-10.6)	24.7 (16.5-34.1)
	Severe	21.2 (12.9-30.6)	-	5.9 (1.2-11.8)
Average	Normal	57.9 (43.9-70.2)	94.7 (89.5-100)	71.9 (59.6-82.5)
	Moderate	28.1 (17.5-40.4)	5.3 (0.0-10.5)	26.3 (15.8-38.6)
	Severe	14.0 (5.3-22.8)	-	1.8 (0.0-5.3)
Rich	Normal	68.8 (43.8-87.5)	100.0	75.0 (50.0-93.8)
	Moderate	18.8 (0.0-37.5)	-	25.0 (6.3-50.0)
	Severe	12.5 (0.0-31.3)	-	-
Richest	Normal	85.2 (70.4-96.3)	100.0	100.0
	Moderate	11.1 (0.0-22.2)	-	-
	Severe	3.7 (0.0-11.1)	-	-

3.4. Markers of Inflamation

In the present study and in concordance with the above reports, two acute phase proteins were used as biomarkers for infection: C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) as previously described by Thurnham *et al.*, 2010. This method requires the categorization of AGP and CRP for the adjustment of the influence of infection on micronutrient deficiencies in to the following categories:

- Category 0: No infection, aapparently healthy reference group (CRP ≤5 mg/L and AGP ≤1 g/L)
- Category 1: incubation(CRP >5 mg/L and AGP ≤1 g/L)
- Category 2: presence of symptoms / early convalescence (CRP >5 mg/L and AGP >1g/L),
- Category 3: late convalescence(CRP ≤5 mg/L and AGP >1 g/L)

For the purpose of more detailed analysis, a 5th category was added:

- Category 4: both CRP and AGP elevated (CRP ≥5 mg/L and AGP ≥1 g/L)

3.4.1. Distribution of Infection category by region

According to table 26 below, the mean CRP and AGP are higher for the North Region compared to the Far North region and this is reflected in the proportion of children in convalescence state higher for the North (46.1%) than the Far North (33.8%).

Table 26: Distribution of Infection category by region

Variable	Far North Region	North Region	Total
n	160	154	314
CRP (mg/l) (Mean, 95% CI)	4.91 (3.98, 5.91)	6.41 (5.35, 7.52)	5.64 (4.95, 6.37)
AGP (g/l) (Mean, 95% CI)	0.94 (0.98, 0.97)	0.98 (0.94, 1.02)	0.96 (0.93,0.99)
CRP <5 mg/L and AGP <1 g/L, %	56.9 (48.8, 64.4)	46.8 (39.0, 54.5)	51.9 (46.8, 57.6)
CRP ≥5 mg/L and AGP <1 g/L, %	33.8 (26.3, 41.3)	41.6 (34.4, 49.4)	37.6 (32.2, 42.7)
CRP ≥5 mg/L and AGP ≥1 g/L, %	24.4 (17.5, 31.3)	34.4 (27.3, 42.2)	29.3 (24.2, 34.4)

CRP <5 mg/L and AGP ≥1 g/L, %	33.8 (26.3, 41.3)	46.1 (38.3, 53.9)	39.8 (34.4, 44.9)
CRP ≥5 mg/L and/ or AGP ≥1 g/L, %	43.1 (35.6, 51.2)	53.2 (45.5, 61.0)	48.1 (42.4, 53.2)

3.4.2. Distribution of Infection category by age group

The age distribution of infection category indicates that children less than 47 months old are more infected compared to the children between 48 and 59 months. Table 27 shows that the proportion of children in the late convalescence state is highest when the child is between 24 and 47 months.

Table 27: Distribution of Infection category by age group

Age group (months)	12-23	24-35	36-47	48-59
n	105	85	75	49
CRP (mg/l) (Mean, 95% CI)	4.9 (3.8, 6.2)	6.5 (4.9, 8.0)	5.9 (4.5, 7.4)	5.1 (3.4, 6.9)
AGP (g/l) (Mean, 95% CI)	0.95 (0.91, 1.01)	0.99 (0.94, 1.04)	0.96 (0.91, 1.01)	0.89 (0.84, 0.96)
CRP <5 mg/L and AGP <1 g/L, %	54.3 (44.8, 62.9)	51.8 (41.2, 62.4)	45.3 (34.7, 56.0)	57.1 (42.9, 71.4)
CRP ≥5 mg/L and AGP <1 g/L, %	32.4 (23.8, 41.9)	42.4 (31.8, 52.9)	41.3 (30.7, 52.0)	34.7 (20.4, 49.0)
CRP ≥5 mg/L and AGP ≥1 g/L, %	25.7 (17.2, 34.3)	35.3 (24.7, 44.7)	29.3 (20.0, 40,0)	26.5 (14.3,40.0)
CRP <5 mg/L and AGP ≥1 g/L, %	39.0 (29.5, 48.6)	41.2 (30.6, 51.8)	42.7 (32.0, 54.7)	34.7 (20.4, 49.0)
CRP ≥5 mg/L and/ or AGP ≥1 g/L, %	45.7 (37.1, 55.2)	48.2 (37.6, 58.8)	54.7 (44.0, 65.3)	42.9 (28.6, 57.1)

3.4.3. Distribution of Infection category by SES quintile

Mean CRP and AGP values decrease with SES quintile from Poorest to Richest (Table 28). This confirms the social affirmation that that the poor are more exposed to infection compared to the Rich. Another reason that can explain this drop is the small representation of the rich and richest groups in the study population. However, the differences observed were statistically significant suggesting that the negative role of the small sample size was mitigated by the sampling methodology.

Table 28: Distribution of Infection category by SES quintile

SES Quintile	Poorest	Poor	Average	Rich	Richest
N	131	85	58	15	25
CRP (mg/l) (Mean, 95% CI)	6.7(5.5, 7.9)	6.1 (4.7, 7.6)	3.7 (2.4, 4.9)	4.3 (1.4, 7,9)	3.6 (1.8, 5.6)
AGP (g/l) (Mean, 95% CI)	0.99 (0.94, 1.03)	0.99 (0.92, 1.04)	0.89 (0.84, 0.95)	0.86 (0.77, 0.95)	0.87 (0.79, 0.94)
CRP <5 mg/L and AGP <1 g/L, %	45.8 (37.4, 55.0)	49.4 (38.8, 60.0)	56.9 (44.8, 70.7)	66.7 (40.0, 86.7)	72.0 (52.0, 88.0)
CRP ≥5 mg/L and AGP <1 g/L, %	44.3 (35.9, 52.7)	40.0 (29.4, 50.6)	27.6 (15.5, 39.7)	26.7 (6.7, 46.7)	24.0 (8.0, 40.0)
CRP ≥5 mg/L and AGP ≥1 g/L, %	34.4 (26.0, 42.7)	32.9 (22.4, 42.4)	20.7 (10.3, 31.0)	20.0 (30.0, 40.0)	16.0 (4.0, 32.0)
CRP <5 mg/L and AGP ≥1 g/L, %	44.3 (35.9, 53.4)	43.5 (32.9, 52.9)	36.2 (24.1, 48.3)	26.7 (6.7, 46.7)	20.0 (4.0, 36.0)

CRP ≥5 mg/L and/ or AGP ≥1 g/L, %	54.2 (45.0, 62.6)	50.6 (40.0, 61.2)	43.1 (29.3, 55.2)	33.3 (13.3, 60.0)	28.0 (12.0, 48.0)

3.4.4. Distribution of Infection category by Stunting, Wasting and Underweight categories

There is a well-recognized association between risk of infection and nutrient status. Poor nutrition increases susceptibility to infection, and infection, in turn, has an adverse effect on nutritional status. Aging is associated with a functional decline in many physiological systems including the immune system, and it might be that aging and poor nutritional status exert cumulative influences on immune status and resistance to infection in older children.

Table 29 below showed that the expression of Acute Phase Protein increases with the severity of stunting but not wasting and underweight. The severely stunted have higher mean values than the mildly stunted and also higher prevalence of children in category 1 (CRP ≥5 mg/L and AGP <1 g/L, 45.5% vs 32.0%) and category 2 (CRP ≥5 mg/L and AGP ≥1 g/L, 36.4% vs 25.3%). It has been documented that stunting predisposes children to infection and vice versa. Thus this is an indication that the nutritional status of the children affect their health status.

Table 29: Stunting Category and infection

Stunting Category	Normal	Moderately stunted	Severely Stunted
N	175	75	55
CRP (mg/l) (Mean, ±SEM)	5.5 ±0.5	5.1±0.7	6.5±0.9
AGP (g/l) (Mean, ±SEM)	0.93±0.02	0.97±0.03	1.02±0.03
CRP <5 mg/L and AGP <1 g/L, %	56.0	49.3	47.3
CRP ≥5 mg/L and AGP <1 g/L, %	36.6	32.0	45.5
CRP ≥5 mg/L and AGP ≥1 g/L, %	27.4	25.3	36.4
CRP <5 mg/L and AGP ≥1 g/L, %	34.9	44.0	43.6
CRP ≥5 mg/L and/ or AGP ≥1 g/L, %	44.0	50.7	52.7

The severely underweight children have lower mean values than the mildly underweight children and also lower prevalence of children in category 1 (CRP ≥5 mg/L and AGP <1 g/L, 15.8% vs 40.3%) and category 2 (CRP ≥5 mg/L and AGP ≥1 g/L, 15.8% vs 39.9%). It has been documented that stunting predisposes children to infection and vice versa. Thus this is an indication that the nutritional status of the children affect their health status.

Underweight is not a disease, but rather a symptom, which has many possible causes. The causes of poor weight gain include the following:

- Not consuming an adequate amount of calories or not consuming the right combination of protein, fat, and carbohydrates
- Not absorbing an adequate amount of nutrients

- Requiring a higher than normal amount of calories

Underweight can occur as a result of a medical problem, a developmental or behavioral problem, lack of adequate food, a social problem at home, or most frequently, a combination of these problems. Common causes of underweight can be Prenatal (low birth weight), Neonatal (Poor quality of breast feeding), and underfeeding (sometimes associated with poverty or not understanding dietary needs of infants) amongst others. Consequently, underweight most often is not caused by infection explaining the low levels of infection biomarkers in children with underweight.

Table 30: Underweight Category and infection

Underweight Category	Normal	Moderately Underweight	Severely Underweight
N	224	62	19
CRP (mg/l) (Mean, ±SEM)	5.7±0.4	5.9±0.8	2.7±1.1
AGP (g/l) (Mean, ±SEM)	0.94±0.02	1.01±0.03	0.93±0.06
CRP <5 mg/L and AGP <1 g/L, %	53.1	45.2	73.7
CRP ≥5 mg/L and AGP <1 g/L, %	37.9	40.3	15.8
CRP ≥5 mg/L and AGP ≥1 g/L, %	28.1	33.9	15.8
CRP <5 mg/L and AGP ≥1 g/L, %	37.1	48.4	26.3
CRP ≥5 mg/L and/ or AGP ≥1 g/L, %	46.9	54.8	26.3

Severe wasting just like stunting have higher mean values than the moderate group and also higher prevalence of children in category 1 and 2 (33.3% vs 22.2%) – table 31. It has been documented that wasting just like stunting predisposes children to infection and other studies have shown that infection is the underlying cause of wasting.

Table 31: Wasting Category and infection

Wasting Category	Normal	Moderately Wasted	Severely Wasted
N	284	18	3
CRP (mg/l) (Mean, ±SEM)	5.7±0.4	3.7±1.4	5.6±3.6
AGP (g/l) (Mean, ±SEM)	0.96±0.01	0.89±0.06	1.11±0.22
CRP <5 mg/L and AGP <1 g/L, %	51.4	72.2	66.7
CRP ≥5 mg/L and AGP <1 g/L, %	38.0	22.2	33.3
CRP ≥5 mg/L and AGP ≥1 g/L, %	28.9	22.2	33.3
CRP <5 mg/L and AGP ≥1 g/L, %	39.4	27.8	33.3
CRP ≥5 mg/L and/ or AGP ≥1 g/L, %	48.6	27.8	33.3

3.4.5. Distribution of Infection category by Level of education of Caregivers and HH heads.

A strong association between infection category and level of education of both HH heads and Caregivers was noticed ($p<0.01$) - Table 32 & 33. The higher the level of education of the parents, the lower the prevalence of children in infection categories 2 and 3. This can be attributed to the fact that the more educated parents adopt better health practices that reduces the exposure of their children to infection.

Table 32: Distribution of Infection category by Level of education of Caregivers

Care giver Level of Education	No schooling	Primary	Secondary	Higher
N	202	79	32	1
CRP (mg/l) (Mean, ±SEM)	6.2±0.4	5.2±0.7	3.2±0.9	0.3±0.0
AGP (g/l) (Mean, ±SEM)	0.99±0.02	0.93±0.03	0.87±0.03	0.84±0.00
CRP <5 mg/L and AGP <1 g/L, %	47.5	55.7	68.8	100.0
CRP ≥5 mg/L and AGP <1 g/L, %	41.6	35.4	18.8	0.0
CRP ≥5 mg/L and AGP ≥1 g/L, %	33.7	26.6	9.4	0.0
CRP <5 mg/L and AGP ≥1 g/L, %	44.6	35.4	21.9	44.6
CRP ≥5 mg/L and/ or AGP ≥1 g/L, %	52.5	44.3	31.3	0.0

In agreement with the earlier findings of others (Doherty *et al*., 1993, Shelp *et al*., 1979), tables 32 and 33 showed that the plasma concentrations of C-reactive protein, α_1-acid glycoprotein, in the Malnourished children were markedly higher than the uninfected-malnourished and recovered values.

Table 33: Distribution of Infection category by Level of education of HH heads

Head of Household Level of Education	No schooling	Primary	Secondary	Higher
N	158	76	70	10
CRP (mg/l) (Mean, ±SEM)	6.2±0.5	6.2±0.8	4.3±0.7	1.8±0.8
AGP (g/l) (Mean, ±SEM)	1.00±0.02	0.97±0.02	0.88±0.03	0.86±0.05
CRP <5 mg/L and AGP <1 g/L, %	46.2	48.7	64.3	80.0
CRP ≥5 mg/L and AGP <1 g/L, %	43.0	40.8	24.3	20.0
CRP ≥5 mg/L and AGP ≥1 g/L, %	35.4	28.9	18.6	10.0
CRP <5 mg/L and AGP ≥1 g/L, %	46.2	39.5	30.0	46.2
CRP ≥5 mg/L and/ or AGP ≥1 g/L, %	53.8	51.3	35.7	20.0

3.5. Vitamin A status

For the evaluation of the Vitamin A status in this study, two biomarker were used: ***Plasma RBP and Plasma ROH***

3.5.1. ROH and RBP Plasma concentrations

Plasma concentrations of ROH and RBP are relatively stable across agegroup (Table 34), increases as SES quintile moves from poorest to richest (Table 35), while the plasma RBP concentrations are significantly higher in the Far North Region compared to the North Region (p<0.05) – Table 36 . This may be associated to the difference in food consumption patterns as presented in the food consumption section above (table 33).

Table 34: Mean ROH and RBP plasma concentration by age group

Age group (months)	12-23	24-35	36-47	47-59
N	105	85	75	49
Plasma RBP (µmol/l) (Mean±SEM)	0.86±0.02	0.82±0.03	0.78±0.02	0.83±0.03
N	13	7	8	7
Plasma ROH (µmol/l) (Mean±SEM)	0.64±0.06	0.78±0.18	0.60±0.05	0.70±0.09
ROH:RBP molar Ration (Mean±SEM)	0.79±0.02	0.84±0.05	0.78±0.03	0.83±0.02
Apo-RBP (µmol/l) (Mean±SEM)	0.15±0.01	0.14±0.05	0.16±0.01	0.14±0.02

Table 35: Mean ROH and RBP plasma concentration by SES Quintile

SES quintile	Poorest	Poor	Average	Rich	Richest
N	131	85	58	15	25
Plasma RBP (µmol/l) (Mean±SEM)	0.81±0.02	0.78±0.03	0.85±0.04	0.97±0.06	0.92±0.04
N	16	10	6	3	0
Plasma ROH (µmol/l) (Mean±SEM)	0.60±0.04	0.73±0.07	0.71±0.10	0.78±0.16	-
ROH:RBP molar Ration (Mean±SEM)	0.80±0.02	0.81±0.01	0.83±0.04	0.82±0.04	-
Apo-RBP (µmol/l) (Mean±SEM)	0.14±0.01	0.16±0.01	0.13±0.02	0.17±0.05	-

Table 36: Mean ROH and RBP plasma concentration by Region

Region	Far North	North	Total
N	160	154	314
Plasma RBP (µmol/l) (Mean±SEM)	0.86±0.02a	0.79±0.02 b	0.83±0.01 a
N	20	15	35
Plasma ROH (µmol/l) (Mean±SEM)	0.69±0.05 a	0.65±0.04 a	0.67±0.03 c

ROH:RBP molar Ration (Mean±SEM)	0.80±0.02	0.81±0.01	0.81±0.01
Apo-RBP (µmol/l) (Mean±SEM)	0.15±0.01	0.14±0.01	0.15±0.01

3.5.2. Effect of sampling process ROH and RBP Plasma concentrations

Spearman correlations between Plasma RBP and ROH and the total time of sample collection, the time of plasma separation and the freezing time were non-significant (Table 37). This is a demonstration of the fact that the process indicators did not influence the plasma concentrations of RBP and ROH

Table 37: Spearman correlations between Plasma RBP, ROH and processing factors

	Time of blood collection		Time from plasma separation to freezing		Total time of freezing	
	r	p-value	r	p-value	r	p-value
Plasma RBP (µmol/l) n=314	-0.011	0.842	0.051	0.381	0.123	0.063
Plasma ROH (µmol/l) n=35	0.039	0.822	0.170	0.336	-0.096	0.656

3.5.3. Effect of infection on Plasma ROH and Plasma RBP concentrations

As shown in table 38, a strong spearman correlation between RBP and ROH (r=0.974, p<0.0001). Also, both AGP and CRP are negatively correlated to RBP while only CRP is significantly correlated with ROH. This table demonstrate that in this study population, the presence of elevated acute phase proteins will potentially reduce the plasma concentrations of both RBP and ROH. The insignificant correlation between ROH and AGP indicates that the influence of combinations on AGP and CRP will influence these plasma proteins to different degrees.

Table 38: Spearman correlation between AGP, CRP, RBP and ROH

	Plasma RBP (µmol/l)		CRP (mg/l		AGP (g/l)	
	r	p-value	r	p-value	r	p-value
CRP (mg/l) n=314	-0.427	<0.0001	-	-	-	-
AGP (g/l) n=314	-0.380	<0.0001	0.695	<0.0001	-	-
Plasma ROH (µmol/l) n=35	0.974	<0.0001	-0.398	0.0179	-0.28	0.1003

3.5.4. Adjusting for Elevated Acute phase proteins.

The relationship between RBP and ROH in plasma has been examined by using either absolute molar ratio of the two indicators or regression analysis. Results seem to vary according to the

population studied. Although the ratio of ROH:RPB is close to but consistently less than 1:1, several factors may influence this ration such as VA status, the prevalence of elevated acute phase proteins, iron status, protein energy malnutrition and pregnancy. Also, RBP has recently gained attention as an adipokine (referred to as RBP4) associated with obesity and indices of insulin resistance. These factors may confound VA status assessment using RBP. The acute phase response to infection can also temporarily lower plasma RBP and ROH values, confounding VA status assessment.

Mathematical adjustment for elevated CRP and/or AGP was done using the method published by (Thurman *et al*., 2011) as follows:

1. The observations were divided into four categories reflecting the stage of infection (Cat 0, 1, 2, 3) as presented in the Material and methods Section,
2. Observations in each of the three infection groups (1,2 and 3) were multiplied by the ratio of the geometric mean of the healthy group to that of the infection group to raise individual concentrations of RBP or ROH by an amount specific to that group.

As seen in table 39 below, the adjusted RBP and ROH levels are higher than the unadjusted values. This confirms the inverse correlation noted above. The molar ROH:RBP ratio does not significantly change with increasing Age, region or SES quintile (Tables 40 and 41).

Table 39: Adjusted Mean ROH and RBP plasma concentration by age group

Age group (months)	12-23	24-35	36-47	47-59	Total
n	105	85	75	49	314
Adjusted Plasma RBP (µmol/l) (Mean±SEM)	0.93±0.02	0.90±0.03	0.86±0.03	0.91±0.04	0.90±0.01
n	13	7	8	7	35
Adjusted Plasma ROH (µmol/l) (Mean±SEM)	0.74±0.07	0.84±0.07	0.67±0.05	0.79±0.10	0.75±0.04
Adjusted ROH:RBP molar Ration (Mean±SEM)	0.80±0.02	0.84±0.02	0.78±0.03	0.83±0.03	0.81±0.01
Adjusted Apo-RBP (µmol/l) (Mean±SEM)	0.17±0.02	0.16±0.02	0.19±0.02	0.15±0.02	0.17±0.01

Table 40: Adjusted Mean ROH and RBP plasma concentration by region

Region	Far North	North	Total
n	160	154	314
Adjusted Plasma RBP (µmol/l) (Mean±SEM)	0.93±0.02	0.87±0.02	0.90±0.01
n	20	15	35
Adjusted Plasma ROH (µmol/l) (Mean±SEM)	0.74±0.05	0.77±0.06	0.75±0.04
Adjusted ROH:RBP molar Ration (Mean±SEM)	0.81±0.02	0.81±0.02	0.81±0.01
Adjusted Apo-RBP (µmol/l) (Mean±SEM)	0.17±0.01	0.17±0.01	0.17±0.01

Table 41: Adjusted Mean ROH and RBP plasma concentration by SES Quintile

SES quintile	Poorest	Poor	Average	Rich	Richest

n	131	85	58	15	25
Adjusted Plasma RBP (µmol/l) (Mean±SEM)	0.90±0.02	0.86±0.03	0.91±0.04	1.02±0.05	0.96±0.01
n	16	10	6	3	0
Adjusted Plasma ROH (µmol/l) (Mean±SEM)	0.69±0.05	0.81±0.08	0.77±0.09	0.91±0.10	-
Adjusted ROH:RBP molar Ration (Mean±SEM)	0.80±0.02	0.81±0.02	0.82±0.04	0.85±0.03	-
Adjusted Apo-RBP (µmol/l) (Mean±SEM)	0.16±0.01	0.18±0.01	0.16±0.03	0.17±0.04	-

3.5.5. Vitamin A Deficiency (VAD)

For the evaluation of the proportion of Vitamin A Deficiency in this study, two references were used:

- Plasma RBP <0.83 µmol/L, %
- Plasma ROH <0.70 µmol/L, %

Vitamin A deficiency by Region

Un-adjusted prevalence of VAD with respect to WHO standards (ROH <0.70 µmol/L) is 54.3% and after adjustment, this proportion is 37.1%. On the other hand, the overall proportion of VAD as defined by unadjusted plasma RBP<0.83 µmol/L is 32.8%. After adjusting for acute phase proteins, this proportion is 40.8%. Though the unadjusted proportions for ROH and RBP were significantly different ($p<0.001$), there was no statistical difference between the adjusted values ($p=0.1400$) when the two indicators are compared (table 42).

Table 42: Vitamin A deficiency by Region

Region	Far North	North	Total
N	160	154	314
Plasma RBP <0.83 µmol/L, %	27.5%	38.3%	32.8%
Adjusted Plasma RBP <0.83 µmol/L, %	35.6%	46.1%	40.8%
N	20	15	35
Plasma ROH <0.70 µmol/L, %	50.0%	60.0%	54.3%
Adjusted Plasma ROH <0.70 µmol/L, %	40.0%	33.3%	37.1%

Age wise, the proportion of VAD was highest in the 36-47 age groups for all indicators but for Adjusted Plasma RBP <0.83 µmol/L (table 43). also, VAD significantly relatively decreases with increasing SES quintile (table 44).

Table 43: Vitamin A deficiency by Age group

Age group (months)	12-23	24-35	36-47	47-59	Total
N	105	85	75	49	314
Plasma RBP <0.83 µmol/L, %	25.7%	35.3%	40.0%	32.7%	32.8%
Adjusted Plasma RBP <0.83 µmol/L, %	32.4%	44.7%	42.7%	49.0%	40.8%
N	13	7	8	7	35
Plasma ROH <0.70 µmol/L, %	61.5%	28.6%	62.5%	57.1%	54.3%
Adjusted Plasma ROH <0.70 µmol/L, %	46.2%	14.3%	50.0%	28.6%	37.1%

Table 44: Vitamin A deficiency by Age SES quintile

SES quintile	Poorest	Poor	Average	Rich	Richest	Total
N	131	85	58	15	25	314
Plasma RBP <0.83 µmol/L, %	32.1%	40.0%	34.5%	13.3%	20.0%	32.8%
Adjusted Plasma RBP <0.83 µmol/L, %	41.2%	45.9%	44.8%	20.0%	24.0%	40.8%
N	16	10	6	3	0	35
Plasma ROH <0.70 µmol/L, %	68.8%	30.0%	50.0%	66.7%	0.0%	54.3%
Adjusted Plasma ROH <0.70 µmol/L, %	50.0%	30.0%	33.3%	0.0%	0.0%	37.1%

Comparison of the ROH and RBP cut offs

Comparing the WHO cutoffs (Plasma ROH <0.70 µmol/L) and the newly published cut off by Engle-stone *et al.*, 2014 (Plasma RBP <0.83 µmol/L), it was noticed that these two cutoffs where significantly correlated for the total population (r=0.878, p<0.0001) and for all age groups, there was a significant positive correlation between the adjusted proportions (Table 45). This supports the fact that the Engle-stone *et al.*, cut off for RBP was a good estimate for the Cameroonian population.

Table 45: Spearman correlation between RBP and ROH per age group

Age group	r	p-value
12-23	0.986	<0.0001
24-35	0.950	<0.0001
36-47	0.866	0.005
47-59	0.977	<0.0001
Overall	0.878	<0.0001

3.6. Iron deficiency (ID) and Anemia

For this study, the following biomarkers of Iron status and Anemia were considered:

- Hemoglobin
- Plasma sTFR
- Plasma ferritin
- Body Iron Stores

Body iron store was calculated as describe by Cook *et al.*, 2003

$$Body\ iron(mg/kg) = \frac{-[\log(R/F\ ratio) - 2.8229]}{0.1207}$$

3.6.1. Plasma concentrations of biomarkers of Iron and anemia

Results showed that the mean global hemoglobin, sTfR, Ferritin and Body Iron Stores levels are respectively 99.9g/l, 15.6mg/l, 43.1 µg/l and 1.02 mg/kg (Table 46). This is an indication that a good proportion of the children may be anemic. These are significantly higher for the North Region compared to the Far North region (p<0.05).

Table 46: Mean Hemoglobin, sTfR, Ferritin and BIS by Region

Region	*Far North*	*North*	*Total*
N	154	155	309
Hemoglobin (g/l) (Mean±SEM)	98.77±1.34	101.12±1.59	99.95±1.04
N	160	154	314
Plasma sTfR (mg/l) (Mean±SEM)	14.54±0.63	16.67±1.09	15.59±0.63
Plasma ferritin (µg/l) (Mean±SEM)	35.38±3.03	51.05±4.12	43.07±2.58
Body Iron Stores (mg/kg) (Mean±SEM)	0.52±0.32	1.54±0.37	1.02±0.25

It has been documented that hemoglobin levels increase with age. Thus the relative increase in the mean hemoglobin levels with age as observed in table 47 is in concordance with previous findings. As for the markers of iron status, it was observed that Plasma ferritin and Body iron stores significantly increased with age while plasma sTfR significantly reduces with increasing age. This is an interesting finding because it further documents the fact that sTfR plasma concerntrations begin to increase with the depletion of body iron stores. The higher the concentration of body iron stores, the lowest the concentration of sTfR (Table 47). A relative insignificant increase was observed when the mean concentrations of hemoglobin was compared to SES Quintile (Table 48).

Table 47: Mean Hemoglobin, sTfR, Ferritin and BIS by Agegroup

Age group (months)	*12-23*	*24-35*	*36-47*	*47-59*
N	98	89	73	49
Hemoglobin (g/l) (Mean±SEM)	96.73±2.11	100.04±2.10	99.45±1.79	106.94±1.61
N	105	85	75	49
Plasma sTFR (mg/l) (Mean±SEM)	17.65±1.26	16.02±1.33	14.53±1.02	12.03±0.83
Plasma ferritin (µg/l) (Mean±SEM)	35.27±3.93	44.51±6.02	48.78±5.13	48.54±5.40
Body Iron Stores (mg/kg) (Mean±SEM)	-0.08 ±0.40	0.86±0.47	1.67±0.53	2.66±0.57

Table 48: Mean Hemoglobin, sTfR, Ferritin and BIS by SES Quintile

SES quintile	*Poorest*	*Poor*	*Average*	*Rich*	*Richest*
N	131	83	57	14	24
Hemoglobin (g/l) (Mean±SEM)	95.01±1.45	98.11±2.00	103.00±2.16	110.64±3.18	119.79±3.98
N	131	85	58	15	25
Plasma sTFR (mg/l) (Mean±SEM)	16.98±1.12	16.12±1.05	15.28±1.46	9.69±0.82	10.73±1.03
Plasma ferritin (µg/l) (Mean±SEM)	43.87±3.81	49.94±5.77	35.07±5.86	35.12±5.42	38.86±8.17
Body Iron Stores (mg/kg) (Mean±SEM)	0.93±0.37	1.29±0.47	-0.10±0.69	2.69±0.71	2.19±0.73

3.6.2. Effect of infection on Biomarkers of Iron status and Anemia

As described above, the plasma concentration of biomarkers of iron status are expressed differently depending on the infection status of the subjects. In order to evaluate the effect of infection of biomarkers of iron status, spearman correlation between acute phase proteins and Iron Markers was done.

Results (Table 49) showed strong positive correlation between APPs and sTfR, Ferriting and Body Iron Stores and a negative correlation between APPs and hemoglobin. Apart from hemoglobin that drops with increase in APPs, high levels of inflammation characterized by high AGP and CRP values will potentially increase the plasma concentrations of sTfR, Ferritin and BIS.

Table 49: spearman correlation between acute phase proteins and Iron Markers

	CRP (mg/l		AGP (g/l)	
	r	p-value	r	p-value
Hemoglobin (g/l) n=300	-0.329	<0.0001	-0.401	<0.0001
Plasma sTfR (mg/l) n=314	0.180	0.001	0.276	<0.0001
Plasma ferritin (µg/l) n=314	0.546	<0.0001	0.492	<0.0001
Body Iron Stores (mg/kg) n=314	0.384	<0.0001	0.258	<0.0001

In order to mitigate the confounding effects of APPs, Adjustment of the sTFR and Ferritin levels are essential for the accurate assessment of the body iron levels. Plasma concentrations of ferritin were adjusted for the presence of inflammation using 2 methods: one published by published by Thurnham *et al*., 2010 and another method by using APPs as continuous variables published by Engle-Stone *et al*., 2013

Thurnham *et al.*, 2010 adjustment for inflammation (T)

This method involves first stratifying individuals into 4 categories based on elevated CRP and/or AGP:

- Category 0:apparently healthy reference group (CRP ≤5 mg/L and AGP ≤1 g/L)
- Category 1: incubation(CRP >5 mg/L and AGP ≤1 g/L)
- Category 2: early convalescence (CRP >5 mg/L and AGP >1g/L),
- Category 3: late convalescence(CRP ≤5 mg/L and AGP >1 g/L)

Individual values were then adjusted by multiplying by the ratio of the median of the apparently healthy group to the median of that individual's inflammation group. Because plasma ferritin is falsely elevated during infection, plasma ferritin concentrations of individuals with elevated CRP and/or AGP were adjusted downward using a similar procedure.

Adjustment for inflammation using acute phase proteins as continuous variables (C)

This second method published by Engle-Stone *et al*.,2013 examined whether adjusting the results using CRP and AGP as continuous variables, rather than categorical variables, would improve the adjustment for inflammation. In this method, individuals were divided into the same categories as for the Thurnham *et al*., 2010 method based on elevated CRP, AGP, neither, or both. Three separate linear regression equations were developed with ferritin as the dependent variable (after transformation to achieve a normal distribution); the independent variables in the 3 equations were CRP only, AGP only, or both CRP and AGP. The linearity of the relationships between CRP and AGP and each iron indicator was confirmed using visual examination of scatter plots and evaluation of higher order terms and interactions in the linear regression models. Using the regression coefficients from these models, individual ferritin values were adjusted based on whether that person's CRP and/or AGP values were elevated. Thus, for individuals with only CRP elevated, coefficients from the model containing CRP only were used. If only AGP was elevated, coefficients for the model containing AGP only were assessed. If both CRP and AGP were elevated, the coefficients from the equation containing both proteins were used. Ferritin values for individuals without elevated CRP and/or AGP were not adjusted. The reference CRP and AGP concentrations used for the adjustment were CRP = 3.75 mg/L and AGP = 0.75 g/L. As an example, the formula for adjustment of results for an individual with both CRP ≥5 mg/L and AGP ≥1 g/L would be:

$$Fer_Adj = Fer_Unadj - (CRP\ Reg\ Coef)x(CRP - 3.75) - (AGP\ Reg\ Coef)x(AGP - 0.75)$$

Where:

$Fer_Adj = Adjusted\ Ferritin$

$Fer_Unadj = Unadjusted\ Ferritin$

$CRP\ Reg\ Coef = Regression\ coefficient\ when\ CRP\ alone\ is\ continous\ variable$

$AGP\ Reg\ Coef = Regression\ coefficient\ when\ AGP\ alone\ is\ continous\ variable$

Although sTfR concentrations were significantly correlated with CRP and AGP, adjustment of sTfR concentrations for the presence of elevated CRP and/or AGP was not done, because acute-phase proteins are nonspecific indicators of conditions, such as malaria, that could cause elevated sTfR concentrations in the absence of tissue Iron deficiency (ID). Moreover, despite the significance of the relationship between the acute-phase proteins and sTfR, the magnitude of the effect of inflammation on sTfR was small (Table 49) and the presence of inflammation had minimal impact on the estimated prevalence of ID using this indicator.

3.6.3. Adjusted Plasma concentrations of biomarkers of Iron and anemia

The mean adjusted global Ferritin and Body Iron Stores levels are respectively 26.4µg/l and -2.11mg/kg (Table 50). The mean plasma concentrations of adjusted markers are significantly higher for the North region compared to the far North region ($p<0.05$)

Table 50: Adjusted mean Hemoglobin, sTfR, Ferritin and BIS by Region

Region	*Far North*	*North*	*Total*
N	*160*	*154*	*314*
Adjusted Plasma Ferritin (C), (µg/l) (Mean±SEM)	23.05±1.59	29.79±2.10	26.36±1.32
Adjusted Plasma Ferritin (T), (µg/l) (Mean±SEM)	23.17±1.60	29.92±2.11	26.48±1.33
Adjusted Body Iron (C), (mg/kg) (Mean±SEM)	-2.12±0.32	-2.09±0.37	-2.11±0.24
Adjusted Body Iron (T), (mg/kg) (Mean±SEM)	-2.29±0.30	-2.26±0.33	-2.28±0.24

C, Adjustment for inflammation using acute phase proteins as continuous variables
T, Thurnham adjustment for inflammation

A key finding is the increase of iron markers with increasing SES quintile (Table 51). It is probable that iron deficiency will most affect the poor in this study population.

Table 51: Adjusted mean Hemoglobin, sTfR, Ferritin and BIS by SES Quintile

SES quintile	*Poorest*	*Poor*	*Average*	*Rich*	*Richest*
N	*131*	*85*	*58*	*15*	*25*
Adjusted Plasma ferritine (C), (µg/l) (Mean±SEM)	25.79±1.87	27.85±2.41	21.77±2.74	27.69±4.08	34.06±8.19
Adjusted Plasma ferritine (T), (µg/l) (Mean±SEM)	25.94±1.88	27.98±2.41	21.86±2.75	27.80±4.10	34.15±8.19
Adjusted Body Iron (C), (mg/kg) (Mean±SEM)	-2.65±0.35	-2.28±0.44	-2.44±0.60	0.54±1.09	0.48±0.97
Adjusted Body Iron (T), (mg/kg) (Mean±SEM)	-2.82±0.33	-2.45±0.45	-2.61±0.61	0.37±1.09	0.31±0.97

(C), Adjustment for inflammation using acute phase proteins as continuous variables, (T), Thurnham adjustment for inflammation

According to table 52, all irons marker increase with age group. This may be an indication of the strong probability of age influencing the iron status.

Table 52: Adjusted mean Hemoglobin, sTfR, Ferritin and BIS by Age group

Age group (months)	*12-23*	*24-35*	*36-47*	*47-59*
N	*105*	*85*	*75*	*49*
Adjusted Plasma ferritin (C), (µg/l) (Mean±SEM)	22.13±1.88	24.57±2.49	28.90±2.64	34.60±4.37
Adjusted Plasma ferritin (T), (µg/l) (Mean±SEM)	22.22±1.89	24.68±2.50	29.10±2.66	34.71±4.38
Adjusted Body Iron (C), (mg/kg) (Mean±SEM)	-2.65±0.38	-2.62±4.15	-1.96±0.47	-0.28±0.72
Adjusted Body Iron (T), (mg/kg) (Mean±SEM)	-2.82±0.39	-2.79±4.16	-2.13±0.45	-0.45±0.73

3.6.4. Anemia and Iron deficiency (ID) and Iron Deficiency Anemia (IDA)

The cutoffs used for Anemia and iron deficiency used in this study were as follows (WHO recommendation (WHO 2011);:

- Hemoglobin <110 g/L for children - Anemia
- Ferritin, <12 mg/L for children without infection;

- Ferritin, <30 mg/L for children with infection
- Body Iron, <0 mg/kg for children;
- sTfR, >8.3 mg/L for children;
- Iron deficiency anemia was calculated as the prevalence of IDA divided by the prevalence of all anemia

Prevalence of ID, IDA and the proportion of anemia associated with ID by iron indicator

Table 53 below presents the prevalence of Iron Deficiency (ID), Iron Deficiency Anemia (IDA) and the proportion of anemia associated with ID by iron indicator and adjustment for inflammation

The prevalence of ID and IDA and the proportion of anemia associated with ID varied widely by indicator and adjustment for inflammation. The prevalence of ID was lowest according to unadjusted ferritin (22.7%) whereas the prevalence of ID was highest when defined by elevated sTfR (77.3%). Adjustment of ferritin and BIS for inflammation increased the measured prevalence of ID, regardless of the method of adjustment. The proportion of anemia associated with ID ranged from 30.3 to 88% in the target population, depending on the indicator applied. The prevalence of ID was positively related to the proportion of anemia that was associated with ID.

Table 53: Prevalence of ID, IDA and the proportion of anemia associated with ID by iron indicator

Anemia , Hemoglobin <110g/L, %(95% CI)	63.7 (58.3-68.9)				
	ID		IDA		Anemia associated with ID
	%	CI	%	CI	%
Unadjusted ferritin <12 µg/L	22.70	18.1-27.2	19.30	15.1-23.9	30.30
Unadjusted ferritin <30 µg/L	51.70	46.2-56.8	32.60	27.8-37.5	51.18
Adjusted Plasma ferritin (C) <12 µg/L	29.60	24.5-34.7	24.50	19.9-29.0	38.46
Adjusted Plasma ferritin (T) <12 µg/L	29.30	24.8-34.1	24.20	19.3-28.7	37.99
Unadjusted Body Iron < mg/kg	33.80	28.4-39.3	26.90	22.7-31.7	42.23
Adjusted Body Iron (C) < 0 mg/kg	61.00	55.6-66.2	48.90	43.5-54.1	76.77
Adjusted Body Iron (T) < 0mg/kg	63.10	57.7-68.0	49.50	44.4-54.7	77.71
sTfR>8.30 mg/L	77.30	72.5-81.9	56.20	51.1-61.6	88.23

Values are percent (95% CI) or percent, N=331

3.6.5. Comparison of ID among potential risk groups

The physiology of iron balance has been implicit in the selection of risk factors or the discussion of results in previous studies. Using current understanding of the physiology of infant iron status to propose a physiologic model that postulates that iron status in infancy should be determined in large part by 4 factors: the iron the infant is born with (which is related to maternal iron

status), the infant's postnatal needs for iron, the external sources of bioavailable iron, and iron losses. As shown in Figures 10, 11 and 12, ten risk factors were identified to significantly influence Iron deficiency to different degrees. These include: Age, Vitamin A status, Educational level of Caregiver, socioeconomic status, Milieu, nutritional status (stunting, wasting an underweight) region of origin and infection status.

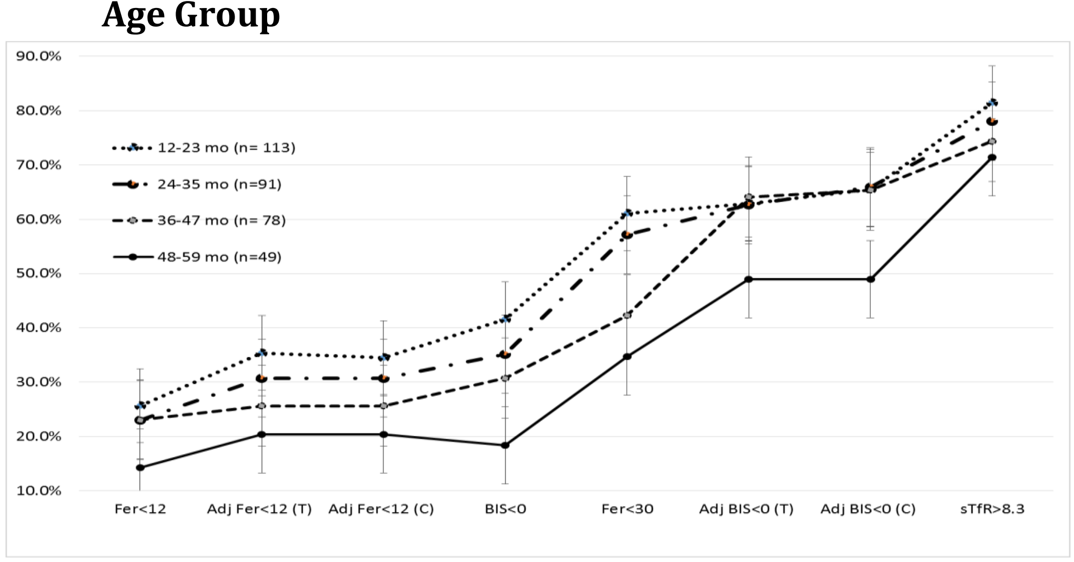

Figure 7: Prevalence of ID by age group among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals

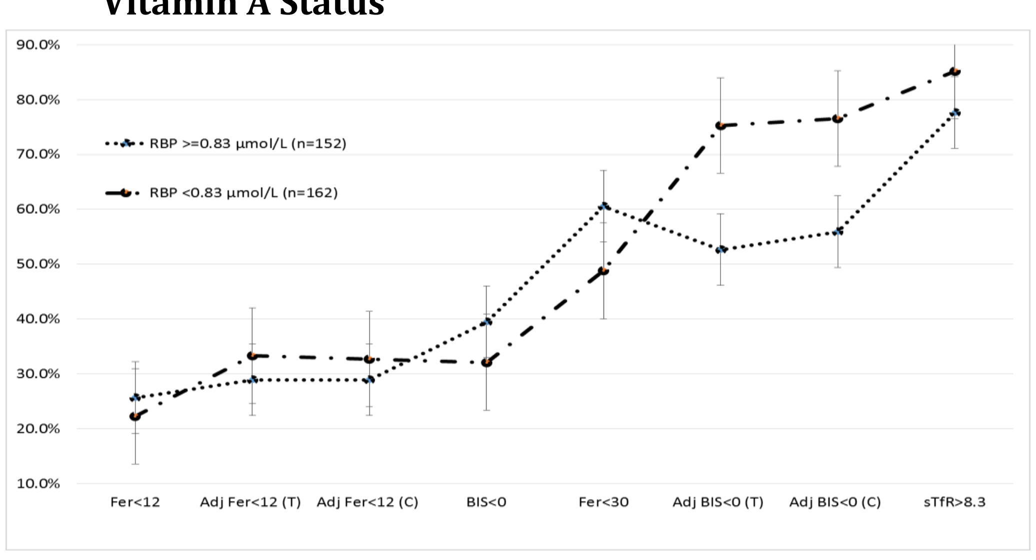

Figure 8: Prevalence of ID by Vit A status Category among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals.

Caregiver educational Level

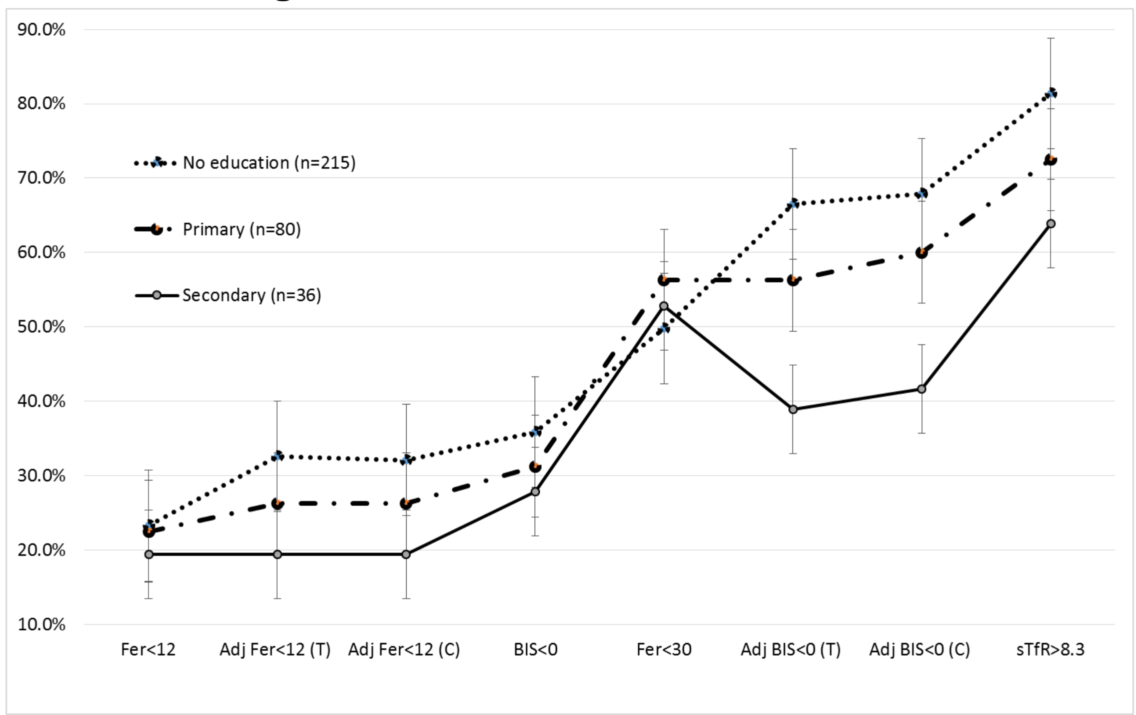

Figure 9: Prevalence of ID by caregiver educational level among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals.

Socio Economic Status Quintile

As documented in figures 13-19, the prevalence of iron deficiency varied by SES (p=0.03), with the lowest anemia prevalence in the highest SES quintile. Prevalence of iron deficiency was 17.9% in the highest quintile compared to 29.1% in the remaining four SES quintiles (estimated prevalence decreased significantly with increasing SES quintile).

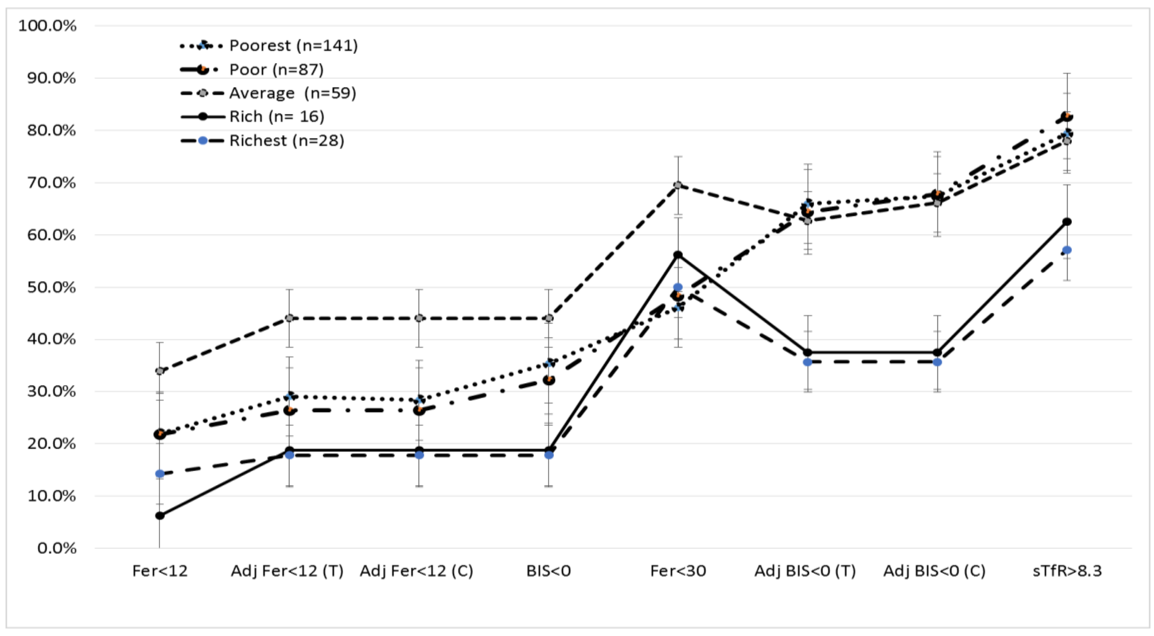

Figure 10: Prevalence of ID by Socio-economic status quintile among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals

Milieu (Rural & Urban)

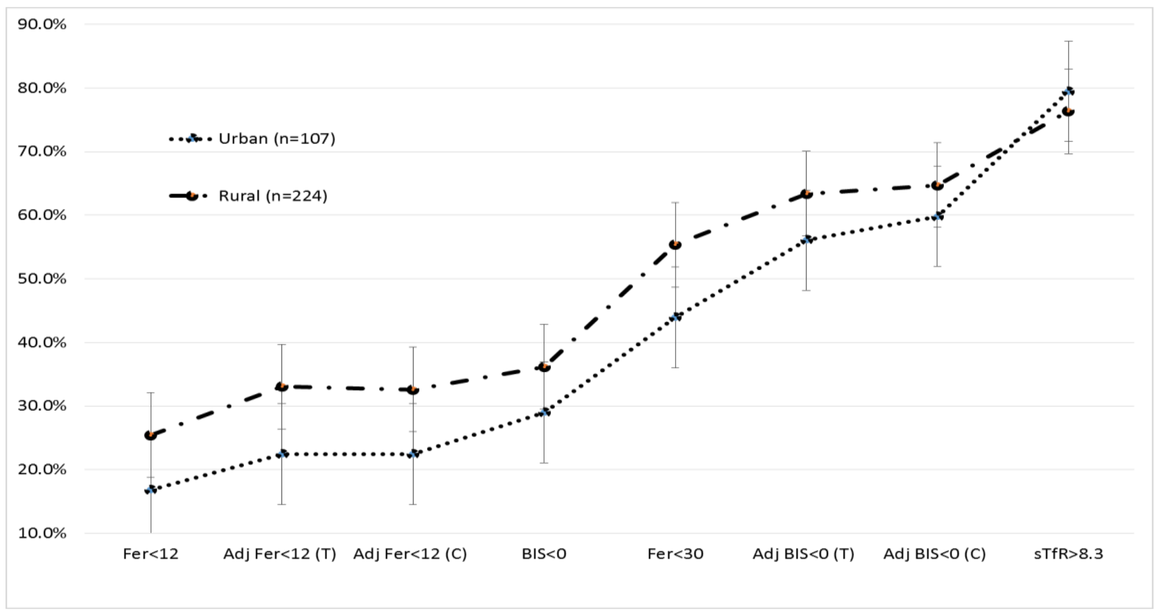

Figure 11: Prevalence of ID by Urban and rural milieu among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals.

Child Stunting

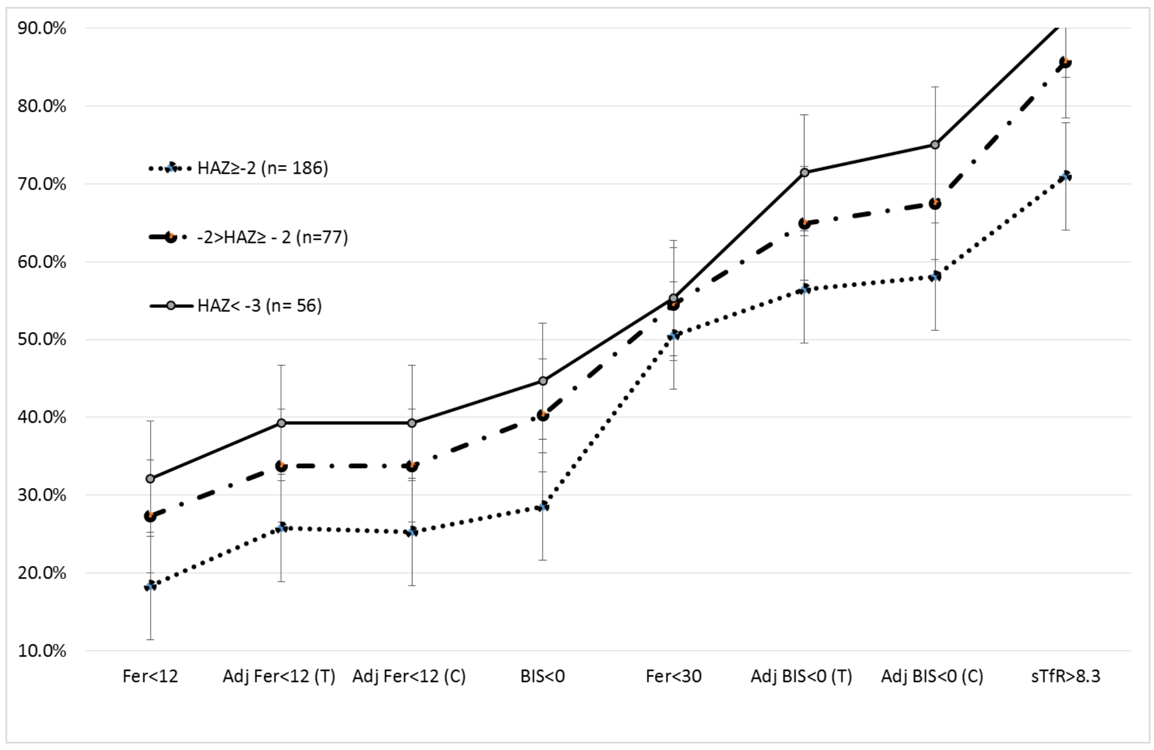

Figure 12: Prevalence of ID by child stunting among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals.

Child Wasting

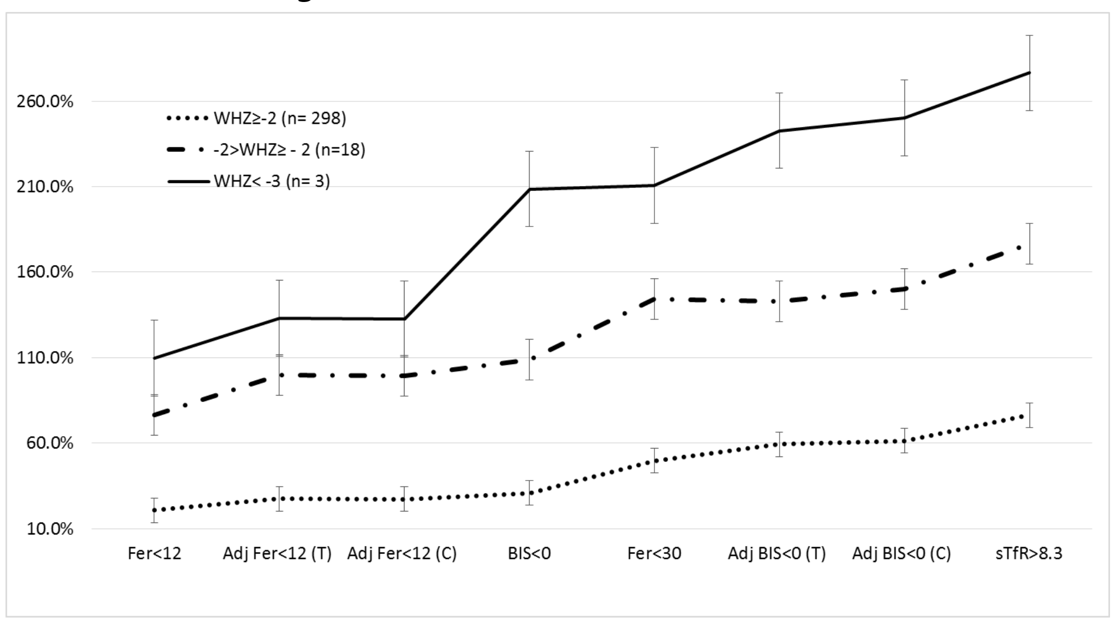

Figure 13: Prevalence of ID by child wasting among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals

Child Underweight

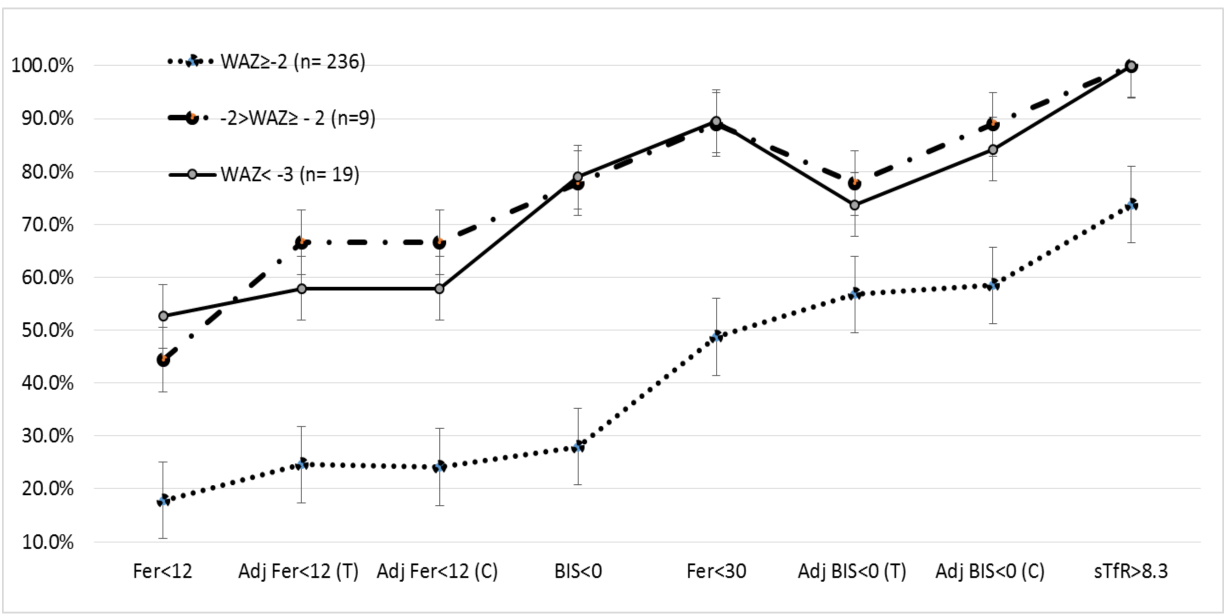

Figure 14: Prevalence of ID by child Underweight among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals.

Region

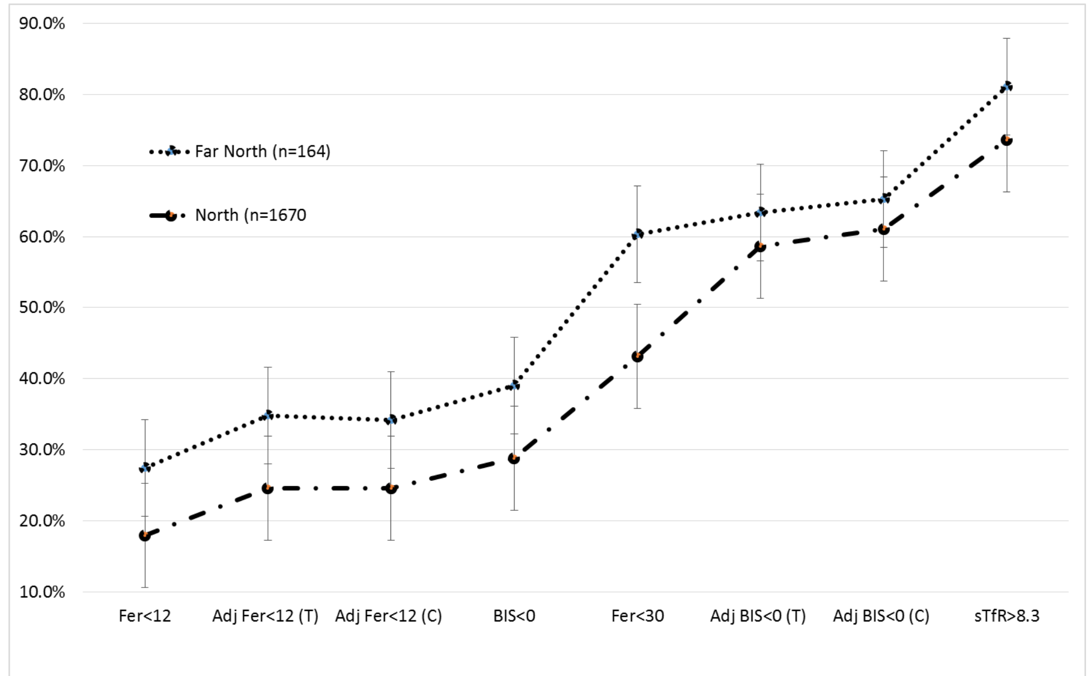

Figure 15: Prevalence of ID by study region among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals

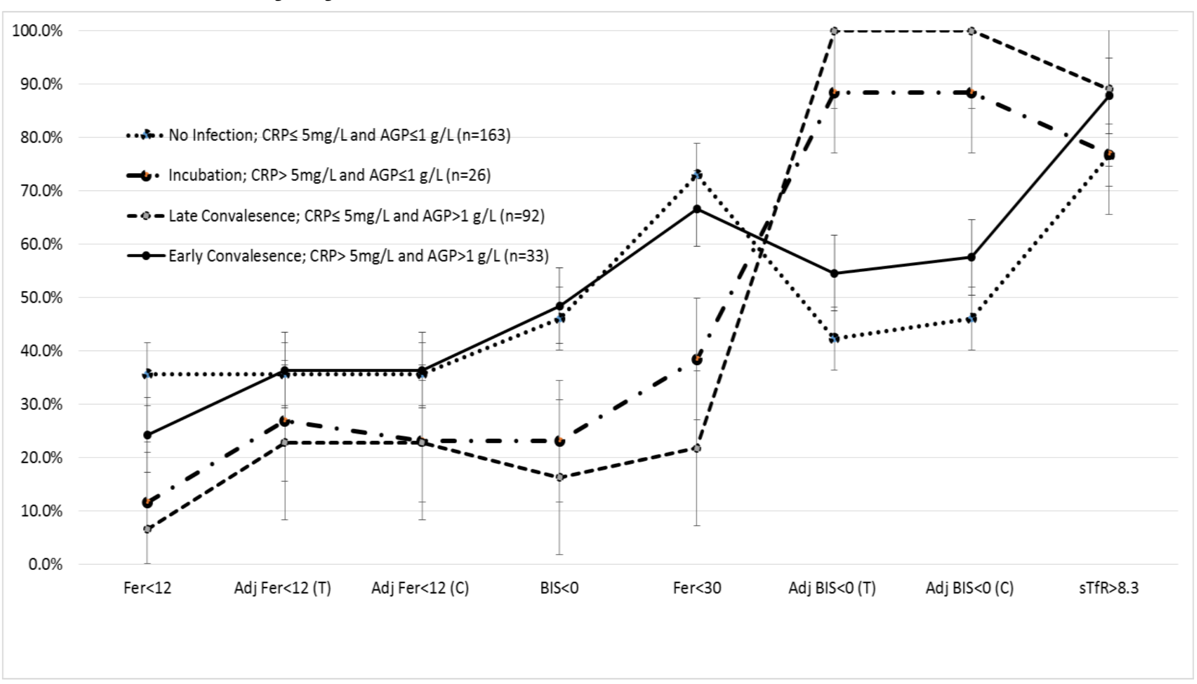

Figure 16: Prevalence of ID by presence of inflammation among children according to various iron status indicators and adjustments for inflammation. Error bars indicate 95% confidence intervals.

3.7. ZINC Status

3.7.1. Plasma Zinc Concentration

Effect of sampling process on PZC

The effects of sampling process and markers on infection on the plasma Zinc concentration and sampling factors: time of sample collection, time from blood collection to centrifugation and time from previous meal were assessed (Table 54).

Table 54: Effect infection and sampling process on plasma Zinc Concentration

	Unadjusted PZC, (µg/dL)	
	r	p
CRP (mg/l)	-0.225	0.00006
AGP (g/l)	-0.283	<0.00001
Time of Sample collection	-0.088	<0.05
Time from Blood collection to Centrifugation	0.982	<0.001
Time from previous meal	-0.062	<0.001

Bivariate correlation analysis (Table 54) showed a significant correlation between CRP and the levels of Zinc (r=-0.225, p<0.0001), AGP (r=-0.283, p<0.00001), Time of sample collection (r=-0.088, p<0.05), time from blood collection to centrifugation (r=0.982, p<0.001) and time from previous meal (r=-0.062, p<0.001).

PZC was thus adjusted for inflammation and methodologic factors using multivariate linear regression analysis. PZC was adjusted to the following values:

- CRP = 3.75mg/L,
- AGP = 0.75 g/L,
- Sample collection at 1000 m above sea level,
- Time from collection to centrifugation =20min
- Time between blood collection and the previous meal = 4 h.

Distribution of mean PZC by Region

The average unadjusted PZC was 49.25±0.79 µg/dl while the APP adjusted PZC was 51.38±0.81 µg/dl. There mean PZC was significantly higher for the North Region compared to the Far North region for both Adjusted and unadjusted markers of PZC. No significant difference was observed between AGP Adjusted PZC and CRP adjusted PZC. However, APP Adjusted PZC was significantly higher than both APP and CRP adjusted PZC (Table 55).

Table 55: Distribution of mean PZC by Region

Region	*Far North*	*North*	*Total*
N	***159***	***155***	***314***
Unadjusted PZC, (µg/dL) (Mean±SEM)	48.28±1.33$_a$	50.24±0.83 $_b$	49.25±0.79 $_a$

AGP adjusted PZC, (µg/dL) (Mean±SEM)	49.70±1.35 a	52.17±0.86 b	50.92±0.81 a
CRP adjusted PZC, (µg/dL) (Mean±SEM)	49.80±1.35 a	52.07±0.83 b	50.92±0.80 a
APP adjusted PZC, (µg/dL) (Mean±SEM)	50.15±1.36 a	52.63±0.85 b	51.38±0.81c

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at p< 0.05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test.

Distribution of mean PZC by SES Quintile

A progressive relative increase in the mean PZC was observed as the SES Quintile moves from Poorest to Richest irrespective of the adjustment that was done. However, the increase was only significant for APP adjusted PZC (Table 56).

Table 56: Distribution of mean PZC by SES Quintile

SES quintile	*Poorest*	*Poor*	*Average*	*Rich*	*Richest*
N	*131*	*85*	*58*	*15*	*25*
Unadjusted PZC, (µg/dL) (Mean±SEM)	48.04±1.55	46.76±1.06	51.57±1.29	49.94±2.63	58.20±1.99
AGP adjusted PZC, (µg/dL) (Mean±SEM)	49.94±1.61	48.40±1.06	53.14±1.32	51.11±2.71	59.31±2.22
CRP adjusted PZC, (µg/dL) (Mean±SEM)	50.04±1.59	48.37±1.05	52.84±1.29	51.13±2.62	59.52±2.11
APP adjusted PZC, (µg/dL) (Mean±SEM)	50.50±1.61	48.83±1.05	53.40±1.31	51.46±2.67	59.78±2.21

Distribution of mean PZC by Age group

Results showed that the mean PZC was highest for the 24-35 months age-group compared to the other age groups. Globally, there was a decrease in the PZC as the age increased (Table 57).

Table 57: Distribution of mean PZC by Age group

Age group (months)	*12-23*	*24-35*	*36-47*	*48-59*
N	*104*	*85*	*76*	*49*
Unadjusted PZC, (µg/dL) (Mean±SEM)	50.24±1.05	50.99±2.16	47.00±1.29	47.59±1.60
AGP adjusted PZC, (µg/dL) (Mean±SEM)	51.88±1.08	52.70±2.18	48.88±1.33	48.95±1.64
CRP adjusted PZC, (µg/dL) (Mean±SEM)	51.63±1.04	52.92±2.19	48.88±1.29	49.09±1.66
APP adjusted PZC, (µg/dL) (Mean±SEM)	52.17±1.06	53.30±2.19	49.38±1.32	49.42±1.66

3.7.2. Prevalence of Low Plasma Zinc Concentration

Zinc deficiency as assessed by estimation of plasma zinc showed that 85.8% of the children were zinc-deficient (plasma zinc <65 µg/dL). Younger children were at a lower risk of zinc deficiency than older children; however, the prevalence was similar among children from the North and Far North Regions. Children of the richest quintile had a higher risk of zinc deficiency compared to the other SES quintiles. For all the three indices of nutritional status children in the Moderate and sever groups where more predisposed to zinc deficiency compared to the Normal group. (Table 58).

Table 58: Prevalence of Low Plasma Zinc Concentration

		N	Low Unadjusted PZC, (µg/dL), %	Low APP adjusted PZC, (µg/dL), %
Region	Far North	164	90.9%	89.0%
	North	167	85.0%	82.6%
	Total	331	87.9%	85.8%
Age group (months)	12-23	113	82.3%	79.6%
	24-35	91	86.8%	85.7%
	36-47	78	93.6%	91.0%
	48-59	49	93.9%	91.8%
SES quintile	Poorest	141	87.2%	84.4%
	Poor	87	95.4%	94.3%
	Average	59	88.1%	86.4%
	Rich	16	87.5%	87.5%
	Richest	28	67.9%	64.3%
Height-for-Age	Normal	186	85.5%	83.3%
	Moderate	77	92.2%	88.3%
	Severe	56	92.9%	92.9%
Weight-for Height	Normal	298	88.3%	85.9%
	Moderate	18	88.9%	88.9%
	Severe	3	100.0%	100.0%
Weight-for-Age	Normal	236	86.4%	83.9%
	Moderate	9	88.9%	88.9%
	Severe	19	94.7%	94.7%

3.8. Folate and Vitamin B12

In this study, the concentrations used for defining folate and vitamin B12 deficiencies based on metabolic indicators for children are:

Plasma vitamin B12 < 75 pmol/L : severe deficiency

Plasma vitamin B12: 75-150 pmol/L : moderate deficiency

Plasma vitamin B12: 150-210 pmol/L : mild deficiency

Plasma vitamin B12 : < 210 pmol/L : total deficiency

And

Plasma folate <10 nmol/L

3.8.1. Plasma Concentration of Folate and vitamin B12

Plasma Vitamin B12 and Folate were analyzed just for a subset of the children (133) in all. Overall, the mean plasma folate was 21.14±0.90 nmol/L while the mean plasma vitamin B12 was 294.39±16.62 pmol/L. These means are greater that the WHO cut offs (10nmol/L for folate and 210 pmol/L for Vitamin B12) indicating that the prevalence of Folate and Vitamin B12 deficiencies might be very low in this subset of study population.

The mean plasma folate concentration was significantly higher for the North Region compared to the Far North region but the mean plasma Vitamin B12 concentration did not significantly differ between the two regions. This shows that once more, plasma folate deficiency may be more acute in the Far North region compared to the North Region (Table 59).

Table 59: Mean plas ma concentrations of Folate and Vitamin B12 by Region

Region	*Far North*	*North*	*Total*
N	***66***	***67***	***133***
Plasma Folate, (nmol/L), (Mean±SEM)	19.29±1.29	22.96±1.22	21.14±0.90
N	***66***	***66***	***132***
Plasma Vitamin B12 (pmol/L), (Mean±SEM)	294.83±21.88	293.95±25.20	294.39±16.62

Furthermore, the mean plasma folate concentration increase with increasing SES Quintile between 21.78nmol/L and 23.44 nmol/L while a steady increase was also observed in the plasma Vit B12 concentration. However, a peak was observed for the rich Category that presented a very high mean concentration of 521.90 pmol/L (Table 60)

Table 60: Mean plas ma concentrations of Folate and Vitamin B12 by SES Quintile

SES quintile	*Poorest*	*Poor*	*Average*	*Rich*	*Richest*
N	***57***	***35***	***25***	***4***	***12***
Plasma Folate, (nmol/L), (Mean±SEM)	21.78±1.50	19.76±1.69	20.11±1.98	23.64±3.64	23.44±2.68
N	***56***	***35***	***25***	***4***	***12***
Plasma Vitamin B12 (pmol/L), (Mean±SEM)	244.43±20.02	310.14±36.22	302.41±35.69	521.90±139.60	389.02±62.70

Age wise, it was noticed that plasma folate decreased significantly with age ($p<0.05$) while plasma vitamin B12 increase significantly with age ($p<0.01$). This shows that older children might be more prone to folate deficiency and less affected by Vitamin B12 deficiency (Table 61).

Table 61: Mean plas ma concentrations of Folate and Vitamine B12 by Agegroup

Age group (months)	*12-23*	*24-35*	*36-47*	*48-59*
N	***37***	***36***	***35***	***25***
Plasma Folate, (nmol/L), (Mean±SEM)	24.62±1.89	22.17±1.77	18.52±1.64	18.16±1.52
N	***37***	***36***	***34***	***25***
Plasma Vitamin B12 (pmol/L), (Mean±SEM)	284.84±33.78	286.29±30.38	289.41±34.33	326.95±35.07

3.8.2. Prevalence of low Folate and vitamin B12 deficiency

The overall prevalence of Low plasma folate was 9.8%, with a very high proportion in the Far North Region (16.7%) compared to the North Region (3.0%). Age wise, a gradual increasing

the prevalence of low folate with Age was observed (Table 62). Children in the 36-47 months age group presented the highest proportion of low folate. It was interesting to notice that only children in the average, poor and poorest SES quintile categories presented low folate proportions. This may be linked to the quality of the diet of the children. As discussed above, the mean food consumption score and dietary diversity score increased with increasing SES quintile, thus affirming the fact that they might be a strong relationship between food consumption patterns and prevalence of low folate.

Table 62: Prevalence of low Folate and Folate and vitamin B12 deficiency

		Low Plasma Folate, nmol/L		***Low Plasma Vitamin B12 , pmol/L, %***					
		N	***%***	***N***	***Normal***	***Mild***	***Moderate***	***Sever***	***Global***
Region	Far North	66	16.7%	66	65.2%	16.7%	13.6%	4.5%	34.8%
	North	67	3.0%	66	56.1%	15.2%	21.2%	7.6%	43.9%
	Total	133	9.8%	132	60.6%	15.9%	17.4%	6.1%	39.4%
Age group (months)	12-23	37	5.4%	37	56.8%	18.9%	16.2%	8.1%	43.2%
	24-35	36	5.6%	36	55.6%	13.9%	25.0%	5.6%	44.4%
	36-47	35	17.1%	34	58.8%	17.6%	20.6%	2.9%	41.2%
	48-59	25	12.0%	25	76.0%	12.0%	4.0%	8.0%	24.0%
SES quintile	Poorest	57	12.3%	56	53.6%	14.3%	25.0%	7.1%	46.4%
	Poor	35	14.3%	35	60.0%	22.9%	8.6%	8.6%	40.0%
	Average	25	4.0%	25	64.0%	16.0%	16.0%	4.0%	36.0%
	Rich	4	-	4	100.0%	-	-	-	-
	Richest	12	-	12	75.0%	8.3%	16.7%	-	25.0%
Height-for-Age	Normal	73	11.0%	73	71.2%	16.4%	9.6%	2.7%	28.8%
	Moderate	31	9.7%	30	53.3%	16.7%	23.3%	6.7%	46.7%
	Severe	26	7.7%	26	42.3%	11.5%	30.8%	15.4%	57.7%
Weight-for Height	Normal	123	9.8%	122	63.9%	15.6%	13.9%	6.6%	36.1%
	Moderate	7	14.3%	7	14.3%	14.3%	71.4%	-	85.7%
	Severe	94	10.6%	94	64.9%	16.0%	13.8%	5.3%	35.1%
Weight-for-Age	Normal	2	-	2	-	-	100.0%	-	100.0%
	Moderate	8	-	8	25.0%	25.0%	37.5%	12.5%	75.0%
	Severe	-	-	-	-	-	-	-	-

The distribution of the proportion of low folate by the various indices of nutritionals status showed that the proportion of children with low folate did not significantly vary with stunting category. However, the proportion of children who were severely underweight was significantly higher than those who were moderately underweight. As discussed above, one of the key causes of underweight is sudden decrease in food intake thus this variation may be attributed to low food intake characterized by low FCS and DDS for children who are moderately and severely underweight.

The prevalence of global plasma Vitamin B12 deficiency was 39.4% with the North region presenting a higher proportion (43.9%) compared to the Far North region (34.8%). A significant decrease in the proportion of Vitamin B12 deficiency with age was observed. This shows that younger children are more affected by Vitamin B12 deficiency than older children. As expected, the proportion of low plasma vitamin B12 decreased with increasing SES Quintile. Also, the proportion of low plasma vitamin B12 was higher amongst severely stunted children (57.7%) compared to moderately stunted children (46.7%). This proportion was lower for severely underweight children (35.1%) compared to moderately underweight children (85.7%).

These findings indicate that vitamin b12 and folate deficiency may be strongly related to the nutritional status of the child especially stunting and underweight.

3.9. Trace Elements (Copper, Calcium, Magnesium, and Zinc)

Children are most vulnerable to under nutrition due to low dietary intake, inaccessibility to food, inequitable distribution of food within the household, improper food storage and preparation, dietary taboos and infectious diseases. Especially, micronutrient deficiencies are a result of inadequate intake or inefficient utilization of available micronutrients due to infections and parasitic infestations. However, information on the serum levels of multiple micronutrients in human biological tissues is scarce. For many essential elements, baseline levels in the general population, and especially in children, are lacking.

3.9.1. Plasma Concentration of Trace Elements

Mean concentration of Trace element

Table 63 below shows the concentrations of serum magnesium, calcium, copper, and zinc in relation to region, SES quintile and age-group status. The mean levels of Calcium, magnesium, copper and zinc were respectively 80.7±0.3, 19.9±0.1, 200.6±32.8, and 49.2±0.8 µg/dl. The mean serum level of Calcium and copper was significantly lower in the North region compared to the Far North Region ($p<0.05$) while the serum concentrations of Magnesium and zinc were significantly lower in the Far North Region ($p<0.05$). Serum concentration of calcium, magnesium and copper decreased significantly with increasing age ($p<0.05$) while the serum concentration of zinc decreased with increasing age.

Table 63: Mean Values of Trace Elements by region, SES quintile and Agegroup

		N	*Ca (µg/dl)*	*Mg (µg/dl)*	*Ca/Mg Ratio*	*Cu (µg/dl)*	*Zinc (µg/dl)*	*Cu/Zn Ratio*
Region	Far North	159	81.3±0.4	19.9±0.1	4.1±0.3	234.1±64.5	48.3±1.3	4.8±1.0
	North	155	80.1±0.5	19.9±0.1	4.0±0.1	166.3±4.4	50.2±0.8	3.4±0.1
	Total	314	80.7±0.3	19.9±0.1	4.1±0.1	200.6±32.8	49.2±0.8	4.1±0.5
SES Quintile	Poorest	131	80.6±0.4	20.0±0.1	4.1±0.1	181.4±7.1	48.0±1.6	4.0±0.2
	Poor	85	80.4±0.7	20.0±0.2	4.0±0.1	161.5±4.4	46.8±1.1	3.6±0.1
	Average	58	81.0±0.5	19.9±0.2	4.1±0.1	336.8±17.6	51.6±1.3	6.0±2.1

	Rich	15	78.6±0.9	19.0±0.3	4.1±0.1	143.8±5.6	49.9±2.6	3.0±0.2
	Richest	25	82.4±1.7	19.8±0.4	4.2±0.1	152.8±5.9	58.2±1.9	2.7±0.2
	total	314	80.7±0.3	19.9±0.1	4.1±0.1	200.6±32.8	49.2±0.8	4.1±0.5
Age group (months)	12-23	104	82.3±0.7	20.3±0.2	4.1±0.1	173.6±5.9	50.2±1.1	3.6±0.1
	24-35	85	80.9±0.6	19.9±0.2	4.1±0.1	172.9±10.3	51.0±2.2	3.6±0.2
	36-47	76	79.3±0.4	19.8±0.2	4.0±0.1	166.6±4.5	47.0±1.3	3.8±0.1
	48-59	49	79.1±0.4	19.4±0.2	4.1±0.1	358.9±20.9	47.6±1.6	6.7±3.4
	total	314	80.7±0.3	19.9±0.2	4.1±0.1	200.6±32.8	49.2±0.8	4.1±0.5

As shown in table 64, significant correlations were found between serum zinc and SES quintile (r=0.183, p<0.01) serum Zinc and Food consumption score (r=-0.002, p<0.05) and between serum Zinc and Diet diversity score (r=0.088, p<0.05). Copper was only correlated with diet diversity score (r= -0.009, p<0.05) while both Serum calcium and magnesium were correlated with age (r= -0.230, p<0.01 and r = -0.181, p<0.01 respectively).

Table 64: Correlations between Trace Elements, FCS, and DDS

	SES Quintile	Age	FCS	DDS
Zinc (µg/dL)	0.183**	-0.093	-0.002*	0.088*
Copper (µg/dL)	0.023	0.081	-0.062	-0.009*
Cu/Zn Ratio	-0.005	0.089	-0.072	-0.024
Calcium (mg/dL)	0.046	-0.230**	-0.044	-0.031
Magnesium (mg/dL)	-0.064	-0.181**	-0.046	-0.002
Ca/Mg Ration	0.096	-0.010	0.006	-0.018

**Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).*

Serum levels Trace elements and Nutritional status

Table 65 below shows the concentrations of serum magnesium, calcium, copper, and zinc in relation to nutritional status. The mean serum level of Calcium, Magnesium, copper and zinc was significantly lower in severely stunted compared to moderately stunted children (p<0.05). Serum concentration of copper was significantly higher in moderately wasted children (p<0.05) compared to normal. However, serum concentration of zinc was significantly lower in moderately wasted children (p<0.05) compared to normal. On the contrary, severely wasted children had higher concentration of copper, although not statistically significant. As a result, the copper-to-zinc ratio was significantly higher in mildly wasted children (*p*<0.05) compared to normal children.

Table 65: Levels of Serum Micro-nutrients (Mean±SEM) in relation to Nutritional status of children of the North and Far North regions of Cameroon

		N	*Ca (µg/dl)*	*Mg (µg/dl)*	*Ca/Mg Ratio*	*Cu (µg/dl)*	*Zinc (µg/dl)*	*Cu/Zn Ratio*
Height-for-Age	Normal	176	81.1±0.4	20.0±0.1	4.1±0.03	225.4±58.3	50.3±0.8	4.5±0.9
	Moderate	75	80.8±0.6	20.0±0.4	4.1±0.04	163.1±4.8	48.9±1.3	3.5±0.1
	Severe	54	79.6±0.9	19.6±0.2	4.1±0.04	176.9±11.7	47.1±3.2	4.0±0.2
	total	305	80.7±0.3	19.9±0.1	4.1±0.02	201.5±33.7	49.4±0.8	4.1±0.5
Weight-for Height	Normal	285	80.7±0.3	19.9±0.1	4.1±0.02	204.1±36.1	49.2±0.7	4.2±0.6
	Moderate	17	82.6±1.1	20.0±0.4	4.2±0.10	170.8±8.8	55.0±9.2	3.6±0.3

	Severe	3	76.2±1.5	21.1±0.2	3.6±0.07	133.3±34.3	36.3±1.7	3.6±0.8
	total	305	80.7±0.3	19.9±0.1	4.1±0.02	201.5±33.7	49.4±0.8	4.1±0.5
Weight-for - Age	Normal	225	80.7±0.4	19.9±0.1	4.1±0.03	213.1±45.6	49.9±0.8	4.3±0.7
	Moderate	8	83.0±0.7	20.4±0.6	4.1±0.10	190.5±10.7	48.2±3.0	4.1±0.4
	Severe	19	82.6±1.2	19.9±0.3	4.2±0.10	150.3±7.6	52.8±8.4	3.3±0.2
	total	252	80.9±0.4	19.9±0.1	4.1±0.02	207.6±40.8	50.1±0.9	4.2±0.7

Wasting was significantly correlated with zinc (r=0.094, p<0.05), Cu/Zn ratio (r=-0.085, p<0.05), Body iron stores (r=0.140, p<0.01) and serum ferritin (r=0.168, p<0.01). Stunting was correlated to same micronutrients like wasting while underweight was only correlated to serum magnesium (r=-0.101, p<0.01), body iron stores (r=0.106, p<0.01) and serum ferritin (r=0.166, p<0.01) – Table 66.

Table 66: Correlation between Micro-nutrients and Nutritional status in children of the North and Far North regions of Cameroon

	Weight-for - Age	Height-for-Age	Weight-for Height
Zinc (µg/dL)	0.094*	0.093*	0.05
Copper (µg/dL)	-0.041	-0.015	-0.048
Cu/Zn Ratio	-0.085*	-.088*	-0.034
Calcium (mg/dL)	-0.017	0.029	-0.071
Magnesium (mg/dL)	-0.053	0.008	-0.101**
Ca/Mg Ration	0.053	0.024	0.051
Body Iron Stores (mg/Kg)	0.140**	0.111**	0.106**
Serum Ferritin (µg/l)	0.168**	0.105**	0.166**

**Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).*

3.10. Multiple Micronutrient deficiency

Undernourished children are more likely to develop severe infections secondary to compromised immune responses (Rayhan *et al.*, 2006). Under nutrition influences several aspects of immunity, including cell-mediated immune responses (Chandra *et al.*, 1997), cytokine production (Grimble *et al.*, 1994) and antibody responses (Brussow *et al.*, 1995) particularly those that require T cell support (Redmond *et al.*, 1995). The high prevalence of bacterial and parasitic diseases in poor countries contributes greatly to under nutrition (Girma *et al.*, 2002). Children are most vulnerable to under nutrition due to low dietary intake, inaccessibility to food, inequitable distribution of food within the household, improper food storage and preparation, dietary taboos and infectious diseases (Bárány *et al.*, 2002). Especially, micronutrient deficiencies are a result of inadequate intake or inefficient utilization of available micronutrients due to infections and parasitic infestations. However, information on the serum levels of multiple micronutrients in human biological tissues is scarce. For many essential elements, baseline levels in the general population, and especially in children, are lacking (Bárány *et al.*, 2002).

3.10.1. Prevalence of all micronutrient deficiencies

Table 67 presents the prevalence of selected plasma micronutrient deficiencies. It was observe that the most prevalent micro nutrient deficiency is low PZC (85.8%) followed by anemia (63.75), then vitamin A deficiency (40.8%) and low vitamin B12 (39.4%). Iron deficiency was present in 29.3% of the study population while the least prevalent micronutrient deficiency is low folate (9.8%).

Table 67: Prevalence of Major Micronutrient deficiencies

Micronutrient	Far North	North	Total
Vitamin A deficiency, Adjusted Plasma RBP <0.83 µmol/L, %	35.6%	46.1%	40.8%
Iron Deficiency, Adjusted Plasma ferritin (T) <12 µg/L	34.1%	24.6%	29.3%
Anemia , Hemoglobin <110g/L, %(95%	66.5%	61.1%	63.7%
Low PZC, APP adjusted PZC < 65 µg/dL, %	89.0%	82.6%	85.8%
Low Folate, Plasma Folate <10nm/L, %	16.7%	3.0%	9.8%
Vitamin B12 Deficiency, Plasma Vitamin B12 < 210 pmol/L , %	34.8%	43.9%	39.4%

3.10.2. Prevalence of multiple micronutrient deficiencies

Of all the children who took part in the study, 94.3% had at least one form of micronutrient deficiency with 73.8% having at least 2 micronutrient deficiencies. The prevalence of multi-micronutrient deficiency was high with up to 70.4% of the children presenting two or more forms of micronutrient deficiency. The proportion of children presenting two, three, four, five and six micronutrient deficiencies were respectively 20.5%, 26.3%, 29.3%, 14.8%, 3.0% and 0.3% (Table 68). The difference in the prevalence of multiple micronutrient deficiency was not significant by region, sex, age group or SES quintile

Table 68: Prevalence of Multiple micronutreint deficiencies

	Far North	***North***	***Total***
n	***164***	***167***	***331***
No Micronutrient	3.0%	8.4%	5.7%
1 Micronutrient	21.3%	19.8%	20.5%
2 Micronutrients	25.6%	26.9%	26.3%
3 Micronutrients	30.5%	28.1%	29.3%
4 Micronutrients	16.5%	13.2%	14.8%
5 Micronutrients	3.0%	3.0%	3.0%
6 Micronutrients	0.0%	0.6%	0.3%

3.10.3. Characterization of multiple micronutrient deficiencies

Of the children who presented between two and five types of MD, the most prevalent of MDs were Vitamin A, Iron and Anemia Table 69 and figure 20.

Table 69: Characterization of Multiple micronutrient deficiencies

	Vitamin A deficiency	Iron Deficiency	Anemia	Low PZC	Low Folate	Vitamin B12 Deficiency
1 Micronutrient	20.7%	21.7%	22.0%	20.5%	18.0%	18.2%
2 Micronutrients	27.4%	27.7%	28.2%	26.3%	22.6%	22.0%

3 Micronutrients	30.9%	30.9%	31.4%	29.3%	27.1%	27.3%
4 Micronutrients	15.6%	15.6%	15.9%	14.8%	21.8%	22.0%
5 Micronutrients	3.2%	3.2%	3.2%	3.0%	7.5%	7.6%
6 Micronutrients	0.3%	0.3%	0.3%	0.3%	0.8%	0.8%

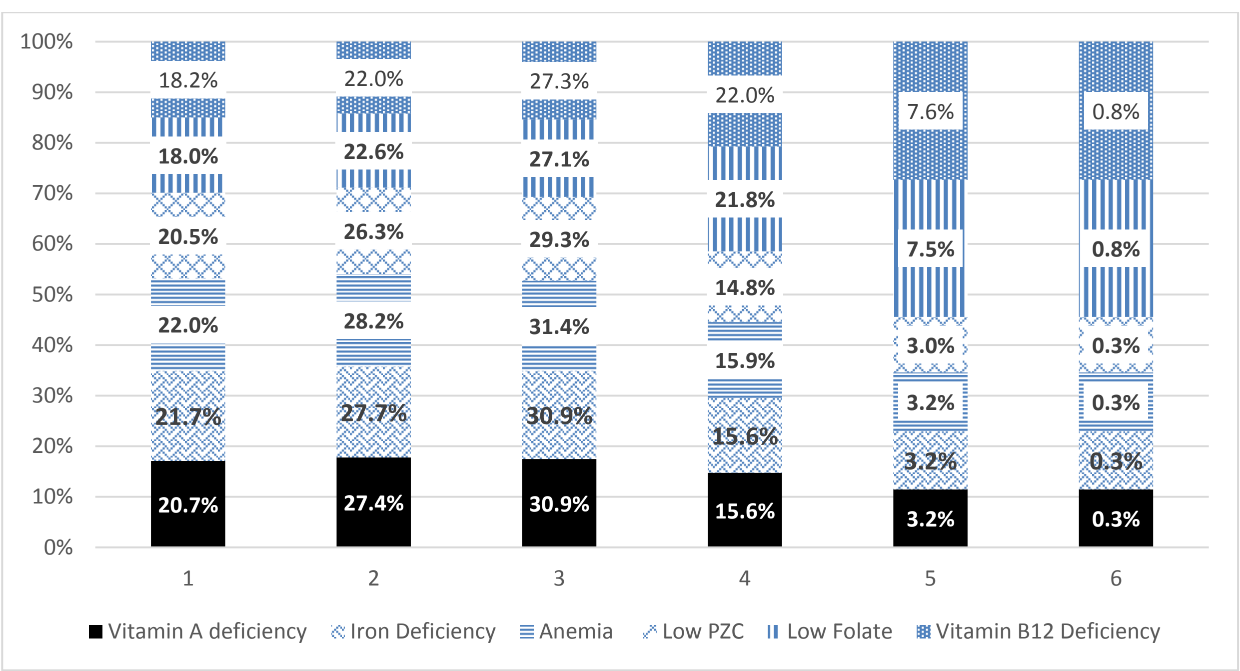

Figure 17: Characterization of Multiple micronutrient deficiencies

3.11. Determinants of Micronutrient deficiency

Regression models are relationships between categorical or continuous predictors and one response, and models are used to predict response values for new observations.

Stepwise regression is an automated tool used in the exploratory stages of model building to identify a useful subset of predictors. The process systematically adds the most significant variable or removes the least significant variable during each step.

With more than 100 predictor variables, finding the most significant models could be a time consuming task. The Stepwise regression approach automatically outputs the most significant models along with the R^2, adjusted R^2, and predicted R^2 values for the models.

Standard stepwise regression both adds and removes predictors as needed for each step. Analysis stops when all variables not in the model have p-values that are greater than the specified alpha-to-enter value and when all variables in the model have p-values that are less than or equal to the specified alpha-to-remove value.

Forward selection starts with no predictors in the model and adds the most significant variable for each step. Analysis stops when all variables not in the model have p-values that are greater than the specified alpha-to-enter value.

Backward elimination starts with all predictors in the model and Minitab removes the least significant variable for each step. Analysis stops when all variables in the model have p-values that are less than or equal to the specified alpha-to-remove value.

In this study, the backward stepwise elimination Regression was use to assess the key determinants of each micronutrient deficiency.

3.11.1. Determinants for vitamin A deficiency

Distribution of Vitamin A Deficiency by Nutritional Status

The relationship between Vitamin A deficiency defined as Adjusted plasma RBP<0.83µmol/L was examined and it was found that the proportion of VAD was significantly higher amongst children with Severe stunting, Moderate underweight, moderate and severe wasting ($p<0.05$) – Table 70. This indicates that there is a higher probability of wasted children to present VAD compared to stunted and underweight children.

Table 70: Distribution of Vitamin A Deficiency by Nutritional Status

		Adjusted Plasma RBP <0.83 µmol/L,			
		N	***No***	***Yes***	***Total***
Stunting	Normal	176	63.5%$_a$	52.2%$_b$	57.7%
	Moderate	74	21.6%$_a$	26.8%$_a$	24.3%
	Severe	55	14.9%$_a$	21.0%$_b$	18.0%
	total	305	100%	100%	100.0%
Underweight	Normal	284	93.9%$_a$	92.4%$_a$	93.1%
	Moderate	18	4.7%$_a$	7.0%$_b$	5.9%
	Severe	3	1.4%$_a$	0.6%$_a$	1.0%
	total	305	100%	100%	100.0%
Wasting	Normal	224	87.9%$_a$	89.8%$_a$	88.9%
	Moderate	9	1.6%$_a$	5.5%$_b$	3.6%
	Severe	19	10.5%$_a$	4.7%$_b$	7.5%
	total	252	100%	100%	100.0%

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at p< 0.05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction

Determinants of Vitamin A Deficiency

In a multivariate model, VAD among children was positively associated with food consumption score, Age, stunting, wasting, and underweight and caregiver level of education (Table 71). The model explained 81.1% of the variance in VAD. Further analysis in the univariate model shows that the key determinants of VAD is the study population are poor food consumption score, being between 12-23 months of age, being severely stunted, underweight and wasted. Interestingly, it was noticed that VAD was more associated to mothers who have done primary education compared to mothers who have not schooled before. Also the caregiver level of education was strongly associated to VAD more than the other variables.

Table 71: Determinants of Vitamin A Deficiency

Determinants[1] (Variable)	Univariate Model[2]				Multivariate Model[3]		
	R^2	***β - Coefficient***	***SE***	***p Value***	***β - Coefficient***	***SE***	***p Value***
Food Consumption Score	0.031				-0.066	0.001	0.030
Poor		0.156	0.078	0.008	-	-	-
Borderline		0.099	0.046	0.008	-	-	-
Acceptable		Ref	-	-	-	-	-
Age Group	0.029				0.060	0.000	0.029
12-23		0.278	0.056	0.006	-	-	-
24-35		0.136	0.041	0.007	-	-	-
36-47		0.251	0.043	0.006	-	-	-
47-59		Ref	-	-	-	-	-
Stunting	0.070				0.170	0.001	0.000
Normal			-	-	-	-	-
Moderate		-0.796	0.058	0.017	-	-	-
Severe		-0.818	0.064	0.020	-	-	-
Underweight	0.022				0.508	0.002	0.003
Normal		Ref	-	-	-	-	-
Moderate		-	-	-	-	-	-
Severe		-0.187	0.117	0.009	-	-	-
Wasting	0.047				-0.473	0.002	0.004
Normal		Ref	-	-	-	-	-
Moderate		1.265	0.112	0.026	-	-	-
Severe		0.912	0.138	0.051	-	-	-
Care Giver Level of Education	0.111				-0.073	0.001	0.000
No schooling		0.046	0.564	0.009	-	-	-
Primary		0.152	0.671	0.008	-	-	-
Secondary		Ref	-	-	-	-	-
R^2					0.811	-	-

[1]Models included 331 children. Dash signifies the variable was not included in the model due to insignificance. [2] Linear regression models using SPSS GzLM with overall prevalence of VAD as the dependent variable and one independent variable. [3]Linear regression model with overall VAD as the dependent variable and the independent variables that remained significant, $p<0.05$

3.11.2. Risk factors for iron deficiency

The distribution of the proportion of Iron deficiency (ID) defined by adjusted Plasma Ferritin <12 µg/L by nutritional status showed that ID was significantly higher amongst severely stunted, moderately underweight, moderate and severely wasted children. However, it was also found that there was a significantly higher proportion of ID in children of the normal category as per Stunting, wasting and underweight (Table 72). This may be attributed to the high prevalence of ID (29.3%) in the overall population. Also this also shows that ID may be a health problem independent of the nutritional status of the child. Thus these results suggest that apart from nutritional status, there are other factors that account for ID in the population. Results showed that 37.9% of iron deficiency was associated to anemia and that there was a strong correlation between ID and indicators of feeding patterns: Diet diversity score ($r=0.089$, $p<0.05$) and Food consumption score($r=0.131$, $p<0.05$).

Table 72: Distribution of iron deficiency by Nutritional status

		Adjusted Plasma Ferritin <12 µg/L			
		N	*No*	*Yes*	*Total*
Stunting	Normal	186	62.1% a	49.5% b	58.3%
	Moderate	77	22.8% a	27.4% a	24.1%
	Severe	56	15.2% a	23.2% b	17.6%
	total	319	100%	100%	100.0%
Underweight	Normal	298	96.9% a	85.3% b	93.4%
	Moderate	18	2.2% a	13.7% b	5.6%
	Severe	3	0.9% a	1.1% a	0.9%
	total	319	100%	100%	100.0%
Wasting	Normal	236	94.2% a	77.0% b	89.4%
	Moderate	9	1.6% a	8.1% b	3.4%
	Severe	19	4.2% a	14.9% b	7.2%
	total	264	100%	100%	100.0%

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at p< 0.05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction

Univariate and multiple regression analysis showed that seven key factors were associated with ID which explain 68.1% of the overall variance in the ID. Thus children most likely to have Iron deficiency are children who are anemic, suffering from one or more forms of malnutrition (underweight, wasting or stunting), having low folate and Vitamin A with a limited dietary diversity in their nutritional habits (Table 73).

Table 73: Determinants of Iron Deficiency

Determinants	Univariate Model				Multivariate Model		
	R^2	*β – Coefficient*	*SE*	*p Value*	*β – Coefficient*	*SE*	*p Value*
Underweight	0.159				-0.100	0.001	0.058
Normal		Ref	-	-	-	-	-
Moderate		-0.637	0.066	0.003	-	-	-
Severe		-0.210	0.067	0.007	-	-	-
Wasting	0.211				0.184	0.001	0.007
Normal		Ref	-	-	-	-	-
Moderate		-0.859	0.065	0.001	-	-	-
Severe		-0.127	0.111	0.009	-	-	-
Stunting	0.352				-0.053	0.071	0.0063
Normal		Ref	-	-	-	-	-
Moderate		-0.796	0.577	0.016	-	-	-
Severe		-0.818	0.636	0.019	-	-	-
Anemia	0.739				0.150	0.095	<0.001
No		Ref	-	-	-	-	-
Yes		0.51	0.019	0.007	-	-	-
Adjusted Plasma RBP	0.052	0.451	0.284	<0.001	0.291	0.005	0.001
Plasma folate <10nm/L	0.158				0.256	0.001	0.005
No		Ref	-	-	-	-	-
Yes		0.821	0.052	0.001	-	-	-
Dietary diversity Score Adequacy	0.031				0.092	0.001	0.024
NO		-0.513	0.043	0.002	-	-	-
Yes		Ref	-	-	-	-	-
R^2					0.681		

[1]Models included 331 children. Dash signifies the variable was not included in the model due to insignificance. [2] Linear regression models using SPSS GzLM with overall prevalence of VAD as the dependent variable and one independent variable.

[3]*Linear regression model with overall VAD as the dependent variable and the independent variables that remained significant, p<0.05*

3.11.3. Risk factors for Low PZC

Looking at the relationship between Low PZC and Nutritional status, it was noticed that the prevalence of Low PZC was higher only among stunted children (moderate or severe), and severely wasted children (Table 74).

Table 74: Distribution of Low Plasma Zinc concentration by nutritional status

		APP adjusted PZC, <65µg/dL			
		N	*No*	*Yes*	*Total*
Stunting	Normal	186	70.5% a	56.4% a	58.3%
	Moderate	77	20.5% a	24.7% b	24.1%
	Severe	56	9.1% a	18.9% b	17.6%
	total	319	100%	100%	100.0%
Underweight	Normal	298	95.5% a	93.1% a	93.4%
	Moderate	18	4.5% a	5.8% a	5.6%
	Severe	3	0.0% [1]	1.1% a	0.9%
	total	319	100%	100%	100.0%
Wasting	Normal	236	95.0% a	88.4% a	89.4%
	Moderate	9	2.5% a	3.6% a	3.4%
	Severe	19	2.5% a	8.0% b	7.2%
	total	264	100%	100%	100.0%

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at p< 0.05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction. [1]*This category is not used in comparisons because its column proportion is equal to zero or one.*

Eight determinants are strongly associated with plasma zinc concentration, explaining 78.6% of the total variance observed. These are Body iron, plasma ferritin, the consumption of food sources of Zinc, the age of the child, the plasma Vitamin B12 content and anemia (table 75 below)

Table 75: Determinants of Low plasma Zinc Concentration

Determinants	Univariate Model				Multivariate Model		
	R^2	*β - Coefficient*	*SE*	*p Value*	*β - Coefficient*	*SE*	*p Value*
Adjusted Body Iron < 0 mg/kg	0.047				0.390	0.001	0.000
No		Ref	-	-	-	-	-
Yes		-0.091	0.038	0.081	-	-	-
Adjusted Plasma ferritin <12 µg/L	0.027				0.038	0.000	0.001
No		Ref	-	-	-	-	-
Yes		0.002	0.040	0.010	-	-	-
Consumed food groups sources of Zinc	0.045				0.105	0.001	0.024
No		-0.057	0.030	0.008	-	-	-
Yes		Ref	-	-	-	-	-
Age of Child	0.101				0.036	0.000	0.025
12-23		0.010	0.028	0.010	-	-	-
24-35		-0.034	0.029	0.009	-	-	-
36-47		0.031	0.029	0.009	-	-	-

	R^2	β - Coefficient	SE	p Value	β - Coefficient	SE	p Value
47-59		Ref	-	-	-	-	-
Plasma vitamin B12 : < 210 pmol/L	0.037				0.085	0.001	0.023
No		Ref	-	-	-	-	-
Yes		0.051	0.020	0.008	-	-	-
Hemoglobin < 110g/l	0.187				0.110	0.001	0.015
No		Ref	-	-	-	-	-
Yes		0.042	0.225	0.009	-	-	-
R^2					0.786		

[1]Models included 331 children. Dash signifies the variable was not included in the model due to insignificance. [2] Linear regression models using SPSS GzLM with overall prevalence of VAD as the dependent variable and one independent variable. [3]Linear regression model with overall VAD as the dependent variable and the independent variables that remained significant, p<0.05

3.11.4. Risk factors for Low Folate

The prevalence of low folate did not significantly differ by nutritional status. However, the prevalence of low folate was slightly higher in the moderate underweight group (Table 76).

Table 76: distribution of Low folate by Nutritional Status

			Low Folate		
		N	***No***	***Yes***	***Total***
Stunting	Normal	73	55.6% a	61.5% a	56.2%
	Moderate	31	23.9% a	23.1% a	23.8%
	Severe	26	20.5% a	15.4% a	20.0%
	total	130	100%	100%	100.0%
Underweight	Normal	123	94.9% a	92.3% a	94.6%
	Moderate	7	5.1% a	7.7% a	5.4%
	total	130	100%	100%	100.0%
Wasting	Normal	94	89.4% a	100.0%[1]	90.4%
	Moderate	2	2.1% a	0.0%[1]	1.9%
	Severe	8	8.5% a	0.0%[1]	7.7%
	total	104	100%	100%	100.0%

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at p< 0.05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction. 1This category is not used in comparisons because its column proportion is equal to zero or one.

Seven determinants are strongly associated with Low folate concentration, explaining 53.7% of the total variance observed. (Table 77)

Table 77: Determinants of low folate

Determinants of low Folate	Univariate Model				Multivariate Model		
	R^2	***β - Coefficient***	***SE***	***p Value***	***β - Coefficient***	***SE***	***p Value***
Adjusted Body Iron < 0 mg/kg	0.105				0.061	0.007	0.033
No		Ref	-	-	-	-	-
Yes		-25.606	0.134	0.010	-	-	-
Age of Child	0.231				0.031	0.003	0.022
12-23		-0.781	1.392	0.005	-	-	-
24-35		0.252	1.116	0.008	-	-	-
36-47		-0.294	1.345	0.008	-	-	-
47-59		Ref	-	-	-	-	-
Hemoglobin < 110g/l	0.059				0.077	0.006	0.021
No		Ref	-	-	-	-	-
Yes		-0.550	1.239	0.065	-	-	-
Dietary diversity Score Adequacy	0.104				-0.114	0.005	0.003

NO		0.492	0.989	0.061	-	-	-
Yes		Ref	-	-	-	-	-
Underweight	0.016				0.373	0.014	0.008
Normal		Ref	-	-	-	-	-
Moderate		-0.822	1.453	0.057	-	-	-
Severe		-	-	-	-	-	-
Adjusted Plasma RBP	0.110	1.451	1.284	0.025	-0.191	0.006	0.001
Adjusted Plasma ferritin	0.063	-0.833	0.906	0.035	0.119	0.007	0.007
R^2					0.537		

[1]Models included 331 children. Dash signifies the variable was not included in the model due to insignificance. [2] Linear regression models using SPSS GzLM with overall prevalence of VAD as the dependent variable and one independent variable. [3]Linear regression model with overall VAD as the dependent variable and the independent variables that remained significant, p<0.05

3.11.5. Risk factors for vitamin B12 deficiency

Vitamin B 12 deficiency was found to be more prevalent in moderately and severely stunted children. This prevalence was also found to be higher in moderately underweight Children. The prevalence of Vitamin B12 deficiency did not considerably vary with wasting (Table 78).

Table 78: Distribution Vitamin B12 Category by Nutritional status

			Vitamin B12 category				
		N	**Normal**	**Mild**	**Moderate**	**Severe**	**Total**
Stunting	Normal	73	65.8% $_{a}$	60.0% $_{a,b}$	31.8% $_{b}$	25.0% $_{a,b}$	56.6%
	Moderate	30	20.3% $_{a}$	25.0% $_{a}$	31.8% $_{a}$	25.0% $_{a}$	23.3%
	Severe	26	13.9% $_{a}$	15.0% $_{a}$	36.4% $_{a}$	50.0% $_{b}$	20.2%
	total	129	100%	100%	100%	100%	100.0%
Underweight	Normal	122	98.7% $_{a}$	95.0% $_{a,b}$	77.3% $_{b}$	100.0%[1]	94.6%
	Moderate	7	1.3% $_{a}$	5.0% $_{a,b}$	22.7% $_{b}$	0.0%[1]	5.4%
	total	129	100%	100%	100%	100%	100.0%
Wasting	Normal	94	96.8% $_{a}$	88.2% $_{a,b}$	72.2% $_{b}$	83.3% $_{a,b}$	90.4%
	Moderate	2	0.0%[1]	0.0%[1]	11.1% $_{a}$	0.0%[1]	1.9%
	Severe	8	3.2% $_{a}$	11.8% $_{a}$	16.7% $_{a}$	16.7% $_{a}$	7.7%
	total	104	100%	100%	100%	100%	100.0%

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction. [1]This category is not used in comparisons because its column proportion is equal to zero or one

In the multivariate model, vitamin B12 deficiency among children was positively associated with Age, anemia, stunting, VAD and caregiver level of education (Table 79). The model explained 74.1% of the variance in vitamin B12 defficiency. Further analysis in the univariate model shows that the key determinants of VAD is the study population are being between 12-23 months of age, being moderately or severely stunted, having anemia, and low plasma levels of Vitamin A. Also, as was the case with VAD that vitamin B12 deficiency was more associated to mothers who have done primary education compared to mothers who have not schooled before.

Table 79: Determinants of vitamin B12 Deficiency

Determinants	Univariate Model	Multivariate Model

	R2	β - Coefficient	SE	p Value	β - Coefficient	SE	p Value
Age of Child	0.333				-0.023	0.004	0.060
12-23		-0.048	0.414	0.091	-	-	-
24-35		-0.057	0.382	0.088	-	-	-
36-47		-0.142	0.396	0.072	-	-	-
47-59		Ref	-	-	-	-	-
Hemoglobin < 110g/l	0.196				0.088	0.010	0.040
No		Ref	-	-	-	-	-
Yes		-0.162	0.310	0.006	-	-	-
Stunting	0.218				0.121	0.006	0.005
Normal		Ref	-	-	-	-	-
Moderate		-0.321	0.271	0.024	-	-	-
Severe		-0.149	0.281	0.060	-	-	-
Care Giver Level of Education	0.194				-0.037	0.007	0.059
No schooling		0.056	0.652	0.093	-	-	-
Primary		-0.082	0.728	0.091	-	-	-
Secondary		Ref	-	-	-	-	-
Adjusted Plasma RBP,	0.139	-0.048	0.279	0.009	0.098 0.741	0.009	0.029

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at p< 0.05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction. 1This category is not used in comparisons because its column proportion is equal to zero or one

Table 80 below presents the determinants of folate and Vitamin B 12 showing three commont predictors (age, haemoglobin and Vitamin A status).

Table 80: Determinants of both folate and Vitamin B 12

Variable	Folate				Vitamin B12			
	R2	β	SE	p	R2	β	SE	p
Adjusted Body Iron	0.105	0.061	0.007	0.033	-	-	-	-
Age of Child	0.231	0.031	0.003	0.022	0.333	-0.023	0.004	0.060
Haemoglobin	0.059	0.077	0.006	0.021	0.196	0.088	0.010	0.040
DDS Adequacy	0.104	-0.114	0.005	0.003	-	-	-	-
Underweight	0.016	0.373	0.014	0.008	-	-	-	-
Adjusted Plasma RBP	0.110	-0.191	0.006	0.001	0.139	0.098	0.009	0.029
Adjusted Plasma Ferritin	0.063	0.119	0.007	0.007	-	-	-	-
Stunting	-	-	-	-	0.218	0.121	0.006	0.005
Care Giver Level of Education	-	-	-	-	0.194	-0.037	0.007	0.059

As shown in Figure 22 below, this study also demonstrated for the first time that with respect to nutritional status, the patterns of Vitamin B12 and Folate are very similar.

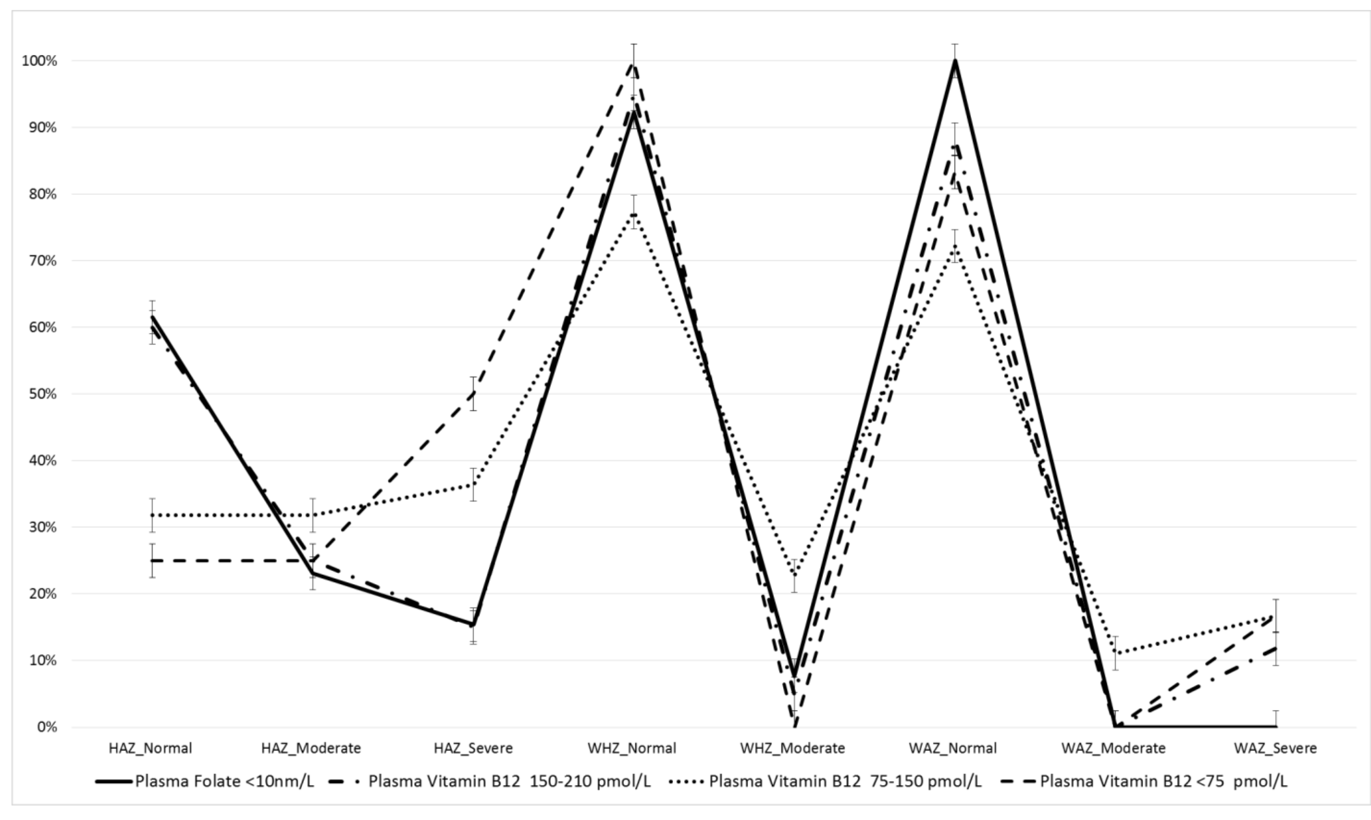

Figure 18: Patterns of both Folate and Vitamin B12 deficiency with Nutritional status

DISCUSSION

3.12. Vitamin A

3.12.1. Vitamin A Deficiency

Choice of marker for Vitamin A deficiency

Vitamin A is required throughout life and participates in numerous cellular activities involved in reproduction, embryonic development, vision, growth, cellular differentiation and proliferation, tissue maintenance and lipid metabolism.

Though it is widely accepted that in response to tissue demand, it is released from the liver in a 1:1 ratio with its carrier protein, retinol-binding protein. This study found that the ROH: RBP ratio was consistently less than 1:1. This is because several factors may influence this ratio, such as VA status, the presence of elevated acute phase proteins (Baeten *et al*., 2004), iron status (Jang *et al*., 2000), and protein energy malnutrition.

All vitamin A in the body originates from the diet and is absorbed in the small intestine, where it is packaged along with other dietary fats into chylomicrons, which are secreted into the lymphatic system (Goodman *et al*., 1984). After entering the general circulation, chylomicrons undergo lipolysis and are eventually taken up by the liver, where the bulk of the body's vitamin A is stored as retinyl esters (Blaner *et al*., 1994). To meet constant tissue needs despite day-to-day variability in dietary vitamin A intake, a steady concentration of circulating retinol is maintained by drawing on hepatic reserves. This is accomplished through consecration from the liver of retinol bound to its specific carrier protein, retinol binding protein (RBP) (Blaner *et al*., 1989, , Goodman *et al*., 1984). Circulating retinol is taken up by target tissues, where it is converted to its active forms, retinoic acid (the ligand for nuclear retinoid receptors (Petkovich *et al*., 1987, Giguere *et al*., 1987, Benbrook *et al*., 1988) or 11-*cis*-retinal (the chromophore of the visual pigment rhodopsin (Wald G 1969, Wald G 1968). If the liver becomes depleted of retinol stores, as occurs in the late stages of VAD, apo-RBP is retained in the endoplasmic reticulum of the hepatocyte and is not secreted. This retention may result in an underestimation of the levels on ROH.

Also, the short life span of ROH makes its assessment difficult. The results of this study corroborates the results of a recent study (Mary *et al*., 2001) that found a very strong correlation between the serum level of RoH and RBP. A very strong significant correlation between RPB and ROH ($r=0.974$, $p<0.0001$) was noticed. This suggests that RBP can be used as a surrogate for ROH in the evaluation of VAD. This study was able to measure serum ROH only in 35 children while serum RBP was measured in 314 children. Thus for the evaluation of Vitamin A Deficiency, serum RBP was preferred.

Secondly, there was a very significant negative correlation between markers of infection and serum levels of both ROH (r=-0.398, p=0.017 for CRP; r=-0.280, p=0.100 for AGP) and RBP (r=-0.427, p<0.0001 for CRP; r=-0.380, p<0.0001 for AGP) supporting the findings of previous studies which showed that infection significantly reduces the serum levels of RPB and ROH. Thus these Acute phase proteins (APPs) are susceptible to influencing serum levels of biomarkers of Vitamin A.

The APPs are a highly heterogeneous group of plasma proteins both in respect of the physicochemical properties as well as in respect of their biological actions, which can include anti-proteinase activity, coagulation properties, transport functions, immune response modulation and/or miscellaneous enzymic activity. However, one feature that they all have in common is a role in the function of restoring the delicate homeostatic balance disturbed by injury, tissue necrosis or infection (Koj *et al*., 1985).

Infectious diseases depress circulating retinol and contribute to vitamin A depletion. Enteric infections may alter the absorptive surface area, compete for absorption-binding sites, and increase urinary loss (Solomons *et al*., 1981, Feachem *et al*., 1987). Febrile systemic infections also increase urinary loss (Thurnham *et al*., 1991) and metabolic utilization rates and may reduce apparent retinol stores if fever occurs frequently (Campos *et al*., 1987). Thus the serum concentrations of both ROH and RBP are reduced in the presence of high levels of CRP and AGP.

Distribution of Vitamin A deficiency

This study found that the proportion of children who with Adjusted Plasma RBP <0.83 µmol/L was 40.8%. This proportion increase with Age group from 32.4% in children 12-23 month to 49.0% in children 47-59 months. Also, this proportion decrease with increasing Socio – economic status Quintile from 41.2% in the poorest category to 24.0% in the richest category. This results showed that VAD is a major micronutrient deficiency in the study population. The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency (VAD). In addition, goblet cell numbers are reduced in epithelial tissues and as a consequence, mucous secretions (with their antimicrobial components) diminish. Cells lining protective tissue surfaces fail to regenerate and differentiate, hence they flatten and accumulate keratin. Both factors—the decline in mucous secretions and loss of cellular integrity—reduce the body's ability to resist invasion from potentially pathogenic organisms. Thus VAD renders the child susceptible to infection.

Vitamin A deficiency and nutritional status

The proportion of VAD in this study was significantly higher amongst children with Severe stunting, Moderate underweight, moderate and severe wasting ($p<0.05$). Significant correlations between VAD and stunting ($r=0.113$, $p<0.05$) but not with wasting ($r=0.014$, $p=0.808$) and underweight ($r=-0.070$, $p=0.266$) was also found.

For the first time, this study demonstrated the strong association between nutritional status and VAD in Cameroon. VAD was strongly associated to stunting (a phenomenon that takes a longer time to set in) but not with underweight and wasting (which are often caused by sudden weight loss due to infection or low food intake). These findings support the fact that VAD is a common cause of malnutrition. This can be explained by the fact that the mechanism of vitamin A action within cells outside the visual cycle is that cellular functions are mediated through specific nuclear receptors. Binding with specific isomers of retinoic acid (i.e. all *trans*-and 9-*cis*-retinoic acid) activates these receptors. Activated receptors bind to DNA response elements located upstream of specific genes to regulate the level of expression of those genes (Pemrick *et al*., 1994). These retinoid-activated genes regulate the synthesis of a large number of proteins vital to maintaining normal physiologic functions.

Severe protein–energy malnutrition affects many aspects of vitamin A metabolism, and even when some retinyl ester stores are still present, malnutrition—often coupled with infection—can prevent transport-protein synthesis, resulting in immobilization of existing vitamin A stores (Arroyave *et al*., 1961).

Vitamin A deficiency and Anemia

Vitamin A deficiency was strongly correlated to anemia in this study ($r=0.876$, $P<0.05$). This can be explained by the fact that Vitamin A is also involved in the production of red blood cells, which are derived from stem cells that are dependent upon retinoids for their proper differentiation. In addition, vitamin A appears to facilitate the mobilisation of iron stores to developing red blood cells, where it is incorporated into the oxygen carrier haemoglobin

Association between different VAD cut-offs

It was demonstrated for the first time that the relationship between the WHO (Plasma ROH <0.70 µmol/L) and the Engle-stone *et al*., 2014 (Plasma RBP <0.83 µmol/L) cutoffs in the Cameroon context. The strong correlation ($r=0.878$, $p<0.0001$) between these two cut-offs suggests that both cut offs can be used for the Cameroonian population without major concerns. However, Correcting for sample size, it was noticed that the adjusted RBP cut-off gave higher prevalence of VAD (40.8%) compared to the adjusted ROH cutoff (37.1%). This study thus recommends that these cutoffs can be used in Cameroon for the population estimation of VAD.

Vitamin A deficiency and food consumption patterns

This study established that 83.7% of the study population consumed food group sources of vitamin A and this proportion decreases with increasing SES quintile. This means that the rich and richest groups consumed less food sources of Vitamin A. Also, the proportion of children who consumed food sources of vitamin A decreased with age. This indicates that the proportion of children within the 47-59 months age group consumed less sources of Vitamin A. A strong association was observed between serum RBP and Food Consumption score ($r=0.603$, $p<0.01$). The decrease in the consumption of Vitamin A sources with age group may partly explain the increase VAD with age group. The main source of Vitamin A consumed by the study population is from animal source (38.1%). This study ***demonstrates for the first time,*** the relationship between food consumption and VAD in the North and Far North regions of Cameroon.

3.12.2. Determinants of Vitamin A Deficiency

The important dietary sources of pre-formed vitamin A are animal products such as egg and liver. In addition, β-carotene, which can be found in orange-yellow vegetables and fruits such as mango as well as in red palm oil can be converted to retinoids (34). All of the above foods can be found in the North and Far North Regions of Cameroon. A strong variation in the prevalence of VAD with Health district and a possible explanation could be food availability. Mango, carrots and tomatoes supply might partly explain seasonal variation in vitamin A status.

It seems likely that vitamin A status follows the intake of vitamin A rich foods and to a lesser degree age and nutritional status since Food consumption score was a strong predictor for VAD, but age and nutritional status were not. If the WHO guidelines were to be followed, special attention should be given to improving vitamin A status in risk situations, e.g. in the dry season when Vitamin A rich foods are scarce.

For the first time, this study found out that in Cameroon, VAD is most common in populations consuming most of their vitamin A needs from provitamin carotenoid sources and where minimal dietary fat is available. In this study, Food consumption score was one of the predictors of VAD ($\beta=-0.066$, $p=0.030$). The negative β coefficient indicates that the higher the food consumption score, the lower the probability of VAD. This can be explained by the fact that about 90% of ingested preformed vitamin A is absorbed, whereas the absorption efficiency of provitamin A carotenoids varies widely, depending on the type of plant source and the fat content of the accompanying meal (Erdman *et al*., 1988). Where possible, an increased intake of dietary fat is likely to improve the absorption of vitamin A in the body.

Another determinants of VAD was nutritional status. All the three indices of nutritional status were positive predictors for VAD. But stunting was highly associated with VAD compared to wasting and underweight. This can be explained by the fact that retinol and retinoic acid are important signalling molecules that act to alter the transcriptional activation or repression of numerous genes. Several of these retinoid-controlled genes are involved in growth and differentiation, such as those involved in the differentiation of the three germ layers, organogenesis and limb development during embryogenesis. Retinoic acid exerts its effect through its binding to retinoic acid receptors (members of the steroid hormone superfamily of proteins), where the vitamin-receptor complex interacts with the genes.

The fact that age was a predictor of VAD was due to the fact that age was very strongly stunting and in this model, Multiple co-linearity was present between stunting, wasting and age. This explains why age was one of the predictors for VAD. The increase in VAD with age can also be explained by the fact that the proportion of children consuming food sources of Vitamin A decreased with increasing age-group.

The last predictor for VAD observed in this study was the primary caregiver level of education. This can be explained by the fact that the level of education of the primary care is related to the food frequency consumption of the children and the diet diversity. The higher the level of education the better the knowledge on the use of sources of Vitamin A.

Vitamin A is required for the formation of the photoreceptor rhodopsin, which is a complex of retinal and the vision protein opsin, where retinal functions as the chromophore. Rhodopsins are found in animals and green algae where they act as regulators of light-activated photochannels, and in archaea where they act as light-driven ion pumps. In animals, the light-sensitive pigment rhodopsin occurs embedded in the membrane of rod cells in the retina at the back of the eye. When light passes through the lens, it is sensed in the retina by both rod cells (black and white vision) and cone cells (colour vision). In rod cells, the exposure of rhodopsin to light causes 11-cis-retinal to be released from opsin, resulting in a conformational change in the photoreceptor that activates the G-protein transducin. Transducin activation leads to the closure of the sodium channel in the membrane and the hyperpolarisation of the rod cell, which propagates a nerve impulse to the brain that is perceived as light. Rod cells are especially important for night vision as they can detect very small amounts of light. Inadequate amounts of retinol can led to Night Blindness and corneal malformations, therefore eating carrots does let you see better in the dark!

In gene expression, Retinol and retinoic acid are important signalling molecules in vertebrates that act to alter the transcriptional activation or repression of numerous genes. Several of these retinoid-controlled genes are involved in growth and differentiation, such as those involved in the differentiation of the three germ layers, organogenesis and limb development during embryogenesis. Retinoic acid exerts its effect through its binding to retinoic acid receptors (members of the steroid hormone superfamily of proteins), where the vitamin-receptor complex interacts with the genes. Two families of receptors interact with vitamin A: the retinoic acid receptor (RAR) family that bind all-trans-retinoic acid (and 9-cis-retinoic acid), and the retinoic acid X receptor (RXR) family that bind only 9-cis retinoic acid. Together these receptors can regulate the rate of gene expression. Both vitamin A deficiency and excess can cause birth defects.

At the level of the immune system, Vitamin A is required for the normal functioning of the immune system. Retinol and its derivatives are required for the maintenance of the skin and mucosal cells that function as a barrier against infection, and are also required for the development of white blood cells that play a critical role in mounding an immune response. For example, the activation of T-cell lymphocytes requires the binding of the RAR receptor to retinoic acid. A deficiency in vitamin A can cause the mucosal membranes to atrophy, decreasing resistance to infection, and can increase the severity of infection. As such, vitamin A deficiency can be regarded as a nutritionally acquired immunodeficiency disease.

Furthermore, Vitamin A intake has a complex relationship with cancer prevention: while small doses of vitamin A or beta-carotene appear to help prevent cancer, higher doses seem to have the reverse effect. The anti-cancer effects of beta-carotene appear to stem from its anti-oxidative ability to scavenge for reactive oxygen species, as well as through its conversion to vitamin A, which can improve immune function in addition to eliciting an anti-proliferative effect through the RAR and RXR receptors, thereby acting to block certain carcinogenic processes and inhibit tumour cell growth. However, an excessive intake of beta-carotene appears to have carcinogen effects, possibly through its promotion of the eccentric (or asymmetric) pathway of beta-carotene cleavage, which produces breakdown products that might lead to the destruction of retinoic acid through the activation of the P450 enzyme, which in turn could decrease retinoid signalling leading to enhanced cell proliferation. Therefore dosage seems to be an important factor in beta-carotene action.

3.13. Anemia and Iron deficiency

3.13.1. Anemia

NO significant difference was observed between the mean Hemoglobin values for the North and Far north region. As expected, the mean Heamoglobin significantly increase with age. This can be attributed to the fact that as the child grows, the energy needs are increasing and the amount of Heamoglobin required to meet up with the rapidly increasing metabolism is on the rise. This justifies the increase in heamoglobin with age. Also, this study showed that that the mean value of heamoglobin increased with increasing SES Quintile indicating that the rich and richest quintiles presented the lowest prevalence of anemia. This can be explained by the fact that the rich and richest groups consume more of meat and fish that ameliorate the bioavailability of iron for absorption.

About half of the basal iron losses are from blood and occur primarily in the gastrointestinal tract. Both these losses are influenced by the haemoglobin level; during the development of an iron deficiency, menstrual and basal iron losses will successively decrease when the haemoglobin level decreases.

An elevated CRP (>5 mg/L) was negatively associated with hemoglobin, notwithstanding the relatively small number of subjects ($n = 302$) with elevated values indicative of underlying acute infection or inflammation. The latter is often accompanied by anemia, commonly termed anemia of chronic disease (ACD), which results from the effects of cytokines. Apparently normal or increased iron stores are present in ACD (Means *et al*., 1992).

Plasma ferritin was also a significant predictor of hemoglobin, although its importance, based on the absolute value of its standardized coefficient, was less than that for plasma zinc. Dietary factors probably account in part for the positive association between plasma ferritin and hemoglobin noted here.

Unlike ferritin, neither plasma vitamin B-12 was a significant predictor of hemoglobin. This discrepancy is attributed to nonfunctional vitamin B-12 analogs present in the plasma of children consuming fermented cereals, as discussed earlier, as well as the absence of any hematopoietic defects associated with vitamin B-12-deficiency anemia. Plasma folate was also not a significant predictor of hemoglobin. This finding may indicate some folate (or pyridoxine) deprivation, although plasma folate may not reflect true folate status in malarial endemic areas such as Garoua and Maroua, where levels may be elevated through erythrocyte hemolysis induced by malaria.

3.13.2. Distribution of Iron Deficiency

Choice of marker for Iron deficiency

In this study and in consonance with WHO guidelines, three markers of iron status (Plasma Ferritin, Plasma Soluble Transferrin Receptor (sTfR), and body iron stores (BIS)) were used in order to understand the iron status of the sampled population. The fact that these three markers reflect different metabolic processes, the results of these markers could not be compared directly. The WHO recommended Plasma ferritin to be used for the assessment of iron status in population. Given that this is an acute phase protein that is influenced by infection, plasma concentrations of ferritin was adjusted for the presence of infection.

According to table 53, the prevalence of iron deficiency varied from one indicator to another. The highest prevalence was according to sTfR>8.30 mg/L (77.3%) followed by Adjusted Body Iron (T) < 0mg/kg (63.1%), then Adjusted Plasma ferritin (T) <12 µg/L (29.3%). This difference can be explained by the fact that plasma concentrations of ferritin, an iron storage protein, decrease with falling iron stores; however, ferritin concentrations are insensitive to further change during severe ID or negative iron balance (Skikne *et al*., 1990). In contrast, soluble transferrin receptor (sTfR) concentrations generally begin to change only after iron stores (in the form of ferritin) are depleted (). Body iron stores (BIS) can be estimated using the ratio of sTfR: ferritin20 and this indicator is thought to reflect iron status over a wider range of iron stores (i.e., from decreased storage iron to functional tissue ID).Also, no significant difference was obtained with iron markers were adjusted for inflammation using acute phase proteins as continuous variables and when they were adjusted using the Thurnham adjustment method.

Prevalence of Iron deficiency

Presence of inflammation

This is the first time that a study in the North and Far North regions is demonstrating a strong negative association between markers of iron deficiency and markers of inflammation and infection. This indicates that iron deficiency is one of the primary risk factors for infection and inflammation in the study population. This can be justified by the implication of iron in the regulation of the immune system.

Iron is a fundamental element for normal development of immune system. Iron is essential for proper cell differentiation and cell growth. It is an important component of peroxide-generating enzymes and nitrous oxide-generating enzymes that are critical for proper enzymatic functioning of immune cells (Beard *et al*., 2001). It is also involved in regulation of cytokine production and action as well as in the development of cell mediated immunity. Spear and Sherman demonstrated that iron is an integral component of enzyme myeloperoxidase (MPO),

which produces reactive oxygen intermediates responsible for intracellular killing of pathogens (Spear *et al*., 1992). Humoral and cell mediated immunity both have been studied extensively, mainly in vitro, in relation to iron deficiency in both humans and animals. Impairment of cell mediated immunity have been well described in iron-deficient humans. Various abnormalities of cellular defences observed in iron deficiency include:

- Reduced neutrophil function with decreased myeloperoxidase (MPO) activity
- Impaired bactericidal activity
- Depression of T-lymphocyte numbers with thymic atrophy
- Defective T lymphocyte-induced proliferative response
- Impaired natural killer cell activity
- Impaired interleukin-2 production by lymphocytes
- Reduced production of macrophage migration inhibition factor
- Reversible impairment of delayed cutaneous hypersensitivity including tuberculin reactivity.

Decreased MPO activity gets reversed on correcting iron deficiency. Neutrophil and macrophage dysfunction has been associated with low iron levels, as evidenced by deficient nitroblue tetrazoleum reduction and hydrogen peroxide formation in these respective cell lines (Markel *et al*., 2007).

Ribonucleotide reductase activity has been discovered to be iron dependent. Iron levels have also been shown to alter the proliferation of T helper (Th)-1 and Th-2 subsets, likely related to the difference in dependence of cells on transferrin-related iron uptake. Th-2 clones possess larger pools of iron susceptible to chelation, as compared with Th-1 cells, making Th-1 immune pathways more susceptible to changes in ambient iron concentrations.

In summary, iron deficiency depresses certain aspects of cell-mediated immunity and innate immunity but the significance of hypoferremia (as opposed to normal transferrin saturation) on growth of microorganisms remains to be clarified

Age

Results showed that the mean values of both plasma ferritin and Body iron stores increase significantly with age (Figure 11) while the mean concentration of plasma sTfR decrease significantly with Age. This can be explained by the fact that in the term infant, iron requirements rise markedly after age 4–6 months and amount to about 0.7–0.9 mg/day during the remaining part of the first year. These requirements are very high, especially in relation to body size and energy intake. In the first year of life, the term infant almost doubles its total iron stores and triples its body weight. The increase in body iron during this period occurs mainly during the latter 6 months. Between 1 and 6 years of age, the body iron content is again doubled. The requirements for absorbed iron in infants and children are very high in relation to their energy requirements.

Region

Also, the prevalence of iron deficiency was significantly higher for the Far North Region compared to the North Region (34.8% vs 24.6%). This can be attributed to the fact that the Far North region closer to the Sahelian belt with respect to the North region presents relatively high annual temperatures and low humidity. Iron is not actively excreted from the body in urine or in the intestines. Iron is only lost with cells from the skin and the interior surfaces of the body—intestines, urinary tract, and airways. The total amount lost is estimated at 14µg/kg body weight/day (Green *et al*., 1968). In children, it is probably more correct to relate these losses to body surface. The range of individual variation has been estimated to be $\pm 15\%$. Earlier studies suggested that sweat iron losses could be considerable, especially in a hot, humid climate (FAO/WHO 1988).

Vitamin A status

An association between serum vitamin A levels and iron status, as have others (Ahmed *et al*., 1996, Wolde-Gebriel *et al*., 1993). Children with low vitamin A status had significantly reduced ferritin and Hb levels. Thus, this study agrees with the suggestions that have been made that programmes designed to reduce anaemia should include efforts to improve vitamin A status (Bloem *et al*., 1995). These data suggest that both severe vitamin A deficiency and iron deficiency are independent risk factors for anemia among preschool children in the Marshall Islands.

This is the first study to characterize the association of vitamin A deficiency, iron deficiency, and inflammation with anemia among preschool children in Cameroon. Severe vitamin A deficiency was independently associated with anemia among preschool children, both in univariate models and in multivariate models that adjusted for iron deficiency, sex, age, and inflammation. Iron deficiency is a major cause of anemia among preschool children (Allen *et*

al., 2001), and as would be expected, iron deficiency was also independently associated with increased risk of anemia.

Socioeconomic status and Milieu

Prevalence of iron deficiency varied by SES (p=0.03), and Prevalence of iron depletion was strongly associated with SES (p=<0.01) with the lowest anemia prevalence in the highest SES quintile. Prevalence of iron deficiency was 17.9% in the highest quintile compared to 29.1% in the remaining four SES quintiles (estimated prevalence decreased significantly with increasing SES quintile). This is most probably due to the fact that those in the rich group have a high food Diversity score and consume most of their iron in meat and fish which increases the bioavailability of iron for absorption. ***This is the first study to characterize the association of Iron deficiency with Milieu and the socio economic status quintile in the North and Far North Regions of Cameroon***.

3.13.3. Iron deficiency and nutritional status

The results of this study showed that two markers of iron status (Body iron stores and plasma ferritin) were significantly associated with all indices of malnutrition. Both the univariate and multivariate regression models identified stunting, wasting and underweight as risk factors for Iron Deficiency. Also the univariate models demonstrated that association of severe forms of all indices of malnutrition with iron deficiency was higher compared to the moderate forms.

Once more, this demonstrates for the first time the association between nutritional status and iron deficiency in the North and Far North regions of Cameroon.

Studies in animals have clearly shown that iron deficiency has several negative effects on important functions in the body (Dallman *et al*., 1986). The physical working capacity of rats is significantly reduced in states of iron deficiency, especially during endurance activities (Edgerton *et al*., 1972, Finch *et al*., 1976). This negative effect seems to be less related to the degree of anaemia than to impaired oxidative metabolism in the muscles with an increased formation of lactic acid. Thus, the effect witnessed seems to be due to a lack of iron-containing enzymes which are rate limiting for oxidative metabolism (Scrimshaw *et al*., 1984). Further to this, several groups have observed a reduction in physical working capacity in human populations with longstanding iron deficiency, and demonstrated an improvement in working capacity in these populations after iron administration.

The relationship between iron deficiency and brain function and development is very important to consider when choosing a strategy to combat iron deficiency (Lozoff *et al*., 1991, Youdim *et al*., 1988, Beard *et al*., 1993, Pollitt *et al*., 1993). Several structures in the brain have a high iron

content; levels are of the same order of magnitude as those observed in the liver. The observation that the lower iron content of the brain in iron-deficient growing rats cannot be increased by giving iron at a later date strongly suggests that the supply of iron to brain cells takes place during an early phase of brain development and that, as such, early iron deficiency may lead to irreparable damage to brain cells. In humans about 10% of brain-iron is present at birth; at the age of 10 years the brain has only reached half its normal iron content, and optimal amounts are first reached between the ages of 20 and 30 years.

Iron deficiency also negatively influences the normal defence systems against infections. In animal studies, the cell-mediated immunologic response by the action of T-lymphocytes is impaired as a result of a reduced formation of these cells. This in turn is due to a reduced DNA synthesis dependent on the function of ribonucleotide reductase, which requires a continuous supply of iron for its function. In addition, the phagocytosis and killing of bacteria by the neutrophil leukocytes is an important component of the defence mechanism against infections. These functions are impaired in iron deficiency as well. The killing function is based on the formation of free hydroxyl radicals within the leukocytes, the respiratory burst, and results from the activation of the iron-sulfur enzyme NADPH oxidase and probably also cytochrome b (a haem enzyme) (Brock *et al*., 1994).

Several groups have demonstrated a relationship between iron deficiency and attention, memory, and learning in infants and small children. In the most recent well-controlled studies, no effect was noted from the administration of iron. This finding is consistent with the observations in animals.

3.13.4. Determinants of Iron Deficiency

For the first time this study was able to identify key risk factors that are associated with Iron deficiency. Bivariate analysis revealed that the following factors were associated with iron deficiency:

- Age:
- Vitamin A status
- Caregiver level of education: this associated with Food Frequency consumption and dietary diversity
- Socio economic status
- Nutritional status (stunting, wasting and underweight)
- Region and milieu (urban vs rural)
- Presence of infection and inflammation

The univariate and bivariate regression models identified two additional determinants of iron deficiency:

- Plasma folate and
- Diet diversity

Thus children most likely to have Iron deficiency are children who are anemic, suffering from one or more forms of malnutrition (underweight, wasting or stunting), having low folate and Vitamin A with a limited dietary diversity in their nutritional habits.

These associations can be attributed to the fact that the absorption of iron is dependent on the body's iron stores, hypoxia and rate of erythropoiesis. These factors are related to a multitude of other biochemical mechanisms required to the regulation of iron metabolism. Two models have been proposed to explain how the absorption of iron is regulated. These models have been termed i) the crypt programming model and ii) the hepcidin model (Pietrangelo *et al*., 2004). The crypt programming model proposes that the crypt cells sense body iron levels, which in turn regulate the absorption of dietary iron via the mature villus enterocytes. The second model proposes that liver hepcidin, which is regulated by a number of factors such as liver iron levels, inflammation, hypoxia and anaemia, is secreted into the blood and interacts with villus enterocytes to regulate the rate of iron absorption. There is evidence to support both models and it is possible that both control mechanisms may contribute to the regulation of iron absorption. The crypt programming model proposes that enterocytes in the crypts of the duodenum take up iron from the plasma. The intracellular iron level of the crypt cells corresponds to the body's iron stores, which in turn determines the amount of iron absorbed from the gut lumen as these crypt cells migrate upwards to become absorptive cells at the brush border. The crypt cells express both TfR1 and TfR2 which mediate the cellular uptake of transferrin-bound iron from plasma.

3.14. Low plasma Zinc Concentration (PZC)

3.14.1. Prevalence of Low PZC deficiency

Region

For the first time, this study was able to document that the prevalence of APP adjusted Low plasma zinc concentration was higher for the Far North regions compared to the North region. This significant difference can be attributed to the feeding patterns that are widely different between the two regions given that results showed that the proportion of households consuming food sources of Zing is significantly higher for the North region compared to the far North region (82.6% versus 77.4%, $p<0.05$). Though the proportion of households with acceptable food consumption is higher in the Far North region, the proportion of acceptable diet

diversity is lower in this region. Thus it is highly probable that the difference in low PZC can be attributed to Food consumption patterns.

Biochemically, lean red meat, whole-grain cereals, pulses, and legumes provide the highest concentrations of zinc: concentrations in such foods are generally in the range of 25–50mg/kg (380–760mmol/kg) raw weight. – These are the main foods consumed in these regions. Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc, having concentrations <10mg/kg (<150mmol/kg) (Sandström *et al.*, 1989). Saturated fats and oils, sugar, and alcohol have very low zinc contents. The utilization of zinc depends on the overall composition of the diet. Experimental studies have identified a number of dietary factors as potential promoters or antagonists of zinc absorption (Sandström *et al.*, 1989). Soluble organic substances of low relative molecular mass, such as amino and hydroxy acids, facilitate zinc absorption. In contrast, organic compounds forming stable and poorly soluble complexes with zinc can impair absorption. In addition, competitive interactions between zinc and other ions with similar physicochemical properties can affect the uptake and intestinal absorption of zinc.

Nutritional Status

Secondly, these results documented for the first time a very significant relationships between stunting (n=303, r=0.134, p<0.01) and wasting (n=303 r=0.147, p<0.01) and Plasma zinc concentration in the North and Far North regions of Cameroon. However, the association of plasma zinc concentration with underweight was not signification.

More than 200 enzymes require zinc as a functional component, and these enzymes affect most major metabolic processes. Despite the diversity of functions that zinc metalloenzymes affect, correlations between loss of enzyme activity and characteristics of zinc-deficiency have proved unsuccessful. In animal models, growth inhibition occurs before changes occur in tissue zinc concentrations. Hence, changes in zinc metalloenzymes are not considered the first limiting effect on growth in zinc deficiency (Chesters *et al.*, 1991). A direct role for zinc in DNA and protein synthesis is also evident. Zinc is present in the cell nucleus, nucleolus and chromosomes, and zinc stabilizes the structure of DNA, RNA and ribosomes (Wu *et al.*, 1987). Numerous enzymes associated with DNA and RNA synthesis are also zinc metalloenzymes, including RNA polymerase, reverse transcriptases and transcription factor IIIA. The zinc in these enzymes is tightly bound and forms a variety of structures that are functionally important to the enzyme. One common structure is the zinc finger domains in which the zinc ion forms a loop in the polypeptide chain by creating a bridge between cysteine and histidine residues. Many proteins containing zinc fingers have been discovered, with this motif being one of three considered fundamental for eukaryotic regulatory proteins to bind specific DNA sequences.

Bunce *et al*., 1994) reviewed the relationships among the clinical effects of zinc deficiency on embyrogenesis, growth and differentiation and regulation of the nuclear hormone receptor superfamily. Because these receptors are regulated by zinc finger domains, limited zinc availability may impair their responsiveness and thereby explain the reproductive effects of zinc deficiency. It appears likely that transcription factor control of gene expression is a site for zinc regulation.

Also, the pituitary contains a higher concentration of zinc than other organs, and zinc enhances pituitary hormone function (Henkin *et al*., 1976). As the pituitary is the source of GH, a primary endocrine regulator of somatic growth, several studies have investigated the role of GH in the inhibition of growth due to zinc deficiency. Zinc deficiency caused failure of GH secretion from the pituitary (Root *et al*., 1979), and circulating GH concentrations are decreased by zinc deficiency in rats (Roth *et al*., 1997). The level of GH in the blood of rats fed a zinc-deficient diet or pair-fed for 2 d was lower than zinc-adequate controls.

Thirdly, growth hormone contains a zinc-binding site that is structurally and functionally important (Cunningham *et al*., 1991). At concentrations of zinc greater than micromolar, zinc promotes the formation of a GH dimer. The high concentrations of zinc in the pituitary, therefore, may provide for formation of dimerized GH, which is less susceptible to degradation. Dimerized GH has a low affinity for GH receptors, so the presence of high concentrations of zinc in pituitary secretions may prevent the association of GH with cellular receptors proximal to the pituitary. This may be necessary to ensure GH reaches receptors in the periphery. The binding of GH to the prolactin receptor, but not to the GH receptor, requires zinc (Cunningham *et al*., 1990). The presence of 50 μmol/L zinc resulted in an 8000-fold increase in binding affinity of GH to the prolactin receptor. In contrast, these concentrations of zinc slightly inhibited GH binding to the GH receptor. Because prolactin receptors mediate lactogenic responses and GH receptors mediate somatogenic responses, the dependence of prolactin receptors on zinc does not fully correlate with the observed growth inhibition of zinc-deficient animals. However, GH and prolactin receptors belong to the cytokine superfamily of receptors (Cunningham *et al*. 1990), so future work may demonstrate other roles for zinc in mediating the activity of these hormones.

Results from zinc supplementation studies suggest that a low zinc status in children not only affects growth but is also associated with an increased risk of severe infectious diseases (Black *et al*., 1998). Episodes of acute diarrhoea were characterized by shorter duration and less severity in zinc-supplemented groups; reductions in incidence of diarrhoea were also reported. Other studies indicate that the incidence of acute lower respiratory tract infections and malaria

may also be reduced by zinc supplementation. Prevention of suboptimal zinc status and zinc deficiency in children by an increased intake and availability of zinc could consequently have a significant effect on child health in developing countries.

3.14.2. Determinants of Low PZC deficiency

Results identifed for the first time risk factors associated with zinc and other micronutrients in the North and Far North regions of Cameroon. Amongst the eight determinants strongly associated with low plasma zinc concentration and explaining 78.6% of the total association observed, are Body iron stores, Plasma ferritin, Plasma vitamin B12 and Hemoglobin. The other determinants of low PZC were the consumption of food sources of Zinc and the age of the child.

Hemoglobin concentrations were the strongest predictor of Plasma zinc, followed by the indicator variables of food consumption, body iron stores (<0mg/kg), and plasma ferritin (≥12 μg/L). Moreover, there were no significant pairwise correlations linking the independent variables, including plasma ferritin and zinc (data not shown). The strong positive association between plasma zinc and hemoglobin was unexpected, although it has been reported in some earlier studies (Jameson *et al*., 1976, de Jong *et al*., 2002, Folin *et al*., 1994). There have also been a few intervention studies in which the addition of zinc alone (Smith *et al*., 1999), or zinc and iron relative to iron alone (Alarcon *et al*., 2004), has improved the hematological response of young children who were thought to be zinc deficient. Several mechanisms may be involved whereby zinc affects hemoglobin concentrations. Zinc is implicated in hemoglobin synthesis through the activity of several zinc-dependent enzyme systems, including aminolevulinic acid dehydrase that mediates a step in the synthesis of heme (Garnica *et al*., 1981) and thymidine kinase and DNA polymerase, which are involved in DNA synthesis. More recently, the zinc-finger transcription factor, GATA-1, has also been confirmed as essential for normal erythropoiesis (Labbaye *et al*., 1995). Other potential mechanisms may involve the stimulation of hematopoiesis by zinc-induced increases in plasma insulin-like growth factor-1 levels (Nishiyama *et al*., 1999) and the role of zinc in stabilizing cell membranes (Dash *et al*., 1974).

The synergy between markers of iron status (body iron stores and ferritin) and plasma zinc can be explained by the fact that Iron and zinc are essential micronutrients for human growth, development, and maintenance of the immune system. Iron is needed for psychomotor development, maintenance of physical activity and work capacity, and resistance to infection (Stoltzfus *et al*., 2001). Zinc is needed for growth and for maintenance of immune function, which enhances both the prevention of and recovery from infectious diseases (Black *et al*., 2003). Meat products are the best source of both iron and zinc. Consequently, iron and zinc deficiencies may coexist in populations that consume diets with insufficient amounts of animal-

source foods. The intake of these 2 micronutrients would ideally be improved through enhanced dietary quality, but food fortification or supplementation programs may also be needed.

The association between plasma zinc concentration and the consumption of food sources rich in zinc can be justified by the fact that a number of dietary factors can act as potential promoters or antagonists of zinc absorption (Sandström *et al*., 1989).Soluble organic substances of low relative molecular mass, such as amino and hydroxy acids, facilitate zinc absorption. In contrast, organic compounds forming stable and poorly soluble complexes with zinc can impair absorption. In addition, competitive interactions between zinc and other ions with similar physicochemical properties can affect the uptake and intestinal absorption of zinc. The risk of competitive interactions with zinc seems to be mainly related to the consumption of high doses of these other ions, in the form of supplements or in aqueous solutions.

3.15. Plasma Folate and Vitamin B 12

This is the first study to assess the determinants of Folate and Vitamin B12 deficiencies in the target population. The following variables were identified as predictors for Vitamin B 12 and Folate

- Adjusted Body Iron < 0 mg/kg
- Age of Child
- Haemoglobin < 110g/l
- Dietary diversity Score Adequacy
- Underweight
- Adjusted Plasma RBP
- Adjusted Plasma ferritin
- Stunting
- Care Giver Level of Education

Though vitamin B 12 was associated with Stunting, this was not the case with Folote. Howerver, folate was associated with underweight. This coincidence in the predictors of both vitamin B12 and Folate can be explained by the role of both micronutrients in the methylation cycle. One of the vitamin B12-dependent enzymes, methionine synthase, functions in one of the two folate cycles, namely, the methylation cycle. This cycle is necessary to maintain availability of the methyl donor, S-adenosylmethionine. Interruption of the cycle reduces the level of S-adenosylmethionine. Disruption of the methylation cycle also causes a lack of DNA biosynthesis and anaemia. The methyl trap hypothesis is based on the fact that once the cofactor 5,10-methylenetetrahydrofolate is reduced by its reductase to form 5-methyltetrahydrofolate, the reverse reaction cannot occur. This suggests that the only way for the 5-

methyltetrahydrofolate to be recycled to tetrahydrofolate, and thus to participate in DNA biosynthesis and cell division, is through the vitamin B12-dependent enzyme methionine synthase.

Results thus confirm the fact that folic acid and Vitamin B-12 serve as coenzymes in one-carbon metabolism have strong interactions. Specifically, a carbon unit from serine or glycine reacts with tetrahydrofolate (THF) to form methylene-THF. This may be used for the synthesis of thymidylate, a DNA nucleotide, or for purine synthesis. Folate deficiency-related macrocytic anemia is due to failure of precursor blood cells to divide because of a lack of DNA. The adverse effect of vitamin B-12 deficiency on DNA synthesis is explained by the "methylfolate trap hypothesis" (Selhub *et al*., 2009). Vitamin B-12 acts as a cofactor for methionine synthase, which catalyzes the remethylation of homocysteine to methionine. The methyl group is donated by methyl-THF, which is derived by the irreversible reduction of methylene-THF to methyl-THF by methylene-THF reductase. If methionine synthase is inactivated by a lack of vitamin B-12, the result is a functional folate deficiency (ie, a lack of the nonmethylated folates needed for serine-glycine interconversion and the synthesis of purines and pyrimidines) as folate becomes increasingly "trapped" as methyl-THF.

CONCLUSION AND PERSPECTIVES

4.1. Conclusion

This study set out with the following key objectives: to determine the food consumption patterns among children 12-59 months of age, to assess the nutritional Status and prevalence of micronutrient deficiencies (iron, zinc, folate, and vitamins A and B12, Calcium, Magnesium and Copper) of these children and finally to determine the predictors (or risk factors) of micronutrient deficiencies in the target group. Consequently, the following conclusions were arrived at:

As per the determination of the food consumption patterns among children 12-59 months,:

- The three main food groups consumed in the North and Far North regions are Cereals (91.2%), vegetables (51.7%), and meat (38.1%). This high consumption of these three food groups was attributed to the feeding habits of this area who have cereals as the base and Meat and vegetables as the complement. Participants from the North region presented a relatively better food frequency and Diet diversity when compared to participants from the Far North Region.

Regarding the assessment of the nutritional Status of children 12-59 months in the north and Far North Regions of Cameroon,:

- The mean HAZ indicated that stunting is a major issue but the mean WHZ and WAZ confirmed that stunting is more prevalent in the population compared to wasting and underweight. The prevalence of stunting, wasting and underweight were respectively 42.5%, 6.6% and 26.0%. The prevalence of stunting, wasting and underweight were all significantly higher for the Far North Region compared to the North region ($P<0.001$).

As for the evaluation of the micronutrient deficiencies among children 12-59 months,:

- The mean CRP and AGP are higher for the North Region compared to the Far North region and this is reflected in the proportion of children in convalescence state higher for the North (46.1%) than the Far North (33.8%). Mean CRP and AGP values decrease with SES quintile moves from Poorest to Richest. The severely stunted have higher mean values than the mildly stunted and also higher prevalence of children in category 1 and category 2. The severely underweight have lower mean values than the mildly underweight and also lower prevalence of children in category 1 and category 2 .Severe wasting just like stunting have higher mean values than the moderate group and also higher prevalence of children in category 1 and 2.
- Un-adjusted prevalence of VAD with respect to WHO standards (ROH <0.70 µmol/L) is 54.3% and after adjustment, this proportion is 37.1%. On the other hand, the overall proportion of VAD as defined by unadjusted plasma RBP<0.83 µmol/L is 32.8%. After

adjusting for acute phase proteins, this proportion is 40.8%. The proportion of VAD was highest in the 36-47 age groups for all indicators but for Adjusted Plasma RBP <0.83 µmol/L. Also, VAD significantly relatively decreases with increasing SES quintile. This study demonstrates for the first time that the relationship between the WHO (Plasma ROH <0.70 µmol/L) and the Engle-stone *et al.*, 2014 (Plasma RBP <0.83 µmol/L) cutoffs in the Cameroon context.

- Results showed that the mean global hemoglobin, sTfR, Ferritin and Body Iron Stores levels are respectively 99.9g/l, 15.6mg/l, 43.1 µg/l and 1.02 mg/kg. These are significantly higher for the North Region compared to the Far North region ($p<0.05$).
- Zinc deficiency as assessed by estimation of plasma zinc showed that 85.8% of the children were zinc-deficient. Younger children were at a lower risk of zinc deficiency than were older children; however, the prevalence was similar among children from the North and Far North Regions. The average unadjusted PZC was 49.25±0.79 µg/dl while the APP adjusted PZC was 51.38±0.81 µg/dl. There mean PZC was significantly higher for the North Region compared to the Far North region for both Adjusted and unadjusted markers of PZC. For the first time, this study documented that the prevalence of APP adjusted Low plasma zinc concentration was higher for the Far North regions compared to the North region.
- The overall prevalence of Low plasma folate was 9.8%, with a very high proportion in the Far North Region (16.7%) compared to the North Region (3.0%). Overall, the mean plasma folate was 21.14±0.90 nmol/L. Age wise, a gradual increase of the prevalence of low folate with age was observed. Children in the 36-47 months age group presented the highest proportion of low folate.
- The prevalence of global plasma Vitamin B12 deficiency was 39.4% with the North region presenting a higher proportion (43.9%) compared to the Far North region (34.8%). A significant decrease in the proportion of Vitamin B12 deficiency with age was observed. Overall, the mean plasma vitamin B12 was 294.39±16.62 pmol/L.
- The mean levels of Calcium, magnesium, copper and zinc were respectively 80.7±0.3, 19.9±0.1, 200.6±32.8, and 49.2±0.8 µg/dl. The mean serum level of Calcium and copper was significantly lower in the North region compared to the far North Region ($p<0.05$) while the serum concentrations of Magnesium and zinc were significantly lower in the Far North Region ($p<0.05$). Serum concentration of calcium, magnesium and copper decreased significantly with increasing age ($p<0.05$) while the serum concentration of zinc decreased with increasing age.
- The most prevalent micro nutrient deficiency is low PZC (85.8%) followed by anemia (63.75), then Vitamin A deficiency (40.8%) and low vitamin B12 (39.4%). Iron

deficiency was present in 29.3% of the study population while the least prevalent micronutrient deficiency is low folate (9.8%).

Concerning the determination of the predictors of micronutrient deficiencies (iron, zinc, folate, and vitamins A and B12) and Nutritional status among children 12-59 months of age

For the first time, risk factors that are associated with VAD were identified. In the multivariate model, VAD among children was positively associated with food consumption score, Age, stunting, wasting, and underweight and caregiver level of education. The model explained 81.1% of the variance in VAD.

Results showed strong positive correlation between APPs and sTfR, Ferriting and Body Iron Stores and a negative correlation between APPs and hemoglobin. The prevalence of ID was lowest according to unadjusted ferritin (22.7%) whereas the prevalence of ID was highest when defined by elevated sTfR (77.3%). Adjustment of ferritin and BIS for inflammation increased the measured prevalence of ID, regardless of the method of adjustment. The proportion of anemia associated with ID ranged from 30.3 to 88% in the target population, depending on the indicator applied.

Also, results documente for the first time a very significant relationships between stunting ($n=303$, $r=0.134$, $p<0.01$) and wasting ($n=303$ $r=0.147$, $p<0.01$) and Plasma zinc concentration in the North and Far North regions of Cameroon.

Univariate and multiple regression analysis showed that seven key factors were associated with ID which explain 68.1% of the overall variance in the ID. Thus children most likely to have Iron deficiency are children who are anemic, suffering from one or more forms of malnutrition (underweight, wasting or stunting), having low folate and Vitamin A with a limited dietary diversity in their nutritional habits.

This is the first study to characterize the association of vitamin A deficiency, iron deficiency, Nutritional status and inflammation with anemia among preschool children alongside the determination of risk factors for iron deficiency in Cameroon

Eight determinants are strongly associated with plasma zinc concentration, explaining 78.6% of the total variance observed. These are Body iron, plasma ferritin, the consumption of food sources of Zinc, the age of the child, the plasma Vitamin B12 content and anemia. Results identifed for the first time risk factors associated with zinc and other micronutrients in the North and Far North regions of Cameroon.

Seven determinants are strongly associated with Low folate concentration, explaining 53.7% of the total variance observed. These include, body iron stores, age, heamoglobin dietary diversity, Nutritional status (Underweight), plasma concentrations of Vitamin A and Iron

In the multivariate model, vitamin B12 deficiency among children was positively associated with Age, anemia, stunting, VAD and caregiver level of education. The model explained 74.1% of the variance in vitamin B12 deficiency. Further analysis in the univariate model shows that the key determinants of VAD is the study population are being between 12-23 months of age, being moderately or severely stunted, having anemia, and low plasma levels of Vitamin A. This is the first study to assess the determinants of Folate and Vitamin B12 deficiencies in the target population.

Significant correlations were found between serum zinc and SES quintile serum Zinc and Food consumption score and between serum Zinc and Diet diversity score. Copper was only correlated with diet diversity score while both Serum calcium and magnesium were correlated with age

- The prevalence of multi-micronutrient deficiency was high with up to 70.4% of the children presenting two or more forms of micronutrient deficiency. The proportion of children presenting two, three, four, five and six micronutrient deficiencies were respectively 20.5%, 26.3%, 29.3%, 14.8%, 3.0% and 0.3%. The difference in the prevalence of multiple micronutrient deficiency was not significant by region, sex, age group or SES quintile. This is the first time that a study in this target area is demonstrating the presence of multiple micronutrient deficiencies.

4.2. Perspectives

All these results indicate that further studies are required to better understand the impact of nutrition on multiple micronutrient deficiencies. As next steps, the study proposed to:

- Use these findings as evidence to explore des policies and possible mechanisms to strengthen nutritional diversity and Nutritional education in the target regions,
- Extend this study to other regions of the country to better contribute to the Health policy
- Perform further analysis to determine the causes and risk factors for malnutrition
- Characterize further the relationship between multiple micro nutrition deficiency, nutritional status and health.

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ANNEXES

4.3. Data collection Tools

Socio Economic Status Questionnaire

To be completed by Interviewer

Please complete before the Interview, where possible

1.1 Interviewer ID: |___| / |___|

Team / Interviewer

1.2 Date: |___|___| / |___|___| / 2010

Day Month

1.3 Cluster # |___|___| **1.4 Household #** |___|___| **Village/Quartier name:**_______________

1.5 Results code |___|

1 = completed

2 = partly completed

9 = Other, specify:

____

1.6 Language of interview |___|

1 = English **4 = Fufuldé**

2 = French **9 = Other, specify:**

3 = Pidgin _______________

1.7 Interpreter used? |___| **Y = 1, N = 2**

I would now like to ask you a few questions on the composition of your household.

A household is defined as a group of people currently eating from the same pot "under the same roof" (or in same compound if more than one structure). Members having left for more than 6 months and not planning on returning should not be considered part of the household. People having joined the household and planning to stay should be considered part of the household if they have been present for at least one month.

How many individuals live in your household that are __________(insert group below)?

Groups by age and/or sex	Number of individuals per household	Number lactating
0-11 months	**1.8** |__|__|	
12-59 months	**1.9** |__|__|	
5—15 years	**1.10** |__|__|	
Women of reproductive age, 15-49 years	**1.11** |__|__|	**1.12** |__|__|
Men older than 15 years	**1.13** |__|__|	

To be completed by Team Leader:

Signature of Team Leader: ____________

1.17 – Date: |___|___| / |___|___| / 2010

Day Month

1.18 - Team Leader ID: |___| / |___|

Team / Team Leader

Remarks:

To be completed by Data Entry Operators

1.19 Data Entry Date 1:

|___|___|/|___|___|/20|___|___|

Day Month

Women older than 49 years	1.14 |__|__|	
Total	1.15 |__|__|	

1.16	What main religion is practiced by the household? |__|		
	1 Christian – Catholic	5	Other religion, specify:
	2 Christian – Protestant		
	3 Muslim	6	No religion
	4 Traditional/Animist	9	No response

1.20 Data entry operator 1 ID |__|__|

Signature:___________________

1.21 Data Entry Date 2:

|__|__|/|__|__|/20|__|__|

Day Month

1.22 Data entry operator 2 ID |__|__|

Signature: _______________________

Remarks:

____

Now I would like to ask a few specific questions about the child, caregiver, and head of household.

		1.23	1.24	1.25	1.26
		Sex	**Relationship to household head**	**Marital Status**	**Level of education**
House-hold member code		1=Male 2= Female	1 = Household Head 2 = Spouse 3 = Son/Daughter 4 = Other relative 5 = Non related household member 6 = Foster child 9 = don't know/no response	1 = Single 2 = Married 3 = Living together with partner 4 = Divorced 5 = Widow or widower 9 = don't know/no response	1 = None 2 = Primary 3 = Secondary 4 = Higher education 5 = Other, specify 9 = don't know
A	Index child	|__|	|__|		
B	Caregiver of child	2	|__|	|__|	|__|
C	Head of household	|__|	1	|__|	|__|

Cluster: |__|__| Household: |__|__|

2.0	**OBSERVE AND RECORD. Do not ask question!** Describe the housing structure. |__|	1	SEPARATE HOUSE	5	SEVERAL HUTS/BUILDINGS [SAME COMPOUND]
		2	APARTMENT	6	SEVERAL HUTS/BUILDINGS [DIFFERENT COMPOUND]
		3	Boucarou	7	TENT/IMPROVISED HOME
		4	ROOM(S) [OTHER TYPE]	9	OTHER, SPECIFY: ______________________

2.1	**Do not read answers.** Do you or your household own or rent this dwelling? |__|	1	Own	5	Mortgage
		2	Don't own but live for free	6	Other, specify:
		3	Pay Rent	9	Don't know/No response
		4	Squatter		

2.2	**Do not read answers.** What kind of toilet facility do members of your household usually use? |__|	1	Water system toilet (modern toilet)
		2	Ventilated Improved Pit Latrine (VIP)
		3	Pit latrine with slab
		4	Pit latrine without slab/open pit
		5	Bucket/Pan
		6	Composting toilet
		7	No facilities (bush, beach, etc.)
		8	Other, specify:
		9	Don't know/No response

2.3	**Do not read answers.** What is the **main** source of energy for cooking? |__|	1	Electricity	5	Gas
		2	Kerosene	6	Wood
		3	Charcoal	7	Other, specify
		4	Animal dung or agricultural crop residue	9	Don't know/No response

2.4	**Do not read answers.** What is the **main** source of lighting for this house? |__|	1	Oil, kerosene or gas lantern	4	Candles/firewood	7	Solar
		2	Battery flashlights/ fluorescent lights/tube light	5	Electric Company	8	Other, specify:
		3	Electric generator/Invertor	6	No lighting	9	Don't know/No response

2.5	**Do not read answers.** What is the **main** source of drinking water for members of your household? |__|	01	Piped water in/out side	07	Protected spring
		02	Tube well/borehole	08	Unprotected spring
		03	**Un**protected dug well	09	Rain water
		04	Protected dug well	10	Tanker truck
		05	Surface water (river, dam, lake, etc.)	11	Bottled water
		06	Sachet water	12	Other, specify:
				99	Don't know/No response

2.6	Where is the drinking water source located? |__|	1	In own dwelling
		2	In own yard/plot
		3	Elsewhere
		9	Don't know/No response

2.7	How long does it take to go there, get water and come back?	|__|__|__| Minutes	Write 999 if don't know

Cluster: |__|__| Household: |__|__|

2.8	Does your household **own** any of the following assets (should be in good working condition)? **Also observe.** Y = 1, N = 2, don't know/no response = 9					
	A |__|	Sofa	I |__|	Clock	Q |__|	Car
	B |__|	Table	J |__|	Radio	R |__|	Cooker (gas)
	C |__|	Chair	K |__|	Television	S |__|	Refrigerator
	D |__|	Cupboard, Cabinet	L |__|	Cable	T |__|	Freezer
	E |__|	Sewing machine	M |__|	Land-line telephone	U |__|	Motorbike/Scooter
	F |__|	Boat without motor	N |__|	Mobile phone	V |__|	Bicycle
	G |__|	Boat with motor	O |__|	Cutlass	W |__|	Animal-drawn cart
	H |__|	Wristwatch	P |__|	Axe		

2.9	Does your household own any livestock (cattle, small ruminants or poultry)? |__|	1	Yes	2	No → **Skip to next section**

2.10	If yes, how many of each of the following animals do you own?	A. Cows: |__|	F. Other poultry: |__|
	Codes for number of livestock 0 = none 4 = 21-99 1 = 1-5 5 = >100 2 = 6-10 9 = don't know/no resonse 3 = 11-20	B. Goats: |__|	G. Rabbits: |__|
		C. Sheep: |__|	H. Horse/Donkeys/Mules: |__|
		D. Pigs: |__|	I. Grasscutter/other ruminants: |__|
		E. Chickens: |__|	

Now I would like to ask you a few questions about your activities during the day.				
		2.11	**2.12**	**2.13**
		Of the past 7 days, on how many days did you… (enter 1-7, or 0 if did not use, or 9 if don't know)	**When was the last day that you….?** **A-D:** On this day, for how much time did you…? **E only:** One this day, how many times did you make or receive calls on the phone? **Enter 99 if don't know**	If you did not ……. in the past week, why not? **(DO NOT read choices. Mark the first response given)** 1 = can't read 2 = too expensive (to buy newspaper/radio/TV/computer/phone) 3 = don't like to 4 = don't have time to 5 = not available (newspaper/radio/computer/TV/phone) 6 = other, specify 9 = don't know
A	**Read the newspaper?**	|__|	|__|__|__| **minutes**	|__|
B	**Listen to the radio?**	|__|	|__|__|__| **minutes**	|__|
C	**Watch television?**	|__|	|__|__|__| **minutes**	|__|
D	**Use the internet?**	|__|	|__|__|__| **minutes**	|__|
E	**Use a landline or mobile phone?**	|__|	|__|__| **times**	|__|

2.14	**Ask the caregiver: does anyone in your household belong to any community organizations?** |__|	1	Yes	2	No → **Skip to 2.18**
2.15	If yes, which ones? Y = 1, N = 2, don't know/no response = 9	|__|	A. Religious organization	|__|	E. Cooperative group
		|__|	B. Women's group	|__|	F. Njangi group (tontine)
		|__|	C. Sports organization	|__|	G. Dance group
		|__|	D. Cultural organization	|__|	H. Ex-student group (alumni)

Cluster: \|__\|__\| Household: \|__\|__\|

2.16		01	Professional/technical managerial-civil servant	08	Sales or services / business – trader

	Do not read the choices. Code the appropriate category for the occupation given by the respondent. **A. Caregiver:** What is the principle activity of the caregiver of the child? **[What gives you money for yourself?]** |___|___| B. **Head of household**: What is the principle activity of the head of the household? |___|___|	02	Professional/technical/managerial – personal business	09	Small business (petit commerce)
		03	Professional/technical/managerial – private company	10	"Bayam sellam"
		04	Farmer (own land)	11	Other, specify:
		05	Farmer (rented land)	00	Not employed
		06	Farmer (any land)	99	Don't know/No response
		07	Household and domestic		
2.17	**A**. What is the employment status of the **caregiver over the past year?** |___| **B**. What is the employment status of **the head of household over the past year?** |___|	1	No work last 12 months	4	Seasonally
		2	Worked last 12 months, 5+ days/week	5	Occasionally
		3	Worked last 12 months, less than 5 days/week	9	Don't know/No response

Food Frequency consumption questionnaire

CHILDREN 12-59 Months Cluster: |__|__| Household: |__|__|

Ask the **caregiver:** Could you please tell me how many **days** in the **past 7 days the CHILD** has eaten the following food items

	3.14 Food	**Preparation**	**3.15 Number of days eaten in past 7 days**	*(What was the last day the child consumed this food?)* **3.16 On this day you consumed this food, how many times per day did the child consume it?**	**3.17 If not consumed, why not?** (do not read choices)
	Oil Type Codes 0 = No other type eaten 1 = Red palm 2 = refined palm 3 = soybean, 4 = groundnut 5 = cottonseed 6 = vegetable oil 7 = other, specify 9 = don't know		|_/_| = not applicable 1 = 1 day 2 = 2 days 3 = 3 days 4 = 4 days 5 = 5 days 6 = 6 days 7 = 7 days 9 = don't know	|_/_| = not applicable 99 = don't know	|_/_| = not applicable 1 = too expensive 2 = not available 3 = don't like it 4 = don't prepare this food 5 = no time 6 = other, specify 9 = don't know
A1	Oil 1 Type |__|	Sauce/stew	|__|	|__|__|	|__|
B1		Fried foods (eg, eggs, sautéed vegetables, fish, chicken, beignets, etc)	|__|	|__|__|	|__|
D1		Condré (melange)	|__|	|__|__|	|__|
E1		Others with oil	|__|	|__|__|	|__|
A2	Oil 2 Type |__|	Sauce/stew	|__|	|__|__|	|__|
B2		Fried foods (eg, eggs, sautéed vegetables, fish, chicken, beignets, etc)	|__|	|__|__|	|__|
D2		Condré (melange)	|__|	|__|__|	|__|
E2		Others with oil	|__|	|__|__|	|__|
A3	Oil 3 Type |__|	Sauce/stew	|__|	|__|__|	|__|
B3		Fried foods (eg, eggs, sautéed vegetables, fish, chicken, beignets, etc)	|__|	|__|__|	|__|
D3		Condré (melange)	|__|	|__|__|	|__|
E3		Others with oil	|__|	|__|__|	|__|
E	Wheat flour	Fried dough (beignets)	|__|	|__|__|	|__|

<table>
<tr><td>F</td><td></td><td>Bread</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>G</td><td></td><td>Pasta – macaroni, spaghetti</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>H</td><td></td><td>Biscuits</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>I</td><td></td><td>Cakes</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>J</td><td></td><td>Other foods with wheat flour</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>K</td><td>Sugar</td><td>Tea or coffee</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>L</td><td></td><td>dessert (cakes and others)</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>M</td><td></td><td>Top, coke,</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>N</td><td></td><td>juice (sucette)</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>O</td><td></td><td>Bonbon</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>P</td><td></td><td>Pap/bouillie</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>Q</td><td></td><td>Other foods with sugar</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>R</td><td>Bouillon cube</td><td>Sauces and salad creams</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>S</td><td></td><td>Added to meat</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
<tr><td>T</td><td></td><td>Mixed dishes</td><td>|__|</td><td>|__|__|</td><td>|__|</td></tr>
</table>

Picture Chart 24hour recall

Aliment	Consommé? ☑	Aliment	Consommé? ☑	Aliment	Consommé? ☑
Citron/ orange/ mandarine / pamplemousse		Autres fruits (mangue / papaye / ananas, guayaba, banan)		Gombo	
Ail/ Oignon		Tomate (frais ou en boite)		Avocat	
Epices et condiments verts (carrotte, persil, poivron, céleri etc.)		Feuilles vertes (Ndolé, Folon, zom, kpwem, keleng-keleng, foléré)		Tubercules (pomme, taro, igname, macabo, manioc)	

Aliment	**Consommé?** ☑	**Aliment**	**Consommé?** ☑	**Aliment**	**Consommé?** ☑
Haricot (blanc, noir ou rouge)		Plantain (jaunes ou verts)		Njangsa et autres aliments comme les noix	
Autres végétaux		Escargot / chenille		Arachides	
Pistache / Egusi		Kola ou noix de palme		Niebe / koki	
Beignet / Gâteau		Farine (tout type)		Pain	

Aliment	**Consommé? ☑**	**Aliment**	**Consommé? ☑**	**Aliment**	**Consommé? ☑**
Mil / Sorgho		Mais		Riz	
Couscous / Fufu		Bouillie / Pap		Bâton/miondo/Bobolo	
Koki		Tapioca / Gari		Spaghetti/Macaroni	
Biscuits		Céréales pour l'enfant		Bonbon/Chocolat	

Aliment	**Consommé?** ☑	**Aliment**	**Consommé?** ☑	**Aliment**	**Consommé?** ☑
Lait-adultes ou enfants / Yaourt / Fromage		Huile (tout type) / jus de noix de palme		Epices Haoussa / Kanwa	
Arome et cube		Sel / piment / autres épices		Ketchup / moutarde / mayonnaise	
Sucre		Margarine/Beurre		Œufs / Omelette	

Aliment	**Consommé?** ☑	**Aliment**	**Consommé?** ☑	**Aliment**	**Consommé?** ☑
Viande / Saucisse		Poulet / Volaille		Poisson / Crevettes	
Boissons sucrée : Coca et jus		Boisson alcoolisée : whiskey, bière, vin		Boisson chaude comme thé/café	

Ethical Clearance

LIST OF PUBLICATIONS

Multiple Micronutrient Deficiencies are related to the Nutritional status of children 12 – 59 months living in North and Far North Regions of Cameroon. *Am.J.Pharm.Health Res 2015;3(9) p2321-3647*

Estimates of malnutrition in children 6-59monhts of age based on the WHO standard reveal higher prevalence compared to the NCHS reference in a cross-sectional sample of children from North Cameroon. *Am.J.Pharm.Health Res 2016;4(3) p2321-3647*