Consensus Study on

Improved Nutritional Assessment of Micronutrients

July 2013

ASSAf

ACADEMY OF SCIENCE OF SOUTH AFRICA

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The Academy of Science of South Africa (ASSAf) was inaugurated in May 1996. It was formed in response to the need for an Academy of Science consonant with the dawn of democracy in South Africa: activist in its mission of using science for the benefit of society, with a mandate encompassing all fields of scientific enquiry in a seamless way, and including in its ranks the full diversity of South Africa's distinguished scientists. The Parliament of South Africa passed the Academy of Science of South Africa Act (*Act 67 in 2001*), as amended, which came into operation on 15 May 2002. This has made ASSAf the official Academy of Science of South Africa, recognised by government and representing South Africa in the international community of science academies.

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Foreword

This consensus report is in fulfillment of the Academy of Science of South Africa (ASSAf) mandate of providing evidence-based scientific advice to South African policymakers.

It is well known that food and nutrition security, and therefore nutritional status of the population, influence birth outcomes, physical and mental (cognitive) development of children, their ability to benefit from education, human capacity of adults, and their work potential and performance. But the nutritional status of individuals also influences general health, immunity against infectious diseases and protection against the development of chronic non-communicable diseases.

This report was motivated by the findings of the first ASSAf consensus report on *HIV/AIDS, TB and Nutrition* that was published in 2007. The 2007 report indicated that the three major epidemics of malnutrition, HIV/AIDS and TB are closely related, and that the nutritional status of the majority of South Africans is far from optimal. That report also showed that it is especially micronutrient malnutrition that affects the South African population.

In this study, therefore, six key micronutrients (namely, vitamin A, vitamin D, folate, selenium, iron and zinc), that have been shown to contribute to the malnutrition epidemic in South Africa, or that are known and/or suspected to play vital roles in response to infections such as HIV and TB, were selected for in-depth study. The focus of this study was on the most suitable and affordable methods of assessing the status of these six micronutrients.

This consensus report highlights the important issues in nutritional assessment, the challenges and the findings. It offers recommendations that are evidence-based and have been evaluated carefully by the 11-member consensus study panel. The recommendations are for the optimal assessment of nutritional status of individuals and populations to be used in designing interventions that will improve nutritional status, health, human capacity and performance of the South African population.

It is envisaged that the findings and recommendations of this study will impact on all policies within the relevant government departments whose mandates are aimed at improving food and nutrition security and health of all South Africans. It is also hoped that the relevant non-government institutions will implement the report's important recommendations.

The 11-member study panel completed the study and this report is the product of their work. The report was peer-reviewed by four experts: Professor Alan Jackson (United Kingdom), Professor Joyce Kinabo (Tanzania), Professor Rina Swart (South Africa) and Dr Saskia de Pee (Italy), who recommended that the report be published.

The ASSAf Council expresses its great appreciation to the reviewers for their valuable comments which have greatly enriched the report. The Council has reviewed both the report and the reviewers' comments and approved that the report be published. The Council also expresses its gratitude to the panel for their dedication in successfully completing this report.

Prof Daya Reddy
President: Academy of Science of South Africa

Acknowledgements

This report is a result of the collaborative work of the 11-member consensus study panel which included both national and international experts. The national study panel members were: Dr Namukolo Covic, Professor Ali Dhansay, Professor Wieland Gevers, Professor Salome Kruger, Professor Xikombiso Mbhenyane, Professor Barry Mendelow, Professor John Pettifor (Chair) and Professor Esté Vorster. The international panel members were: Professors Tola Atinmo, Jack Metz and Michael Zimmermann.

All panel members agreed on the report's findings, conclusions and recommendations. They are all hereby acknowledged and thanked for their dedication to this study and subsequently this important report.

Professor Esté Vorster is particularly acknowledged for the additional time and effort she dedicated in ensuring that this report is of the highest standard.

The study panel would also like to thank the following individuals for their contribution and assistance in completing this report:

The peer-reviewers, Professor Alan Jackson (United Kingdom), Professor Joyce Kinabo (Tanzania), Professor Rina Swart (South Africa) and Dr Saskia de Pee (Italy) are thanked for inputs that greatly enriched the report.

The ASSAf editorial and production team led by Ms Patricia Scholtz is acknowledged for all the work they did on the report. The ASSAf secretariat, especially Prof Roseanne Diab and Ms Phakamile Mngadi, are also acknowledged for their support. The panel also thanks the ASSAf Council and the ASSAf Standing Committee on Health for their support.

The panel acknowledges the Department of Science and Technology (DST) for the core funding provided to ASSAf and the financial support provided by the United States National Academies (USNA) through their African Science Academy Development Initiative (ASADI) programme.

Prof John Pettifor
Chairperson: ASSAf Consensus Study,
Improved Nutritional Assessment of Micronutrients

Abbreviations and Acronyms

AI	Average intake
AIDS	Acquired immune deficiency syndrome
ALL	Acute lymphoblastic leukaemia
AD	Alzheimer's disease
ADSA	Association for Dietetics in South Africa
ACT	Alpha-1 chymotrypsin
AGP	Alpha-1 acid glycoprotein
AML	Acute myeloid leukemia
ANR	Average nutrient requirement
APP	Acute-phase protein
ASSAf	Academy of Science of South Africa
BMI	Body mass index
CAD	Coronary artery disease
CDC	Centres for Disease Control and Prevention
Chaos-2	Cambridge Heart and Antioxidant Study
CHD	Congenital heart defects
CIC	Conjunctival impression cytology
CRC	Colorectal cancer
CRP	C-reactive protein
CRBP-1	Cellular retinol-binding protein-1
CV	Coefficient of variation
DBS	Dried blood spots
DNA	Deoxyribonucleic acid
DRI	Dietary Reference Intakes
DS	Down syndrome
DXP	Deoxyxylulose 5'phosphate
EAR	Estimated average requirement

Abbreviations and Acronyms

DoH	Department of Health
DST	Department of Science and Technology
ELISA	Enzyme-linked immunosorbent assay
FACIT	Folic acid and carotid intima-media thickness
FANUS	Federation of African Nutrition Societies
FAO	Food and Agriculture Organisation
FAZ	Fractional absorption of zinc
FFF	Folate food fortification
GATA-1	Globin transcription factor 1
GSHPx	Glutathione peroxidase
Hb	Haemoglobin
Hcy	Homocysteine
HLA	Human leukocyte antigen
HOPE-2	Heart Outcomes Prevention Evaluation-2
HPCE	High performance capillary electrophoresis
HPLC	High performance liquid chromatography
1,25-$(OH)_2D$	1,25-dihydroxy vitamin D
IAEA	International Atomic Energy Association
ICSU	International Council for Science
ICT	Impression cytology with transfer
IIH	Idiopathic intracranial hypertension
IgA	Immunoglobulin A
IL-2	Interleukin-2
INL	Individual nutrient level
IPP	Isopentenyl pyrophosphate
IU	International Unit
IUNS	International Union of Nutritional Sciences

Abbreviations and Acronyms

IZINcG	International Zinc Nutrition Consultative Group
IVACG	International Vitamin A Consultative Group
JCEM	*Journal of Clinical Endocrinology and Metabolism*
MCI	Mild cognitive impairment
MDS	Myelodysplastic syndrome
Met	Methionine
MetaHIT	Metagenomics of the Human Intestinal Tract
mRNA	Messenger ribonucleic acid
MTHFR	Methylenetetrahydrofolate reductase
MNM	Micronutrient malnutrition
MRC	South African Medical Research Council
MRDR	Modified relative dose response
MTB	Mycobacterium tuberculosis
MTR	Methionine synthase
MTRR	Methionine synthase reductase
NAD+	Nicotinamide adenine dinucleotide
NADP+	Nicotinamide adenine dinucleotide phosphate
NCDs	Non-communicable diseases
ND DoH	Nutrition Directorate: Department of Health
NFCS	National Food Consumption Survey
NHANES	National Health and Nutrition Examination Survey
NHI	National Health Insurance
NHLS	National Health Laboratory Service
NIRU	Nutritional Intervention Research Unit
NIVs	Nutrient intake values
NK	Natural killer
NNIA	Nestlé Nutrition Institute Africa

Abbreviations and Acronyms

NORVIT	Norwegian Vitamin Interventional Trial
NRIND	National Research Institute for Nutritional Diseases
NSSA	Nutrition Society of South Africa
NTDs	Neural tube defects
NUGAG	World Health Organisation Nutrition Guidance Expert Advisory Group
PBC	Primary biliary cirrhosis
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-para-dioxins
PCDFs	Polychlorinated dibenzofurans
PCR	Polymerase chain reaction
PEM	Protein-energy malnutrition
PUFA	Polyunsaturated fatty acids
QFFQ	Quantitative food frequency questionnaire
RAR	Retinoic acid receptor
RBP	Retinol-binding protein
RCF	Red cell folate
RDA	Recommended dietary allowance
RDR	Relative dose response
RE	Retinol equivalents
RNA	Ribonucleic acid
ROS	Reactive oxygen-containing species
RXR	Retinoid X receptor
SAA	Serum amyloid A
SAFCT	South African Food Composition Tables
Se	Selenium
SEARCH	Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine

Abbreviations and Acronyms

SeCys	Selenocysteine
SeMet	Selenium methionine
SF	Serum folate
TB	Tuberculosis
tHcy	Total homocysteine
T3	Triiodothyronine
TS	Thymidylate synthase
T4	Thyroxine
TTR	Transthyretin
UNICEF	United Nations Children's Fund
UNIDO	United Nations Industrial Development Organisation
UNL	Upper nutrient level
UV	Ultraviolet
USA	United States of America
VAD	Vitamin A deficiency
VDR	Vitamin D receptor
VISP	Vitamin Intervention for Stroke Prevention
WAFACS	Women's Antioxidant and Folic Acid Cardiovascular Study
WENBIT	Western Norway B Vitamin Intervention Trial
WHO	World Health Organisation
Zn	Zinc

Measurements

C	Celsius	mg	Milligramme
dl	Decilitre	ml	Millilitre
g	Gramme	RE	Retinol equivalent
IU	International Unit	µg	Microgramme
kg	Kilogramme	µmol	Micromole
l	Litre		

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Executive Summary

The purpose of this consensus study on improved nutritional assessment of micronutrients was to optimise our approach to the assessment of nutritional status of individuals, specific groups of people and the population in all settings. Information on nutritional status is necessary for recommendations to be used in the design and implementation of research and interventions that will address malnutrition in order to improve human well-being and performance.

The study selected the six micronutrients, *viz*. vitamins A, D, and folate; and the inorganic elements or minerals, selenium, iron and zinc that have been shown in a previous consensus study[1] by ASSAf to have a potential impact on the major epidemics of malnutrition, HIV/AIDS and TB in South Africa. Because the bowel microflora are currently under intense scrutiny as co-determinants of the whole-body physiology and pathophysiology of micronutrients, a further chapter on this topic is included. It is, however, acknowledged that other micronutrient deficiencies are prevalent in South Africa, and that these should also be addressed.

Chapter 1 Introduction

In the introductory chapter, the far-from-optimal nutritional status of South African children and adults, the inter-related causes of malnutrition, and possible causes of the co-existence of under and overnutrition, are briefly reviewed. The chapter then focuses on the modern understanding of the evolutionary development of micronutrient requirements and their coenzyme functions in humans, and the complexities of variability and bioavailability. The chapter makes the important point that different levels of imbalances in intakes and loss, and human heterogeneity in physiology and metabolism, will lead to different levels of depletion: overt clinical symptoms often occur only after severe depletion while mild to moderate depletion, which may be more difficult to assess, might play a major role in influencing health and risk of disease.

The general principles of assessment of nutritional status and specifically micronutrient status are delineated and the relevant recommendations of the previous consensus study[1] which motivated the present study are given and discussed. The main objectives of this study are formulated as the following questions:

- How have modern understandings of the detailed physiology and pathophysiology of the six micronutrients concerned affected the nature of the laboratory/clinical tests and other assessment methods (in-depth dietary assessments, nutritional anthropometry, and clinical signs of malnutrition) that have to date been developed worldwide?

- Which of the tests or methods are maximally informative and reliable as to the actual depletion/repletion status of individuals and populations with respect to these micronutrients?

- Which of these tests are robust, affordable, capable of decentralised operation, and of mass application?

- How are the tests affected, or the interpretation altered, by factors such as varying health status of the subjects under investigation, by other dietary components (nutrients, foodstuffs and non-nutrient endogenous compounds and additives), and by differing composition of gastrointestinal bacteria and other commensals?

The chapter concludes with suggestions on how and to whom the results of this study should be disseminated.

Chapter 2 Vitamin A

This chapter shows that vitamin A deficiency (VAD) is a global and developing-country problem, and that many South African children and possibly also apparently healthy adults suffer from sub-clinical VAD.

The chapter includes discussions on the metabolism of vitamin A (retinol and related substances: retinylaldehyde, retinoic acid and several carotenoids), human requirements and dietary sources, established but also new potential functions and effects (including effects of retinoic acid on genetic expression).

The chapter focuses on the variety of available methods to assess vitamin A status. It is recommended that the biochemical measurement of retinol, retinol-binding protein (RBP) and transthyretin (TTR) in serum, plasma (or possibly dried blood spots in the future), as well as clinical assessment of night blindness (dark adaptation), are at present the most practical and cost-effective methods in use to assess vitamin A status in individuals and populations.

The periodic high dose vitamin A supplementation to address VAD is discussed and the recent policy brief of the South African Medical Research Council (MRC) with a warning that children with adequate vitamin A status should not receive the supplementation, mentioned.

Chapter 3 Vitamin D

In Chapter 3 it is shown that vitamin D, a generic term for a number of seco-steroid compounds that regulate calcium homeostasis and therefore have anti-rachitic properties, is mainly formed from the conversion of 7-hydrocholesterol under the influence of sunlight (ultraviolet (UV) irradiation) in the skin and that dietary sources are limited in developing countries. It is emphasised and evidence is provided that despite the abundance of sunlight in South Africa, some South African children may suffer from sub-clinical vitamin D deficiency. Little information on the vitamin D status of adult South Africans is available. This is important in the light of the effects of vitamin D on immunity and its potential protective role in the treatment of HIV/AIDS and TB.

The chapter delineates vitamin D requirements, possible dietary sources, accepted ranges for serum values, recommended cut-point ranges for vitamin D deficiency and insufficiency, and shows that measurement of 25(OH)D in serum with enzyme-linked immunosorbent assay (ELISA) and/or radioimmuno-tracer assays can be used to assess vitamin D status.

The chapter concludes with a recommendation that more randomised controlled trials and intervention studies are necessary to establish the possible role of vitamin D in the treatment of TB and HIV/AIDS.

Chapter 4 Folate

In this chapter the importance of adequate folate intakes and status on health, the role of folate fortification of foods, and the safety of folate supplementation are critically examined using evidence from a large body of recent publications. In summary, the chapter indicates that inadequate folate function may be a result of low intakes, polymorphisms in genes coding for folate co-enzymes, and/or administration of drugs interfering with folate metabolism.

The chapter further emphasises the role of folate in DNA synthesis, accumulation of homocysteine and possible risk of cardiovascular disease, genetic polymorphisms of folate enzymes, megaloblastic anaemia, risk of neural tube defects (NTDs), low birth weight and possibly congenital heart defects.

The chapter also indicates that the assessment of folate nutritional status in the individual is by standard haematological tests, together with measurement of both serum and red cell folate concentrations. At the population level, measurement of both serum and red cell folate concentrations are required: serum folate to assess the prevalence of inadequate folate intake, and red cell folate for the prevalence of folate deficiency. Doing both tests in tandem is particularly important in countries with mandatory folate food fortification. These tests are in general robust, reliable and affordable. Tests of folate nutrition may be affected by any intercurrent illness that is associated with prolonged reduction in food intake. Abnormal tests probably reflect the development of folate deficiency rather than a direct effect of the illness.

The chapter concludes that although only limited reliable information on folate status of South Africans is available, recent studies indicate that the mandatory food fortification programme has had positive effects on folate intake, status and neural tube defects.

Chapter 5 Selenium

Selenium (Se), a non-metallic element, was shown to be an essential nutrient only in the late 1970s. It has four natural oxidation states and combines with other elements to form selenides, selenites, selenates, oxides, oxyacids and selenoproteins. Se exerts its antioxidant biological effects as a constituent of at least 30 selenium containing proteins in humans.

In this chapter, the bioavailability, absorption and metabolism of Se, markers for Se intake and Se status, the role of Se in the immune system, interaction with other nutrients, factors influencing recommendations for intakes, as well as what is known about selenium intakes and status in South Africa and elsewhere in Africa, are briefly reviewed. The chapter shows that Se

plays a role in innate and acquired immunity and is thought to play a protective role against certain cancers and cardiovascular disease.

Se intakes can be assessed by measuring total Se concentration in plasma and whole blood samples. The total Se level needed to maintain the function of the immune system is probably lower than requirements of people with HIV infection and other disease states. The assessment of Se status by measuring specific functional markers (selonoproteins and enzyme activities in biological samples) is determined by the specific function of Se being investigated.

The limited information on Se intake and status of South Africans, as well as the risk map of possible Se deficient areas, and the lower Se status found in persons with HIV-infection, suggest that Se deficiency amongst groups of South Africans (and also other populations in Africa) is prevalent.

The chapter concludes with recommendations that research on intake and status, and effects of selenium supplementation is urgently needed, and that more research on the Se content of South African foods, as well as Se requirements of vulnerable groups (such as HIV-infected people, and those with increased antioxidant needs) is indicated.

Chapter 6 Iron

In this chapter, attention is drawn to the fact that iron deficiency and resultant anaemia and compromised cognitive development of children is a global problem, being the most prevalent micronutrient deficiency. The bioavailability, absorption, metabolism, turnover, homeostasis, requirements and functions of iron are briefly reviewed.

The internationally recommended variables to assess iron status, namely, haemoglobin, ferritin and transferrin receptor are critically evaluated. It is shown that the diversity of the South African population, as well as the influence of factors such as parasitic infection, inflammation, alcohol consumption and obesity on iron status should be taken into account when iron status variables are interpreted.

The chapter gives a summary of some South African studies in which iron status has been reported and demonstrates different interpretations of the data. A number of recommendations are made for further research and to take confounding factors into account when iron status is assessed in different groups. A plea is made for cooperation among African countries to accumulate data on iron status so that shared solutions and interventions can be designed.

Chapter 7 Zinc

In this chapter the physiology of zinc, its distribution in the body, as well as zinc pathophysiology and consequences of deficiency, are reviewed. Special attention is given to what is known about zinc deficiency in persons living with HIV/AIDS and those suffering from TB. The chapter also reviews the few studies in South Africans in which zinc intake and/or status have been determined, and concludes that zinc deficiency is a problem in South Africa, affecting especially children and the elderly.

The chapter includes discussions on the bioavailability of zinc and emphasises its lower availability in plant-based diets, and in particular when maize is the staple. The low zinc content of maize and low availability due to phytates that inhibit absorption are challenges to improve zinc status in South Africa.

A review of the methods to determine zinc intake and zinc status led to the recommendation that a combination of three methods should be used to assess zinc status, namely: dietary intake, serum (plasma) zinc level, as well as measurement of growth (height-for-age) in children. The zinc concentration in serum is low, and contamination of samples to be analysed should be avoided. The chapter therefore also summarises the technical aspects that should be taken into account when assessing zinc in serum samples. The same three variables should be measured to assess the effect of zinc interventions in populations. On an individual level, serum zinc combined with dietary assessment should be useful for decisions regarding therapy. It should be kept in mind that compromised growth of children is influenced by many factors, in addition to a zinc deficiency.

Serum (plasma) zinc is also influenced by diurnal fluctuations, age, sex, fasting state, and the presence of infections. All these factors should be taken into account when serum values are interpreted. The cut points for serum zinc values, as well as dietary zinc recommendations, are summarised in tables.

The chapter concludes with recommendations and practical advice on the assessment of zinc status of South African groups.

Chapter 8 The Bowel Microbiotica in Relation to Nutritional Assessment

In this chapter, the often neglected contribution of the bowel microflora to micronutrient status is highlighted. In addition to the fermentation of carbohydrates that reach the colon by the large bowel microbiotica, the chapter provides evidence of the production of several water-soluble vitamins by the large bowel microflora, as well as of the presence of transporters for their absorption from the large bowel mucosa. It is mentioned that functional metabolomic methodologies can be used to identify biomarkers in urine that reflect the activities of the microflora in the large bowel. It is emphasised that the impact of the increasingly prescribed and use of prebiotic and probiotic supplementation on health and disease should be further investigated.

The chapter concludes with a recommendation that local expertise should be developed to examine the contribution of the large bowel microflora to nutritional status and health of South Africans.

Chapter 9 Findings, Discussion and Conclusions

The purpose of this chapter is to integrate and discuss the main findings of the study, highlighting the methods for assessment that have been recommended in the individual chapters. The general findings and principles that collectively emerged from the chapters are discussed first, followed by a discussion on the whole-body turnover of micronutrients as a basis for

understanding how they should be assessed and results interpreted. The chapter concludes with a summary of methods recommended for assessment in both field and clinical settings.

The general findings and principles include the following:

- Deficiency of the selected six micronutrients is a problem in South Africa, not only in recognised vulnerable groups, but probably also in many overweight and obese individuals.
- The second observation is confirmation that these micronutrients may be directly or indirectly involved in the susceptibility to and/or development of HIV/AIDS and TB, possibly as a result of effects on the immune system.
- The role of these micronutrients in the optimal function of the immune system, as well as protective effects against some non-communicable diseases (NCDs), can be regarded as non-classical or 'new' functions. The perception that micronutrients have additional benefits at intakes higher than those required to prevent the classical deficiency syndromes, has already impacted on micronutrient intake recommendations.
- With the exception of vitamin D, laboratory (biochemical) methods to assess status should be accompanied by one or more other assessment method (dietary intake, anthropometry and/or functional tests).
- The present advantages and limitations of laboratory tests are highlighted. For most of these micronutrients, laboratory tests are affordable, reliable, feasible, robust and suitable for mass application.
- Different levels of depletion/repletion may need different approaches to assess status.
- Assessment of individuals *versus* populations, and differences in interpretation of results in individuals compared to populations are emphasised, using nutrient intake data as an example.
- Confounders, such as inflammation and the catabolic state, which may be present when nutritional status is assessed, may influence interpretation of assessment results and should be taken into account.

In the second part of this chapter, using a systems approach, our understanding of the paradigm of whole-body micronutrient turnover and how the body responds to different levels of intake is described. This discussion serves as a background for the development of methodology and interpretation of measurement results.

The chapter is concluded by a summary of the recommended assessment methods.

Chapter 10 Recommendations

In this chapter, policy recommendations are made regarding the assessment of the six selected micronutrients, followed by recommendations for further research to refine and develop nutritional status assessment methods.

The policy recommendations are based on and motivated from observations in the individual chapters, and they include:

Recommendation 1: Implementation of a regular nutritional status surveillance system

- The regular nutritional status surveillance of the population, using the laboratory (biochemical) methods identified in this report. Results from the surveillance should inform existing programmes, such as the mandatory food fortification of staples and vitamin A supplementation, which should be adapted if necessary.

- The appointment of an authoritative Task Team to advise on the surveillance and other nutrition recommendations, including advocacy for the employment of more public health nutritionists.

- The establishment of a central nutritional status data bank to monitor changes in micronutrient status over time.

Recommendation 2: Routine assessment of micronutrient status

- Routine assessment of micronutrient status of individual patients in clinical settings to diagnose pre-clinical, as well as overt deficiencies for improved treatment, using protocols and algorithms developed for this purpose.

- Improve the training of all health personnel in the assessment of micronutrient status, and interpretation of test results for improved treatment. This should include re-training of practising health professionals and better training of future professionals.

Recommendation 4: Developing expertise

- Expertise to examine the contribution of the micobiotica to nutritional status should be developed.

Recommendation 5: Investing more in local nutrition research

- An investment in more local research (for example, on the contribution of large bowel microflora to micronutrient status) and development of assessment methodologies, using the rapid developing techniques of nutrigenomics, proteomics and metabolomics.

A corollary of these recommendations is that the National Health Laboratory Service (NHLS) should be in the vanguard of establishing best practice in the assessment of micronutrient status, and should seek to offer the most informative tests as widely as possible and at the lowest possible cost.

References

1. Academy of Science of South Africa (ASSAf). HIV/AIDS, TB and Nutrition: Scientific enquiry into the nutritional influences on human immunity with special reference to HIV infection and active *TB in South Africa,* 2007.

Chapter 1

Introduction

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Introduction

Human nutrition is a multi-disciplinary science in which many established sub-disciplines intersect[1]. Remarkably, new knowledge garnered in one discipline frequently fails to filter through into another; this is most evident in the divide between laboratory and clinical fields. An additional problem is that nutrition has often been a Cinderella discipline amongst other disciplines taught to health professionals, frequently either not formally taught or assigned to junior lecturers and usually consigned to rote-learning that is frozen in old paradigms. The physiological and pathological ways in which nutrients are handled by the body have rarely been as well understood by students as are those relating to drugs or hormones, despite the similarity in the relevant conceptual frameworks.

This consensus study focuses on optimising our approach to the assessment of the nutritional status of individuals and populations in South Africa for specific micronutrients. This is a major national problem because of the subtle and pervasive effects of undernutrition and malnutrition, common in the population, on human well-being and performance.

Clinical and field assessments of food and nutrient intake estimates, and of overt symptoms and signs of nutrient deficiencies, require well-defined signs and symptoms, highly skilled assessors and are labour-intensive. They are also fraught with uncertainties since much is hidden from sight in the sub-clinical phases of depletion states which are compounded by variations in the population associated with mostly ill-defined genetic and environmental determinants. Laboratory measurements can assist greatly in generating more objective and reliable assessments of nutritional status, but present their own difficulties of cost, feasibility, skills, equipment, and interpretation. The situation can obviously be aided by focusing on those tests that are most informative, robust, affordable and feasible in many sites and locations.

In this introduction, we look first at the actual extent of the national problem in respect of undernutrition and overnutrition, then by generalisation, at the physiological and pathological principles underlying laboratory assessment of nutritional status, and last at the recommendations made in a previous ASSAf consensus report[2] concerning the urgent need for this follow-up detailed study on the optimal assessment of the nutritional status of individuals and populations for six micronutrients in South Africa. These six micronutrients were selected because of their likely impact on the massive pandemics of the human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* (MTB) infections in the country.

The consensus study on *HIV/AIDS, TB and Nutrition* published by the Academy of Science of South Africa (ASSAf) in 2007[2] contained an analysis of malnutrition in children and adults in South Africa, which makes a strong case for the present study and is quoted here in parts only (without repeating all the references used).

Nutritional Status of South African Children

In Africa, 50% of children with severe undernutrition die during hospital treatment due to inappropriate care and 40% of preschool children suffer from chronic undernutrition[3] resulting in stunting, which may affect mental and physical development. Inappropriate feeding of infants and children is responsible for one-third of the cases of malnutrition. The most common forms of malnutrition include micronutrient malnutrition (MNM) and severe acute malnutrition. The latter is characterised by protein energy malnutrition (PEM) accompanied by inadequate intake of most micronutrients, disease, and inappropriate care of infants.

MNM is caused by poor-quality diets which are characterised by high intakes of staples[3], but low consumption of animal and fish products, fruits, legumes, and vegetables, which are rich sources of bioavailable minerals and vitamins. Many of the malnourished are those who cannot obtain these foods to supplement staples from their own production in an impoverished subsistence situation. Even mild levels of micronutrient malnutrition may damage cognitive development, lower disease resistance in children, and reduce the likelihood that mothers survive childbirth. The cost of these deficiencies, rooted in poverty and food insecurity, in terms of lives lost and quality of life are staggering.

In South Africa, national food consumption surveys published in 2000 and 2005[4-5] found that stunting (low height-for-age) remained the most common nutritional disorder, affecting 21.6% to 19.3% of children aged one to nine years. The highest prevalence of stunting was found in one to three-year-olds (24.4%), children in rural areas (23.8%), and those living on commercial farms (25.6%). A case-control study conducted in 12-24-month-old children in a high-density urban slum in East London showed that the most important determinants of growth failure were related to the caring capacity, and resultant caring behaviour of mothers or caregivers. No clear picture emerged on the role of dietary intake or disease in the development of growth failure.

A common pattern of growth in disadvantaged children in South Africa, and indeed throughout the developing world, is one of normal weight gain during the first four to six months of life, largely associated with successful breastfeeding. Thereafter, the prevalence of underweight-for-age and stunting increases rapidly after six to 12 months of age (at the time of the introduction of complementary foods into the diet of the breastfed infant). The relative rarity of wasting (low weight-for-height) and the high prevalence of stunting (low height-for-age) in South Africa suggest that the main problem is chronic socioeconomic underdevelopment. Most stunting occurs before the age of three years, and stunted children usually become stunted and often obese adults, as catch-up growth is difficult to achieve. Stunting results primarily from poor feeding practices over long periods, coupled by an increased incidence of infections, which may be aggravated by a lack of food in the household. Between 11 and 17 million South Africans are considered food and nutrition insecure, with 38% of African households often or sometimes going hungry[2].

The health and nutritional status of mothers greatly influences the growth and development of their babies during pregnancy and infancy. If mothers are undernourished, in poor health, or too young, babies have a greater chance of being born underweight. Infants born with low birth weight are undernourished, and therefore at risk of a number of health conditions. These infants may not be able to gain sufficient weight, and may suffer long-term health and developmental defects[2].

There are also new dimensions to the child malnutrition problem and it has been hypothesised that stunted children are at increased risk of non-communicable diseases (NDCs) later in life. Therefore, undernutrition predisposes them to NDCs, in addition to lifestyle issues. The established obesity and diet-related pandemic NDCs in developed countries is spreading to the developing world[6]. This means that health systems in poorer countries increasingly have to cope with the double burden of treating expensive diet-related NCDs (such as diabetes and hypertension) while simultaneously attempting to combat undernutrition and other common communicable diseases (such as diarrhoea and tuberculosis (TB)). Childhood obesity is rapidly emerging as a global epidemic that will have profound public health consequences as overweight children become overweight adults.

Nutritional Status of South African Adults

Research on the nutritional status of South Africans during the past ten years has focused on children and to a certain extent on the nutrition transition associated with urbanisation of black South Africans[7]. No national study has been done to assess the nutritional status other than in children. Nevertheless, a number of *ad hoc* cross-sectional epidemiological surveys in the different provinces of South Africa, as well as the 1998 South African demographic and health survey, have been undertaken, and have provided data on nutritional status variables, markers, and associated risk factors in adults[2]. Research reports indicate that on a population level, the nutritional status of South Africans is far from optimal. The major problems related to malnutrition, the causes and consequences of which have to be addressed by public health policy have been identified in the previous ASSAf study[2] as:

- Inequity between different population groups, related to poverty and the gap between the rich and the poor.
- The co-existence of under and overnutrition in the same household, families and communities.
- The prevalence of 'hidden hunger': the existence of micronutrient deficiencies in undernourished, as well as apparently well-fed, often overweight and obese individuals.
- The increased vulnerability to develop overweight and obesity and other NCDs in adults who had low birth weights and who were undernourished and had stunted growth during infancy and childhood.
- The negative trends in changes of diets and nutrient intakes associated with urbanisation, acculturation, and modernisation.

Analyses of the nutrient intakes of South Africans[8] show that most communities do not meet their requirements (dietary reference values) for calcium, iron (especially black girls and women), zinc, riboflavin, and vitamin B_6 (most groups), folate (Indian and black women) and vitamin C (Indian, coloured and black communities). The nutrition transition amongst black South Africans is characterised by increases in animal protein and fat intake, decreases in carbohydrate and dietary fibre consumption, as well as improved but still not optimal micronutrient intakes[8-9].

It is not known what percentage of overweight and obese men and women suffer from micronutrient deficiencies (hidden hunger). However, given the low intakes of calcium, iron, zinc, riboflavin, vitamins B_6 and C, it is estimated that substantial numbers of South Africans that meet or exceed their energy needs, do not meet their micronutrient requirements. Using body mass index (BMI) as an indicator and combining men and women, less than half of adult South Africans maintain a normal body weight for their height, reflecting imbalances in energy intake and expenditure.

Causes of Undernutrition in South Africa

The multi-factorial and inter-related causes of undernutrition are characterised by its consequences, which often aggravate the primary causes. This forms a vicious cycle of poverty and undernutrition from which it is difficult to escape[9-10]. Undernourished individuals often have low energy intake and expenditure levels, as well as micronutrient deficiencies, associated with low human capital and an inability to optimally benefit from education and development programmes to create a sufficiently healthy socio-economic environment for their families to prevent undernutrition in the next generation. Poverty is a fundamental or root cause of undernutrition, because it is associated with unemployment, inability to pay for nutritious food, health care and basic services, disintegration of family life, inability to care for children, vulnerability, homelessness and despair[10].

Food insecurity, defined as the lack of the human right of access to adequate, affordable, safe and nutritious food, is a major determinant of undernutrition. Although South Africa is food secure on a national basis, and even in a position to export food, many households experience hunger and food and nutrition insecurity because of all the factors contributing to poverty and underdevelopment. The HIV/AIDS pandemic, often associated with children becoming heads of households and main 'breadwinners', also contributes to food insecurity[2].

The standard of housing, occupational density, access to clean, safe water and sanitation, as well as the availability of adequate cooking and refrigeration facilities, combine to determine the risk of malnutrition. While much has been done during the past years to improve housing conditions in South Africa, many South Africans in transition still reside in informal settlements in conditions not conducive to optimal nutrition.

Several studies have shown that disruption of family units and broken homes, with less support from fathers as heads of households, are associated with malnutrition. In the past, migrant workers were probably a main contributor to this situation. Although labour migration continues to play a major role at present, the HIV/AIDS pandemic is one of the main reasons for the disruption of family life with resultant malnutrition[2].

Repeated pregnancies may jeopardise the nutritional status of both mother and child. Pregnant women have a high risk of developing iron-deficiency anaemia. A short duration of exclusive breastfeeding and the early introduction of complementary foods predispose to undernutrition and increased infections in infants. Several South African studies have indicated that one of the major reasons for childhood undernutrition is inappropriate weaning practices, with possible long-term consequences in adulthood[11].

The excessive consumption of alcoholic beverages may influence nutritional status directly and indirectly: directly, by providing energy without micronutrients (diluting micronutrient density of the diet), and indirectly, as a result of psycho-social problems affecting household resources to buy food[12].

The Interrelationship between Under and Overnutrition

There is now an increasing awareness that maternal malnutrition, foetal undernutrition (low birth weight and small for gestational age), as well as infant and childhood undernutrition (underweight, stunting and wasting), may be related to an increased risk of obesity and other NCDs in adulthood[2]. The mechanism of this phenomenon (foetal origins of adult disease) is thought to be related to a nutritional influence on epigenetics (DNA methylation) that leads to changed expressions of specific genes and metabolic programming resulting in increased vulnerability to NCDs in later life. An association between adverse early life exposures and propensity to obesity has been observed in several studies, which could explain overweight and obesity occurring without rectifying underlying micronutrient undernutrition. In all population groups, increased exposure to cheaper energy-dense, high-fat and sweet foods is leading to food choices that contribute to overnutrition in respect of macronutrients, and undernutrition in respect of micronutrients. In addition, many poor households are characterised by undernutrition in children and overweight or obesity in the mothers (caregivers). This co-existence of under and overnutrition can be addressed by ensuring optimal nutrition of pregnant women, household food security, education regarding healthy food choices, and creating an environment where these choices are available and affordable[2].

Micronutrients: Vitamins and Minerals (Inorganic Elements)

The following description of vitamins and inorganic micronutrients is quoted from a chapter in the 2007 ASSAf consensus report on *HIV/AIDS, TB and Nutrition*[2] as this furnishes the most appropriate background to the topic of the present study. (Note: The detailed references given in the previous report are not repeated.)

"A large domain of nutrition science and practice is that concerned with micronutrients, which are usefully sub-divided into organic substances (vitamins) and inorganic elements, usually referred to as minerals. Both of these are defined as nutrients which have been definitively shown to be required in milligramme or microgramme quantities in medium to long-term human diets for optimum health and to avoid asymptomatic deficiency states or full-blown diseases.

Vitamins

Most of the vitamins are precursors of some of the co-enzymes of cellular metabolism, while a few have less well-defined, but potentially important, roles such as anti-oxidant function. In evolutionary terms, the progression to complex, multicellular, multi-organ, predatory animal life (such as that of humans) appears to be have been accompanied by the deletion (or mutational loss-of-function) of many of the genes required for the often multi-step and complex pathways leading to the biosynthesis of many co-enzymes. This evolution has occurred either through natural selection or genetic drift, as the foods habitually consumed began adequately to provide what was required by the populations concerned. Frequently, the evolutionary genetic change left in place a variable number of enzymes involved in the final stages of co-enzyme biosynthesis, to increase the 'catchment' of vitamin materials by enabling food consumers to use a number of prevalent precursors rather than only the finished co-enzyme itself; co-enzyme molecules in foods need, in any case, to be partly disassembled to be absorbed during the bulk processes of the digestion and absorption of food. All compounds that can be transformed to the active intracellular co-enzymes from food are called the 'vitamers' of the vitamin concerned.

The vitamer concept is crucial in nutrition because it partly explains the variable bioavailability of vitamins present in food sources of different kinds, subjected to different forms of storage and types of preparation, and mixed with other food constituents. The other main cause of differential bioavailability is the complexity of the physico-chemical environment of the vitamer molecules in the food being ingested – many forms of strong or weak binding to other molecules or structures affect the rates at which they can be made available to intestinal absorptive mechanisms. A well-known example in pellagra prevention is the binding of vitamin B_3 *(niacin) to a variety of complex polymeric carbohydrates in staple maize products, but rendered freely available to the body by prior treatment with alkaline solutions. Another factor in the bioavailability of some vitamers is the concomitant consumption of certain drugs or alcohol. A key factor in the absorption of fat-soluble vitamins (vitamins A, D, E, and K) is the need for fat to be present in the food being ingested, so that co-absorption with the bulk lipid phase can occur.*

Because of the enormous complexity and variety of vitamin digestion, absorption, transport, storage and cellular metabolism, vitamin deficiencies have necessarily to be seen as the consequence of medium to long-term negative balance between whole-body intake and loss, in a spectrum of features typical of mild, moderate and severe imbalance. Characteristically, mechanisms exist to protect vital functions, and decreased urinary excretion is often the first indication of a shrinking body pool, blood levels remaining unchanged. As depletion progresses, still asymptomatically, urinary excretion of vitamins or their inactive metabolites virtually ceases, intestinal absorptive elements may be induced and blood concentrations of vitamins and their metabolites begin to fall, reflecting lowered content of tissues and decreased metabolic transformation. The next stage is reached when the measurable activity significantly falls of tissue or cell systems dependent on, or actually involving co-enzymes derived from vitamins; this may be accompanied by subjective symptoms of ill-health (malaise, anorexia or psychological changes) and/or detectable dysfunction of certain body systems and/or early clinical signs of a deficiency state.

It is important to remember that vitamin-derived co-enzymes are shared inside living cells as co-catalysts by many enzymes, with differing concentrations, affinities and turnover rates: there will invariably be a hierarchy of ways in which functions are lost, and these will differ in different cell types or organs. Eventually, the body's 'defence' of its crucial co-enzyme supply fails, and severe morphological and functional abnormalities ensue, usually quickly correctable by high doses of replacement vitamins, and more slowly by doses closer to the recommended daily allowances (RDAs), now called the 'nutrient intake values' (NIVs) of the vitamin in question. If uncorrected, the by now severely ill bodies of deficient subjects will develop the serious features of classical deficiency syndromes, which, unless reversed by energetic, usually hospital-based therapy, will be followed by death. The above account is necessarily highly generalised, and will vary with different vitamins, populations, individuals and situations, but it is sufficiently applicable to be of considerable value in approaching any given person who may have nutritional inadequacy of any kind, especially in clinical trials involving nutritional interventions.

Causes of vitamin insufficiency in any given subject can thus be one or more of the following sustained states: primary food shortage; diminished food intake; decreased absorption; increased requirements; and increased losses. The last two reflect the dynamic status of co-enzymes and other vitamins or vitamin-derived compounds in different body situations, such as heavy physical activity, pregnancy, lactation, rapid growth and infections which are accompanied both by systemic 'inflammation' and by specific pathophysiological disturbances associated with the type and stage of infection concerned (for example, the rapid turnover of lymphocyte populations in long-term HIV infections, or acute illness caused by secondary infections). The capacity of the body to 'store' vitamins that can be mobilised in times of shortage consists in some cases of an actual evolved mechanism to create a reserve (for example, special cells in the liver that store vitamin A as retinol esters) but is more often simply a reflection of the complex inter-organ, intercell or even intracellular devices, already described above, that serve to protect essential body functions for as long as possible when the total pool of micronutrient falls for any of the above reasons.

Modern understanding of human physiology at the molecular level has begun to reveal extensive genetic heterogeneity in populations, not so much rare point mutations giving rise to specific disorders in vitamin-related pathways or processes, but common variant alleles whose frequency during human evolution has reached some kind of genetic equilibrium for reasons that may or may not have been already deciphered. Such alleles may be systematically associated with different rates of gene transcription, in different challenge situations, if the substitution is in the promoter region; alternatively, there may be a structural (and functional) difference between the products of the alternative genes. Important examples of this phenomenon have been elucidated in the field of inflammatory cytokines, haemoglobins and histocompatibility antigens of the human leukocyte antigen (HLA) system. There is no reason to doubt that similar allelic frequency differences will be found in the case of human vitamin metabolism, and that many conclusions based on the assumption of genetic homogeneity in test or trial populations will be found to reflect composite response behaviours of subsets of the subjects concerned.

The classification of vitamins depends on two factors: the acceptance by the international nutritional community of particular organic micronutrients as being usually or always essential (though only in small quantities relative to macronutrient needs), in the long-term diets of

human populations; and the water or fat-solubility of the vitamer(s) concerned that is most easily utilised by human bodies (for example, nicotinic acid or nicotinamide in the case of vitamin B_3, are water-soluble compounds but many of their unprocessed food forms are water-insoluble). Only 13 vitamins (including all the known vitamers in each case) are recognised in human nutrition – the nine water-soluble vitamins are thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin, folate, cobalamin (B_{12}) and vitamin C; while the four fat-soluble vitamins are vitamins A, D, E and K. Three of these vitamins are really only 'conditionally essential' micronutrients, as they can be synthesised in most or all human bodies: – vitamin D by the action of sunlight on skin, vitamin K made available from intestinal bacteria and niacin can be biosynthesised from the essential amino acid tryptophan, provided the latter itself is present in the diet in adequate amounts.

Since the dietary intake is a primary determinant of whether a state of vitamin insufficiency may develop in any person, the stability of the vitamers present in food before they are eaten is important. Storage losses are proportional to temperature, pH and moisture content (enzymatic decomposition), to light exposure (riboflavin, folate), or to bacterial or fungal contamination. Processing losses are caused by the same physical factors, enhanced by physical leaching into cooking water (some processing gains may occur, of course, such as the release of bioavailable niacin from maize foods under alkaline conditions). Modern pre-treatments that inactivate degradative enzymes (for example, rapid heat exposure or pasteurisation) assist materially in conserving the effective vitamin content of many foods."

Assessment of Vitamin Deficiency Status of Individuals and Populations

Assessment of nutritional status usually includes a combination of dietary intake determinations, anthropometic measurements, clinical examinations and biochemical laboratory tests[13]. Because dietary intake measurements combined with food tables to translate food data into nutrient values, while important, is not a wholly reliable guide to the nutritional status of individuals, clinical acumen and laboratory tests are mandatory in assessments, such as are needed in persons who suffer from subacute or chronic infections, and who may be involved in clinical trials. Clinical anthropometric measures, like body weight, body-mass indices, standardised skin-fold thickness, and body composition (e.g. fat percentage, lean mass and water content) are especially valuable if observed over time. Other clinical features include symptoms and signs of systemic inflammation, such as fever, muscle pains, anorexia and nausea; indications of intestinal macronutrient malabsorption and symptoms and signs of all the specific vitamin deficiency syndromes.

Laboratory tests have become preferred for reasons of affordability, feasibility, reliability, relevance and conclusiveness. These tests have usually been derived from a detailed understanding of the physiological phenomena underlying the absorption, intravascular transport, organ metabolism and pre-excretory biotransformations of the vitamin concerned. As our knowledge increases, the tests available will undoubtedly become better and more informative, and will include genetic assessments now in their infancy. In general, there is still at present a lack of useful markers for the detailed vitamin status of individuals. Complex and expensive tests that demonstrate functional deficits directly correctable with vitamin administration or addition are superior to simpler and cheaper 'snapshot' measurements of the levels in blood,

plasma and/or urine of micronutrients or their metabolites. There is no doubt that the ready availability of tests that are both accurate and informative, and affordable and usable in field settings, is a high priority for South Africa, a country where 'hidden hunger' (equivalent to functional deficiencies of one or more vitamins or micronutrients) is very common, and where clinical research relies heavily on establishing valid inclusion criteria, baselines and outcomes for nutritional interventions in chronic infectious diseases like HIV infection and clinical tuberculosis.

An example of how improved understanding can lead to better nutritional assessment is especially relevant to the topic of this report. Persons who are in a state of systemic 'inflammation' as they respond to pathogen invasions undergo a large-scale, short-term redirection of hepatic protein synthesis (due to cytokine stimulation) towards making and secreting less albumin, plasma retinol-binding protein (RBP), transthyretin and apolipoprotein A1, and much more of the acute-phase proteins (APPs), C-reactive (CRP), serum amyloid A (SAA), fibrinogen and alpha-antitrypsin. The other 'chronic phase proteins', made in elevated amounts, are ferritin, alpha-1 chymotrypsin (ACT) and alpha-1 acid glycoprotein (AGP). As a result, certain serum values that are normally indicative of nutrient status lose much of their value, and even the use of complicated multi-variate analysis incorporating acute-phase protein levels have proved problematic because of variations in the impact of the acute-phase situation on measured levels of different micronutrients or their proxies[14]. In HIV-infected subjects, the plasma concentrations and both the fractional and absolute synthetic rates of various positive APPs were all elevated, but the plasma concentrations of some usually negative APPs[15] were not reduced despite displaying faster fractional synthesis rates; the overall pattern was thus not similar to that typical of other chronic viral infections.

A second confounder of laboratory measurement for nutritional assessment in respect of many vitamins is the fact that vitamin-derived co-enzymes are present in normal working tissues and organs as a particular optimum percentage of the cell mass concerned. Catabolic states, such as those found in infection-associated systemic 'inflammation', can cause a rapidly progressive fall in the mass of certain bulky tissues, especially skeletal muscle and white fat. While bulk tissue components are broken down to small molecules and re-distributed to cells and tissues needing them in the body's defence (e.g. glutamine from protein catabolism for energy production in lymphocytes, and fatty acids for energy generation in bodies displaying an increased metabolic rate), the surplus vitamin-derived compounds released by the 'scale-down' in tissue mass are mostly lost from the body in the urine, sweat and faeces. While this is happening, the concentrations of vitamins and metabolites in body fluids may not reflect the 'real' nutritional status of the subjects concerned.

Bioavailability is determined by comparing the effectiveness, in terms of a selected measurable parameter(s), of vitamins present in different foodstuffs with synthetic/pure compounds administered (usually singly) in the same amounts. Multiple-vitamin supplementation is complicated by the fact that the bioavailabilities of the component substances may not equate to those determined individually; some may be lower because of competition for carriers, and others may be higher, for reasons of synergism in absorptive mechanisms. In addition, some supplements require additional components for absorption (e.g. bulk fat for fat-soluble vitamins), and natural foods typically contain large numbers of uncharacterised compounds that may also be nutritionally beneficial in as yet unknown ways.

The main reservations about multivitamin supplementation are the huge cost differentials between natural foods and synthetic pharmaceutical products, and dosage safety. Particularly, the possibility that states of functional hypervitaminosis may be induced, or, even more seriously, that surplus vitamins will become involved in drug interactions that attenuate the efficacy of therapies (e.g. anti-retroviral drug regimens) or that enhance them by competing for shared disposal pathways and prolonging half-lives in the body are of concern.

Mineral Micronutrients: Inorganic Elements

Inorganic micronutrients are a number of elements present in foods that are required to be present in human diets over medium to long periods, in sufficient quantities to replace the base-line or accelerated net losses from body stores that are unavoidable as a result of excretory and secretory processes, such as urination, defaecation and sweating. A subset of these inorganic micronutrients has been shown to be especially relevant to human immunity and resistance to infections, and will be briefly discussed here.

In general, they resemble vitamins being present in particular food sources in notable amounts; in varying bioavailability because of differential binding to other food constituents, either in the native food (e.g. phytic acid) and/or in the digestive mixtures generated in the gut; and in each having extremely complex and highly regulated mechanisms for their absorption, intravascular transport, and tissue uptake. In addition, like most vitamins, they are also bound with varying affinities to intracellular molecules, creating a kind of graduated 'storage' system, while their egress mechanisms are often also specialised. All of this reflects evolved homeostatic devices to 'protect' the essential functions subserved by the particular metals or other inorganic elements concerned. Again, depletion of one of the essential inorganic micronutrients from the body (i.e. a steady-state negative balance between intake and losses over time) leads in most instances to complex compensatory rearrangements throughout the body, which 'protect' vital functions by enhancing capture from food, changing transport patterns, prioritising cell types in terms of supply, and diminishing rates of net excretion. As in the case of most vitamins, the order of events during progressive depletion will usually first consist of diminished urinary/faecal losses without a change in blood levels; next, lowered blood concentration without change in tissue content; followed by next, symptomatic deficiency associated with lowered tissue content; and finally, serious disorder and death.

Assessment of Nutritional Status with Respect to Inorganic Micronutrients

As in the case of vitamins, this requires a combination of clinical acumen and laboratory measurements. Detailed knowledge of the relevant physiology in each case has made it possible to devise feasible, reliable and accurate tests ('deficiency markers') that involve mixes of direct assays of the particular element in body fluids or tissues; indirect measures based on proteins that bind the substance or are necessary for its transport or uptake, or on the activity of enzymes requiring the substance for their catalytic functioning. The acute phase of infections (systemic 'inflammation'), as in the case of several vitamins, involves perturbations in the relative concentrations of plasma proteins and bulk tissue catabolism, that may lead to data being collected in respect of certain metals or other inorganic elements that are misleading in terms of the 'true' nutritional status of the persons concerned. Genetic micro-heterogeneity

in the complex systems responsible for handling inorganic micronutrients is likely to be prevalent in human populations, even if little is as yet known about this factor, apart from the prevalent genetic iron-overload conditions that have been well-characterised.

Relevant Recommendations of the 2007 ASSAf Consensus Report

The 2007 ASSAf consensus report on *HIV/AIDS, TB and Nutrition*[2] made a number of recommendations regarding the prevention and treatment of malnutrition, HIV/AIDS and TB. Some of these recommendations motivated the present study and are briefly discussed below:

Recommendation 1

One of the main recommendations of the 2007 ASSAf report was that the indicators of both vitamin and inorganic micronutrient depletion and repletion in individuals and populations needed to be much better defined. Examination of the evidence included in that report revealed that many, perhaps most, of the studies had proceeded without a reasonable understanding of the actual nutritional status of the subjects included in the studies concerned, nor had the complexity of what was meant by 'nutritional status' been adequately explored.

Because of the enormous complexity and variety of vitamin digestion, absorption, transport, storage and cellular metabolism, vitamin deficiencies had necessarily been seen as consequences of medium to long-term negative balance between whole-body intake and loss, in a spectrum of features typical of mild, moderate and severe imbalance.

The major problem that needed to be addressed by investigators in the design and execution of their studies, was to distinguish between the effects of supplemental micronutrients in repleting actually deficient cellular supplies of crucially important metabolites on the one hand, and effects which amount to simply repleting the whole-body supply situation on the other. In the first case, one was likely to see improved functioning (including fighting infection and minimising its deleterious effects), while in the second, beneficial effects in respect of infection were unlikely to ensue as the supplementation mainly brought the body's reserves up to the steady state. This generalised view had to be modified when there was no steady state, for example when extensive losses of micronutrients were occurring on a regular basis as a result of chronic diarrhoea. It had also to be remembered that each of the micronutrients would have an individualised effect on the spectrum of depletion/repletion. In pioneer studies, where all the stops had to be pulled out in order to establish the mechanisms involved in an intervention, for later simplification in the implementation phases, such information was essential.

It was evident, for example, that antiviral treatment of HIV infection, which almost always had markedly positive effects on the state of well-being of the subject (with increased appetite, decreased bowel disorder and associated malabsorption, etc.) would itself bring about a 'repletion process' if there was deficiency at the time when treatment was started and if an adequate diet was followed. Careful characterisation of this kind of whole-body process would be useful in developing comprehensive dietary guidelines.

Recommendation 2

Obesity was common in South Africa despite the frequency of below-par micronutrient intakes and research was needed to assess possible interactions between low-grade 'inflammation', now known to occur in apparently healthy obese persons, micronutrient deficiencies and HIV infection at various stages.

Recommendation 3

In cases where micronutrients were not precursors of cellular co-enzymes, such as the vitamins which acted as antioxidants in various locations, the above-described model of a depletion/repletion spectrum had to be modified, as the dose-response curves were not likely to be saturational, but rather continuously linear or (more usually) peaking to a maximum benefit before becoming toxic. There may be cases, such as vitamin A (See below) where there was a mix of both kinds of systems due to a multiplicity of pathways and active metabolites.

The above considerations applied similarly to the case of inorganic micronutrients, where the assessment situation was also not very different to that of vitamins.

Recommendation 4

In addition, the confounding of measured blood levels by marked changes in the various acute-phase proteins, as well as by losses of lean tissue mass, needed to be understood and better evaluated in South African populations. The chronicity of the situation had also be determined in order to sharpen the assessment, as had the assessment of protein/energy nutritional status before the onset of the infection concerned. Both these factors strongly coloured the interpretation of the findings.

Recommendation 5

A good example of an obvious need in the system was the full exploration of the possibility that improvements in nutritional status because of better food security had, on balance, been negated by the impact of widespread HIV/AIDS; a thorough re-assessment, with finer grain tools, of the current and evolving situation with respect to population nutritional status in the light of the HIV/AIDS and TB epidemics was urgently necessary.

Motivation for the Present Study from these Recommendations

The 2007 ASSAf study panel accordingly attached prime importance to the rapid development of knowledge-based, affordable, available and reliable tests of actual status with respect to individual micronutrients, applicable both to individuals and to populations, especially in intervention studies where study designs could be improved and interpretational difficulties overcome. This could not be done without encouraging relevant basic research and teamwork in clinical studies, plus energetic attempts to produce national consensus guidelines to best practice, supported by the necessary attention to human resource capacity, as well as access and affordability issues.

Some Issues Relating to the Provision of Appropriate Laboratory Tests in Both the Public and the Private Sectors

This study is predicated on the notion that micronutrient malnutrition is common in South Africa and that it is a contributing cause of much ill-health, under-performance and misery. The accurate measurement, treatment and monitoring of the micronutrient status of individuals and populations is accordingly something that needs to be prioritised, and its provision widened and optimised, as best possible. Like immunisation, micronutrients as cheap and generally available chemical compounds have the potential to become sustainable, affordable and effective preventive and therapeutic agents in promoting the health of many people, especially the poor.

The findings of the previous ASSAf study panel, as summarised above, of a high prevalence in South African communities of inadequate intakes of many micronutrients, point to the relevance and importance of this agenda, which is particularly pressing in a developing country with high rates of poverty, unemployment, changing lifestyles, and widespread household food insecurity.

One of the other key findings of the previous ASSAf report supports the same conclusion, namely, the (surprising) national deficit in modern nutritional assessment methods, particularly tests of micronutrient status that are grounded in the most up-to-date understanding of relevant physiology and pathological chemistry, and are reliable, affordable and practically helpful.

Main Objectives of the Present Study

In essence, the present study aims to answer the following four questions:

1. How have modern understandings of the detailed physiology and pathophysiology of the six micronutrients concerned, affected the nature of the laboratory/clinical tests and other assessment methods (in-depth dietary assessments, nutritional anthropometry, and clinical signs of malnutrition) that have to date been developed worldwide?

2. Which of the tests or methods are maximally informative and reliable as to the actual depletion/repletion status of individuals and populations with respect to these micronutrients?

3. Which of these tests are robust, affordable, capable of decentralised operation, and of mass application, in both clinical and population-based settings?

4. How are the tests affected, or the interpretation altered, by factors such as varying health status of the subjects under investigation, by other dietary components (nutrient foodstuffs and non-nutrient endogenous compounds and additives), and by differing composition of gastrointestinal bacteria and other commensals?

Concluding Remarks: Dissemination of the Study Results

The assumption that answers to all these questions can be provided, begs the question as to how the new conclusions and other information produced by the study can be made available to the broader health system in South Africa? That system is divided into the public sector (dominated by the National Health Laboratory Service(NHLS) servicing public clinics, smaller hospitals and large academic complexes), and the private sector (dominated by a number of large, for-profit companies serving private general and specialist practitioners and private hospitals). The medical aid companies form another crucial part of the system that needs to be considered, as they impose limits on testing and in other ways regulate the provision of particular tests, mainly but not only in the private sector.

The possible implementation of National Health Insurance (NHI) may become paramount in the evolution of effective and affordable health care for the nation, and much work needs to be done to crystallise the optimal mix of fee-for-service *versus* capitation-based cost recoveries for health care provision, especially with reference to laboratory testing. Whatever emerges as best for South Africa, it is increasingly apparent that appropriate laboratory testing will be a vital step in defining and correcting the highly prevalent nutritional deficiencies that beset much of our population. This study is aimed at providing the scientific evidence base for the definition of policy in future efforts to improve the nutritional health of the nation.

References

1. Vorster HH. A global perspective of Food and Nutrition. In: Gibney MJ, Lanham-New S, Cassidy A, Vorster HH. Eds. *Introduction to Human Nutrition: The Nutrition Society Textbook Series:* (Second Edition). Chichester, Wiley-Blackwell. 2009; 1-11 (Chapter 1).
2. Academy of Science of South Africa (ASSAf). *HIV/AIDS, TB and Nutrition: Scientific enquiry into the nutritional influences on human immunity with special reference to HIV infection and active TB in South Africa, 2007*.
3. Faber M, Wenhold F. Nutrition in contemporary South Africa. *Water SA*. 2007; 33(3):393-400.
4. Labadarios D, Maunder E, Steyn, MacIntyre UE, Swart R, Gericke, Nesamvuni AE, Huskisson J, Vorster HH, Dannhauser A. 2001. National Food Consumption Survey in children aged 1-9 years. (In: Elmadfa I, Anklam E, JS König. Eds. IUNS Joint Research Centre: *Modern Aspects of Nutrition. Present Knowledge and Future Perspectives.* 2001; 56:106 – 109.
5. Labadarios, D *et al.*, (2008). Executive summary of the National Food Consumption Survey Fortification Baseline (NFCS-FB-I) South Africa, 2005. *South African Journal of Clinical Nutrition,* 2008; 21(3) (Suppl 2): 245-300.
6. Boutayeb A, Boutayeb S. The burden of non communicable diseases in developing countries. *Int J Equity Health*. 2005; 4(2): doi:10.1186/1475-9276-4-2 (open access).
7. Vorster HH, Venter CS, Wissing MP, Margetts BM. The nutrition and health transition in the North-West province of South Africa: a review of the THUSA (Transition and Health during Urbanisation of South Africans) study. *Public Health Nutr.* 2005; 8(5):480-490.
8. Vorster HH, Oosthuizen W, Jerling JC, Veldman F, Burtger HM. *The nutritional status of South Africans: A review of the literature from 1975-1996*. Health Systems Trust, Durban. 1997: 1-22; 1-120.

9. Vorster, HH, Bourne, LT, Venter, CS, Oosthuizen, W. Contribution of nutrition to the health transition in developing countries: a framework for research and intervention. *Nutr Rev*, 1999; 57(11): 341-349.

10. Vorster HH. The link between poverty and undernutrition: a South African perspective. *Health SA Gesondheid*. 2010; 15(1):6p ISSN: 2071-9736 (on line).

11. Kruger R, Gericke G. Breastfeeding practices of mothers with children (aged 0-36 months in a rural area of South Africa: A qualitative approach. *J Fam Ecol Cons Sci.* 2001; 29:60-70.

12. Pisa PT, Loots DuT, Nienaber C. Alcohol metabolism and health hazards accociated with alcohol abuse in a South African context: a review. *SA J Clin Nutr.* 2010; 23(3):S4-S10.

13. Gibson RS. *Principles of Nutritional Assessment*. Second Edition. Oxford University Press. 2005; 908p.

14. Tomkins A. Assessing micronutrient status in the presence of inflammation. *J Nutr.* 2003; 133(5):16495-16555 (on line).

15. Ogunro PS, Idogun ES, Ogungbamigbe TO, Ajala MO, Olowu OA. Serum concentration of acute-phase protein and lipid profile in HIV-1 seropositive patients and its relationship to the progression of the disease. *Nig Postgrad med J*. 2008; 15(4):219-224.

Chapter 2

Vitamin A

ASSAf
ACADEMY OF SCIENCE OF SOUTH AFRICA

Introduction

Each individual is unique in terms of his or her precise metabolic situation at any given time. The particular pre-history of food intake, body growth and activity, disease and injury interact cumulatively in their effects on a person. Additionally, unique genetic and epigenetic determinants may also exist. Together with this individuality comes the complexity of the forms of vitamin A present in the body, their locations, amounts, inter-relationships and subtle effects on multiple processes. Systematic but individually varying responses in respect of these variables will occur when the supply of this essential dietary nutrient diminishes; similarly, typical but also varying responses will be found when surplus quantities are ingested acutely or over time.

Nutritional assessment in respect of vitamin A, in individuals, groups and populations, therefore requires knowledge of the chemistry, food composition, physiological handling, metabolism and disposal of the group of substances known collectively as 'vitamin A'. It also requires a best-possible understanding of individual variability as described in the first paragraph. Constraints imposed by many factors make it necessary to select those assessment methods that are robust and reliable, affordable and feasible in different settings including in disease-endemic populations and maximally informative for the purposes of the particular investigations underway, based on up-to-date conceptual frameworks and knowledge.

This chapter seeks to review available information and evidence that bears on nutritional assessment in respect of vitamin A, ensuring that all recent advances in the field are taken into account.

Definition, Description and Chemistry of Vitamin A

Vitamin A was the first vitamin to be discovered, almost simultaneously by two American groups of researchers in 1913. It was initially known for its essential role in growth and survival, and later for its role in vision.

Vitamin A is a collective name for two groups of compounds with vitamin activity, namely, (i) retinol, the alcohol form, originally isolated from the retina, and related compounds, retinaldehyde and retinoic acid, all with 20 carbon atoms; retinoic acid is an oxidised form of retinol and has important roles in the genetic control of metabolism; and (ii) a variety of 40-carbon atom carotenes and related compounds, known as the carotenoids. Beta-carotene, alpha-carotene, beta-cryptoxanthin, lutein, and lycopene are the carotenoids most

commonly found in human plasma. These carotenoids, together with zeaxanthin, have been shown to have health-promoting effects; beta-carotene, alpha-carotene and beta-cryptoxanthin are provitamins A. The latter two exhibit about 50% of the vitamin A activity of beta-carotene. The minimum requirement for a carotenoid to have vitamin A activity is an unsubstituted beta-ring with an 11-carbon polyene chain.

Epidemiology of Vitamin A Deficiency

Vitamin A deficiency (VAD) is common in many developing countries, being second only to iron deficiency anaemia as the highest public health nutrition-related problem in these countries. Globally, more than 200 million children under five years are vitamin A deficient and VAD is still the leading cause of blindness in children. Women in developing countries are also at risk of VAD, especially during pregnancy and lactation. Because the vitamin A content of breast milk is often low in vitamin A-depleted women, infants of these women are at greater risk of becoming VAD early in life. There is a strong association of VAD with overall undernutrition. Both are prevalent in deprived poor people with low food intake, especially of meat and milk products (e.g. liver, eggs and butter), sources rich in vitamin A, and of oils and fats, which are necessary for the absorption of vitamin A. It is generally accepted that correcting VAD in populations at risk of deficiency is an investment in improving human development.

In South Africa, several national studies[1,2,3] have indicated that VAD is a significant public health problem. In 1995, 3% of children aged six to 71 months were adjudged vitamin A deficient (serum retinol values < 10 ug/dL) while 33% were thought to be marginally deficient (serum retinol < 20 ug/dL). In the 2005 National Fortification Baseline Survey[3] it was found that two out of three children, and one out of four women, had a poor vitamin A status.

Public health programmes to control VAD include supplementation with therapeutic doses of the vitamin, food fortification, and dietary diversification. Biofortification to enhance the content of provitamin A in staple crops has been recognised recently as a viable and feasible alternative.

Supplementation with vitamin A reduces the risk of child morbidity and mortality, as well as the risk of severe diarrhoea and measles. It may also reduce maternal mortality[4]. There is convincing evidence that vitamin A given via fortified food or in pharmaceutical doses as supplementation has resulted in a significant reduction in overt clinical deficiency. However, the effect of supplementation on improving marginal vitamin A status, which is more prevalent, is difficult to assess. This is important because populations with marginal vitamin A status are more susceptible to infections, and a single bout of infection can rapidly deplete vitamin A stores further. The importance of other health problems, such as infection and systemic inflammation or gastrointestinal helminths has to be considered in any strategy for prevention or treatment of VAD, as well as the complex interactions with other nutrients, e.g. zinc, iron, riboflavin, vitamin D and methyl donors.

Metabolism, Physiology and Storage of Vitamin A and Provitamin Carotenoids

Metabolism

The metabolism of vitamin A is illustrated in Figure 2.1

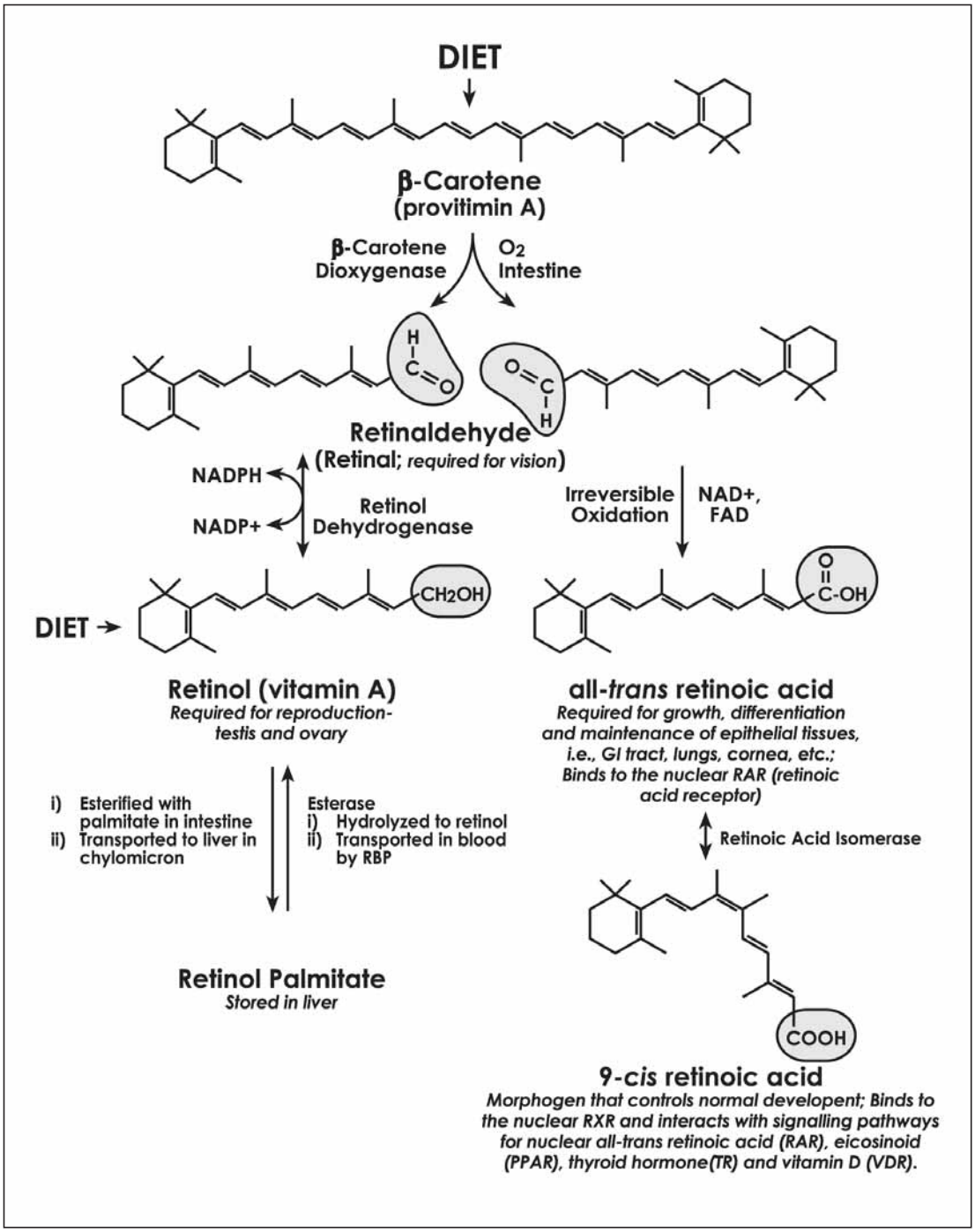


Figure 2.1 Diagramme of vitamin A metabolism and function

The preformed natural retinoids (compounds structurally related to retinol), and precursor carotenoids that possess the biological activity of retinol, are absorbed from the small intestine, incorporated into chylomicrons, and are then transported via the lymphatic system to the liver. The amount of dietary fat present determines the quantity of vitamin A that will be absorbed. Vitamin A is stored in the liver as retinyl esters (retinol-palmitate) in intracellular lipid droplets. Retinyl esters are hydrolysed in the liver and secreted into the plasma bound to retinol-binding protein (RBP), which itself is bound to a second plasma protein, transthyretin, for transfer to the required sites in the body. Hepatic synthesis of RBP is dependent on the availability of zinc and an adequate overall intake of protein; it is diminished during the generalised body response to shock and infection, the acute-phase response.

Cellular uptake of both retinol and its oxidation product, retinoic acid, may involve a cell-surface receptor for the retinal-RBP-transthyretin complex and/or an endocytic cycle releasing the retinol inside the cell. Uptake is facilitated by a number of intracellular retinoid-binding proteins, the 'cellular retinol-binding protein-1' (CRBP-1) and 'retinoic acid-binding protein-1' (CRAB-1). These proteins are constitutive, i.e. their tissue levels do not change in vitamin A-deficiency states. Measuring them in tissue samples is therefore not helpful in assessment of VAD.

Intracellular retinol (released in the case of the liver by de-esterification of retinyl esters by a microsomal hydrolase) is oxidised to retinaldehyde while bound to CRBP-1, through the action of a number of nicotinamide adenine dinucleotide (NAD+) or nicotinamide adenine dinucleotide phosphate (NADP+) dependent microsomal dehydrogenases, and retinaldehyde in turn, is oxidised by a number of retinaldehyde dehydrogenases to retinoic acid. This reaction takes place in a large number of locations in the body. Both retinol and retinoic acid (in their two respective isomeric all-trans and 11-cis forms) are important and highly pleiotropic transcriptional regulators, with diverse biological activities, many of which are inter-dependent with other transcription-controlling hormones. These effects are mediated by a complex set of nuclear retinoid receptors, in two classes, retinoid X receptors (RXRs) for retinal and retinoic acid receptors (RARs) for retinoic acid.

In general, the way in which the expression of the enormous range of proteins controlled by the two retinoids is hierarchically ordered at different concentrations of their regulator retinoid is as yet not understood. At present, an all-or-nothing approach assumes that expression of any given target protein in any given target cell type will fall if the plasma retinol level falls. (A topical example is the recent work on the role of dendritic cells in the intestinal mucosa in generating retinoic acid from circulating retinol in the regulation of the 'homing' of immunoglobulin A (IgA)-producing B lymphocytes to the mucosa in question - See below.) Understanding the hierarchy of transcriptional control is obviously a matter of great importance in future attempts to refine the assessment of VAD at the tissue level.

In hypervitaminotic states, some of the surplus retinol may be excreted as retinoyl glucoronide via the bile. At high intakes, a microsomal cytochrome P450-dependent oxidation pathway is utilised for catabolism of retinol in the liver to other metabolites, which are excreted via the bile and in the urine. At very high intakes, this pathway becomes saturated and the excess vitamin A becomes toxic.

Physiology: Functions and Effects of Vitamin A

Progressive depletion of vitamin A in animals has been shown to result in histological and functional abnormalities in cells throughout the body, alterations in immune function[5], wasting[6], severe infections, and death[7]. Night blindness is an early and universal phenomenon, and total blindness very common in late stages.

Modern molecular biology has begun to unravel the mechanisms by which vitamin A exerts its powerful, pervasive effects. It is now known that vitamin A and/or its metabolites (especially retinoic acid) directly affect the expression of at least 300 different genes – a number that is likely to grow – which, in turn, affect cellular differentiation, the integrity of epithelial structures, and immunologic function. While many of the precise mechanisms by which vitamin A and retinoic acid manifest their impact have yet to be delineated, the biological plausibility of these effects is well established.

Vitamin A is essential for vision, and plays a role in gene expression and tissue differentiation. Retinoic acid supports most of the functions of vitamin A. However, it cannot be reduced back to retinol in the body. Animals maintained on retinoic acid only will be blind and not able to reproduce successfully. Therefore, vitamin A is needed by all body tissues systemically, to maintain growth and the well-being of cells. With this crucial role, VAD has grave consequences for all stages of the human lifecycle. The functions of vitamin A are summarised in Box 2.1 below.

Box 2.1 Summary of Functions of Vitamin A in the Human Body

- Component of rhodopsin for night vision.
- Essential in the differentiation process of epithelial tissues.
- Required for normal ciliary function.
- Important in the synthesis of glycoproteins and glycosaminoglycans.
- Assists in stabilisation of lysosomal membranes.
- Essential for cell-mediated immunity and host defence.
- Facilitates process of reproduction and control of embryonic development.
- Essential for normal growth (together with many other nutrients).
- Essential for haematopoiesishematopoiesis (in conjunction with iron).

Provitamin A and other carotenoids may have additional influences on human health, such as enhancement of the immune response and reduction of the risk of degenerative diseases, such as cancer, cardiovascular diseases, cataracts, and macular degeneration[8-14]. The action of carotenoids against these diseases has been attributed to an antioxidant property, specifically, their ability to quench singlet oxygen and interact with free radicals. However, other mechanisms have been reported: modulation of carcinogen metabolism, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of cell-to-cell communication, and filtering of blue light[13].

The ability of carotenoids to quench singlet oxygen has been linked to the conjugated double-bond system, the maximum efficiency being shown by carotenoids with nine or more conjugated double bonds[15]. The acyclic carotenoid lycopene was found to be more efficient than the dicyclic beta-carotene[16], despite both compounds possessing 11 conjugated double bonds.

Vitamin A and Possible Prevention of Non-communicable Diseases (NCDs)

Prostate Cancer

Reports from several studies have suggested that carotenoids, and in particular lycopene, could be prostate cancer-preventive agents. Although this has stimulated extensive research, and enthusiasm, the epidemiologic evidence remains inconclusive. Peters *et al*.[17] investigated the association between prediagnostic serum carotenoids (lycopene, alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, and zeaxanthin) and risk of prostate cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, a multicentred study designed to examine methods of early detection and risk factors for cancer. The study revealed that high serum beta-carotene concentrations were associated with increased risk for aggressive, clinically relevant prostate cancer. Lycopene and other carotenoids were unrelated to prostate cancer. Consistent with other recent publications, these results suggest that lycopene or tomato-based regimens will not be effective for prostate cancer prevention.

Schenk *et al*.[18] examined whether serum concentrations of retinol were associated with the risk of prostate cancer in a nested case-control study in the above-mentioned trial and found serum retinol concentrations not to be associated with overall prostate cancer risk. However, the highest *versus* lowest concentrations of serum retinol were associated with a 42% reduction in aggressive prostate cancer risk, with the strongest inverse association for high-grade disease. Their results suggested that "higher circulating concentrations of retinol are associated with a decreased risk of aggressive prostate cancer".

Atherosclerosis

Another possibility is that vitamin A and the carotenoids may be involved in protection against atherosclerosis. Riccioni *et al.*[19] found that low plasma concentrations of antioxidant vitamins and provitamins (A, E, beta-carotene and lycopene) were associated with early carotid atherosclerotic lesions, and that regular intake of foods rich in lycopene and antioxidant vitamins may slow the progression of atherosclerosis.

Vitamin A Deficiency (VAD)

The Role of VAD in Child Survival

The evidence that VAD increases childhood morbidity and mortality and that this can be prevented by improving vitamin A status is overwhelming. The more severe the VAD, the more common and severe are the consequences. For over 60 years physicians have reported increased rates of infection and greater severity of measles in children who were VAD – all

conditions which could be cured or prevented with vitamin A supplementation. Hospital-based, randomised, controlled trials on the treatment of measles, one in London in 1932[20], and two in the 1990s in Africa[21, 22,] showed that children who received vitamin A died at less than half the rate of children who received routine therapy and that measles complications, in both incidence and severity, were reduced.

As mentioned above, VAD is associated not only with increased morbidity, but also mortality in children. A child may die from a weakened immune system when vitamin A is lacking. Improving vitamin A status can strengthen a child's resistance to disease and decrease the likelihood of childhood mortality[23]. According to Aguayo and Baker[24], the public health importance of VAD came to the fore in the early 1980s, when community-based studies of Sommer and co-workers[25] showed that "the rates of morbidity and mortality from diarrhoea and respiratory infections were higher in children with mild xerophthalmia than in children without any VAD-related eye signs".

Several meta-analyses[26-28] have consistently shown a strong relationship between VAD and child survival. These meta-analyses were based on intervention trials, carried out between 1986 and 1993, that assessed the contribution of vitamin A deficiency to child mortality and which involved more than 165 000 children globally. The findings showed that "in areas where VAD is prevalent, child mortality is reduced by 23% to 34% after vitamin A intervention, and that the reduction in childhood mortality is attributable largely to the reduction in mortality from measles, severe diarrhoea, dysentery and possibly falciparum malaria".

Visual Impairment

Vitamin A plays a role in visual development of infants. The earliest clinical sign of VAD is a loss of sensitivity to green light, followed by an impairment to adapt to dim light. This is eventually followed by night blindness. Severe and persistent VAD results in xerophthalmia, (keratinisation of the cornea, ulceration and blindness) (summarised by Bender[23] in 2009).

VAD therefore causes severe visual impairment and blindness and has been recognised as the leading cause of preventable paediatric blindness in developing countries[29]. However, perhaps more disheartening is the fact that 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. Thus, there are several more millions of children who are health-compromised as a result of VAD[30-31].

In pregnant women, VAD causes night blindness and may increase the risk of maternal mortality. For pregnant women in high-risk areas, VAD occurs especially during the last trimester when demand by both the unborn child and the mother is highest. The mother's deficiency is demonstrated by the high prevalence of night blindness during this period.

Weakened Epithelial Integrity

VAD leads to weakened epithelial integrity and loss of some functions, such as mucus production, thereby increasing vulnerability of affected individuals to infections such as diarrhoea and measles[31-32].

Reduced Immune Response and Haemopoiesis

VAD plays an important role in the differentiation of immune system cells and even mild deficiencies increase susceptibility to many infectious diseases. At the same time, the synthesis of RBP is reduced in response to infections with escalating impairment of immune responses. Both vitamin A and iron are necessary for normal haemopoiesis. However, vitamin A also has independent effects on haemoglobin in the absence of iron, possibly mediated through effects of retinoic acid on genetic control[32]. The syndrome of VAD is therefore characterised by increased susceptibility to infections, anaemia and elevated acute-phase proteins[32].

Recent work has defined some of the mechanisms by which mucosal immunity (particularly in the intestines) is influenced by retinol[33]. It appears that the local synthesis of retinoic acid from food-borne or blood-borne retinol, in dendritic cells (and possibly also in epithelial cells) is necessary for homing of activated B-lymphocytes to the mucosa, and for the protective generation of IgA. The rate of retinoic acid synthesis is proportional to the supply of retinol; thus VAD diminishes IgA-formation and consequently mucosal immunity.

The relationship between impaired vitamin A status and infections appears to be synergistic, the occurrence of one aggravating the other. Infections of the upper and lower respiratory tracts often become apparent early on in VAD, long before the clinical manifestations of xerophthalmia are observed. Respiratory disease prevalence increases in a linear fashion with increasing severity of xerophthalmia[34]. Of the infections associated with VAD the evidence base for measles is the strongest. Vitamin A status affects both the severity of and mortality from measles [21].

Growth Failure in Children

VAD is associated with growth failure in children, particularly with stunting and wasting[35]. However, the inconsistent results on growth of studies in which young children at risk of VAD received vitamin A supplements (summarised by Thurnham[32]) demonstrate that growth is dependent on many nutrients.

VAD in the Elderly

Oxidative damage is involved in the ageing process and also plays a role in many age-related degenerative diseases. Adequate dietary intake of antioxidants may be vital to the promotion of health, prevention of disease, well-being and longevity of the elderly. Recent studies show that low plasma carotenoids are an independent risk factor for mortality among elderly[36,37]. Meydani[38] pointed out that while antioxidants are important in improving immune function in the elderly, there is also literature to support the hypothesis that longevity is associated with favourable antioxidant status. Mecocci *et al*.[39] observed that high levels of vitamin A and vitamin E appeared to be playing a role in the extreme longevity of the subjects in their study.

VAD during Pregnancy and Mother-to-child Transmission of HIV

Vitamin A is transferred in two ways from mother to child: via the placenta during gestation, and via breast milk during lactation. Adequate transfer of vitamin A is essential during both of these periods of development. Thus, maternal vitamin A deficiency during pregnancy

results in placental dysfunction, stillbirths, and congenital malformations. Maternal vitamin A deficiency during lactation rapidly predisposes the nursling to severe vitamin A deficiency. Semba *et al*.[40] showed that among pregnant women in Malawi, infants born to mothers whose serum retinol values were in the lowest quartile had a three-fold greater mortality risk than those born to mothers with serum retinol in the higher quartiles. In rural Nepal, women who became night blind near the end of pregnancy had two to three times more urinary tract infections, diarrhoea and dysentery, eating problems, pre-eclampsia and eclampsia, as well as anaemia. Vitamin A or beta-carotene supplementation reduced perinatal mortality by 44% in a group of more than 2 000 Nepali women[41]. However, Kirkwood *et al.*[42] recently indicated that vitamin A supplementation of pregnant women in Ghana could not confirm the results from Nepal. Their study showed no reduction in maternal mortality with vitamin A supplementation.

All these underscore the importance of good vitamin A nutriture during pregnancy. However, vitamin A can be teratogenic and supplementation during pregnancy should not exceed 10 000 IU (3 mg RE) per day. Thurnham[32] emphasises that embryogenesis is under control of retinoic acid isomers which are probably responsible for observed teratogenic effects. Therefore, women who are pregnant or who may become pregnant should not receive retinoid therapy for skin conditions or as supplements.

Low serum retinol concentrations are very common in HIV infection[43], and are often associated with high viral loads, increased progression of HIV to AIDS and mortality[44], as well as an elevated risk of mother-to-child HIV transmission[45]. However, some trials in Africa showed that vitamin A supplementation of HIV-infected pregnant women increased mother-to-child transmission of the virus. The latest WHO Guideline (2011) states: *Vitamin A supplementation in HIV-positive pregnant women is not recommended as a public health intervention for the prevention of mother-to-child transmission of HIV (strong recommendation). All pregnant women, including those living with HIV/acquired immune deficiency syndrome (AIDS), should be encouraged to receive adequate nutrition through consumption of a healthy balanced diet*[46].

Sources, Requirements and Reference Intakes of Vitamin A

Sources of Vitamin A

Foods

Humans normally obtain preformed vitamin A from animal products in the diet and provitamin A carotenoids (mainly beta-carotene) from deep green, yellow and orange fruits, vegetables and some oils, such as red palm oil as shown in Figure 2.1.

Carotenoids in foods are generally 40 carbon atom tetraterpenoids formed from eight 5-carbon atom isoprenoid units joined head-to-tail, except at the centre where a tail-to-tail linkage reverses the order, resulting in a symmetrical molecule. An important feature is a centrally located, extended conjugated double-bond system, which constitutes the light-absorbing chromophore that gives carotenoids their attractive colour and provides the visible absorption spectrum that serves as a basis for their identification and quantification. The basic skeleton may be modified in many ways, including cyclisation, hydrogenation, dehydrogena-

tion, introduction of oxygen functions, rearrangement, chain shortening, or combinations thereof, resulting in a multitude of structures.

Hydrocarbon carotenoids (e.g. beta-carotene, lycopene) are known as carotenes, and oxygenated derivatives are called xanthophylls. Common oxygen substituents are the hydroxy (as in beta-cryptoxanthin), keto (as in canthaxanthin), epoxy (as in violaxanthin), and aldehyde (as in beta-citraurin) groups. Carotenoids can be acyclic (e.g. lycopene), monocyclic (e.g. gamma-carotene), or dicyclic (e.g. alpha and beta carotene). In nature, carotenoids exist primarily in the more stable all-trans (or all-E) form, but small amounts of cis (or Z) isomers do occur[47].

Because plants are able to synthesise carotenoids *de novo*, the carotenoid composition of plant-derived foods is enriched by low levels of biosynthetic precursors and derivatives of the main components. Carotenoids are not as widely distributed in animal-derived foods and are present at much lower levels. Animals are incapable of carotenoid biosynthesis, and hence depend on dietary carotenoids, which are selectively or unselectively absorbed, converted to vitamin A, deposited as such or slightly altered to form carotenoids typical of animal species.

Consumption of natural sources of vitamin A rarely results in toxicity. The exception is toxicity resulting from excessive high intakes of carnivore liver on a continued basis. Liver contains 3 000 – 5 000 µg of retinol per 100 g. A case study reported vitamin A toxicity symptoms in a seven-month-old infant who was given ~ 12 100 µg of retinol/d, mostly in the form of chicken liver, although this level of intake is rare[48].

Supplements and Massive Dosing

Massive vitamin A supplement dosage of pre-school children from vulnerable communities was first tried in India in the 1960s, using 200 000 to 300 000 IU (60 000 – 90 000 µg RE) vitamin A. In countries with a public health problem of VAD, periodic high dose vitamin A supplementation is recommended to address the problem[32].

In South Africa, the present preventive supplementation protocol of the Department of Health[49] follows international recommendations, with the exception that all post-partum women receive only a single dose of 200 000 IU vitamin A within eight weeks after delivery. However, the department recently (3 August 2012) endorsed the 2011 WHO Guidelines which did not recommend the use of vitamin A supplementation as a public health intervention for the prevention of maternal and infant morbidity and mortality in postpartum women.

Children aged six to 11 months receive a single vitamin A dose of 100 000 IU, while children aged 12 to 60 months receive 200 000 IUs every six months. The capsules with different dosages have different colours to assist health personnel in delivering the correct dosages (50 000 IU capsules are white, 100 000 IU capsules are blue and the 200 000 IU capsules either red or yellow). The protocol is very detailed with specific instructions on when and how to administer the capsules at the health facility, as well as guidelines on curative vitamin A supplementation. However, in a recent policy brief (February 2012) the South African Medical Research Council (MRC) warned that vitamin A supplentation may not be necessary in children who meet vitamin A requirements by eating 40-50 g sheep liver twice a month, a dietary practice followed in certain areas.

Fortified Foods

Fortified foods provide additional vitamin A in some settings. In industrialised countries important sources of preformed fortificant vitamin A include fortified milk (about 150 µg per cup), fortified breakfast cereals (10 – 100% of the recommended intake per serving) and margarine (approximately 50 µg per pat)[50]. In some developing countries, approaches to increasing vitamin A intake include fortification of sugar, oil, margarine, milk, maize meal, wheat flour, corn flour, instant noodles and rice. Infant formulas and prepared infant cereals are usually fortified with vitamin A. In South Africa, in addition to the availability of some of the above-mentioned fortified products, the Department of Health instituted a mandatory fortification scheme of two staple foods, maize meal and bread flour, with a vitamin-mineral mix containing vitamin A.

Based on the potential for individuals to consume large amounts of vitamin A from one or more fortified sources, it is important to ensure that the total intake of the vitamin from all available sources is unlikely to be excessive, given the push toward vitamin A fortification of more foods and the possibility that the current maternal iron-folate supplements available through the United Nations Children's Fund (UNICEF) will be reformulated to include the daily recommended intake of vitamin A[35].

Factors Influencing Body Utilisation of Vitamin A

Infections of the gut, worm infestations and particularly giardiasis, decrease vitamin A absorption. Laboratory studies have shown that many contaminants, including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), in the environment can disrupt vitamin A physiology and alter the distribution of its essential metabolites in dietary sources of vitamin A such as fish.

Effect of Food Processing on Vitamin A

Carotenoids and retinol are affected by pH, enzymatic activity, light, and oxidation associated with the conjugated double bond system[51]. The chemical changes occurring in carotenoids during processing have been reviewed by Simpson[52]. Fresh plant tissue may contain enzymes that are only activated during and following processing. Therefore, the preformed and provitamin A content of the raw form of a food item may be reduced as a consequence of food preparation. The most dramatic example of this is found in red palm oil, which in its raw form is considered one of the richest sources of provitamin A[53]. After heating to 200°C for thirty minutes, the beta-carotene content becomes negligible.

Numerous reports document changes in carotenoid content attributed to various cooking methods. As a general rule, foods boiled in an open container show the greatest losses. Regardless of the method used, most studies found that dehydration significantly reduces the carotene content in vegetables, which has implications for storage of seasonally available foods. However, Khachik *et al*.[54] reported no significant changes in the beta-carotene content in several green vegetables after microwaving, steaming, or boiling. Likewise, the carotenoid content of tomatoes did not change when they were dehydrated by sundrying.

Sweeney and Marsh[55] reported that processing of fruits and vegetables induced isomerisation of carotenoids, resulting in an estimated 15% to 20% reduction in vitamin A potency in green leafy vegetables, and 30% to 35% in yellow vegetables.

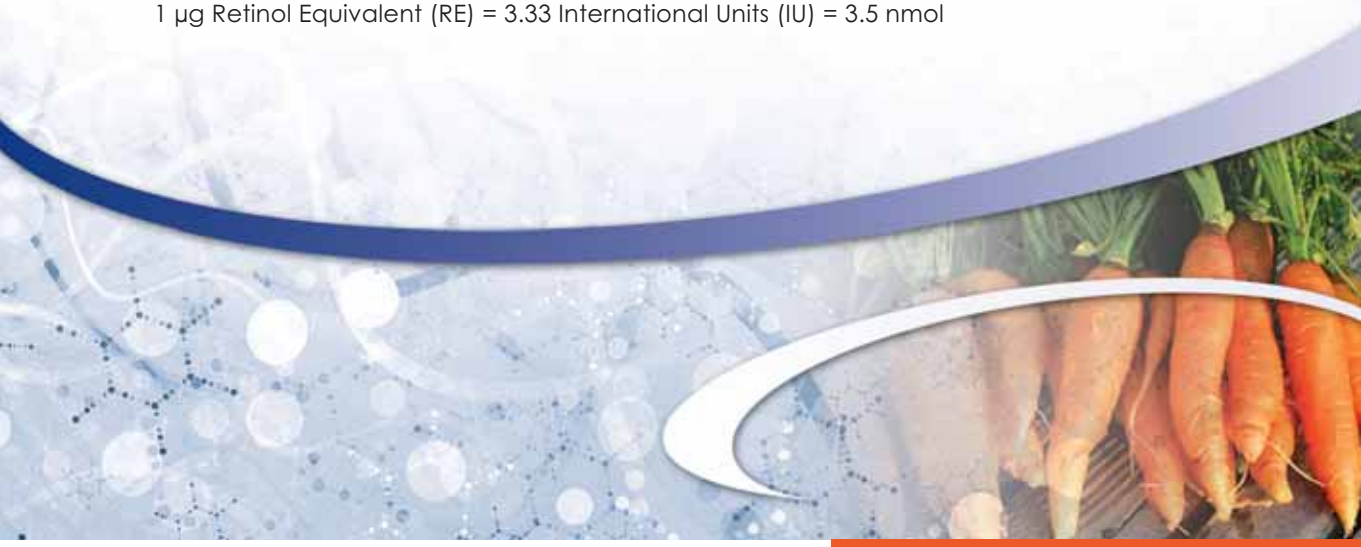
Requirements and Reference Intakes of Vitamin A

Bender[23] summarised the prudent upper levels and reference intakes for vitamin A from various international authorities, as shown in Table 2.1. It is important to take into account that infections can increase vitamin A demands dramatically. Some investigators even calculated the increased need during infections in the order of 3 000 IU per day. Children with measles are particularly likely to develop a rapidly progressing keratomalacia. The mechanisms leading to increased requirements during infection are complex and include decreased synthesis of RBP[23] (a negative acute-phase protein), urinary excretion of retinol, as well as malabsorption and impaired transport.

Table 2.1 Upper limit and reference intakes for vitamin A (adapted from Bender[23])

Age and lifestyle group	Upper limit of intake (µg RE/day)	Reference intake (µg RE/day)
Infants	900	350 – 375
Children 1 – 3 years	1 800	400
Children 4 – 6 years	3 000	400 – 500
Children 7 – 12 years	4 500	500 – 700
Adolescents 13 – 20 years	6 000	600 – 700
Adult women	7 500	600 – 800
Adult men	9 000	600 – 1 000
Pregnant women	3 000 – 3 300	700

1 µg Retinol Equivalent (RE) = 3.33 International Units (IU) = 3.5 nmol



Vitamin A Toxicity and Teratogenicity

General or Chronic Toxicity

Chronic hypervitaminosis A is due to ingestion of large doses of the vitamin on a daily basis, exceeding the upper limit of intake shown in Table 2.1. Because there is only a limited capacity for metabolism of vitamin A, excess intake accumulates in the liver and other tissues. This can lead to hepatitis, cirrhosis, hair loss, dry scaling skin, hyperpigmentation, hyperostosis, bone pains, hepato-splenomegaly and anaemia. It is therefore recommended not to exceed a daily intake of 3 000 µg RE in children and 7 500 µg RE in adults.

Acute Hypervitaminosis

Ingestion of a large dose of vitamin A, exceeding the upper limit of intake as shown in Table 2.1, can give rise to transient signs and symptoms of toxicity, which are self-limiting and completely reversible. No deaths have been reported after the ingestion of the doses used in treatment or prevention. Common complaints include headaches and bulging fontanelle in young children. Nausea, vomiting, dizziness, headaches and loss of appetite have been described in adults. Desquamation of the skin, bone pains and hair loss can occur in the following days. Vitamin A supplementation of children and post-partum pregnant women with recommended amounts is generally regarded as safe, but the recommended amounts for individuals and groups should be determined based on usual intake of vitamin A and upper limits advised in Table 2.1.

Teratogenicity

Vitamin A overdose during the first trimester of pregnancy is teratogenic. According to Thurnham[32] the effects include spontaneous abortions or foetal abnormalities of the cranium (microcephaly), the face (hare lip), heart, kidney, thymus and central nervous system (deafness and lower learning ability). It is always recommended not to give large doses of vitamin A during pregnancy and not to exceed 10 000 IU per day as treatment dose. It is suspected that the influence of retinoic acid isomers on the control of embryogenesis is responsible for the teratogenic effects. The synthetic retinoids used in dermatology are especially teratogenic and women treated with them are advised to take contraceptive precautions for at least 12 months after treatment. Vitamin A, given as liver, increases plasma retinoic acid less than supplements in similar doses, and may be less likely to reach blood concentrations that are teratogenic.

Primary Biliary Cirrhosis

Vitamin A may be implicated in primary biliary cirrhosis (PBC), a chronic, cholestatic disease of unknown aetiology commonly affecting women. It is characterised by progressive destruction of the small intra-hepatic bile ducts and portal inflammation, leading to fibrosis and cirrhosis. The major signs and symptoms of PBC closely resemble the manifestations of hypervitaminosis A. Based on a review of the literature and other observations connecting PBC with retinoid metabolism (vitamin A and its derivatives), Erickson and Mawson[56] proposed the hypothesis that exposure to excess endogenous retinoids contributes to the pathogenesis of PBC. However, recent literature suggests that PBC is primarily an autoimmune disease, with the possible influence of environmental factors[57].

Idiopathic Intracranial Hypertension (IIH)

This is a disorder seen in obese women and is characterised by papilloedema and increased intracranial pressure, without evidence of venous thrombosis, and in whom no obvious cause can be found. While the disorder has been recognised for more than 100 years, little headway has been made in determining the pathophysiology of the condition. A connection to vitamin A was made when polar explorers observed that intracranial hypertension could be acquired by eating vitamin A-rich livers of polar bears. Other observations regarding effects of vitamin A-containing medications, and retinol levels in patients with IIH support a connection, but much more research is needed to clarify a possible role of vitamin A in IIH.

Assessment of Vitamin A Status and Deficiencies

Direct Method: Measuring Hepatic Vitamin A Reserves

Because vitamin A is stored in the liver, direct measurement of liver vitamin A concentration is considered to be the best indicator of vitamin A status. For obvious reasons, this is not a feasible method for routinely assessing status and hepatic reserve of vitamin A is only measured for research purposes.

Measurement of Retinol, Retinol-binding Protein (RBP) and Transthyretin (TTR) in Blood Samples (Plasma, Serum, Dried Blood Spots)

Vitamin A status and VAD without clinical manifestations can be assessed using biochemical techniques. The measurement of serum retinol concentration by high performance liquid chromatography (HPLC) techniques is the most commonly used biochemical technique, and is recommended by the World Health Organisation[35]. During normal vitamin A intake, plasma retinol level is closely regulated. During manifest deficiency, the level of retinol decreases and is a reliable marker of the vitamin A status of individuals in the absence of infection[58].

Retinol-binding protein (RBP) is accepted as a surrogate biochemical marker for retinol to determine vitamin A status. Retinol is released from the liver, bound to RBP. RBP has a molecular weight of 21 000, and has one binding site for retinol. The RBP-retinol complex, known as holo-RBP, is released from the liver bound to another protein, transthyretin (TTR), which has one binding site for holo-RBP. This large complex (1:1:1) enables the body to transport fat-soluble retinol in plasma. It protects retinol from oxidation, and regulates retinol mobilisation inter alia by delivering retinol to specific sites on target cell surfaces. An advantage of measurement of RBP is that it is more stable than plasma retinol. However, retinol and RBP are depressed by inflammation, thus acute-phase proteins should also be monitored simultaneously. The RBP thresholds for inadequacy are the same as those for retinol (See Table 2.2).

Measurement of retinol in dried blood spots (DBS) is a new method, which is still in development. Collecting and preparing venous blood samples in field settings may be problematical. An alternative is to use DBS prepared from finger prick capillary samples collected onto collection cards. The measurement of vitamin A in DBS was first described by Shi *et al.*[59] using high performance capillary electrophoresis (HPCE) with laser-enhanced fluorescence detection. Using a modification of the HPCE method of Ma *et al.*[60], Shi *et al.*[59] optimised the

separation conditions and improved the reliability of the method. These researchers also developed sample preparation methods for the elution of holo-RBP from DBS, ultimately demonstrating the stability of the complex in dried blood. This observation was an important milestone since the holo-RBP complex was thought to be unstable when exposed to air and iron from the red blood cells. However, the method requires highly technical expertise and specific equipment and there is also limited availability and high costs. Therefore, the possibilities for use of this method are limited[32].

Normal Ranges for Plasma Retinol

The normal ranges of plasma retinol used for classification of vitamin A status are summarised in Table 2.2 (adapted from [23,32]). Serum and plasma retinol are used interchangeably, as there is no difference in retinol concentrations in either.

Table 2.2 Classification of plasma retinol levels

Plasma retinol levels		Classification: vitamin A status
µg/dL	µmol/L	
>= 30	>=1.05	Normal
29.9 – 20	1.04 – 0.7	Mild deficiency
19.9 – 10	0.69 – 0.35	Associated with night blindness, Bitot spots; moderate deficiency
< 10	< 0.35	Low liver stores of vitamin A; definitely low status; severe deficiency

Multiply µg/dL by 0.0349 to convert to µmol/L

Serum retinol concentrations have been used extensively to identify populations at risk of VAD. However, serum retinol concentrations are homeostatically controlled and do not reflect liver vitamin A stores until liver reserves are dangerously low. Serum retinol is also affected by recent dietary vitamin A and provitamin A intake, and may change due to seasonal variation in fruit and vegetable intake even though a true change in liver stores of the vitamin may not occur. As mentioned, RBP is a reverse or negative-acute-phase protein; thus, serum retinol and RBP concentrations decline during subclinical infection. Deficiencies of other nutrients, particularly iron, may also negatively affect serum retinol concentrations.

Measurement of Retinol in Breast Milk

Breast milk retinol concentrations have been proposed as a population measure of vitamin A status[61,62]. Breast milk collection is less invasive and usually easier than blood drawing. Breast milk samples do not have to be further processed at the field station, thus shortening sample preparation. While a unique indicator to lactating women, the status of the mother can usually be predictive of the vitamin A status of the nursing infant[61]. There is agreement that if lactating women of a community have a marginal vitamin A status, the chances are high that the children of that community are also at risk of vitamin A depletion. The breast milk retinol assay had been simplified by using 3,4-didehydroretinyl acetate as an internal standard[63]. Thurnham[26] points out that the creamatocrit of milk (an estimation of the fat: g/100mL milk) should also be determined. The normal ranges for milk retinol palmitate are 1.75 to 2.45 µmol/L but decline as lactation continues and vary during the day. Levels of 1.05 µmol/L retinol or less than 27.9 µmol/g fat are indicative of a risk of VAD.

Measurement of Retinoic Acid

Lo *et al.*[64] measured both retinol and retinoic acid in serum samples from a number of human populations. The level of retinoic acid was 1.25% of that of retinol, and appeared not to be directly related to retinol concentrations, suggesting separate homeostatic control mechanisms, and thus being of limited use in routine measurements

Clinical Methods

Impression Cytology

Among the physiologic indicators of vitamin A status currently available, only impression cytology and dark adaptometry have received sufficient attention to evaluate their performance as practical tools for population assessment of vitamin A-dependent function. Conjunctival impression cytology (CIC) and impression cytology with transfer (ICT), a modification of the initial method, are based on well-described histopathological changes due to VAD. Both these methods, combined with other measures, are used to assess overt VAD and nutritional status and have limited application in population assessments.

Dark Adaptation

Measurement of night blindness during pregnancy and impairment of dark adaptation are indirect methods for assessing clinical symptoms and an adverse functional effect of VAD, respectively. However, it is not certain whether these tests are useful in populations in which marginal VAD is prevalent without clinical symptoms of night blindness.

It has been demonstrated that a maternal history of night blindness can be an accurate indicator of night blindness and impaired vitamin A status in a child[65]. Where local terms exist, an oral history can thus be of great benefit in the population assessment of vitamin A status. However, local terms are not available for all populations, and dark adaptation testing has suggested that deficient persons may have measurably abnormal thresholds and yet not complain of clinical night blindness[66].

Conventional, laboratory-based testing of dark adaptation usually requires at least 30 minutes, the period for a fully light-adapted eye to become completely dark-adapted. During this process, the eye becomes more sensitive to low light levels, in large part because of a shift from photopic vision, based on the cones, to scotopic vision, based on the rods.

In field settings, some conflicting results from rapid testing, the need for special training of observers to recognise a pupillary response, and the requirement of a sufficiently dark testing area, are factors which could limit the practicality of these methods.

Functional Dose-response Tests

The relative dose response (RDR) test has been developed during the past two decades as an indirect method to reflect liver reserves of vitamin A. It involves giving a small oral dose (1 800 IU) of retinyl ester and taking a blood sample at time 0 and 5 hours after the dose and calculating a percent increase of plasma retinol. Plasma retinol is not influenced by this loading dose in vitamin A adequate persons. In VAD, liver retinol binding protein (RBP) binds rapidly to dietary vitamin A and appears in the plasma within five hours. If the response (RDR) is greater than 20%, VAD is present. The response is calculated as RDR = A5-A0 x 100/A5, where A0 and A5 are plasma retinol concentrations at baseline and after five hours, respectively[32].

The modified relative dose response (MRDR) is a modification of the RDR, using 3,4-didehydroretinyl acetate as the challenge dose. Because circulating concentrations of 3,4-didehydroretinol are normally very low in human plasma, a single blood sample is all that is required four to six hours after dosing and a ratio of 3,4-didehydroretinol to retinol is calculated. An elevated ratio of dehydroretinol to retinol (greater than 0.06 according to Thurnham[32]) is indicative of inadequate liver vitamin A reserves. The MRDR test has been used extensively throughout the world to assess marginal vitamin A status in populations and to determine the efficacy of interventions. Both the RDR and the MRDR give information about liver stores of vitamin A, but not of the total amount of vitamin A in the body.

Tracer (Stable Isotope) Methods

The tracer dilution technique has emerged as a select method for estimating total body vitamin A pool size, and for answering specific biological questions related to vitamin A metabolism. An advantage is that results of this method are probably not influenced by inflammation[32]. The tracer dilution technique consists of:

- Administering an oral dose of an appropriate tracer, usually deuterium-labelled retinol, to subjects.
- Collecting a blood sample after the tracer has mixed with endogenous vitamin A, usually after 21 days.
- Dilution of the label (tracer) in total retinol is measured to assess total body stores, using a prediction equation. The ratio (dilution) of the tracer in plasma is determined by using gas-chromatography-mass spectrometric methods[4].

The tracer dilution technique is the only indirect assessment technique that provides a quantitative estimate of total body vitamin A pool size. The technique is responsive to food or pharmaceutical supplementation with vitamin A. It can be used to evaluate the efficacy or effectiveness of intervention programmes by assessing the change in total body vitamin A stores in response to the intervention. An additional advantage is that the technique can estimate total body vitamin A status over a wide range – from deficient to sub-toxic levels. It is therefore not necessary to select subjects with deficient or marginally depleted initial status to detect a change in vitamin A status in response to an intervention. The tracer dilution technique is thus useful for assessing change in vitamin A status in populations with low but adequate initial status, in comparison with other indirect assessment techniques which are only useful for detecting a change in status when initial status is deficient or marginally depleted[4].

Vitamin A tracer studies have been successfully applied for assessing vitamin A status of populations, and for assessing efficacy and effectiveness of interventions in population groups at risk of deficiency in several different countries[4]. Disadvantages are that the method is time-consuming, costly, and requires expensive equipment.

Measurement of Dietary Intakes

Food Composition Tables for Vitamin A

Food composition data are needed to calculate the vitamin A intake of a population from dietary surveys and to select food items rich in this nutrient for education programmes. Food tables contain nutrient values from chemical analyses of foods, with no allowance for the biological utilisation of the nutrient[67]. Therefore food composition values are only estimates of active vitamin A. The limitations of vitamin A nutrient values in food composition tables have been reviewed by Booth *et al*.[68], showing that most contain inconsistencies in preformed and provitamin A values, mainly because of differential use of units and conversion rates, and reliance on outdated analytical techniques, particularly of carotenoid sources.

The carotenoid values of foods consumed in the United States were recently re-evaluated[69]. An artificial intelligence system was developed to evaluate existing carotenoid data, including indicators of data quality. Only HPLC-generated data were incorporated into the database to eliminate overestimation associated with analytical methods that quantify total instead of individual carotenoids. A modified version of this artificial intelligence system, with less stringent criteria, was used by West and Poortvliet[70] to evaluate existing carotenoid data for developing countries. Most carotenoid values reported are for vegetables and fruits, although there are limited data for meat, fish, fats, eggs, cereals, and dairy products. The South African Food Composition Tables give vitamin A values in µg RE and used conversion factors of 6 for beta-carotene and 12 for other provitamin A carotenoids. The latest recommended conversion factors are 12 and 24, respectively. Therefore, no individual levels for carotenoids appear in the tables[71].

Questionnaire Techniques for Assessment of Intakes

All dietary assessment techniques for vitamin A intake require estimations of the amount of food consumed, the vitamin A content of the food, and frequency with which it is consumed.

From this information, it is possible to calculate intake and potential risk of deficiency in percentages of the population in various age and gender groups[72]. An important consideration when evaluating vitamin A intake is that it has seasonal fluctuations and thus large intra-individual variations, dependent on food availability, especially in many developing countries where subsistence farming is a major activity.

Despite extensive research on diet survey research methodology, an ideal technique for estimating individual food consumption has yet to be developed[73]. 'Intrusive' methods, such as direct observation, weighed diet records, or diet histories, are more accurate in terms of the nutrient intake estimates generated[74] but are too expensive and time-consuming to use at the community level in developing areas at risk for vitamin A deficiency. Moreover, they only reflect the actual intake of those days recorded and they are not representative of the usual diet. The 24-hour recall and the quantitative food frequency questionnaire (QFFQ) methods, each with its unique advantages and disadvantages, are mostly used to assess population dietary and nutrient intakes. The 24-hour recall method costs less in time and manpower, but it only reflects intakes over the last 24 hours. If interviews are repeated many times for the same individual, the method will give some information on usual intakes, but this is seldom feasible in field settings. Food frequency questionnaires are considered valuable epidemiological tools because of their simplicity and because they measure usual or habitual consumption over a long time. However, they tend to overestimate intakes and are not always statistically comparable in nutrient estimates to those obtained from other dietary survey methods.

Simple frequency forms for summarising community data and estimating percentages of risk for those who consume vitamin A-rich food groups (for example, dark green, leafy vegetables or foods of animal origin) in frequency categories (for example, greater than or less thanthree times/week) can be prepared from brief household surveys[75]. The International Vitamin A Consultative Group (IVACG) developed a simplified approach to dietary assessment of vitamin A intake of preschool children, which was validated in Bangladesh using weighed dietary evaluation for three consecutive days as standard[76].

Other research tools have been used to ascertain food available and consumed in communities at risk for vitamin A deficiency. Market surveys, garden surveys, and additional information can be used to generate a seasonal calendar of vitamin A-rich food items. Information from public health records or questionnaires can provide data on the extent of breastfeeding and patterns of infant feeding and weaning, all of which are important for periods when a population may be vulnerable to vitamin A deficiency[77].

Other Methods to Assess Vitamin A Status

Measurement of Protein Metabolism Indicators

The role of vitamin A in regulating nitrogen retention and amino acid metabolism has prompted the exploration of changes in plasma and urinary indicators of protein metabolism, such as the measurement of urea and ammonium nitrogen, in relation to conventional indicators of vitamin A status[78,79]. These physiological indicators are often evaluated in comparison with one or more conventional indicators, such as clinical signs and symptoms, plasma retinol concentration and relative liver adequacy of vitamin A.

Vital Staining

This is a method of detecting the degree of conjunctival metaplasia and it is conducted by putting dye (Lissamon green or Bengal rose) on the conjunctiva. This method lacks specificity, and is not recommended for routine evaluation of vitamin A status in populations.

Discussion: Interpretation and Limitations

The advantages and disadvantages of the different methods available to assess vitamin A status were mentioned in the preceeding section. In this section, the limitations of these methods will be briefly discussed to evaluate their use on population level.

Limitations of Biochemical Methods

Serum retinol, RBP and TTR:

In healthy individuals, serum retinol concentrations are homeostatically controlled and do not begin to decline until liver reserves of vitamin A are dangerously low. Serum retinol values can therefore only indicate severe vitamin A deficiency, a high risk of deficiency, or in adults, a possible risk of deficiency.

Retinol-binding protein (RBP) is a negative acute-phase protein and levels of serum retinol, RBP and transthyretin (TTR) fall during times of infection. Because of the high prevalence of infection in children at risk of vitamin A deficiency and the above-mentioned control of serum levels, serum retinol does not always respond to vitamin A interventions[80].

The status of other nutrients, particularly iron deficiency, which decreases mobilisation of vitamin A from liver storage, may negatively affect serum retinol concentrations[81,82]. Another major drawback of measuring serum retinol, RBP and TTR is that venous blood samples are required. The sampling process is invasive and impractical to carry out in many field settings. Typically, venipuncture is required to obtain the volume of blood necessary (>500 µL of whole blood). Fear of needles, possible disease transmission and/or religious beliefs, exclude many from participating. Added to this is the need for electricity for centrifugation and long-term freezer storage. A recently developed enzyme immunoassay for RBP uses serum or whole blood stored as dried blood spots[83] which address blood sampling problems to a certain extent.

Advantages and Limitations of Clinical Methods

Depletion of vitamin A induces early disturbances in dark adaptation that can be detected by non-invasive testing. Much work has been done to develop indicators to detect early visual disturbances through dark adaptometry before clinical or behavioural recognition of night blindness. As a tool for population assessment, pupillary dark adaptation offers several advantages over other techniques: it is rapid, non-invasive, inexpensive and highly acceptable to target populations, and it does not require transport of samples. Furthermore, it appears able to detect sub-clinical vitamin A deficiency diseases[30]. However, the observers should be well trained to recognise pupillary responses.

Arranging for a comfortable, darkened testing facility, although possible, can pose logistic challenges in hot, rural environments. Night-time testing, where practical, might offer a practical alternative. Less cumbersome ways to achieve dark adaptation will make the test more practical.

The technique lends itself to baseline population assessment and evaluation of the effect of intervention programmes. To make maximum use of this technique, it is necessary to build a database of tested populations to develop a meaningful context for interpretation of future results.

Practical field-based tests of vitamin A status should be reliable when used among preschool-aged children, the group most at risk for vitamin A deficiency. Traditional psychophysical dark adaptations testing, including the rapid methods discussed above, are not well suited to testing young children. Duncan *et al*.[84] recently reported a dark adaptation test that required young subjects (aged six to 67 months) to follow a dim light projected on a wall after ten minutes of dark adaptation, with movement of the head tracked by means of head-mounted illumination. Although dark adaptation thresholds measured in this way did not correlate significantly with serum retinol for all subjects, the correlation was significant ($P < 0.05$) for subjects with serum retinol <0.35 µmol/L.

The time course for recovery of the normal pupillary response after dosing of deficient individuals with vitamin A may be as long as four to six weeks, hence programmes wishing to use pupillary dark adaptation as an outcome indicator in programme assessment likely need to wait at least this long before re-testing treated individuals[85].

The time required to test a subject with this technique, including explanation, bleaching, dark adaptation and testing, is about 20 minutes. Thus, rapid testing of large numbers of persons is not practical. However, sample size calculations reported by Sanchez *et al*.[85] suggest that in very deficient populations no more than half a dozen subjects would need to be tested to demonstrate that the group mean differed significantly from normal, although considerably larger numbers would be needed to ensure a representative sample. In even mildly deficient populations, testing of fewer than 100 subjects would be sufficient.

When to Use Tracer (Stable Isotope) Methodology

The tracer dilution technique is the method of choice when a quantitative estimate of vitamin A-pool size is important for critical decision-making regarding responses to vitamin A interventions. For example, the tracer dilution technique can be used to estimate quantitatively the amount of vitamin A retained in the body in response to supplementation with different dietary sources of vitamin A (e.g. animal source *versus* plant source foods) and to estimate the relative impact of consumption of these foods on total body vitamin A stores. The modified relative dose response (MRDR) test would not be as useful in this situation because it can only determine if vitamin A status has improved in response to a food-based intervention; it cannot estimate and compare how much retinol was derived from the various vitamin A-containing foods. Determination of the amount of vitamin A retained in the body in response to an intervention is required for this type of study and tracer methodology is the only way to obtain this information.

Limitations of Measuring Food and Vitamin A Consumption

The limitation of food and nutrient-consumption data is determined by the inaccuracy of food composition tables and difficulties in reliable measurement of reported intakes.

The factors influencing the inaccuracy and variation of vitamin A and carotene values in food composition tables have been reported by a number of researchers and are summarised in Box 2.2.

Box 2.2 Factors Influencing Vitamin A Values in Food Composition Tables

- Sampling method, including collection of food samples, number and representivity of samples, handling and storage of samples, exposure to light and air, etc. Analytical method, including type of method used, time between sampling and analysis, sample treatment and extraction procedures, etc.
- Natural heterogeneity, including soil pH, rainfall, season, genetic diversity, stage of maturation when harvested, different cultivars, time of marketing, etc.
- Processing methods (for processed foods).

The difficulties and limitations in measuring reported food intakes have been extensively examined and debated. These problems also introduce inaccuracies in vitamin A-consumption data, which should always be interpreted with care. Even the time-consuming weighed record method, which is regarded as the 'gold standard', change food consumption to facilitate weighing and cannot be regarded as completely accurate. To measure food consumption on population or group level, well-designed and validated questionnaires, as well as well-trained and experienced interviewers, using standardised methodologies to assist subjects to estimate food portions sizes and frequency of intake are necessary. Factors, such as cultural differences, the order of items on the questionnaire, under and over-reporting because of psychological influences, and many others, should be controlled.

Factors Influencing the Interpretation of Vitamin A Assessment Results for Planning Interventions

Because of the limitations in both the measurement of vitamin A intakes and of vitamin A status (except in cases of overt VAD), results from these processes should be interpreted taking other factors, observations and measurements into account. These are the following:

Socio-cultural Factors

The socio-cultural and environmental factors that affect vitamin A intake and responses to vitamin A deficiency must be taken into account when evaluating vitamin A-status measurements. This knowledge about the socio-cultural and environmental contexts of vitamin A is essential for instituting and sustaining food-based prevention of vitamin A deficiency. Nutritionists and anthropologists should collaborate to create protocols to evaluate socio-cultural factors influencing utilisation of the natural food sources of vitamin A in areas at risk of deficiency.

Food Security: Availability of Vitamin A-rich Foods

All people involved in improving vitamin A status of populations, such as programme planners, researchers and development leaders in health, agriculture, education, and other sectors, must understand the factors influencing food availability and consumption at the local level. This understanding will assist in making recommendations that will lead to improvements in the dietary quality and quantity of vitamin A through dietary modification and food-fortification programmes. Regarding food supplies, it requires understanding of the species of vitamin A-rich foods that are culturally acceptable and available, their seasonality, methods of preservation and preparation, and barriers to their use due to cost, health beliefs, or other reasons of accessibility are also important. As pointed out by Wasantwisut and Attig[86], "only when these factors are known will agricultural, food-processing, social marketing, and public health education programmes have a sustained impact on behaviour change and in improving dietary modification for vitamin A".

Bioavailability of Vitamin A

Data on the assessment of availability and consumption of vitamin A-rich food from either plant or animal sources (more bioavailable) in the community or at the household levels, should be supplemented by data on the presence of other dietary factors needed for bioavailability, absorption, and metabolism of vitamin A, such as sufficient fat, protein, zinc, and other essential nutrients that are known to influence bioavailability of vitamin A[68].

Vitamin A Data from Food Composition Tables

HPLC is becoming the preferred method of retinol analysis in foods. However, the complexity of carotenoids, their isomers, and other chemical substances in foods have prevented the development of a single HPLC method for carotenoid analysis[87] until recently. Also, while the methodologies using HPLC for carotenoid and retinol analyses are evolving, standardisation among and within different laboratories is difficult to attain. In a recent study on the inter-comparison of methods used for vitamin A determination of foods, the results for retinol analyses in milk agreed very well[88]. However, comparison of beta-carotene contents in green beans analysed by different laboratories showed poor agreement. Another limiting factor for all analytical methods, particularly HPLC, is the cost of equipment and solvents, which is prohibitive in most developing regions[89].

Recommendations

It was mentioned in the introduction that to assess vitamin A status of vulnerable parts of populations, it is necessary to have a robust, reliable, affordable, non-invasive, rapid and feasible method for different settings that will give maximal information on vitamin A status. This information is required to determine the extent of vitamin A interventions needed, and also to monitor the effect of implementing interventions.

Currently, one ideal method does not exist, and it is recommended that a combination of different less-than-optimal methods are used to obtain the best information possible. It is therefore recommended that for assessing vitamin A status in populations, the following methods should be combined:

- Monitor growth of children and general health of the population of interest. Look for clinical signs of vitamin A deficiency and community perceptions about these signs.
- Assess dietary patterns and identify local food sources of vitamin A and all factors influencing the availability of these sources (such as food security, socio-economic, cultural and traditional practices, how foods are acquired, stored, and prepared).
- Measure total dietary and nutrient intakes to assess the bioavailability of vitamin A in particular diets.
- Do a functional test if indicated in vulnerable groups, such as dark adaptation or night blindness in pregnant women where feasible.
- Measure serum retinol and RBP if it is feasible to get venous blood samples.
- Measure retinol in dried blood samples obtained from capillary blood (finger pricks), if feasible, but keep in mind that the methodological variation for this method is high.

From the above, it is clear that each particular circumstance will dictate which methods, in which combinations, should be used and that, especially the method of measuring retinol (and/or RBP) in dried blood spots, is promising if methodology could be further refined.

References

1. South African Vitamin A Consultative Group (SAVACG). Anthropometric, vitamin A, iron and immunisation coverage status in children aged 6-71 months in South Africa, 1994. S *Afr Med* J 1996; 86: 354-357.

2. Labadarios D (Ed), Steyn NP, Maunder E, et al. *The National Food Consumption Survey (NFCS): children aged 1-9 years, South Africa, 1999*. Pretoria: Department of Health, 2000. www.sahealthinfo.org/nutrition/nfcs/chapter4.

3. Labadarios D, Swart R, Maunder EMW, *et al. The National Food Consumption Survey Fortification Baseline (NFCS-FB-1):* South Africa, 2005. Pretoria: Directorate Nutrition, Department of Health, 2007.

4. Haskell MJ, Ribaya-Mercado JD and the Vitamin A Tracer Task Force. *Handbook on Vitamin A Tracer Dilution Methods to Assess Status and Evaluate Intervention Programmes, 2005.* http://www-naweb.iaea.org/nahu/nahres/pdf-files/vitatracer.pdf.

5. West CE, Rombout JHWM, Van der Zipp AJ, *et al*. Vitamin A and immune function. *Proc Nutr Soc* 1991; 50: 294-260.

6. Fawzi WW, Herrera MG, Willet WC, et al. Dietary vitamin A intake in relation to child growth. *Epidemiology* 1997; 8(4): 402-407.

7. Sommer A. Mortality associated with mild, untreated xerophthalmia. *Trans Am Ophthalmol Soc* 1983; 81: 825-853.

8. Astrog P. Food carotenoids and cancer prevention: An overview of current research. *Trends Food Sci Technol* 1997; 81: 406-413.

9. Bendich A. Recent advances in clinical research involving carotenoids. *Pure Appl Chem* 1994; 66:1017-1024.

10. Burri BJ. Beta-carotene and human health: A review of current research. *Nutr Res* 1997; 17: 547-580.

11. Gaziano JM, Hennekens CH. The role of beta-carotene in the prevention of cardiovascular disease. *Ann New York Acad Sci* 1993; 691: 148-155.

12. Krinsky NI. Actions of carotenoids in biological systems. *Annu Rev Nutr* 1993; 13: 561-587.

13. Olson JA. Carotenoids. In Shils ME, Olson JA, Shike M, Ross AC (eds). *Modern nutrition in health and disease, 9th edition*. Williams and Wilkins, Baltimore, 1999: 525-541.

14. Olson JA, Krinsky NI. The colorful, fascinating world of the carotenoids: Important physiologic modulators. *FASEB J* 1995; 9: 1547-1550.

15. Foote CS, Chang YC, Denny RW. Chemistry of singlet oxygen. X1. Cis-trans isomerisation of carotenoids by single oxygen and a probable quenching mechanism. *J Am Chem Soc* 1970; 92(17): 5218-5219.

16. Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 1989; 274: 532-538.

17. Peters U, Leitzman MF, Chatterjee N *et al*. Serum lycopene, other carotenoids, and prostate cancer risk: a nested case-control study in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. 2007. *Cancer Epidemiol Biomarkers Prev 2007*; 16(5): 962-968 (Abstract).

18. Schenk JM, Riboli E, Chatterjee N *et al.* Serum retinol and prostate cancer risk: a nested case-control study in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Cancer Epidemiol Biomarkers Prev* 2009; 18(4): 1227-1231.

19. Riccioni G, Bucciarelli T, D'Orazio N *et al*. Plasma antioxidants and symptomatic carotid atherosclerotic disease. *Ann Nutr Metab* 2008; 53(2): 86-90 (Abstract).

20. Ellison JB. Intensive vitamin therapy in measles. *Br Med* J 1932; Oct 15: 708-777.

21. Hussey G. Preventing measles deaths. *Child Health Dialogue* 1996; 3-4: 14.

22. Coutsoudis A, Broughton M, Coovadia HM. Vitamin A supplementation reduces measles mortality in young African children: a randomised, placebo controlled double-blind trial. *Am J Clin Nutr* 1991; 54(5): 890-895.

23. Bender DA. The vitamins. In: Gibney MJ, Lanham-New SA, Cassidy A *et al*. (eds) *Introduction to Nutrition*. 2nd edition. Oxford: Wiley-Blackwell, 2009.

24. Aguayo VM, Baker SK. Vitamin A deficiency and child survival in sub-Saharan Africa: A reappraisal of challenges and opportunities. *Food Nutr Bull* 2005; 26(4):348-355

25. Sommer A, Tarwotjo I' Hussaini G *et al*. Increased mortality in children with mild vitamin A deficiency. *The Lancet* 1983; 2: 585-588.

26. Fawzi WW, Chalmers TC, Herrera MG *et al*. Vitamin A supplementation and child mortality. A meta-analysis. *JAMA* 1993; 269: 898-903.

27. Glasziou PP, Mackerras DEM. Vitamin A supplementation and infectious diseases: a meta-analysis. *BMJ* 1993; 306: 366-370.

28. Tonascia JA. Meta-analysis of published community trials: impact of vitamin A on mortality. Proceedings of the *Bellagio Meeting on vitamin A deficiency and childhood mortality*. New York: Helen Keller International, 1993.

29. Sommer A, West KP, Jr. *Vitamin A Deficiency: Health, Survival, and Vision*. New York: Oxford University Press, 1996.

30. Christian P, West KP, Jr, Khatry SK *et al*. Vitamin A or beta-carotene supplementation reduces but does not eliminate maternal night blindness in Nepal. *J Nut* 1998; 128(9): 1458-1463.

31. International Vitamin A Consultative Group (IVACG). *Policy Statement on Vitamin A, Diarrhoea, and Measles.* Final report, 1996. www.micronutrientforum.org/ PDF/IVACG-Peru Report.pdf

32. Thurnham DI. Vitamin A and carotenoids. In: Mann J, Truswell AS, (eds) *Essentials of Human Nutrition*. 3rd edition. New York: Oxford University Press, 2007.

33. Mora JR, Iwata M, Eksteen B *et al*. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 2006; 314(5802): 1157-1160.

34. Sommer A. Nutritional factors in corneal xerophthalmia and keratomalacia. *Arch Ophthalmol* 1982; 100(3): 399-403.

35. WHO. Micronutrient deficiencies. *Vitamin A deficiency*. http://www.who.int/ nutrition/ topics/vad/en/index.html.

36. Akbaraly TN, Favier A Berr C. Total plasma carotenoid and mortality in the elderly. Results of the EVA Study. *Br J Nutr* 2008; 101: 86-92.

37. Lauretani F, Semba RD, Dayhoff-Brannigan M *et al.* Low total plasma carotenoidsare independent predictors of mortality among older persons: the InCHIANTI study. *Eur J Nutr* 2008; 47: 335-340.

38. Meydani M. Dietary antioxidants modulation of aging and immune-endothelial cell interaction. *Mech Ageing Dev* 1999; 111:1123-132.

39. Mecocci P, Polidori MC, Troiao L *et al*. Plasma antioxidants and longevity: a study on healthy centenarians. *Free Radical Biology and Medicine* 2000; (28)8: 1243–1248.

40. Semba RD, Miotti PG, Chiphangwi JD *et al.* Maternal vitamin A deficiency and infant mortality in Malawi. *J Trop Pediatr* 1998; 44: 232-234.

41. West KP, Jr, Katz J, Khatry SK *et al*. Double blind, cluster randomised trial of low dose supplementation with vitamin A or beta carotene on mortality related to pregnancy in Nepal. The NNIPS 2 Study Group. *BMJ* 1999; 318.

42. Kirkwood BR, Hurt L, Amenga-Etego S, *et al*. Effects of vitamin A supplementation in women of reproductive age on maternal survival in Ghana (ObaapaVitA): a cluster-randomised, placebo-controlled trial. *The Lancet* 2010; 375(9726): 1640-1649.

43. Beach RS, Mantero-Atienza E, Shor-Posner G *et al*. Specific nutrient abnormalities in asymptomatic HIV1 infection. *AIDS* 1992; 6: 701-708.

44. Tang AM, Graham NMH, Kirby AJ *et al*. Dietary micronutrient intake and risk of progressionto acquired immunodeficiency syndrome (AIDS) in human immunodeficiency. *Am J Epidemiol* 1993; 138: 937-951.

45. Coutsoudis A. The relationship between vitamin A deficiency and HIV infection: review of scientific studies. Report prepared for the *WHO Consultation on Vitamin A in the First Six Months of Life*. Geneva:WHO, 2000.

46. WHO Guideline: Vitamin A supplementation in pregnancy for reducing the risk of mother-to-child transmission of HIV. Geneva, World Health Organisation, 2011.

47. Rodriguez-Amaya DB, Kimura M. *HarvestPlus Handbook for Carotenoid Analysis*. Washington, DC: HarvestPlus, 2004: 6-7.

48. Mahoney CP, Margolis MT, Knauss TA *et al*. Chronic vitamin A intoxication in infants fed chicken liver. *Pediatrics* 1980; 65: 893-897.

49. Department of Health (DOH). Directorate Nutrition. *Immunisation awareness campaign,* 2001. Epi(SA) and Aventis Pasteur Partnership. Integrated Nutrition Programme for Health. Pretoria:DOH.

50. West CE, Eilander A, van Lieshout M. Consequences of revised estimates of carotenoid bioefficacy for dietary control of vitamin A deficiency in developing countries. *J Nutr* 2002; 132(9 suppl): 290S-296S.

51. Elkins ER, Dudek JA. Sampling for vitamin analyses. In: Augustin JA, Klein BP, Becker D *et al.* (eds). *Methods of vitamin assay*. New York: John Wiley and Sons, 1985.

52. Simpson KL. Chemical changes in natural food pigments. In: Finley JW, (ed). *Chemical Changes in Food During Processing*. Westport, CT: AVI Publishing Company 1986: 409-441.

53. Cottrell RC. Introduction: nutritional aspects of palm oil. *Am J Clin Nutr* 1991; 53: 989S-1009S.

54. Khachik F, Goli MB, Beecher GR *et al.* Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *J Agric Food Chem* 1992; 40:390-398.

55. Sweeney JP, Marsh AC. Effect of processing on provitamin A in vegetables. *J Am Diet Assoc* 1971; 59: 238-243.

56. Erickson JM, Mawson AR. Possible role of endogenous retinoid (vitamin A) toxicity in the pathophysiology of primary biliary cirrhosis. *J Theor Biol* 2000; 7: 47-54.

57. Selmi C. Environmental factors in primary biliary cirrhosis. *Hepatology research* 2007; 37: S370-S376.

58. Erhardt JG, Craft NE, Heinrich F *et al*. Rapid and simple measurement of retinol in human dried whole blood spots. *J Nutr* 2002; 132: 318-321.

59. Shi H, Ma Y, Humphrey JH *et al*. Determination of vitamin A in dried human blood spots by high-performance capillary electrophoresis with laser-excited fluorescence detection. *J Chromatogr* B 1995; 665: 89-96.

60. Ma Y, Wu Z, Furr HC *et al.* Fast minimicroassay of serum retinol (vitamin A) by capillary zone electrophoresis with laser-excited fluorescence detection. *J Chromatogr* 1993; 616:31-37.

61. Rice AL, Stoltzfus RJ, De Francisco A *et al*. Evaluation of serum retinol, the modified-relative-dose-response ratio, and breast milk vitamin A as indicators of response to postpartum maternal vitamin A supplementation. *Am J Clin Nutr* 2000; 71: 799-806.

62. Rice AL, Stoltzfus RJ, De Francisco A *et al*. Maternal vitamin A or beta-carotene supplementation in lactating Bangladeshi women benefits mothers and infants but does not prevent sub-clinical deficiency. *J Nutr* 1999; 129: 356-365.

63. Tanumihardjo SA, Penniston KL. Simplified methodology to determine breast milk retinol concentrations. *J Lipid Research* 2002; 43: 350-355.

64. Lo CS, Wahlqvist ML, Horie Y. Determination of retinoic acid and retinol at physiological concentration by HPLC in Caucasians and Japanese women. *Asia Pacific J Clin Nutr* 1996; 5(3): 173-174.

65. Sommer A, Hussai G, Muhilal S *et al*. History of night blindness: a simple tool for xerophthalmia screening. Am *J Clin Nutr* 1980; 33:887-891.

66. Congdon N, Sommer A, Severns M *et al*. Pupillary and visual thresholds in young children as an index of vitamin A status. *Am J Clin Nutr* 1995; 61: 1076-1082.

67. Ferrando R. From analysis to reality: bioavailability in nutrition and toxicology – a misunderstood concept. *World Rev Nutr Diet* 1987; 53: 26-68.

68. Booth SL, Johns T, Kuhnlein HV. Natural food sources of vitamin A and provitamin A. *Food Nutr Bull* 1992; 14(1): 6-19.

69. Mangels AR, Holden JM, Beecher GR *et al*. Carotenoid content of fruits and vegetables: an evaluation of analytical data. *J Am Diet Assoc* 1993; 93: 284-296.

70. West CE, Poortvliet EJ. *The Carotenoid Content of Foods with Special Reference to Developing Countries*. Virginia: International Science and Technology Institute, 1993.

71. Wolmarans P, Danster N, Dalton A *et al*. (eds) *Condensed Food Composition Tables for South Africa*. Parow Valley, Cape Town: MRC, 2010.

72. International Vitamin A Consultative Group (IVACG). *Guidelines for the development of a simplified dietary assessment to identify groups at risk for inadequate intake of vitamin A*. Washington, DC: International Life Sciences Institute, The Nutrition Foundation, 1989.

73. Bingham SA. 1991. Limitations of the various methods of collecting dietary intake data. *Ann Nutr Metab* 1991; 5:117-127.

74. Barrett-Connor E. Nutrition epidemiology: how do we know what they ate? Am *J Clin Nutr* 1991; 54:182S-187S.

75. WHO/UNICEF. *Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes*. Review version, May, 1994. Geneva: World Health Organisation, Nutrition Unit, 1994.

76. Abdullah M, Ahmed L. Validating a simplified approach to the dietary assessment of vitamin A intake in preschool children. *Eur J Clin Nutr* 1993; 47: 115-122.

77. Rosen DA, Haselow NJ, Sloan NL. How to Use the *HKI Food Frequency Factors as Method to Assess Community Risk of Vitamin A Deficiency*. New York: Helen Keller International, Vitamin A Technical Assistance Programme, 1993.

78. John A, Jyoti A, Sivakumar B. Ammonium nitrogen to creatinine ratio (Am-N/CR) in random urine samples as an index of vitamin A nutriture in children. *Nutr Res* 1989; 9: 153-160.

79. Panth M, Shatrugna V, Ravinder P, Sivakumar, B. Assessment of vitamin A status in pregnant women as reflected by *in vitro* destruction of vitamin A by hemolysates and urinary ammonium nitrogen to creatinine ratio. *Int J Vit Nutr* 1993; 3: 168-172.

80. Tanumihardjo SA, Permaesih D, Muherdiyantiningsih *et al*. Vitamin A status of Indonesian children infected with *Ascaris lumbricoides* after dosing with vitamin A supplements and albendazole. *J Nutr* 1996; 126: 451-457.

81. Jang JT, Green JB, Beard JL *et al*. Kinetic analysis shows that iron deficiency decreases liver vitamin A mobilization in rats. *J Nutr* 2000; 130: 1291-1296.

82. Rosales FJ, Jang JT, Pinero DJ *et al*. Iron deficiency in young rats alters the distribution of vitamin A between plasma and liver and between hepatic retinol and retinyl esters. *J Nutr* 1999; 129: 1223-1228.

83. Fujita M, Brindle E, Shofer J *et al.* Retinol-binding protein stability in dried blood spots. *Clin Chem* 2007; 53: 1972-1975.

84. Duncan B, Canfield L, Greivenkamp J *et al*. The night vision threshold test (NVTT): a simple instrument for testing dark adaptation in young children. *J Trop Pediatr* 2000; 46: 30-35.

85. Sanchez A, Congdon N, Sommer A *et al.* Pupillary threshold as an index of population vitamin A status among children in India. *Am J Clin Nutr* 1997; 65: 61-66.

86. Wasantwisut E, Attig G. *Empowering vitamin A Foods. A Food-based Process for Asia and the Pacific region*. Food and Agriculture Organisation Regional Office for Asia and the Pacific, Institute of Nutrition of Mahidol University, and South and East Asia Nutrition Research-cum-Action Network. Bangkok, Thailand: Institute of Nutrition, Mahidol University, 1995.

87. Lee CY, Simpson KL, Gerber L. Vegetables as a major vitamin source in our diet. *New York's Food Life Sci Bull* 1989; 126: 1-11.

88. Hollman PCH, Slangen JH, Wagstaffe PJ *et al*. Intercomparison of methods for the determination of vitamins in foods. *Analyst* 1993; 118: 475-480.

89. Rodriguez-Amaya DB. Critical overview of provitamin A determination in plant foods. *J Micronutrient Anal* 1989; 5: 191-225.

Chapter 3

Vitamin D

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Introduction

Vitamin D is classified as one of the fat-soluble vitamins, but it is regarded by some as a hormone, because it can be endogenously synthesised by humans and its mechanisms of action are similar to those of steroid hormones. In this chapter the metabolism, accepted and known functions of vitamin D, requirements, measurement, recommended normal ranges, and what is known about vitamin D status of South Africans are briefly reviewed. The potential role of vitamin D in the prevention and treatment of tuberculosis and HIV infection, based on its effects on the immune system and response, is emphasised.

Metabolism

Vitamin D is the generic term for a number of secosteroid compounds with anti-rachitic properties. The two important forms are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Ergocalciferol is formed by the UV irradiation of ergosterol, a plant sterol, while cholecalciferol in formed in the skin of humans from 7-dehydrocholesterol (an intermediate in the synthesis of cholesterol that accumulates in the skin but not other tissues) under the influence of UV irradiation with wavelengths between 290 – 315 nm[1]. Once formed in the skin, vitamin D_3 is transported attached to the vitamin D-binding protein (Gc-globulin) to the liver where it is converted to 25-hydroxyvitamin D (25-OHD), a reaction catalysed by a cytochrome P450 25-hydroxylase. The enzyme or enzymes responsible for this step are not clearly established although it is likely that a microsomal 25-hydroxylase (CYP2R1) plays a critical role as a mutation in the gene responsible for this enzyme results in very low concentrations of 25-OHD[2]. Ingested vitamin D (either D_2 or D_3) is absorbed as a fat-soluble nutrient and transported in chylomicrons to the liver, where it undergoes 25-hydroxylation. The resultant 25-OHD is the major circulating form of the vitamin and its serum concentration is a good indicator of the vitamin D status of an individual. 25-OHD, like vitamin D, is biologically inactive and must be further hydroxylated in the kidney to 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$) before becoming active[3] (See Figure 3.1).

Regulation of Vitamin D Activity

The activity of the renal 1a-hydroxylase (CYP27B1- cytochrome P450, family 27, subfamily B, polypeptide 1), which hydroxylates 25-OHD to 1,25-$(OH)_2D$, is tightly controlled mainly by parathyroid hormone (PTH) and serum inorganic phosphorus (Pi) concentrations, with high PTH and/or low Pi stimulating activity and increasing 1,25-$(OH)_2D$ concentrations. Low serum calcium and elevated calcitonin concentrations also stimulate 1a-hydroxylase activity. Fibroblast growth factor 23 suppresses its activity[4]. It is apparent that the renally formed 1,25-$(OH)_2D$

is by far the major contributor to circulating levels of the hormone, as anephric (kidney failure) patients have very low serum concentrations of the active metabolite, although in certain granulomatous diseases such as sarcoidosis the extrarenal production of 1,25-$(OH)_2D$ may contribute to circulating levels[5]. The major catalytic pathway of 25-OHD and 1,25-$(OH)_2D$ begins with the 24-hydroxylation of the metabolites by 24-hydroxylase (CYP24A1- cytochrome P450, family 24, subfamily A, polypeptide 1), whose activity is induced by 1,25-$(OH)_2D$, thus producing a negative feed-back system[6] . The 24-hydroxylase is found in almost all tissues but needs the presence of 1,25-$(OH)_2D$ and the vitamin D receptor to induce its mRNA. The major excretory pathway for 25-OHD and 1,25-$(OH)_2D$ is via their 24-hydroxylation and conversion to calcitroic acid, which is mainly excreted through the hepatic/biliary system and gastrointestinal tract.

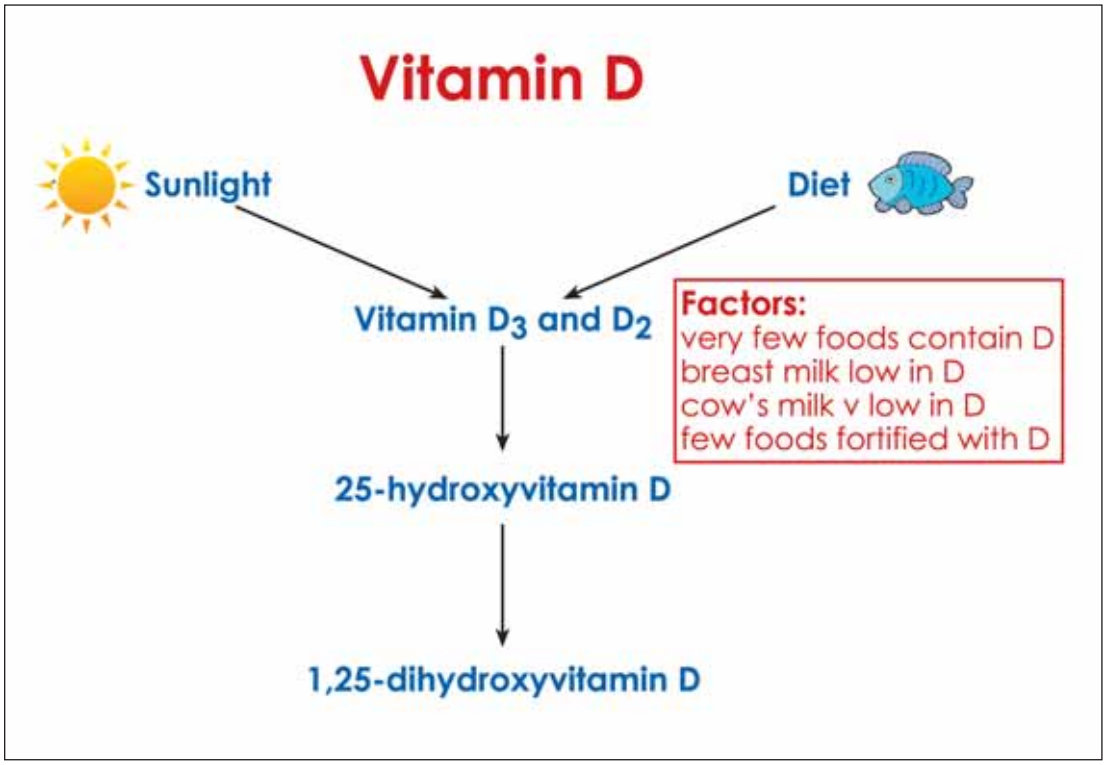

Figure 3.1 The metabolism of vitamin D, indicating sources and pathway of activation

Mechanism of Action of Vitamin D

The active metabolite of vitamin D behaves in a manner similar to that of other steroid hormones, having its effect on protein synthesis through its regulation of vitamin D responsive genes. The intracellular receptor for 1,25-$(OH)_2D$ is the vitamin D receptor (VDR), which has two major domains – the N terminal Zn finger DNA-binding domain and the C terminal ligand (1,25-$(OH)_2D$)-binding domain. When attached to the ligand, VDR heterodimerises with the retinoid X receptor (RXR) allowing the complex to bind to vitamin D responsive elements (VDRE) situated in the promoter region of vitamin D regulated genes[7]. These genes encode proteins that control intestinal calcium absorption, bone growth and remodelling, phosphate homeostasis, the mammalian hair cycle, cell differentiation and proliferation, and lipid detoxification.

Extrarenal Sites of 1a-hydroxylase

At least ten extrarenal tissues are able to express 1a-hydroxylase and thus may be able to produce 1,25-$(OH)_2D$ in a autocrine/paracrine fashion. These tissues include dendritic cells, keratinocytes, breast tissue, colon, prostate and pancreatic islets[3]. Locally produced 1,25-$(OH)_2D$ acting on these tissues through the VDR may suppress IL-2 in T-cells, induce defensin and cathelicidin as local antimicrobial effectors, stimulate involucrin synthesis in the skin, and CYP3A4 and p21 in epithelial cells such as in the colon, stimulate insulin secretion from the islet cells of the pancreas, and promote cell differentiation and suppress proliferation in many of these tissues[7].

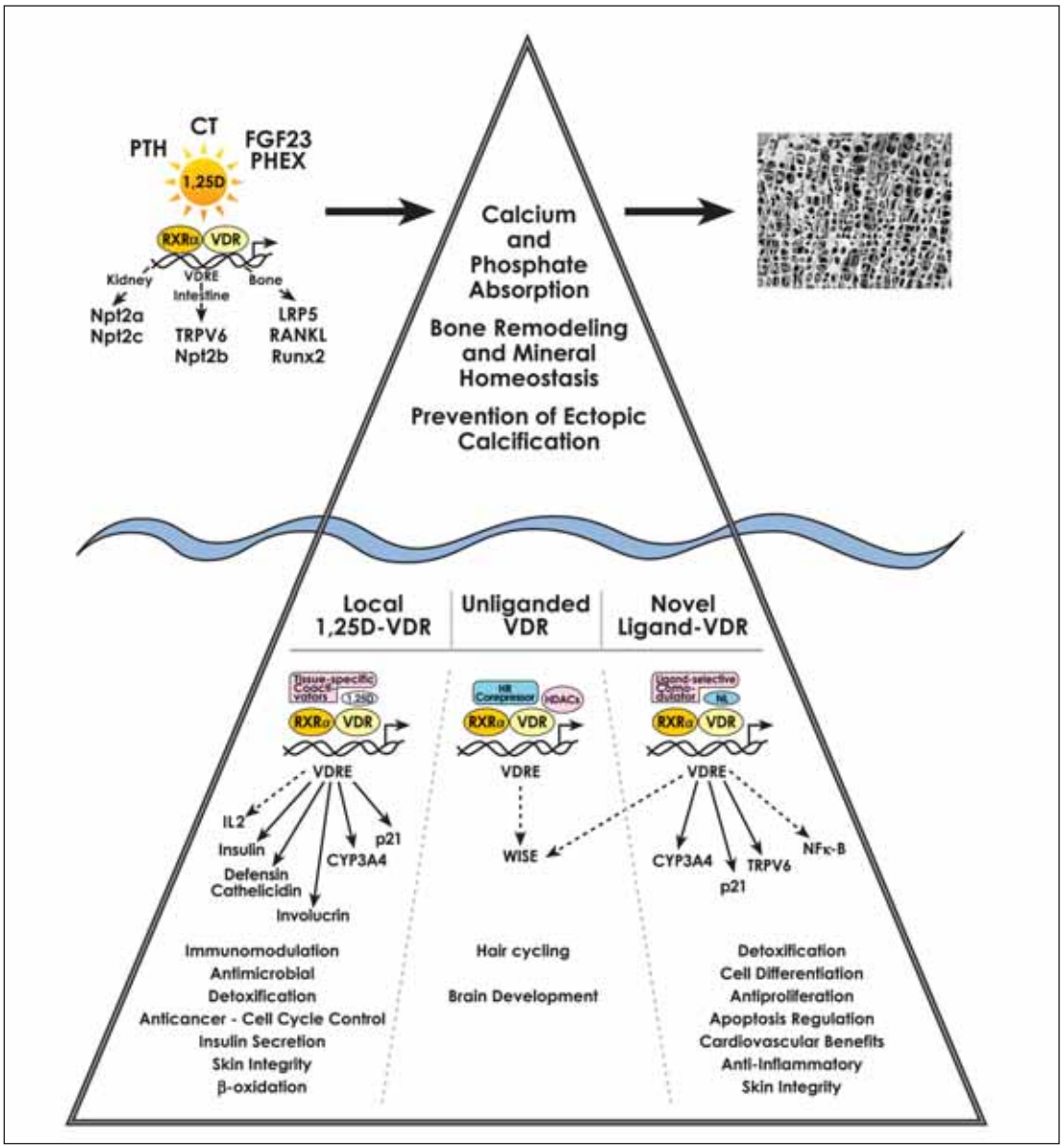

Figure 3.2 The classical and non-classical actions of the 1,25-$(OH)_2D$-VDR complex. The classical calcium and bone-related actions are depicted as being the visible part of the iceberg, while the non-classical actions are below the surface. (Reproduced from Haussler *et al.*[7])

The Physiological Functions of Vitamin D

Calcium and Bone Homeostasis

As depicted in Figure 3.2, the classical action of vitamin D is its role in the maintenance of calcium, phosphorus and bone homeostasis. It has long been established that vitamin D, provided either as vitamin D orally as in the form of cod liver oil, or through the UV irradiation of skin, is essential to prevent the development of hypocalcaemia and rickets in infants and children or osteomalacia in adults. The major role of vitamin D or more correctly that of 1,25-$(OH)_2D$ is to ensure adequate calcium absorption from the intestine to meet the demands of the growing skeleton in children and the mainly renal losses of calcium in the adult, thus maintaining normocalcaemia.

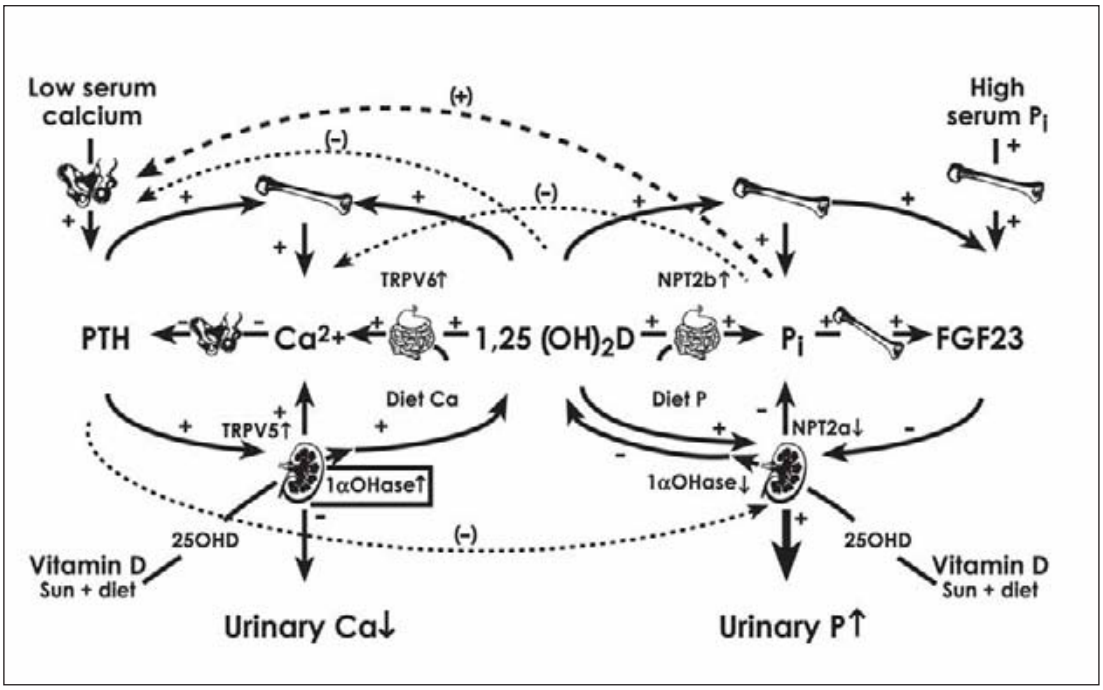

Figure 3.3 The central role played by 1,25-$(OH)_2D$ in calcium and phosphorus homeostasis. FGF23 = Fibroblast Growth Factor 23; PTH = parathyroid hormone; Pi = inorganic phosphate; NPT2b = Na-dependent phosphate transporter 2b. (From Prentice A *et al*.[8])

Figure 3.3 highlights the central but complex role that 1,25-$(OH)_2D$ plays in maintaining normal calcium and phosphorus homeostasis, and by so doing maintaining normal bone mineralisation and growth. A fall in serum calcium stimulates parathyroid hormone secretion which activates renal 1a-hydroxyalse activity and the production of 1,25-$(OH)_2D$, which in turn, increases intestinal calcium and phosphorus absorption, thus elevating circulating calcium concentrations. At the same time the elevated PTH increases renal calcium reabsorption and together with 1,25-$(OH)_2D$ stimulate osteoclastic bone resorption which releases calcium and phosphorus into the blood stream, helping to maintain serum calcium, but elevating phosphorus concentrations. The latter is controlled by the other action of elevated PTH concentrations on the renal tubule, that of increasing renal tubular phosphate clearance, thus reducing serum phosphorus concentrations[8]. Correction of serum calcium through the increased abosorption of calcium from the gut and mobilisation of calcium from bone results in a negative feedback reducing PTH secretion, a thus reducing renal phosphate loss.

Figure 3.3 also depicts a recently described additional loop for the control of serum phosphorus; that of fibroblast growth factor 23 (FGF23). FGF23 was originally discovered as the factor responsible for a rare form of hypophosphataemic rickets[9]. It is now thought that while PTH might play a more acute role in phosphorus homeostasis, FGF23 probably has a more chronic effect. FGF23 which is secreted by osteoblasts/osteocytes in bone osteoblasts, is stimulated by elevated serum phosphorus and 1,25-$(OH)_2D$ levels. FGF23 increases renal phosphorus clearance and reduces 1a-hydroxylase activity, thus providing a negative feed-back loop for the control of 1,25-$(OH)_2D$[10].

Vitamin D and Immunity

The possibility that vitamin D might play a role in preventing or treating infection and modulate immunity, has been considered for many years. According to Martineau *et al.*[11], fish liver oil was used in 1849 in the treatment of tuberculosis with reports of improvement in appetite and strength, and in the 1930s pharmacological doses of vitamin D were used to treat cutaneous tuberculosis. Further, in 1895 Niels Ryberg Finsen used UV light to treat tuberculosis of the skin with excellent results[12]; this research earned him the Nobel Prize in Physiology in 1903. The development of TB sanatoria in mountainous regions of Europe became fashionable so that sufferers could be exposed to the beneficial effects of UV radiation from sunlight.

In young children, rickets is associated with an increased incidence of respiratory and diarrhoeal diseases[13]. The proneness to respiratory infections could possibly be explained on the thoracic cage abnormalities and muscle weakness associated with rickets, however these abnormalities cannot explain the higher prevalence of diarrhoea. Defective neutrophil mobility and impaired phagocytosis, which were documented in vitamin D deficiency In the 1970s[14,15], and more recently the now well-known role of 1,25-$(OH)_2D$ in modulating immune function[16] provide more rational explanations for the increased incidence of infections. Monocytes/macrophages, when activated by bacterial LPS, are able to synthesise and release 1,25-(OH)2D into the pericellular fluid, thus activating VDR in surrounding cells, inhibiting T cell proliferation and indirectly B cell immunoglobulin production. 1,25-$(OH)_2D$ appears to modulate the T cell phenotype by suppressing T-helper cells (Th1) thus favouring T suppressor cells (Th2). These findings suggest that vitamin D (1,25-$(OH)_2D$) might play important roles in suppressing autoimmune diseases and inducing tolerance in host-graft rejection[17]. (Figure 3.4) As both macrophages and dendritic cells contain 1a-hydroxylase (CYP27B1) and are thus able to con-

vert circulating 25(OH)D to 1,25-$(OH)_2$D, they are seen as being central in the control of the immune response at local sites of inflammation.

CYP27B1 (1a-hydroxylase) in extra-renal sites is not under the control of PTH, serum calcium or serum phosphorus, and thus does not appear to have a negative feedback system. As a result the amount of 1,25-$(OH)_2$D produced by these extra-renal tissues is dependent on the amount of activated tissue (the number of activated macrophages) and the serum concentration of the substrate for CYP27B1, 25-OHD. Thus in certain situations hypercalcaemia due to extra-renal synthesis of 1,25-$(OH)_2$D has been reported. Examples of hypercalcaemia due to the excessive production of 1,25-$(OH)_2$D by activated macrophages in granulomatous diseases include conditions such as sarcoidosis, neonatal fat necrosis, Crohn's disease, chronic bacterial and fungal infections (tuberculosis, leprosy, histoplasmosis and coccidiomycosis), and neoplastic diseases (B cell lymphoma, Hodgkin's disease, dysgerminoma)[17].

It appears that Toll-like receptors (TLRs) play an important role in mediating the innate immune response to microbiological ligands. TLRs are present in polymorphonuclear cells, monocytes and macrophages[18].

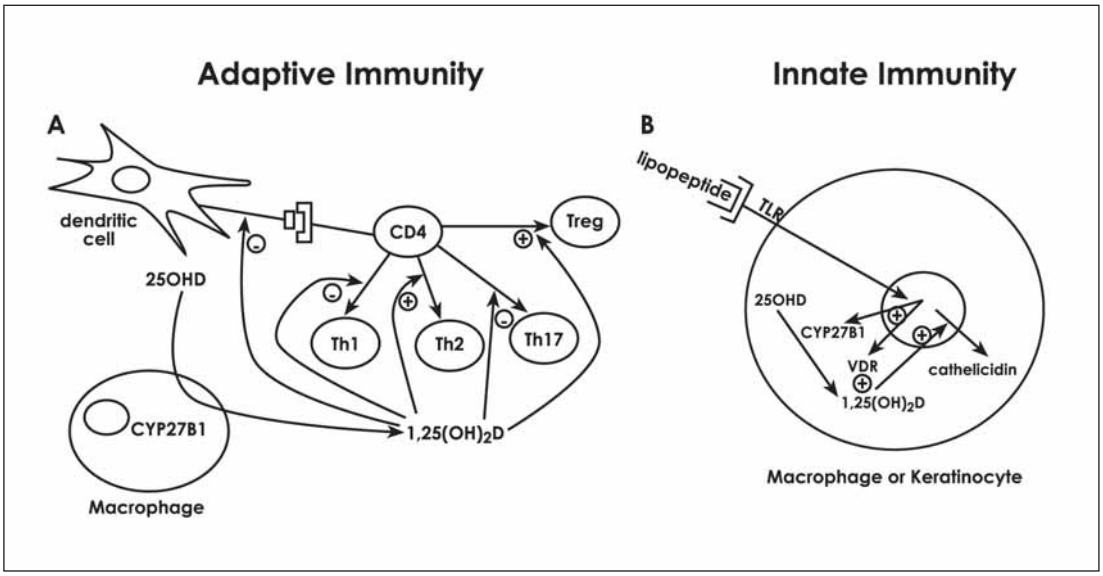

Figure 3.4 Regulation of immune function by 1,25-$(OH)_2$D. 1,25-$(OH)_2$D suppresses adaptive immunity (A) by inhibiting the maturation of dendritic cells, reducing their capacity to present antigen to CD4 cells, by inhibiting the proliferation of CD4 cells into Th1 and Th17 cells, and by promoting the production of Th2 and Treg cells. 1,25-$(OH)_2$D promotes innate immunity (B) by stimulating VDR and 1a-hydroxylase in activated macrophages and stimulates the production of cathelicidin. (Reproduced from Bikle[18])

Recently, Liu and co-workers[19] have described the important role of TLR2/1 in monocytes and macrophages in triggering vitamin D mediated synthesis of cathelicidin and the intracellular killing of *Mycobacterium tuberculosis* through the upregulation of the VDR gene and CYP27B1. 25-OHD is essential for the activation of the TLR-cathelicidin pathway by mycobacterial lipoprotein. Of interest, the same workers using either serum from African American or Caucasian individuals in an *in vitro* study were able to show that cathelicidin mRNA induction was

significantly lower in the monocyte system using African American serum than when Caucasian serum was used. The impaired cathelicidin production in the cell system using African American serum could be normalised by supplementing the serum with 25-OHD to achieve levels similar to those found in Caucasian serum. This *in vitro* study does provide a possible explanation for the higher prevalence of tuberculosis among African Americans than American whites in the USA and could possibly be one of many reasons for the high prevalence of TB among the 'mixed race' community in the Western Cape, as UV synthesis of vitamin D is limited during the winter months in the Western Cape[20]. However this hypothesis needs to be tested before firm conclusions can be drawn.

Vitamin D and Tuberculosis

As mentioned earlier, vitamin D has been used over many years in the management of tuberculosis. With the development of specific anti-tuberculous drugs, the use of vitamin D fell into disrepute, but a number of studies have used vitamin D in conjunction with anti-tuberculous chemotherapy. Martineau *et al*. in 2007[11] reviewed prospective clinical studies using vitamin D in the treatment of pulmonary tuberculosis. One randomised controlled study, using vitamin D 1 000IU/daily for two months in one arm in addition to isoniazid, rifampicin and streptomycin, did not find any clinical difference in response to therapy between the two groups. The other two randomised trials did not report on the effect of vitamin D. There were ten case series, and all reported on the effect of vitamin D on the course of the disease. In four of the studies (all reported over 40-60 years ago), an inflammatory reaction one to four weeks after initiation of therapy was noted in 4-21% of patients, however it is unclear whether or not these reactions could be ascribed to vitamin D. The reaction was reported to consist of worsening pulmonary symptoms, weight loss, pyrexia, an increase in pulmonary infiltrates and in those with skin disease, a worsening of the cutaneous inflammation. These reactions were seen more commonly in patients taking high doses of vitamin D (≥100 000 IU/day), and improved on stopping the vitamin D, which could be started at a lower doses without an exacerbation of the symptoms. In one of the RCTs, hypercalcaemia was reported in 19 of 30 patients at doses of between 400 and 3 800 IU/day; the elevation was noted first after 15 days of therapy and settled after six months despite continuing the vitamin D. Hypercalcaemia was also note in three of the ten case series, and occurred in between 1% and 11% of patients (all were receiving at least 600 000 IU/week). It is of interest to note that many of the case studies reported during the 1950s and 60s used doses of vitamin D which would now be considered to be dangerously high (varying from 50,000 IU/d to 600 000 IU/d for varying periods of time).

With the recent advances in the understanding of the role of vitamin D sufficiency in modulating immune responses, there has been a renewed interest in a possible role for vitamin D in the prevention and treatment of tuberculosis. A recent study of vegetarian Asians with TB living in London, severe vitamin D deficiency (≤10 nmol/L) was associated with a high risk of tuberculosis (odds ratio of 9.9), while vitamin D deficiency had a 2.9-fold increase in risk[21]. The same study also suggested that polymorphisms of the VDR gene might contribute to susceptibility to tuberculosis. Recently, Martineau and co-workers[22] assessed in a double-blind study the effect of a single dose of vitamin D (2.5 mg or 100 000 IU) given to adult TB contacts in the UK on an in vitro assay of anti-mycobacterial activity (using the BCG-*lux assay*) six weeks after the single dose. The vitamin D dose increased the subjects' 25-OHD concentration by 91% and corrected vitamin D deficiency in all subjects at six weeks. There was also a statistical reduction (p=0.03) in BCG-*lux* bioluminescence at 24 h but not 96 h in the assay. These results are

encouraging, but it is possible that the effects might have been greater had better circulating 25-OHD concentrations been obtained with vitamin D supplementation as values only reached a mean of 67 nmol/l and there are suggestions that values greater than 75 nmol/l should be obtained. A similar study employing UV radiation to increase serum 25-OHD concentrations from 11.2 ng/ml (28 nmol/l) to 20.4 ng/ml (51 nmol/l) in eight normal subjects was unable to show any change in whole blood functional assays for anti-mycobacterial immunity associated with the increase in 25-OHD levels[23]. In an attempt to define the level of vitamin D supplementation required to achieve vitamin D sufficiency, the effect of a single dose of 2.5 mg (100 000 IU) vitamin D_2 on serum 25-OHD concentrations was assessed at one and eight weeks post dose in TB patients. Although there was a marked rise in 25-OHD values at one week post dose, the levels had returned to levels <75 nmol/l at eight weeks in nearly all the patients[24]. This study indicates the need for continued vitamin D supplementation in order to maintain adequate vitamin D status in subjects who are not exposed to adequate sunlight or who live in temperate climates where UV radiation is reduced during winter (>40° N or S latitude). It also should be borne in mind that there is some evidence that the pharmacokinetics of intermittent vitamin D_2 treatment is different from that of vitamin D_3[25, 26], with serum 25-OHD_3 concentrations after vitamin D_3 administration being maintained for longer than 25-OHD_2 levels after vitamin D_2 administration.

A number of commentaries on the role of vitamin D status in the prevention and management of tuberculosis have indicated the need for new randomised controlled trials to assess the efficacy of vitamin D supplementation on the prevalence and severity of tuberculosis. A recently published study, using the same dose of vitamin D as was used in the studies listed above (vitamin D_3 100 000 IU at zero, five and eight months), randomised tuberculous patients to received vitamin D as an adjunct to therapy from the onset of treatment[27]. Reduction of TB score, sputum smear conversion rates and mortality did not differ between the two arms. Although this negative outcome is disappointing, it is possible that the dose of vitamin D used was too small to correct vitamin D insufficiency in between the dosage times[28]. This contention is supported by a double-blind controlled study from Indonesia, which used 10 000 IU vitamin D daily[29]. The authors reported 100% sputum conversion in the vitamin D group as compared to 76.7% in the placebo group and more marked radiological improvement in the vitamin D group. A similar result was noted in a paediatric study. However, the dose of vitamin D given is not known (abstract only available)[30].

Thus, although there is some promising evidence to suggest that vitamin D might play a role in reducing the risk of tuberculous infection and that it might also assist in the recovery of infected patients on treatment, there are a number of unanswered questions: what is the optimal level of serum 25-OHD required to confer protection, what dose of vitamin D is required to achieve that concentration and what is the optimum method of administration? In the South African situation, very little is known of the vitamin D status of populations, especially of those communities most at risk of tuberculosis, and this needs to be established before recommendations can be made about whether or not vitamin D supplementation might be an appropriate adjunct to therapy and an efficacious preventative strategy.

Vitamin D and HIV

Because of the suggested role of vitamin D in both the innate and adaptive immune responses, there has recently been considerable interest in its role in HIV. A number of studies have found a high prevalence of vitamin D deficiency among infected subjects in developed countries[31], (however when using the same definition of vitamin D deficiency the prevalence among non-HIV infected controls is similar)[32], but few have assessed the prevalence in developing countries where the scourge of HIV is highest. In one such study conducted in pregnant women in Tanzania, 'low levels' of vitamin D were found in 39%, but the cut-off value for defining hypovitaminosis D was set at a high level of <80 nmol/l[33].

There is some evidence that antiretrovirals may affect vitamin D metabolism and thus may influence 25-OHD and 1,25-$(OH)_2D$ concentrations. *In vitro* studies indicate that protease inhibitors (PIs) inhibit 25- and 1a-hydroxylase activity in a dose dependent manner, but the effects on vitamin D metabolism are unclear in the *in vivo* situation. Non-nucleosidase reverse transcriptase inhibitors (NNRTIs) such as efavirenz may reduce 25-OHD concentrations through their stimulatory effect on 24-hydroxylase. This effect has been substantiated in clinical studies[34].

Low bone mass has increasingly been considered a risk in HIV-infected patients. In one study, 23% of subjects were found to have osteoporosis. Factors found to be important included low body mass index, time on PIs and time on tenofovir, and the current use of PIs[35]. Whether or not these effects could be influenced by vitamin D supplementation is unclear at present.

There is evidence that low vitamin D status is associated with increased morbidity and mortality related to HIV in both developed and developing countries[36, 37]. In the Tanzanian study, vitamin D status was not associated with T-cell numbers, but was inversely related to HIV disease progression, all-cause mortality and the development of anaemia. Randomised trials are urgently required to assess the role of vitamin D supplementation on morbidity and mortality in HIV-infected patients, as it is unclear whether or not these reported positive effects of vitamin D are as a consequence of more ill patients being less exposed to sunlight or having a diet lower in vitamin D.

Assessment of Vitamin D Status

Vitamin D Requirements

As mentioned earlier, humans have two sources of vitamin D; that obtained in the diet and that formed in the skin under the influence of UVB radiation from sunlight. In South Africa, few foods contain significant amounts of vitamin D, as few are fortified with vitamin D (for example, (infant milk formulas, and some powdered milks, yogurts and margarines) and it is only oily fish and possibly egg yolks that contain naturally occurring vitamin D in reasonable amounts. Thus the majority of South Africans are dependent on sunlight exposure to ensure vitamin D sufficiency, unless taking regular vitamin D supplements. A number of factors influence vitamin D formation in the skin, these include the amount of UVB radiation reaching the skin, which is dependent on the latitude, season, time of the day, cloud cover and atmospheric pollution; the surface area of skin exposed, the duration of UVB exposure, the use of sunscreen products

and the degree of melanin pigmentation in the skin[38]. Figure 3.5 depicts the seasonal *in vitro* vitamin D_3 production by sunlight in Cape Town and Johannesburg. It is clearly shown that UVB radiation is sufficient throughout the year in Johannesburg to ensure vitamin D formation, however in Cape Town, due probably to cloud cover and reduced UVB radiation reaching the earth during the winter months because of the greater latitude of Cape Town, vitamin D production during the winter months from March through September is markedly reduced[20]. The impact of the reduced UVB radiation on the vitamin D status of individuals during the winter months in Cape Town is unknown as there is little information on the vitamin D status of different communities and age groups in South Africa, however it might be an explanation for the high prevalence of infantile rickets reported from Cape Town some 50 years ago[39]. Further it must be considered one of many possible factors accounting for the high prevalence of tuberculosis in the Western Cape.

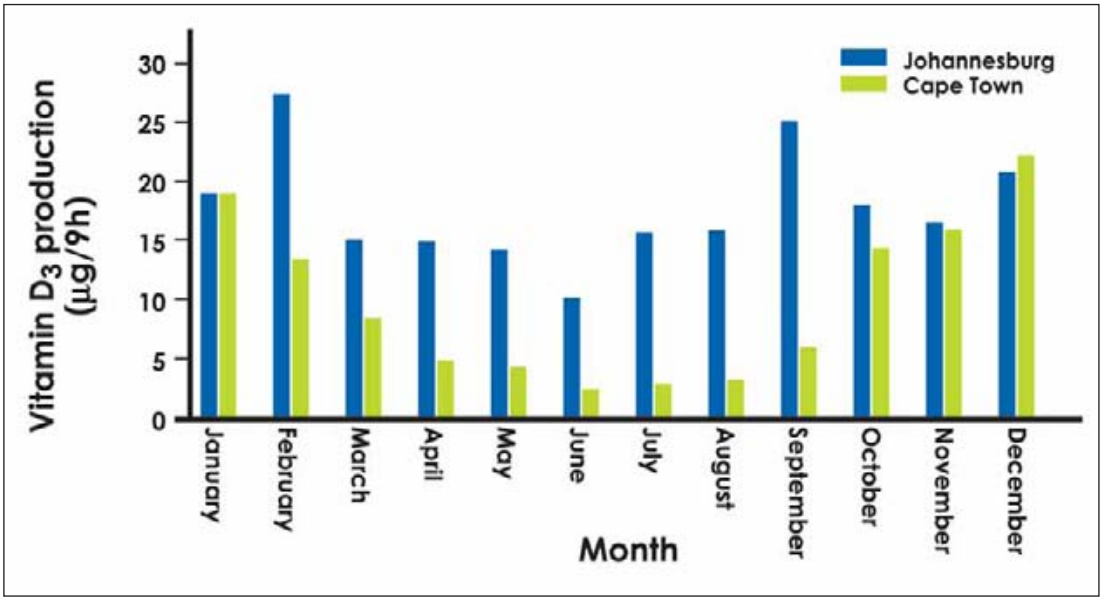

Figure 3.5 Seasonal variation in *in vitro* vitamin D_3 synthesis by sunlight in Johannesburg and Cape Town. Vials containing 7-dehydrocholesterol were exposed to sunlight on one day a month over a year. The conversion of 7-dehydrocholesterol to vitamin D_3 was quantitated by HPLC. Conversion to vitamin D_3 occurred throughout the year in Johannesburg, but little synthesis occurred in Cape Town during the winter months from April through September. (Reproduced from Pettifor *et al*.[20])

With the increasing awareness of the widespread actions of vitamin D in multiple different tissues, there has been a renewed interest in appropriately defining vitamin D sufficiency. In November 2010, the Institute of Medicine of the National Academies of Science in the USA released its draft report on the dietary reference intakes (DRIs) for calcium and vitamin D[40], which had been commissioned by the US and Canadian governments in response to increasing pressure to reassess the DRIs drawn up over ten years previously. Not only did the committee make recommendations on the DRI for vitamin D but it also made recommendations on the assessment of vitamin D sufficiency. The committee confirmed the use of serum 25-OHD as a measure of vitamin D exposure (a combination of dietary intake and skin production), and was able to recommend a concentration that it considered to protect 97.5% of the population from the skeletal effects of vitamin D deficiency. The committee was unable to

provide any recommendation on the serum 25-OHD concentration that would optimise many of the proposed non-skeletal effects of vitamin D, such as on the immune system, cancer prevention, autoimmune diseases and diabetes, as it felt that although there was some evidence of association between vitamin D and the prevention of these diseases, there was a lack of good prospective clinical trial-based evidence that consistently supported the proposed non-skeletal beneficial effects of vitamin D. The committee concluded that a serum 25-OHD concentration of 40 nmol/l (16ng/ml) was the desired level for a population median, while a concentration of 50 nmol/l (20 ng/ml) would cover nearly all the population[40]. Based on these recommendations the committee has provided estimated average requirements (EARs) and recommended dietary allowances (RDAs) for all age groups, except infants, for whom an average intake (AI) was calculated (Table 3.1). It should be noted that these dietary intakes are based on the assumption that negligible amounts of vitamin D are produced in the skin, so as to remove the marked variability in skin production of vitamin D due to latitude, sunlight exposure, etc.

Table 3.1 Vitamin D dietary reference intakes by age group according to the Institute of Medicine[40]

Age group	0-6 months*	6-12 months*	1-3 years	4-8 years	Adolescents and adults	>70 years of age	Pregnancy/ lactation
EAR			400 IU	400 IU	400 IU	400 IU	400 IU
RDA/ AI (infants)	400 IU	400 IU	600 IU	600 IU	600 IU	800 IU	600 IU
UL	1 000 IU	1 500 IU	2 500 IU	3 000 IU	4 000 IU	4 000 IU	4 000 IU

*= Adequate intake rather than RDA; EAR = Estimated average requirement; RDA = Recommended dietary allowance; AI = Adequate intake; UL = Tolerable upper intake level; 40 IU = 1µg vitamin D

A number of studies have assessed the dietary intake of vitamin D in South African communities (both adults and children) and all have found median intakes below 200 IU/day[41-44], confirming the low vitamin D content of typical South African diets. There are however little data on serum 25-OHD concentrations in the same communities. Several studies have been conducted in Johannesburg, the first being reported in 1978, in which 285 children between one and 17 years of age living in the 'coloured' Western Township in Johannesburg were investigated[45]. Mean 25-OHD in pre-teenage children ranged from 30-34 ng/ml (75-85 nmol/l). Values fell over the teenage years to a mean of 22.7 ng/ml (56.7 nmol/l) by 17 years of age. In a more recent study of 10-year old black and white children from the birth-to-twenty cohort, mean 25-OHD concentrations in the black and white children were 93.3nmol/l (37.3 ng/ml) and 120 nmol/l (48 ng/ml) respectively[46]. White children showed a seasonal variation in 25-OHD, which was not seen in the black children. Thus the limited data we have, suggest that the vitamin D status of children in Johannesburg is good, however whether the same holds true for children living in less climatically mild areas of the country is unknown. Personal observation indicates that inner city infants and toddlers living in the high-rise over-crowded flats in central Johannesburg are at risk for vitamin D deficiency, as these children are regularly seen at a clinic at the

Charlotte Maxeke Johannesburg Academic Hospital with bone deformities and active rickets, which respond to vitamin D supplementation. Similarly, little is known of the vitamin D status of at risk groups such as breastfed infants who might not be exposed to sunlight as much as older toddlers and children, and ill children, who do not get outside. However, a study of non-ambulatory children with quadriplegic cerebral palsy living at a residential care centre in Gauteng found a high prevalence of rickets and fractures in the children, who responded dramatically to vitamin D supplementation[47].

Laboratory (Biochemical) Assessment of Vitamin D

Dietary and cutaneous vitamin D is carried in the plasma on a specific alpha-globulin and hydroxilated in liver microsomes to 25-hydroxyvitamin D, 25(OH)D, which has a relatively stable concentration in blood. It circulates at about 1 000 times the concentration of the active metabolite (1,25$(OH)_2$D) and has a half-life of approximately two weeks. Vitamin D status can therefore be assessed by measuring serum or plasma 25(OH)D, using a variety of laboratory methods, such as: radio-iodinated tracer assay (after extraction of all hydroxylated metabolites using acetonitrile), radio-immunosorbent assay or immunoenzymometric assay. Automated immunodiagnostic systems (reagents and analysers) are available for 25(OH)D assays. There are a number of concerns related to the measurement of 25(OH)D by different assay techniques: various methods may not detect the metabolites of vitamin D_2 and D_3 equally, the interassay variation between different assay methods is large, and until recently there has been no gold-standard against which the various assays could be judged. The measurement of plasma or serum 1.25$(OH)_2$D is also possible, but does not indicate vitamin D status. It is a specialised investigation, not recommended for routine assessment of vitamin D status.

Although there is considerable discussion about the cut-offs indicative of deficiency and sufficiency, the following are recommended values for plasma (serum) 25(OH)D:

- < 25 nmol/L indicates deficiency;
- 25-50 nmol/L indicates low or borderline status;
- > 50 nmol/L indicates normal vitamin D status.

However, whether these recommendations are appropriate for the possible non-classical actions of vitamin D (involving immunity, cell differentiation, diabetes, cardiovascular disease, etc.) is unclear, and further studies are required before conclusions can be drawn.

Information on the vitamin D status of adults in South Africa is even less well documented. A study conducted some 30 years ago indicated that elderly patients admitted with hip fractures in Johannesburg had a high prevalence of vitamin D deficiency (defined as <25 nmol/l) varying from 14-21.5% depending on the season[48]. In a more recent survey of a randomly selected cohort of elderly female residents (>60 years of age) in Soweto, 46% had a 25-OHD <20 ng/ml (50 nmol/l). The high prevalence of obesity in the adult is likely to adversely influence the vitamin D status of the adult population[49]. However, a small study of the vitamin D status of black mothers and their babies living in the Eastern Cape found that 25-OHD values were in the normal range[50].

Dietary sources of vitamin D are limited, and in South Africa few foods are fortified with vitamin D. Those fortified are usually processed foods such as some margarines. However, as mentioned below, much more information on vitamin D status of South Africans is necessary before specific recommendations for fortification (such as the mandatory fortification of maize meal and bread flour) can be made.

Conclusions and Recommendations

The recent studies suggesting that vitamin D might have important effects in a number of non-skeletal systems, including possible effects on the immune system, raise exciting prospects that vitamin D supplementation may be a cheap and effective way of reducing morbidity and mortality associated with HIV and TB. However, before such an intervention can be recommended, randomised placebo-controlled trials need to be conducted in a variety of situations to assess the effectiveness of such treatment at different stages of the diseases and in different age groups. Further optimal 25-OHD concentrations need to be determined and the most appropriate means of achieving these levels assessed. There is also an urgent need for the vitamin D status of communities within South Africa to be determined. This is particularly so in those age groups or communities particularly at risk of tuberculosis, such as miners who work underground and specific communities in the Western Cape. There are also other communities which are not exposed to sufficient sunlight and are thus at risk for vitamin D deficiency such as the elderly and women who cover their bodies because of religious or social customs.

References

1. Holick MF. Vitamin D: a D-Lightful health perspective. *Nutr Rev* 2008; 66(10 Suppl 2):S182-S194.

2. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proceedings of the *National Academy of Sciences of the United States of America* 2004; 101(20):7711-7715.

3. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr* 2008; 88(2):491S-499.

4. Perwad F, Zhang MYH, Tenenhouse HS, Portale AA. Fibroblast growth factor 23 impairs phosphorus and vitamin D metabolism in vivo and suppresses 25-hydroxyvitamin D-1{alpha}-hydroxylase expression *in vitro*. *Renal Physiology* 2007; 293(5):F1577-F1583.

5. Barbour GL, Coburn JW, Slatopolsky E, Norman AW, Horst RL. Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. *N Engl J Med* 1981; 305(8):440-443.

6. Omdahl JL, May BK. The *25-hydroxyvitamin D 24-hydroxylase*. In: Feldman D, Pike JW, Glorieux FH, (eds) Vitamin D. 2nd ed. Burlington, MA: Elsevier Academic Press; 2005: 85-104.

7. Haussler MR, Haussler CA, Bartik L *et al*. Vitamin D receptor: molecular signaling and actions of nutritional ligands in disease prevention. *Nutr Rev* 2008; 66(10 Suppl 2):S98-112.

8. Prentice A, Goldberg GR, Schoenmakers I. Vitamin D across the lifecycle: physiology and biomarkers. *Am J Clin Nutr* 2008; 88(2):500S-5506.

9. White KE, Evans WE, O'Riordan JL *et al*. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet* 2000; 26(3):345-348.

10. Fukumoto S. Physiological regulation and disorders of phosphate metabolism–pivotal role of fibroblast growth factor 23. *Intern Med* 2008; 47(5):337-343.

11. Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. Vitamin D in the treatment of pulmonary tuberculosis. *The Journal of Steroid Biochemistry and Molecular Biology* 2007; 103(3-5):793-798.

12. Mandavilli A. The sunshine cure. *Nat Med* 2007; 13(4):396-397.

13. Beser E, Cakmakci T. Factors affecting the morbidity of vitamin D deficiency rickets and primary protection. *East Afr Med J* 1994; 71:358-362.

14. Stroder J, Kasal P. Evaluation of phagocytosis in rickets. *Acta Paediatr Scand* 970; 59: 288-292.

15. Lorente F, Fontan G, Jara P, Casas C, Garcia-Rodriguez MC, Ojeda JA. Defective neutrophil motility in hypovitaminosis D rickets. *Acta Paediatr Scand* 1976; 65:695-699.

16. Bikle DD. Vitamin D and the immune system: role in protection against bacterial infection. Current Opinion in *Nephrology and Hypertension* 2008; 17(4):348-352.

17. Adams JS, Liu PT, Chun R, Modlin RL, Hewison M. Vitamin D in defense of the human immune response. *Ann N Y Acad Sci* 2007; 1117:94-105.

18. Bikle D. Nonclassic Actions of Vitamin D. *J Clin Endocrinol Metab* 2009; 94(1):26-34.

19. Liu PT, Stenger S, Li H et al. Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. *Science* 2006; 311(5768):1770-1773.

20. Pettifor JM, Moodley GP, Hough FS *et al*. The effect of season and latitude on *in vitro* vitamin D formation by sunlight in South Africa. *S Afr Med J* 1996; 86:1270-1272.

21. Wilkinson RJ, Llewelyn M, Toossi Z *et al*. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *The Lancet* 2000; 355(9204):618-621.

22. Martineau AR, Wilkinson RJ, Wilkinson KA *et al*. A Single Dose of Vitamin D Enhances Immunity to Mycobacteria. *Am J Respir Crit Care Med* 2007; 176(2):208-213.

23. Yesudian PD, Berry JL, Wiles S *et al*. The effect of ultraviolet B-induced vitamin D levels on host resistance to Mycobacterium tuberculosis: a pilot study in immigrant Asian adults living in the United Kingdom. *Photodermatol Photoimmunol Photomed* 2008; 24(2):97-98.

24. Martineau AR, Nanzer AM, Satkunam KR *et al*. Influence of a single oral dose of vitamin D(2) on serum 25-hydroxyvitamin D concentrations in tuberculosis patients. *Int J Tuberc Lung Dis* 2009; 13(1):119-125.

25. Armas LAG, Hollis BW, Heaney RP. Vitamin D_2 Is Much Less Effective than Vitamin D_3 in Humans. *J Clin Endocrinol Metab* 2004; 89(11):5387-5391.

26. Heaney RP, Recker RR, Grote J, Horst RL, Armas LAG. Vitamin D_3 is more potent than vitamin D_2 in humans. *J Clin Endocrinol Metab* 2011; 96(3):E447-E452.

27. Wejse C, Gomes VF, Rabna P *et al*. Vitamin D as Supplementary Treatment for Tuberculosis: A Double-blind, Randomised, Placebo-controlled Trial. *Am J Respir Crit Care Med* 2009; 179(9):843-850.

28. Wilkinson RJ, Lange C. Vitamin D and Tuberculosis: New Light on a Potent Biologic Therapy? *Am J Respir Crit Care Med* 2009; 179(9):740-742.

29. Nursyam EW, Amin Z, Rumende CM. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. *Acta Med Indones* 2006; 38(1):3-5.

30. Morcos MM, Gabr AA, Samuel S *et al*. Vitamin D administration to tuberculous children and its value. *Boll Chim Farm* 1998; 137(5):157-164.

31. Lake J, Adams J. Vitamin D in HIV-infected Patients. *Current HIV/AIDS Reports* 2011; 1-9.

32. Overton E, Yin M. The Rapidly Evolving Research on Vitamin D Among HIV-Infected Populations. *Current Infectious Disease Reports* 2011; 13(1):83-93.

33. Mehta S, Spiegelman D, Aboud S *et al*. Lipid-soluble vitamins A, D, and E in HIV-infected pregnant women in Tanzania. *Eur J Clin Nutr* 2010; 64(8):808-817.

34. Dao CN, Patel P, Overton ET *et al*. Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. *Clin Infect Dis* 2011; 52(3): 396-405.

35. Bonjoch A, Figueras M, Estany C *et al*. High prevalence of and progression to low bone mineral density in HIV-infected patients: a longitudinal cohort study. *AIDS* 2010; 24(18): 2827-2833.

36. Viard JP, Souberbielle JC, Kirk O *et al.* Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. *AIDS* 2011; 25(10):1305-1315.

37. Mehta S, Giovannucci E, Mugusi FM *et al*. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. *PLoS One* 2010; 5:e8770.

38. Holick MF, Chen TC, Lu Z, Sauter E. Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res* 2007; 22(Suppl 2):V28-33.

39. Robertson I. Survey of clinical rickets in the infant population in Cape Town 1967 1968. *S Afr Med J* 1969; 43:1072-1076.

40. Institute of Medicine. *Dietary reference intakes for calcium and vitamin D*. Washington, DC: The National Academies Press, 2011.

41. Hattingh Z, Walsh CM, Bester CJ, Oguntibeju OO. An analysis of dietary micronutrient intakes in two age groups of black South African women. West Indian *Med J* 2008; 57(5):431-437.

42. Labadarios D, Steyn NP, Maunder E *et al*. The National Food Consumption Survey (NFCS): South Africa, 1999. *Public Health Nutrition* 2005; 8(05):533-543.

43. Charlton KE, Bourne LT, Steyn K, Laubscher JA. Poor nutritional status in older black South Africans. *Asia Pacific Journal of Clinical Nutrition* 2001; 10(1):31-38.

44. Dannhauser A, van Staden AM, van der Ryst E *et al*. Nutritional status of HIV-1 seropositive patients in the Free State Province of South Africa: anthropometric and dietary profile. *Eur J Clin Nutr* 1999; 53(3):165-173.

45. Pettifor JM, Ross FP, Moodley GP, Margo G. Serum calcium, magnesium, phosphorus, alkaline phosphatase and 25-hydroxyvitamin D concentrations in a paediatric population. *S Afr Med J* 1978; 53:751-754.

46. Poopedi MA, Norris SA, Pettifor JM. Factors influencing the vitamin D status of 10-year-old urban South African children. *Public Health Nutr* 2011; 14(02):334-339.

47. Bischof F, Basu D, Pettifor JM. Pathological long-bone fractures in residents with cerebral palsy in a long-term care facility in South Africa. *Dev Med Child Neurol* 2002; 44(2): 119-122.

48. Pettifor JM, Ross FP, Solomon L. Seasonal variation in serum 25-hydroxycholecalciferol concentrations in elderly South African patients with fractures of femoral neck. *Br Med J* 1978; 1:826-827.

49. Basu D. *Determination of bone mass and prevalence of vertebral deformities in post-menopausal black women in South Africa*. University of the Witwatersrand; 2010.

50. Fairney A, Sloan MA, Patel KV, Coumbe A. Vitamin A and D status of black South African women and their babies. *Hum Nutr Clin Nutr* 1987; 41(1):81-87.

Chapter 4

Folate

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Introduction

Folate (folic acid) is regarded as one of the water-soluble vitamin B group. Folate metabolism is complex, with at least ten known folate-dependent reactions. Folates act as co-enzymes in 1-carbon transfer reactions of purines and pyrimidines for DNA synthesis, in the biosynthesis of methionine, serine and glycine and in the initiation of protein synthesis. With a role in many fundamental biological reactions, adequate amounts of functioning folate are essential for sustaining almost all forms of life. A detailed consideration of the metabolic role of folate is beyond the scope of this review. The review will therefore focus on the role of folate and folate deficiency in health and disease. It will further evaluate and summarise the different methods in use to assess folate nutritional status. Concerns about the folate status of South Africans, especially women in rural areas, have motivated mandatory food fortication of staples in South Africa with *inter alia*, folate. The limited information on folate status of South Africans and preliminary outcomes of the food fortication programme will be briefly reviewed.

Role of Folate in Health and Disease

Inadequate functioning of folate may result from dietary deficiency, polymorphisms in the genes coding for folate co-enzymes, or drugs which interfere with folate metabolism. Many genes involved in folate metabolism are polymorphic. There is some evidence that several single nucleotide polymorphisms, encoded by variant genes, may modulate the risk for a range of diseases. Dietary folate can interact with these proteins and confer protection against the disease. In considering the role of folate in health and disease, folate deficiency, excess intake and the possible impact of gene polymorphisms are all relevant.

The most important polymorphisms are in the enzymes methylenetetra-hydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), and thymidylate synthase (TS). MTHFR is a key enzyme in the folate cycle, irreversibly converting 5,10-methylene tetrahydrofolate to 5-methyl-tetrahydrofolate, the circulating form of folate, and MTHFR polymorphisms have received the most attention. The substrate is vital for DNA synthesis, and the product provides methyl groups for the synthesis of methionine. Several polymorphisms in the MTHFR gene may occur, the commonest being C→T at nucleotide 677, leading to an alanine to valine substitution in the protein, and an A→C in exon 7, causing an alanine to glutamate protein change. These variant alleles may be associated with instability and reduced enzyme activity *in vitro*. There is considerable ethnic and geographic variation in the frequency of these polymorphisms, the C677T variant ranging from 1% in black populations in sub-Saharan Africa to 8-20% in white populations in Europe and the US[1].

A possible role for folate in humans has been investigated in numerous conditions. Many relationships reported are controversial and the evidence is conflicting. In these areas, this review will focus on the most recent publications. To establish a role for folate in a disease, it is necessary to prove a consistent increased prevalence associated with folate deficiency (or folate polymorphisms), as well as consistent reversal by folic acid supplementation. Folate has been implicated in the following conditions.

Haemopoietic

In DNA synthesis, folate in the form of 5,10 methylenetetrahydrafolate provides the methyl group that converts deoxyuridine monophosphate to deoxythymidine monophosphate. This reaction is impaired in folate deficiency, resulting in bone marrow megaloblastosis (many large immature and dysfunctional red blood cells) manifest in the peripheral blood by macrocytic anaemia, neutropenia and thrombocytopenia. Sub-clinical deficiency may occur as macrocytosis without anaemia, and neutropenia and thrombocytopenia are seen usually in severe deficiency only. The anaemia, if left untreated, may be fatal. The administration of folic acid reverses all the haematological effects of folate deficiency.

Developmental Defects

Neural Tube Defects

Second only to its role in the development of megaloblastic anaemia, folate is best known for the prevention of neural tube defects (NTD). The foetal brain and spinal cord develop from the neural tube and NTD (spina bifida, anencephaly and encephalocoele) result from incomplete closure of the neural tube early in pregnancy, with severe mental and physical disability. NTDs are the second most common birth defects in the US. Periconceptual daily maternal folic acid supplements reduce both the recurrence, as well as the first occurrence of NTD. A meta-analysis of trials estimated a 70% reduction in NTD with periconceptual supplementation with folic acid. The mechanism by which folate produces this effect is not known, but current theory is that folate deficiency ultimately leads to depletion of the methyl pool, leaving critical genes unmethylated. It is not known if folate gene polymorphisms per se may result in NTD. In a recent study in Malaysia, the MTHFRC677T genotype was absent in both patient and control groups[2].

Congenital Heart Defects

Congenital heart defects (CHDs) are defined as the structural, functional or positional defects of the heart in isolation or in combination, present from birth, but may manifest at any time after birth or may not manifest at all. CHDs are the most common serious group of birth defects. There is evidence suggesting that the risk of CHDs may be related to maternal folate status, as well as genetic variants in folate-related genes. Folic acid supplementation prenatally may reduce CHDs in offspring. An association between the polymorphism MTHFRA1298C and conotruncal heart defects was reported from the US[3], and a study in China found that the variant genotypes of MTHFRc.1793GA/AA were associated with a significantly decreased risk of CHDs[4]. The maternal 677T allele may be associated with an increased occurrence of CHDs in children with Down syndrome (DS)[5]. However, in the largest to date study reported, there was little evidence of a relationship between left-sided cardiac defects and folate-related

genes[6]. Mandatory folate food fortification in Canada was followed by a decrease in the birth prevalence of severe congenital heart defects; supporting the hypothesis that folic acid has a preventive effect on heart defects[7].

Down Syndrome

Down syndrome (DS) is a congenital disorder, caused by the presence of an extra 21st chromosome, in which the affected person has mild to moderate mental retardation, short stature, and a flattened facial profile. In Brazil, a case control study reported an increased risk of having offspring with DS in mothers with the combined genotype 677CT or TT and 1298AA and a borderline significant association for the C677T polymorphism[5]. Further evidence for an association of folate polymorphisms with DS was provided by a case control study in China which reported that MTHR677C>T and MTRR66A>G are two independent risk factors for DS pregnancies in young Chinese women[8].

Preterm Birth

Preterm birth is the birth of a baby of less than 37 weeks' gestational age. Folic acid supplementation during pregnancy may reduce the rate of preterm births, as was shown originally in a study in South Africa[9]. This observation has been confirmed in many subsequent studies, most recently one from Hungary[10].

Orofacial Clefts

A small but significant decline in the prevalence of orofacial clefts in the US followed the implementation of folic acid fortification[11]. However, orofacial clefts showed no significant decline following folic acid fortification of food in South Africa[12].

Cardiovascular Disease

An association between elevated levels of homocysteine (Hcy) and vascular disease was recognied many years ago in children with inherited homocyteinuria, and a similar association has been reported in some, but not other subsequent studies in adults. As folate is required for the methylation of Hcy to methionine, raised levels of Hcy occur in folate deficiency. Folate is probably the most important dietary determinant of plasma Hcy levels, which are consistently lowered by daily supplementation of folic acid.

The evidence for an association of dietary folate intake with risk of cardiovascular disease is conflicting. Data published in 2007 from eight randomised trials suggested that folic acid supplementation can effectively reduce the risk of stroke in primary prevention[13]. In a subsequent study in Japan, high dietary intakes of folate were associated with reduced risk of mortality from stroke, coronary artery disease (CAD) and heart failure[14], and in a large prospective study in Sweden folate nutritional status was strongly associated with the risk of myocardial infarction[15]. The administration of folic acid in patients with CAD was associated with significantly lower all-cause mortality in patients with elevated Hcy levels, but not in patients with lower levels[16]. A recent review concluded that there is evidence that long-term supplementation with folate was effective in secondary stroke prevention in all patients[17].

There is, however, much evidence to the contrary. The recently reported results of the three large, randomised clinical trials (SEARCH, WENBIT, WAFACS), in combination with those previously reported (Chaos-2, VISP, NORVIT, HOPE-2), effectively rule out the possibility of a large effect of folic acid on risk of CAD, stroke and all-cause mortality[18]. In the WENBIT study from Norway, folic acid showed no beneficial effect on the angiographic progression of CAD and might even have promoted more rapid progression[19]. A meta-analysis of randomised controlled trials to assess the efficacy of folic acid supplementation in the prevention of stroke concluded that supplementation did not demonstrate a major effect in averting stroke[20]. A Cochrane review[21] to assess the clinical effectiveness of Hcy lowering interventions, such as folic acid, included eight randomised clinical trials involving 24 210 patients. It was concluded that Hcy lowering agents did not reduce the risk of non-fatal or fatal myocardial infarction, stroke or death by any cause, suggesting that there is no evidence to support the use of folic acid to prevent cardiovascular events. In a recent report from the US, folic acid supplementation was not associated with a decrease or increase in either cardiac or stroke mortality in haemodialysis patients[22].

It appears then that despite its ability to lower plasma Hcy levels, folic acid supplementation fails to exert significant effects on cardiovascular risk.

Neuropsychiatric

The possible role of folate in neuropsychiatric diseases has been reviewed recently[17]. As in the case of cardiovascular disease, it is the secondary elevation of plasma Hcy in folate deficiency (or with folate polymorphisms) that has been incriminated in a number of studies of the genesis of neuropsychiatric disorders. The literature, however, is conflicting as other studies have failed to show a relationship between folate and neuropsychiatric disorders.

Impairment of Cognitive Functions

In the FACIT trial, folic acid supplementation for three years significantly improved the cognitive functions that tend to decline with age[23]. Decreased cognitive ability has been reported in folate deficiency. An association of cognitive impairment with poor serum folate (SF) concentration was found in elderly people in Malaysia[24], and the results of a study of subjects in rural Korea suggested that folate nutrition influences neuropsychological function test scores significantly[25]. However, in a study in the US, dietary folate intake and supplemental folic acid were unrelated to the incidence of dementia and Alzheimer's disease (AD)[26]. A recent review concluded that there is evidence that low levels of folate pose a risk for mild cognitive impairment (MCI) and dementia, and supplementation with folic acid has beneficial effects for patients with MCI or AD; however, in the light of several negative results, further and long-term studies are necessary to reconfirm previous positive study results[17].

Attempts have been made to link maternal folate status with neurodevelopment in their offspring. Folic acid supplement use in pregnant women was associated with improved neurodevelopment at four years of age in their children in Spain[27], and higher maternal folate concentrations during pregnancy predicted better cognitive ability in children in India[28].

Depression

Folate and homocysteine have been implicated in depression but the results of epidemiologic studies on this issue have been inconsistent. A recent study of Japanese men concluded that low SF may be related to an increased prevalence of depressive symptoms[29] and another reported that decreasing and low levels of SF were associated with greater risk of depressive symptoms in older Chinese adults[30]. One recent review reported that about one third of depressive patients show low levels of folate and in the majority of published results, folate deficiency favours depression and folic acid improves the efficacy of antidepressant drugs[17]. However, a Cochrane review concluded that there was limited evidence that adding folic acid to other antidepressants may be helpful[31].

Today, B vitamins and Hcy are often regarded as past topics that have lost their eligibility in the treatment and prevention of neuropsychiatric disease[17].

Cancer

A role for folate in carcinogenesis is predicated on the DNA damage from the incorporation of uracil in place of thymidine into DNA and aberrant patterns of DNA methylation resulting from inadequate folate supply. Folate deficiency has been reported to be associated with an increased risk of cancer, and a high intake of folate with a reduced risk. In addition, folate-pathway gene polymorphisms have been implicated in several cancers, particularly polymorphisms of the enzyme MTHFR, which regulates influx of folate for methylation reactions for DNA synthesis and repair.

Paradoxically, a high intake of folate has been implicated in accelerating the growth of cancer cells, and folate antagonists are used extensively in the treatment of acute leukaemia. There is thus a potential complex relationship between folate and neoplasia. Folate has been reported to be associated with a number of cancers and leukaemia.

Colorectal Cancer

This cancer has received the most attention in relation to folate nutrition, but the results of recent studies are almost all negative. A large European prospective multicentre study did not show an association of colorectal cancer (CRC) risk with folate status nor with MTHFR polymorphisms[32]. Colorectal adenomas are an established surrogate biomarker for the risk of CRC, and trials of folic acid supplementation have provided no evidence of benefit in terms of the prevention of adenoma recurrence[33-36]. However, positive results continue to be reported, as in women in Korea where a significant relationship between higher dietary folate intake and reduced risk of CRC was found[37].

Breast Cancer

Most recent studies have focused on a possible relationship between folate enzyme polymorphisms and breast cancer. In Sweden, there was a positive association between dietary folate intake and breast cancer in MTHFR677C/TT-1298AA women but an inverse association in 677CT-1298AC women[38]. Further results suggest an association of high plasma folate concentration

with increased risk of postmenopausal breast cancer in carriers of the MTHFR677T allele[39]. In the US, there was increased risk of breast cancer among postmenopausal women with the MTHFR67TT genotype, the most pronounced among women with the lowest intakes of dietary folate, with no significant risks among women with higher intakes[40]. In China, a significant inverse relationship between folate intake and breast cancer risk was observed[41].

Contradictory reports have been published. Dietary intake of folate and genotypes of MTHFR had no overall association with breast cancer risk in Japanese[42] or Brazilian women[43]. However, increased risk was observed in women with the MTR2756GG genotype and in premenstrual women with high folate intake[43]. A meta-analysis strongly suggested that methionine synthesis reductase polymorphism (MTRR A66G) is not associated with breast cancer risk[44].

Prostate Cancer

There are limited data on a possible association between folate status and prostate cancer. A study reported from the US supports an inverse association between dietary folate intake and prostate cancer risk, primarily of high-grade prostate cancer[45]. A meta-analysis suggests that known common folate pathway single nucleotide polymorphisms do not have significant effects on susceptibility to prostate cancer[46].

Other Cancers

The results of studies of pancreatic cancer have been inconsistent. In studies in the US, total folate intake was inversely associated with pancreatic cancer risk[47] and there was an association between higher folate intake and decreased risks of pancreatic cancer in women, but not in men[48]. However, the results of a study in the Netherlands do not support a protective association of folate intake on the risk of pancreatic cancer[49].

A meta-analyses on gene polymorphisms concluded that individuals homozygous for MTHFR677T are significantly at higher risk of gastric cancer[50]. In a single report, patients with MTR2756AG or GG genotypes displayed an increased risk of laryngeal cancer[51]. Supraphysiologic plasma folate concentrations in the post-US folic acid fortification era were associated with a significantly lower risk of cervical intraepithelial neoplasm in premenopausal women[52]. Following mandatory folic acid flour fortification in Canada, no significant change was seen in the incidence of acute lymphoblastic leukaemia, brain cancers or embryonal cancers among children age 0-9 years[53]. Folate supplementation in pregnancy reduced the risk of childhood central nervous system tumours[54].

Leukaemia

It was suggested in a study from Western Australia that maternal folic acid supplementation during pregnancy might reduce the risk of childhood acute lymphoblastic leukaemia (ALL)[55]. However, a subsequent large study of Australian children with ALL found only weak evidence of a protective effect before pregnancy, and none during pregnancy[56]. A meta-analysis including this and two other studies, but not the study that raised the hypothesis, also found little evidence that folate supplementation during pregnancy protects against ALL.

Studies of a potential etiologic role of genetic variation in folate metabolism in childhood leukaemia have yielded conflicting results. Based on the results of 14 studies, it was concluded that it is plausible that polymorphisms in the MTHFR gene 677C<T and 1298A>C are associated with a decreased susceptibility to childhood ALL in non-Asian populations[57]. A significant association between MTHFR CT/TT and reduced risk of ALL was reported in Serbian children[58] and the prevalence of ALL in adult female Chinese was significantly lower for joint MTHFR genotypes 677CC/1298AC than in controls[59]. In a study in the UK of the association between polymorphisms of six key folate metabolism enzymes, including MTHFR677C>T and 1298A>C, in childhood cases of ALL and acute myeloid leukemia (AML), and in their mothers, no evidence of an association with MTHFR677 was observed for ALL or AML, either in children or their mothers[60]. However, in children, an increased risk of ALL was observed with MTR2756 genotype, suggesting that genetic variation in methionine synthetase could mediate risk of childhood leukaemia.

Miscellaneous

Folic acid supplementation was claimed to cause subjective improvement of hot flushes in postmenopausal women[61] in a single yet to be reproduced study.

Folate Food Fortification: Benefits and Possible Deleterious Effects of Supra-Physiological Folate Intake

No consideration of the public health implications of folate nutrition would be complete without reference to folate food fortification (FFF). Following surveys of folate nutritional status in various population groups in South Africa, a series of studies in the 1970s demonstrated that folate fortification of maize meal and flour was feasible and the added folic acid was biologically active[62-66]. The aim of FFF at that time was to reduce the prevalence of folate deficiency in the population, particularly in most vulnerable group, pregnant women. An approach to the milling industry for voluntary fortification was rejected. The demonstration in the 1990s that folic acid supplementation in early pregnancy reduced the prevalence of NTD provided the stimulus to revisit FFF, and in the US folic acid fortification of grain products was mandated by January 1998. Many other countries have followed this lead, including South Africa, when mandatory fortification of maize meal and wheat flour was introduced in October 2003.

Mandatory FFF has produced health benefits including a declining prevalence of NTD and folate deficient anaemia[67]. In South Africa, fortification has raised mean levels of folate intake above the recommended nutrient intake[68], and has been followed by a significant decline of 30.5% in the prevalence of NTDs[12].

However, the wider potential benefits anticipated in the prevention of cardiovascular disease, neoplastic disease and neuropsychiatric disorders in the population have not been realised.

Mandatory fortification raises concerns in that it results in total populations consuming increased amounts of folic acid. In the US, intakes of folic acid from fortified foods are more than twice the level originally predicted[69], but adults who do not consume supplements or who consume an average of <400 µg folic acid/day from supplements are unlikely to exceed the tolerable upper intake level for adults of 1 000 µg/day[70]. The form of folate added to food

is pteroylmonoglutamic acid (PGA), a synthetic folate that is not a normal metabolite, and there is concern at the possible harmful effects of long-term exposure to this form of folate. A post fortification study of older subjects in the US detected circulating unmetabolised PGA in about 33% [71]. The evidence that the increased intake of folic acid might have caused harm in some people has recently been reviewed[72]. The concerns include the following:

Interaction with Vitamin B_{12} Deficiency

The concern most commonly raised is that high folate concentrations may mask the haematological signs of vitamin B_{12} deficiency and lead to missed diagnosis and subsequent serious neurological complications, but this is likely to be very rare. There are now data from the US which demonstrate that rates of B_{12} deficiency, without anaemia, have not increased since fortification was mandated in 1998. Furthermore, macrocytosis, the earliest haematological sign of B_{12} deficiency, is not affected by varying concentrations of serum folate[73]. However, there is a single report that the presence of detectable circulating unmetabolised folic acid was related to lower cognitive test scores in elderly subjects with B_{12} deficiency[71].

Increased risk of colorectal cancer and accelerating the growth of existing cancers

This subject has been considered above. The results of an as yet unpublished pooled analysis of all the randomised control trials to date, combining the results of 35 000 individuals who participated in studies of high-dosage folic acid supplementation around the world, revealed that folic acid had no effect on cancer risk. This included risk for prostate and colorectal cancer. This study is the highest quality analysis extant, and provides the best evidence that there is no increase in cancer risk with high doses of folic acid.

Impairing Therapy with Antifolate Drugs

Antifolate drugs are used in the treatment of neoplastic disease, rheumatoid arthritis, bacterial infections, malaria, psoriasis, ectopic pregnancy and epilepsy. There is justifiable concern that high plasma folate levels may impair the action of antifolate drugs. Many antimalarial drugs are antifolates and there is evidence that folates might modify the response to therapy by antifolate drugs[74]. A recent review of the possible benefits versus harmful effects of folic acid supplementation of children in malarious areas[75] informed the recommendation by the WHO that folic acid should not be included in suplementation of children in these areas.

Increasing Twinning Rates

There has been much debate about whether or not the periconceptual use of folic acid supplements increases the likelihood of twin pregnancies, with public health implications related to both infant and maternal mortality and morbidity. There is an increase in twins resulting from assisted reproduction methods, but not for normal pregnancies.

After 12 years of mandatory FFF, there is no substantial evidence to suggest that the higher intake of folic acid may harm the public, with the exception of the already mentioned WHO recommendation against routine supplementation of children in high malaria areas.

Assessing the Functionally Significant Folate Status of Individuals and Populations

Haematology/Microscopy/Blood Smears

The defective DNA synthesis resulting from folate deficiency affects primarily tissues with rapid cell turnover, such as the bone marrow. This leads to both quantitative and morphological changes in the peripheral blood and the marrow. The quantitative changes in the blood include macrocytic anaemia, neutropenia and thrombocytopenia. Morphologically there is red cell anisopoikilocytosis, oval macrocytosis, and neutrophil hyperlobation. The methods of peripheral blood analysis are robust and reliable, but the findings are not specific, in that identical changes may occur in vitamin B_{12} deficiency and in patients receiving chemotherapy. Furthermore, these changes can be confused with those of myelodysplastic syndrome (MDS) by operators with less experience in blood cell morphology. Specificity is improved by history taking to exclude drug-induced changes, and measurement of serum vitamin B_{12} concentration to exclude B_{12} deficiency. Peripheral blood analysis is labour-intensive and thus expensive, and is applicable to assessing folate status in individuals, but not populations. In assessments at the population level, measurement of haemoglobin concentration is of value when combined with other indices of folate nutrition, as an indication of the prevalence of folate deficiency severe enough to cause anaemia.

The bone marrow counterpart of these changes in the blood is megaloblastic erythropoiesis and abnormally large granulocytic precursors, often with bizarre morphology. As with the peripheral blood, these changes are not specific for folate deficiency. Bone marrow biopsy and examination is an invasive procedure that requires highly skilled operatives, and the cost is exorbitant. It is applicable to the individual only, and is seldom performed today to assess folate status, as it is not an essential component to confirm the diagnosis of folate deficiency.

These haematological changes are late manifestations of folate deficiency and are not of value in the detection of depleted folate stores that predate the haematological changes.

Serum Folate (SF)

Measurement of SF is usually by a competitive binding assay using milk folate binder. The assay is robust and reproducible, provided there is pre-analytic protection of the sample from heat and light. The assay is affordable in that analysis of large batches can be run, but the reagents are expensive. SF is dependent on intake and falls rapidly into the deficient range during deprivation, and is the earliest indicator of folate imbalance, before depletion of folate stores and any morbid effects of deficiency are manifest. The value of SF on its own is to detect deficient folate intake at either individual or population level, rather than establish nutritional status. Furthermore, the value of SF assay as an index of nutritional status has been compromised by the introduction of mandatory folate food fortification in many countries. The ingestion of folate fortified foods will raise SF concentration, but whether these small amounts correct existing deficiency is not known. Thus measurement of SF should always be accompanied by red cell folate (RCF) assay to provide optimum information of folate nutritional status.

Red Cell Folate (RCF)

When the red cell is formed, its folate content reflects available vitamin at that time, and remains constant throughout its 120-day lifespan. RCF is not affected directly by current folate intake, and will fall following the onset of folate deficiency only when a new population of red cells is produced. Measurement of RCF is performed in the same way as SF, but the red cells are lysed before analysis, and the assay is carried out in the presence of relatively large amounts of free haemoglobin. Variable amounts of folate may bind to haemoglobin and are not measured in the assay, thus rendering the technique less robust and reproducible than measurement of SF. It is of concern that in samples from patients with significant anaemia, folate deficiency could remain undetected, as the low haemoglobin concentration would 'lock up' less of the folate for assay. Despite these reservations, measurement of RCF remains the best index of folate nutritional status. It is a better index of tissue folate levels than SF and can be applied to the assessment of folate status both at the individual and population level. The extensive assessments of folate nutritional status in populations in the US as part of the National Health and Nutrition Examination Survey (NHANES) are carried out by combined measurement of both SF and RCF.

The relative sensitivity of the haematological changes and SF and RCF as indices of folate nutritional status was demonstrated in a classic example of self-experimentation[76]. On a diet that was virtually free of folate, SF was subnormal after three weeks, neutrophil hypersegmentation occurred after seven weeks, macroovalocytosis after 18 weeks, subnormal RCF after four months and anaemia after four and a half months.

Total Serum Homocysteine (tHcy)

As folate is required for the conversion of homocysteine to methionine, total homocysteine concentration in plasma is usually raised in folate deficiency. Measurement of tHcy is robust and reproducible, providing the specimen is drawn after an overnight fast, and the assay is carried out within three hours. Specificity is poor, in that hyperhomocysteinaemia may occur in many conditions other than folate deficiency, including vitamin B_{12} deficiency, pyridoxine deficiency, certain metabolic disorders, and is affected by renal function and lifestyle factors, such as smoking and coffee consumption. This lack of specificity renders measurement of tHcy as an index of folate deficiency of limited value. Significant folate deficiency is unlikely in the absence of raised tHcy, and the assay is of value in the individual patient with borderline folate levels and in folate deficient patients with normal serum folate. The assay is expensive.

Detection of the C667T Variant of 5,10-methylene Tetrahydrofolate Reductase Gene

Functioning folate status may be affected not only by folate deficiency, but also by variation of genes that code for folate-dependent enzymes, and particularly by the interaction of these two factors. The commonest polymorphism in the folate enzymes is variant 5,10-methylene tetrahydrofolatereductase (MTHFR). In the assessment of the functionally significant folate status in the individual, detection of this defect may be of importance. The technique is a molecular one, and though robust and reproducible, it is expensive. Its value in assessing the folate status at the individual and population level is yet to be demonstrated.

The role of these various techniques in the assessment of folate nutritional status is summarised in the table.

Table 4.1 Methods for the assessment of folate nutritional status

Method	Robustness	Reliability	Affordability	Informative	Applicability
Haematology	Good	Only in skilled hands	Good	Poor sensitivity and specificity	Individual only
Serum folate	Good	Good	Good	Index of folate intake	Individual and Population; levels < 7nmol/L (3 g/ml) reflect deficiency
Red cell	Reasonable	Reasonable	Good	Index of folate stores	Individual and Population, levels >225 nmol/L (100 ng/ml) normal
Total homocysteine	Good Fasting specimen required	Reasonable	Expensive	Non-specific To exclude deficiency	Individual only

Factors Affecting the Interpretation of Serum and Red Cell Folate Concentrations

Age and Sex

Reference values for SF and RCF are regarded as independent of age or sex. SF, and to a lesser extent, RCF, fall in pregnancy, and return to normal after delivery. It is not clear how much this reflects a physiological phenomenon, and how much is due to reduction of maternal folate stores due to the demands of the foetus. However, the interpretation of low SF and/or RCF in pregnancy should not differ from that in non-pregnant women.

Intercurrent Illness

Many illnesses are associated with reduction in appetite and food intake. As SF falls as early as three weeks following reduced food intake, it may be low in any intercurrent illness and in patients who remain in hospital, from whatever cause, for a prolonged period. SF may be low during both acute and chronic inflammation, and RCF may be low in chronic inflammation. This probably reflects true deficiency. Infection is of relevance to the interpretation of tests of folate nutritional status at the individual level, and at the population level also when the infection is widespread. This could apply to infections, such as HIV and tuberculosis.

HIV Infection

In a study of HIV-infected individuals in France, SF and RCF were significantly decreased in 64% and 57% of the subjects' respectively[77]. Lower SF levels were reported in pregnant women in Zimbabwe[78], in lactating mothers in South Africa[79] and in HIV-infected patients with anaemia in Brazil[80], but SF was normal in HIV-infected children in the US[81]. Impairment of folic acid absorption in patients with HIV infection has been reported[82]. While a direct effect of infection by the virus is possible, it is likely that the fall in folate levels in HIV infection is due to a combination of deficient intake and malabsorption of the vitamin. In population studies, the inclusion of significant numbers of HIV-infected subjects could increase significantly the number of low SF, and possibly, low RCF as well. At the current state of knowledge, it is appropriate to interpret the results of these tests in HIV-infected patients as in non-infected subjects.

Tuberculosis

There are almost no published studies of tests of folate nutrition in TB patients, and none in recent years could be found. In a study of 68 patients in the UK reported in 1966[83], SF was subnormal in 35%. RCF was subnormal in 33% of a small, unselected proportion of the patients tested. This high incidence of abnormalities awaits confirmation in further studies. It is likely that subnormal SF and RCF in patients with TB reflect deficient dietary intake and/or true deficiency, and as with HIV patients, the results of the tests should be interpreted in the same way as non-infected patients.

Folate Intakes and Status of South Africans

Limited information is available on the folate status of South Africans. *Ad hoc* studies indicated that folate intakes in both rural[84] and urban[84, 85] Africans are low, not meeting the 400 µg per day recommended for adults (600 µg in pregnancy and 500 µg in lactation). Low folate status has been reported for an elderly South African population[86]. The low intakes and status have been addressed by the mandatory food fortification of maize meal and bread flour in South Africa, and preliminary indications are that folate intake[68], and folate status[87] have improved, and that neural tube defects have declined[12].

The current practice in South African public health service is to provide a supplement of 5mg (5 000 µg) folic acid daily to pregnant women[88], which is much higher than the recommended intake of 600µg per day and five times the upper level of 1 000µg per day. Concerns about increased circulating unmetabolised folic acid masking the haematological signs of vitamin B_{12} deficiency[72], as well as an association with lower cognitive test scores in persons with B_{12} deficiency[71] ,and impaired immune function[89] have been expressed.

Summary and Conclusions

1 Folate is required for the synthesis of DNA and in the provision of methyl groups via the conversion of homocysteine to methionine. Inadequate functioning of folate results in defective DNA synthesis, reduction in the available methyl pool accumulation of homocysteine.

2 Folate deficiency may result in megaloblastic anaemia, which, if untreated, may be fatal. Folate supplementation pre, peri-conception and during pregnancy reduces the risk of neural tube defects, low birth weight and possibly congenital heart defects in the infant. There is some evidence that maternal supplementation may also improve neuropsychiatric development in the offspring.

3 It has been hypothesised that folate plays a role in cardiovascular and neuropsychiatric disease via the associated hyperhomocysteinaemia. The evidence for this is conflicting. While folic acid reduces the concentration of plasma homocysteine, it has no consistent effect in reducing morbidity or mortality from these diseases, with the possible exception of stroke.

4 As DNA synthesis is defective in folate deficiency, attempts have been made to incriminate folate in carcinogenesis and in leukaemia. The evidence is again conflicting, but the current consensus is that there is no such role for folate.

5 Mandatory folate fortification of staple foods has been introduced in many countries, including South Africa, and has led to a reduction in folate deficient anaemia and in the incidence of neural tube defects. After 12 years of fortification in the US, there is no substantial evidence to suggest that the higher intake of folic acid may harm the general public, all of whom are exposed to a higher folate intake.

6 The assessment of folate nutritional status in the individual is by standard haematological tests together with measurement of both serum and red cell folate concentrations. At the population level, measurement of both serum and red cell folate concentrations are required, serum folate to assess the prevalence of inadequate folate intake, and red cell folate the prevalence of folate deficiency. Doing both tests in tandem is particularly important in countries with mandatory folate food fortification. These tests are in general robust, reliable and affordable.

7 Tests of folate nutrition may be affected by any intercurrent illness that is associated with prolonged reduction in food intake. Abnormal tests probably reflect the development of folate deficiency rather than a direct effect of the illness.

8 There are many genetic polymorphisms of the folate enzymes involved in metabolic reactions. These polymorphisms are usually associated with reduced function. There is evidence that some of these polymorphisms in the mother may confer an increased risk of Down syndrome in their offspring. Association of these polymorphisms with other diseases are inconsistent.

References

1. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. *Am J Epidemiol* 2004; 159:423-43.

2. Hayati AR, Zainal AI, Tan GC et al. MTHFR C677T polymorphism as a risk factor of neural tube defects in Malay: a case control study. *Med J Malaysia* 2008; 63:379-83.

3. Lupo PJ, Goldmuntz E, Mitchell LE. Gene-gene interactions in the folate metabolic pathway and the risk of conotruncal heart defects. *J Biomed Biotechnol* 2010; 2010: 6309402010, DOI:10.1155/2010/630940.

4. Xu J, Xu X, Xue L *et al*. MTHFR c.1793G>A polymorphism is associated with congenital cardiac disease in a Chinese population. *Cardio Young* 2010; 20:318-26.

5. Brandalize AP, Bandinelli E, dos Santos PA *et al*. Evaluation of C677T and A1298C polymorphisms of the MTHFR gene as maternal risk factors for Down syndrome and congenital heart defects. *Am J Med Genet A* 2009; 149A:2080-7.

6. Mitchell LE, Long J, Garbarini J *et al*. Variants of folate metabolism genes and risk of left-sided cardiac defects *Birth Defects Res A Clin Mol Teratol* 2010; 88:48-53.

7. Ionescu-Ittu R, Marelli AJ, Mackie AS *et al*. *Prevalence of severe congenital heart disease after folic acid fortification of grain products: time trend analysis in Quebec,* Canada. *BMJ*. 2009; 338:b1673.

8. Liao YP, Bao MS, Liu CQ *et al.* Folate gene polymorphism and the risk of Down syndrome pregnancies in young Chinese women. *Yi Chuan* 2010; 32:461-6.

9. Baumslag N, Edelstein T, Metz J. Reduction of incidence of prematurity by folic acid supplementation in pregnancy. *Br Med J* 1970; 1:16-17.

10. Czeizel AE, Puhó EH, Langmar Z *et al*. Possible association of folic acid supplementation during pregnancy with reduction of preterm birth: a population-based study. *Eur J Obstet Gynecol Reprod Biol* 2010; 148:135-40.

11. Yazdy MM, Honein MA, Xing J. Reduction in orofacial clefts following folic acid fortification of the US grain supply. *Birth Defects Res A Clin Mol Teratol* 2007; 79:16-23.

12. Sayed AR, Bourne D, Pattinson R *et al.* Decline in the prevalence of neural tube defects following folic acid fortification and its cost-benefit in South Africa. *Birth Defects Res A Clin Mol Teratol.* 2008; 82(4):211-6.

13. Wang X, Qin X, Demirtas H *et al.* Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *The Lancet* 2007; 369(9576):1876-82.

14. Cui R, Iso H, Date C *et al.* Dietary folate and vitamin B_6 and B_{12} intake in relation to mortality from cardiovascular diseases: Japan collaborative cohort study. *Stroke* 2010; 41:1285-9.

15. Van Guelpen B, Hultdin J, Johansson I *et al.* Plasma folate and total homocysteine levels are associated with the risk of myocardial infarction, independently of each other and of renal function. *J Intern Med* 2009; 266:182-95.

16. Mager A, Orvin K, Koren-Morag N *et al.* Impact of homocysteine-lowering vitamin therapy on long-term outcome of patients with coronary artery disease. *Am J Cardiol* 2009; 104:745-9.

17. Stanger O, Fowler B, Piertzik K *et al.* Homocysteine, folate and vitamin B12 in neuropsychiatric diseases: review and treatment recommendations. *Expert Rev Neurother* 2009; 9:1393-412.

18. Bazzano LA. Folic acid supplementation and cardiovascular disease: the state of the art. *Am J Med Sci* 2009; 338:48-9.

19. Løland KH, Bleie O, Blix AJ *et al.* Effect of homocysteine-lowering B vitamin treatment on angiographic progression of coronary artery disease: a western Norway B vitamin intervention trial (WENBIT) substudy. *Am J Cardiol* 2010; 105:1577-1584.

20. Lee M, Hong KS, Chang SC *et al*. Efficacy of homocysteine-lowering therapy with folic acid in stroke prevention: a meta-analysis. *Stroke* 2010; 41:1205-12.

21. Martí-Carvajal AJ, Solà I, Lathyris D. Homocysteine lowering interventions for preventing cardiovascular events. *Cochrane Database Syst Rev*. 2009, Issue 4. CD006612. DOI:10.1002/14651858.CD006612.pub2.

22. Leung J, Larive B, Dwyer J *et al.* The HEMO Study Group. Folic Acid Supplementation and Cardiac and Stroke Mortality among Hemodialysis Patients. *J Ren Nutr*. 2010. DOI:10.1053/j.jrn.2010.01.005.

23. Durga J, van Boxtel MP, Schouten EG *et al.* Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double-blind, controlled trial. *The Lancet* 2007; 369(9557):208-16.

24. Lee LK, Shahar S, Rajab N. Serum folate concentration, cognitive impairment, and DNA damage among elderly individuals in Malaysia. *Nutr Res* 2009; 5:327-34.

25. Chang N, Kim E, Kim KN *et al*. Folate nutrition is related to neuropsychological functions in the elderly. *Nutr Res Pract* 2009; 3:43-8.

26. Nelson C, Wengreen HJ, Munger RG *et al*. Dietary folate, vitamin B_{12}, vitamin B_6 and incident Alzheimer's disease: the cache county memory, health and aging study. *J Nutr Health Aging* 2009; 10:899-905.

27. Julvez J, Fortuny J, Mendez M *et al.* Maternal use of folic acid supplements during pregnancy and four-year-old neurodevelopment in a population-based birth cohort. *Paediatr Perinat Epidemiol* 2009; 3:199-206.

28. Veena SR, Krishnaveni GV, Srinivasan K *et al*. Higher maternal plasma folate but not vitamin B-12 concentrations during pregnancy are associated with better cognitive function scores in 9 to 10- year-old children in South India. *J Nutr* 2010;140:1014-22.

29. Nanri A, Mizoue T, Matsushita Y *et al.* Serum folate and homocysteine and depressive symptoms among Japanese men and women. *Eur J Clin Nutr* 2010; 64:289-96.

30. Ng TP, Feng L, Niti M *et al.* Folate, vitamin B_{12}, homocysteine, and depressive symptoms in a population sample of older Chinese adults. *J Am Geriatr Soc*. 2009; 57:871-6.

31. Taylor MJ, Carney SM, Goodwin GM *et al*. Folate for depressive disorders: systematic review and meta-analysis of randomised controlled trials. *J Psychopharmacol* 2004; 18:251-6.

32. Eussen SJ, Vollset SE, Igland J *et al*. Plasma folate, related genetic variants, and colorectal cancer risk in EPIC. *Cancer Epidemiol Biomarkers Prev* 2010; 19:1328-40.

33. Mathers JC. Folate intake and bowel cancer risk. *Genes Nutr* 2009; 4:173-8.

34. Wu K, Platz EA, Willett WC *et al*. A randomised trial on folic acid supplementation and risk of recurrent colorectal adenoma. *Am J Clin Nutr* 2009; 90:1623-31.

35. Ibrahim EM, Zekri JM. Folic acid supplementation for the prevention of recurrence of colorectal adenomas: metaanalysis of interventional trials. *Med Oncol.* 2009. DOI:10.1007/s12032-009-9306-9.

36. Fife J, Raniga S, Hider PN, Folic Acid Supplementation and Colorectal Cancer Risk; A Meta-analysis. *Colorectal Dis*. 2009. DOI: 10.1111/j.1463-1318.2009.02089.x.

37. Kim J, Kim DH, Lee BH, Folate intake and the risk of colorectal cancer in a Korean population. *Eur J Clin Nutr* 2009; 63:1057-64.

38. Ericson U, Sonestedt E, Ivarsson MI *et al*. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmö Diet and Cancer cohort. *Cancer Epidemiol Biomarkers Prev* 2009; 18:1101-10.

39. Ericson UC, Ivarsson MI, Sonestedt E *et al*. Increased breast cancer risk at high plasma folate concentrations among women with the MTHFR 677T allele. *Am J Clin Nutr* 2009; 90:1380-9.

40. Maruti SS, Ulrich CM, Jupe ER *et al*. MTHFR C677T and postmenopausal breast cancer risk by intakes of one-carbon metabolism nutrients: a nested case-control study. *Breast Cancer Res* 2009; 11:R91.

41. Gao CM, Kazuo T, Tang JH *et al*. MTHFR polymorphisms, dietary folate intake and risks to breast cancer. *Zhonghua Yu Fang Yi Xue Za Zhi* 2009; 43:576-80.

42. Ma E, Iwasaki M, Kobayashi M, Kasuga Y *et al*. Dietary intake of folate, vitamin B_2, vitamin B_6, vitamin B_{12}, genetic polymorphism of related enzymes, and risk of breast cancer: a case-control study in Japan. *Nutr Cancer* 2009; 61:447-456.

43. Ma E, Iwasaki M, Junko I *et al*. Dietary intake of folate, vitamin B_6, and vitamin B_{12}, genetic polymorphism of related enzymes, and risk of breast cancer: a case-control study in Brazilian women. *BMC Cancer* 2009; 9:122.

44. Hu J, Zhou GW, Wang N *et al*. MTRR A66G polymorphism and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat* 2010 Apr 22. DOI: 10.1007/s10549-010-0892-1.

45. Shannon J, Phoutrides E, Palma A *et al*. Folate intake and prostate cancer risk: a case-control study. *Nutr Cancer* 2009; 61:617-28.

46. Collin SM, Metcalfe C, Zuccolo L *et al*. Association of folate-pathway gene polymorphisms with the risk of prostate cancer: a population-based nested case-control study, systematic review, and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2009; 18:2528-39.

47. Gong Z, Holly EA, Bracci PM. Intake of folate, vitamins B6, B12 and methionine and risk of pancreatic cancer in a large population-based case-control study. *Cancer Causes Control* 2009; 20:1317-25.

48. Oaks BM, Dodd KW, Meinhold CL *et al*. Folate intake, post-folic acid grain fortification, and pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr* 2010; 91:449-55.

49. Keszei AP, Verhage BA, Heinen MM *et al*. Dietary folate and folate vitamins and the risk of pancreatic cancer in the Netherlands cohort study. *Cancer Epidemiol Biomarkers Prev* 2009; 18:1785-91.

50. Gianfagna F, De Feo E, van Duijn CM *et al.* A systematic review of meta-analyses on gene polymorphisms and gastric cancer risk. *Curr Genomics* 2008; 9:361-74.

51. Kruszyna Ł, Lianeri M, Rydzanicz M *et al*. Polymorphic variants of folate metabolism genes and the risk of laryngeal cancer. *Mol Biol Rep* 2010; 37:241-7.

52. Piyathilake CJ, Macaluso M, Alvarez RD *et al.* Lower risk of cervical intraepithelial neoplasia in women with high plasma folate and sufficient vitamin $B1_2$ in the post-folic acid fortification era. *Cancer Prev Res* 2009; 2:658-64.

53. Grupp SG, Greenberg ML, Ray JG *et al.* Pediatric Cancer Rates After Universal Folic Acid Flour Fortification in Ontario. *J Clin Pharmacol.* 2010 May 10. DOI:10.1177/00912700 10365553.

54. Ortega-García JA, Ferrís-Tortajada J, Claudio L *et al.* Case control study of periconceptional folic acid intake and nervous system tumors in children. *Childs Nerv Syst* 2010 May 23. DOI: 10.1007/s00381-010-1187-x.

55. Thompson JR, Gerald PF, Willoughby ML *et al.* Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. *The Lancet* 2001; 358(9297):1935-40.

56. Milne E, Royal JA, Miller M *et al*. Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukaemia in the offspring. *Into J Cancer* 2010; 126:2690-9.

57. Koppel IJ, Herman's FJ, Jaspers GJ. Folate related gene polymorphisms and susceptibility to develop childhood acute lymphoblastic leukaemia. *Br J Haematol* 2010; 148:3-14.

58. Damnjanovic T, Milicevic R, Novkovic T *et al.* Association between the methylenetetrahydrofolate reductase polymorphisms and risk of acute lymphoblastic leukemia in Serbian children. *J Pediatr Hematol Oncol* 2010; 32:e148-150.

59. Lv L, Wu C, Sun H *et al*. Combined 677CC/1298AC genotypes of methylenetetrahydrofolate reductase (MTHFR) reduce susceptibility to precursor B lymphoblastic leukemia in a Chinese population *Eur J Haematol.* 2010; 84:506-12.

60. Lightfoot TJ, Johnston WT, Painter D *et al*. Genetic variation in the folate metabolic pathway and risk of childhood leukemia. *Blood* 2010; 115:3923-9.

61. Gaweesh SS, Abdel-Gawad MM, Nagaty *et al*. Folic acid supplementation may cure hot flushes in postmenopausal women: a prospective cohort study. *Gynecol Endocrinol* 2010 Mar 16. DOI: 10.3109/09513591003686288. 10.3109/ 09513591003686288 (on line).

62. Colman N, Barker M, Green R *et al*. Prevention of folate deficiency in pregnancy by food fortification. *Am J Clin Nutr* 1974; 27:339-344.

63. Colman N, Larsen JV, Barker EA *et al*. Prevention of folate deficiency by food fortification. V. A pilot field trial of folic acid-fortified maize meal. *S Afr Med J* 1974; 48:1763-1766.

64. Colman N, Larsen JV, Barker H *et al*. Prevention of folate deficiency by food fortification. III. Effect in pregnant subjects of varying amounts of added folic acid. *Am J Clin Nutr* 1975; 28: 465-470.

65. Colman N, Barker EA, Barker M *et a*l. Prevention of folate deficiency by food fortification. IV. Identification of target groups in addition to pregnant women in an adult rural population. *Am J Clin Nutr* 1975; 28:471-476.

66. Margo G, Barker M, Femandes-Costa F *et al*. Prevention of folate deficiency by food fortification. VII. The use of bread as a vehicle for folate supplementation. *Am J Clin Nutr* 1975; 28,761-763.

67. Shojania AM, von Kuster K. Ordering folate assays is no longer justified for investigation of anemias, in folic acid fortified countries. *BMC Res Notes*. 2010; 3:22

68. Steyn NP, Wolmarans P, Nel JH *et al*. National fortification of staple foods can make a significant contribution to micronutrient intake of South African adults. *Public Health Nutr* 2008; 11:307-13.

69. Quinlivan EP, Gregory JF 3rd. Effect of food fortification on folic acid intake in the United States. *Am J Clin Nutr* 2003; 77:221-5.

70. Yang Q, Cogswell ME, Hamner HC *et al*. Folic acid source, usual intake, and folate and vitamin B12 status in US adults: National Health and Nutrition Examination Survey (NHANES) 2003-2006. *Am J Clin Nutr* 2010; 91:64-72.

71. Morris MS, Jacques PF, Rosenberg IH *et al*. Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. *Am J Clin Nutr* 2010; 91:1733-44.

72. Smith AD, Kim Y, Refsum H. Is folic acid good for everyone? *Am J Clin Nutr* 2008; 87:517-33.

73. Metz, J, McNeil A R, Levin M. The relationship between mean cell volume and serum cobalamin concentration at various serum folate concentrations. Clin Lab Haem 2004; 26:323-325.

74. Carter JY, Loolpapit MP, Lema OE *et al.* Reduction of the efficacy of antifolate antimalarial therapy by folic acid supplementation. *Am J Trop Med Hyg* 2005; 73:166-70.

75. Metz J. Folic acid metabolism and malaria. *Food and Nutrition Bulletin* 2007;28,S540-9.

76. Herbert V. Experimental nutritional folate deficiency in man. *Trans Assoc Am Physicians* 1962; 75:307-20.

77. Boudes P, Zittoun J, Sobel A. Folate, vitamin $B1_2$, and HIV infection. *The Lancet* 1990; 335: 1401-2.

78. Friis H, Gomo E, Koestel P *et al*. HIV and other predictors of serum folate, serum ferritin, and haemoglobin in pregnancy: a cross-sectional study in Zimbabwe. *Am J Clin Nutr* 2001; 73(6):1066-73.

79. Papathakis PC, Rollins NC, Chantry CJ *et al*. Micronutrient status during lactation in HIV-infected and HIV-uninfected South African women during the first 6 months after delivery. *Am J Clin Nutr* 2007; 85:182-92.

80. Castro L, Goldani LZ. Iron, folate and vitamin B12 parameters in HIV-1 infected patients with anaemia in southern Brazil. *Trop Doct* 2009; 39:83-5.

81. Malik ZA, Abadi J, Sansary J *et al*. Elevated levels of vitamin B_{12} and folate in vertically infected children with HIV-1. *AIDS* 2009. 28; 23:403-7.

82. Revell P, O'Doherty MJ, Tang A *et al*. Folic acid absorption in patients infected with the human immunodeficiency virus. *J Intern Med* 199; 230:227-31.

83. Roberts PD, Hoffbrand AV, Mollin DL. Iron and folate metabolism in tuberculosis. *Brit Med J* 1966; 2:198-202.

84. Vorster HH, Venter CS, Wissing MP, Margetts BM. The nutrition and health transition in the North West Province of South Africa: a review of the THUSA (Transition and Health during Urbanisation of South Africans) study. *Public Health Nutrition* 2005; 8(5):480-490.

85. Ubbink JB, Christianson A, Bester MJ, *et al*. Folate status, homosysteine metabolism, and methyl tetrahydrofolate reductase genotype in rural South African blacks with a history of pregnancy complicated by neural tube defects. *Metabolism* 1999; 48(2):269-274.

86. Charlton KE, Kruger M, Labadarios D, Wolmarans P, Aronson I. Iron, folate and vitamin B12 status of an elderly South African population. *Eur J Clin Nutr* 1997; 51(7):424-430.

87. Modjadji SEP, Alberts M, Mamabolo RL. Folate and iron status of South African non-pregnant women of childbearing age, before and after fortification of foods. *SA J Clin Nutr* 2007; 20(3):89-93.

88. Department of Health, South Africa. *Standard treatment guidelines and essential drugs list. Hospital level adults.* Pretoria, Department of Health, 2006.

89. Troen AM, Mitchell B, Sorensen B *et al.* Unmetabolised folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J Nutr* 2006; 136: 189-194.

Chapter 5

Selenium

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Introduction

Selenium (Se), a non-metallic element with properties similar to that of sulphur, was only shown to be an essential nutrient in the late 1970s. It has four natural oxidative states and combines with other elements to form selenides, selenites, selenates, oxides, oxyacids and selenoproteins. Se exerts its antioxidant biological effects as a constituent of at least 30 selenium proteins in humans. In this chapter, the chemistry, bioavailability, absorption and metabolism of Se, markers for Se intake and Se status, the role of Se in the immune system, interaction with other nutrients, factors influencing recommendations for intakes, as well as what is known about selenium intakes and status in South Africa and elsewhere in Africa, will be briefly reviewed. In the light of a possible lower Se status in HIV-infected persons, recommendations for the need for large-scale surveys of selenium status in African countries, as well as risk maps indicating areas of deficiencies are recommended at the conclusion of the chapter.

Chemistry

Selenium, a non-metallic element with four natural oxidative states, combines with other elements to form organic selenides and with oxygen to form oxides and oxyacids. Selenium replaces sulfur to form organic selenium compounds, such as selenocysteine.

Functions

Selenium has an essential role in several metabolic pathways, such as in antioxidant defence systems, thyroid hormone metabolism, and redox control of enzymes and other proteins[1]. Selenium influences these metabolic pathways through selenoproteins, all containing selenocysteine at the active site[1]. These include: at least five glutathione peroxidases which catalyse reduction of hydrogen peroxide and organic hydroperoxides, thus protecting cells from oxidative damage; three types of iodothyronine deiodinases which catalyse activation and inactivation of the thyroid hormones; the thioredoxin reductases which catalyse NADPH-dependent reduction of oxidised thioredoxin; and also other enzymes (selenophosphate synthetase 2, selenoprotein P and selenoprotein W) which may be involved in the regulation of selenium homeostasis, in transport of selenium and in muscle metabolism repectively[1].

Absorption and Metabolism

Absorption of Se compounds takes place in the duodenum and is in general a very efficient process. Various studies have shown similar Se-absorption fractions from food: absorption of selenite is assumed to be >80%, and absorption of selenium methionine (SeMet) and selenocysteine (SeCys) over 90%[1-3]. Absorption is, however, not similar to bioavailability for the body; conversion of the absorbed Se to metabolically active forms is the limiting step[3]. Organic forms are supposed to be re-used by the body more efficiently than inorganic forms[4]. SeMet itself shows no catalytic activity and is not available for functional forms until it is catabolised and converted into SeCys[1].

Inorganic forms of Se are highly soluble and absorbed by passive diffusion. However, storage of selenite and selenate in tissues is low, and due to excretion via urine a relatively low level of inorganic Se is available after consumption. SeMet is absorbed in the same way as methionine (Met)[1]. This means that it is actively transported through the intestinal membranes and accumulated into tissues, e.g. muscle and liver. Little is known about the absorption of SeCys. Absorption studies show that organic and inorganic Se are absorbed independently[5], but possible active transport systems for SeCys have not yet been identified. For bioactivity of the element, Se must be present as an intermediate (selenide) that can be incorporated in SeCys residues at the active site of selenoproteins. Selenide is more readily formed from inorganic than from organic Se. Selenite in the bloodstream is taken up by erythrocytes and reduced to selenide by glutathione[4,6], whereas selenate is taken up by the liver and reduced in the hepatocytes.

The metabolism of organic Se compounds into selenide is more complex, as they are converted to selenide through cleavage of the C-Se binding bond by lyase reactions. SeMet has to be converted to SeCys before it can enter the Se pool and be converted into selenide[6]. Despite this extensive metabolism, organic forms are preferred in interventions because of their lower acute toxicity[7]. Absorbed SeMet will be present in glutathione peroxidase (GSHPx) after conversion to SeCys, but can also be incorporated into other proteins, like haemoglobin and plasma albumin, without being distinguished from Met. These proteins contribute to the body reserve of Se and can be used in conditions of oxidative stress when Se requirement is increased. Proteins including SeMet are called Se-containing proteins, whereas proteins including incorporated SeCys are called selenoproteins[1,6]. Studies with stable isotopes showed that the size of the functional Se pool responds to changes in dietary Se intake. This pool does not include the protein-bound Se in SeMet, which is considered to be a storage pool[8].

Markers of Selenium Intake and Status

A series of functional markers of Se status is available, but choice of the marker depends on which specific function of Se is being investigated. When assessing Se status in relation to disease risk, possible interactions with other antioxidants in the body should be considered.

Plasma and Whole Blood Total Selenium

For international comparisons of Se status, concentration of Se in plasma is used most often and reflects short-term changes in dietary Se intake. Since these values have large variations among countries, no universal normal reference ranges have been defined for plasma Se

concentrations. Whole blood Se concentration is an index of long-term Se intake, corresponding with the 120-day lifespan of erythrocytes, and is relatively constant over time. Studies allowing comparisons of Se concentrations of plasma and whole blood showed that plasma Se concentrations are 75-80% of Se concentrations in whole blood[2,9].

Only a maximum of 15% of blood Se is incorporated in GSHPx. The major part of Se is in the form of SeMet incorporated into haemoglobin, which can be seen as a Se storage pool. The relation of blood Se with intake is complex. Responses on low Se diets or on supplementation are shown after a period of months, as incorporation into erythrocytes requires a long time period. There are several factors influencing both plasma and whole blood Se concentrations, such as pregnancy, disease state and genetic factors. Possible effects of age, gender or race on whole blood Se concentrations are still points of discussion, partly because the analysis of erythrocyte Se is difficult. Changes observed in erythrocytes due to change of dietary intake are in the same range as changes in plasma Se[1,10,11].

Selenoproteins (Enzymes) as Markers of Selenium Status

Se forms an integral part of selenoproteins in the human body[4]. Among the 20-30 selenoproteins, a distinction can be made between different families of Se-containing enzymes:

- The first group, including GSHPx, is involved in control of tissue concentrations of highly reactive oxygen-containing species (ROS) and is therefore essential for maintaining cell-mediated immunity against infections. GSHPx is present in blood cells and blood platelets. The activity of GSHPx enzymes decreases rapidly in the early stages of Se deficiency[3].

- A second group of selenoproteins includes thioredoxin reductase, a major component of redox systems, which are, amongst others, involved in disposal of products of oxidative metabolism and regulation of enzyme transcription factors and receptors. Thioredoxin stimulates expression of a subunit of the interleukin-2 (IL-2) receptor: IL-2 is a cytokine responsible for an early clonal expansion of cytotoxic T-lymphocytes[4,12,13]. Supplement-ation with Se resulted in a significant increase in the number of high affinity IL-2 binding sites[14], whereas Se deficiency has the opposite effect[14].

- A third group of selenoproteins is that of the iodothyronine deiodinases. Se deficiency reduces the activity of the deiodinase enzymes, which are responsible for the production of triiodothyronine (T3), the active thyroid hormone, from thyroxine (T4). Thyroid hormone is involved in processes of growth, development and metabolism. Co-occurrence of Se deficiency and iodine deficiency has been suggested to be causal for myxoedematousmyxedematous cretinism[15,16]. A third factor, thiocyanate overload, could also be involved in this[17].

- A fourth selenoprotein is selenoprotein P, which is necessary for Se transport and distribution and is suggested to play a role in cell membranes. Selenoprotein P possibly participates in antioxidant defence[18].

Other Markers

Other markers of Se status are urinary Se since homeostatic regulation of Se is controlled by excretion in the urine; blood GSHPx and selenoprotein P; and hair and toenail Se concentrations, which are both indices of long-term Se status[1,10]. However, the use of hair samples is limited because of selenium-containing shampoos.

Effects of Selenium on the Immune System

Se has an effect on several cells of both the innate and the acquired immune system. Se deficiency impairs macrophage activity, leading to decreased intracellular killing of pathogens. Se deficiency also influences antibody production, resulting in decreased maturation of T-lymphocytes and natural killer cell activity. Supplementation with Se has a positive effect on these cells. In general, it can be stated that the effect of Se on immune cells is dependent of dose[13]. There appears to be a Se threshold below which Se deficiency results in a weakened immune system and less efficient protection against HIV infection[18]. Whole blood Se concentrations >85 µg/L (≈ 1.08 µmol/L) are considered to be adequate for functioning of the immune system, whereas Se deficiency is defined as a Se concentration below this value[19-21]. However, selenium concentrations were inversely related to immune activation in 244 HIV-infected patients in the USA despite their adequate Se status[22], suggesting that optimal concentrations of Se in whole blood might be well above the currently recommended threshold.

Se can up-regulate expression of IL-2 receptors on the surface of activated lymphocytes and natural killer cells. Low IL-2 levels hinder the maturation processes of lymphocytes in the thymus, resulting in a lack of replacement of T-cells[23]. Since CD4+ T-cells form a key component in stimulating B-cells to synthesise antibodies, this may explain the stimulatory effects of Se on antibody production. Se supplementation could partially reverse age-related decreases in cell-mediated immunity by increased responsiveness to IL-2. Increased T-cell response due to Se supplementation, results in reduced oxidative stress-induced damage to immune cells[24]. Activated T-cells show an improved selenophosphate synthetase activity[7], which is a crucial precursor for synthesis of selenocysteine (SeCys) during selenoprotein synthesis[25].

Interaction between Selenium and Other Nutrients

Se is not the only element that is important in antioxidant defence. Interaction with other nutrients is of great importance to the immune system. The following interactions have been identified[26-27]:

- Se functions in close relationship with vitamin E and it is often the case that a combination of these two nutrients achieves the most optimal effect, as both elements are important in maintaining the efficiency of antioxidant systems.

- Maintenance of glutathione status is affected by pyridoxine (vitamin B_6) and riboflavin (vitamin B_2). Vitamin B_6 is a co-factor in cysteine-synthetase and is thus a limiting factor for glutathione biosynthesis; vitamin B_2 is a co-factor for glutathione reductase. Deficiencies in these two vitamins will produce functional disturbances in the immune response.

- GSHPx-activity is further affected in a negative way by deficiencies in iron, zinc, copper and magnesium. Zinc is able to up-regulate gene expression of GSHPx. Zinc and copper interact with Se in antioxidant defence by conversion of superoxide to oxygen and hydrogen peroxide which in turn can be reduced by GSHPx.

- Se absorption and its erythrocyte concentration are decreased in magnesium-deficient individuals, leading to a lower bioavailability of Se[40]. Increased vitamin A and ascorbic acid levels, however, promote absorption of Se.

Other Factors Affecting Se Function

The antioxidant role of Se can further be affected by factors that increase oxidative stress, such as smoking[2], high intake of polyunsaturated fatty acids (PUFA) and extreme exercise[10]. Requirements of antioxidants – including Se – are increased under these conditions. In addition to that, smoking is shown to be associated with inadequate dietary intake of Se[1,10]. Low dietary intake, increasing age, and diseases other than HIV can all be factors that result in low Se levels[1].

Recommendations for Selenium Intake

Total body values of Se show a wide range of 3 mg (New Zealanders) to 14 mg (some Americans)[3]. Se is distributed throughout the body with 30% of total body Se stored in the liver, 15% in the kidneys, 30% in muscle and 10% in blood plasma[27].

Since soil concentrations of Se differ between (and within) countries, realising a certain intake can be a challenge in one and not difficult at all in another country. Se recommendations can be linked to full expression of GSHPx activity, but for human health two-thirds of full activity is assumed to protect against oxidative stress[28]. A formal Recommended Dietary Allowance (RDA) Committee sets recommendations for Se intake based on intervention trials designed to estimate Se requirements for maximal GSHPx activity. After adjustment for body weight, RDAs for healthy adults (>14 y) were set at 55 µg/day[28]. The Scientific Committee for Food recommends 40 µg Se/day for the European Community, and a recommended 30 µg Se/day should be sufficient for women. This proposed intake was based on results showing that 41 µg Se/day was adequate to express two-thirds of GSHPx activity in healthy men with an average body weight of 60 kg[27].

Bioavailability Studies in Humans

Only a few metabolic Se studies in humans have been published, mostly involving small samples. Below is a short summary of some studies of which results contributed to our understanding of Se bioavailability:

- Xia *et al*. [29] found that in Se-deficient subjects with an average Se intake of 10 µg/day, supplements in the form of SeMet led to maximum plasma GSHPx activity at a dose of 37 µg/day. In case of selenite supplementation a dose of 66 µg/day of selenite was required to express GSHPx completely, almost twice as much as compared to SeMet supplementation.

- Assumed low Se availability from fish was confirmed in a study on 32 healthy subjects. After six weeks of consumption of wheat, fish or control diet, the 'wheat group' showed a significant increase of 17% in serum and 30% in whole blood Se level, whereas the 'fish group' only showed small insignificant increases in Se levels. The amounts of Se in wheat and fish were similar[30].

- In a Dutch study[31] with 28 participants, differences between the effects of a nine-week intervention with wheat bread rolls, minced meat or low-Se bread (control) were determined. Based on whole blood Se levels, bioavailability of Se from bread and meat was similar. Moreover, increases in GSHPx activity were similar for both bread and meat and were significantly different from the control group[31].

- In a similar study[32] from Finland with 50 participants, supplementation with both Se-rich yeast and wheat for 11 weeks resulted in a significant rapid increase of GSHPx activity. Se availability from these sources was higher than that of selenate, considering plasma and whole blood Se concentrations as endpoint. After supplementation had ended, wheat and yeast were better in maintaining GSHPx activity as compared to selenate[32].

- Bioavailability of Se from milk was reported to be reasonable in a rat model[33], as well as in pre-school children[34].

Selenium Intake in sub-Saharan Africa

Almost all Se in foods occurs in proteins. Due to protein malnutrition in sub-Saharan countries, Se intake in Southern Africa can be assumed to be low. However, at present, data that relied on the South African Food Composition Tables should be interpreted with care, since the selenium content of some foods are questionable.

Assessment of micronutrient intake in 249 HIV-infected women and 239 uninfected control subjects in Manguang (Free State, South Africa)35 showed that the median intake of all subjects was lower than the recommended dietary allowances (RDA) of 55 µg/day. About half of the subjects consumed less than 67% of RDA. Consumption of maize products as staple foods, which are assumed to be poor sources of Se, might contribute to this deficient Se intake[35]. In another study[36] it was shown that levels of dietary Se intake in 35 HIV/AIDS patients in the African community of Bloemfontein (Free State, South Africa) were on average higher than the RDA, but median Se intake among women was less than the RDA. Almost half of the women had a Se intake <67% of the RDA[36].

Results of the South African National Food Consumption Survey of 1999 showed that Se intake, assessed by a 24-hour recall to measure consumption levels of children, was constantly low in all age groups and all provinces: 60% of all children had an intake of less than 50% of the RDA. The Northern Province, Free State and Mpumalanga showed the lowest Se ingestion level[37]. A report from Malawi indicated that 43% of children aged four to six years had a Se intake below the RDA (20µg/day)[38].

Blood Concentrations of Selenium in sub-Saharan Africa

Characterisation of antioxidant micronutrient status among 500 adults from Malawi showed that 88% of all subjects was Se deficient (plasma Se concentration <0.89 µmol/L). Non-significant differences between 370 HIV-infected and 130 non-infected subjects were shown. A study done in 29 black South Africans in Soweto (Gauteng Province) with chronic pancreatitis as clinical endpoint showed Se deficiency in patients (mean plasma Se concentration 0.85 µmol/L), but healthy controls had an adequate Se status (mean plasma Se concentration 1.33 µmol/L)[39]. Jaskiewicz *et al*.[40] showed that Se deficiency occurs in South Africans living in Ciskei and Transkei (Eastern Cape), but not in inhabitants of Cape Town (Western Cape).

Combs[2] reported on blood Se concentrations in 69 countries to get an impression of Se inadequacy in the world. Most of the data were obtained from healthy adults. Data from Burundi, Zambia, Nigeria, Niger and South Africa showed mean serum or plasma Se concentrations ranging from 15 -117 µg/L; Se deficiency was found in half of the African populations included. In Zaire, serum Se concentrations were shown to be adequate in Bas-Zaire (80-120 µg/L), sub-optimal in the regions of Badundu and Kasai (55-80 µg/L), and inadequate in Kivu, Haut-Zaire, Equateur and Shaba (< 55µg/L)[41]. Apparently, Se adequacy and inadequacy go hand in hand in Africa and both can occur within a single country, dependent on the target population. Larger scale surveys should indicate exactly how widespread the problem of Se deficiency is.

Risk Maps of Selenium Deficiency in South Africa

'Risk maps' reflecting Se status in sub-Saharan Africa could be helpful to determine areas where Se intake is inadequate. Van Ryssen[42] has compiled data on Se status of grazing herbivores in South Africa to get an impression of Se status in South African soil and plants[42]. Unfortunately, the selenium content is not known for all foods produced and eaten in South Africa[43]. In general, human blood Se levels are comparable to those of livestock in the same region[27]. Deficient regions were defined as areas with whole blood Se concentrations below 50 µg/L, but Se concentrations of 50-80 µg/L blood ('marginally deficient') are included in 'deficient areas' as well. Adequate levels include Se blood concentrations of 80-120 µg/L. Although no information on Se status could be found for North-Eastern KwaZulu-Natal and its coastal areas, North-West Province, Limpopo and the southern part of Eastern Cape, enough data were gathered from other areas to compile a map of Se distribution. Gauteng Province, Mpumalanga and Northern Free State showed variable status between sufficient and deficient, as well as the south-western coastal part of Western Cape and the south of the Kruger National Park. Se status in the upper part of the Kruger National Park appears to be adequate, just as the main part of the Northern Cape and central Karoo (northern part of Western and Eastern Cape). Coastal regions in the Western Cape and a dominating part of KwaZulu-Natal show Se deficiency[42].

Methods of Analysis of Selenium in Body Fluids

Since reliable dietary assessment of selenium intakes in South Africa is not possible at present, selenium in body fluids must be measured to assess selenium status. There are many possible analytical methods available[44-47] and the choice of method will depend on available infrastructure and available body fluid samples. The methods range from atomic absorption spectrometry[44], cell mass spectrometry[45], neutron activation[46] and mass spectrometry[47].

Dietary Sources of Selenium

As mentioned, the selenium content of food depends on the selenium content of the soil in which plant foods are grown and the selenium content of animal feed. Therefore, selenium status of populations can be influenced and changed by importing foods high in selenium.

The major dietary sources of selenium are sea food, organ meat (liver, kidney, lungs, heart), muscle meat, poultry, eggs and grains (cereals). Fruit and vegetables are low in selenium[1].

Recommendations

The limited information on Se intake and status of South Africans, as well as the risk map of possible Se deficient areas, and the lower values found in HIV-infected subjects, suggest that Se deficiency amongst groups of South Africans (and also other populations in Africa) is prevalent. It is recommended that research on intake and status, and effects of selenium supplementation are urgently needed. The data on selenium content of foods is also limited. The most recent South African Food Composition Tables[43] does not show Se values. Intakes should be assessed by measuring total plasma and/or blood Se as biological marker, while assessment of functional markers of Se status will depend on the specific function of Se being investigated. Large-scale surveys of Se intakes and status, leading to 'risk maps' for South Africa (and the whole of Africa) will be useful to plan necessary interventions. More research on the Se content of South African foods, as well as Se requirements of vulnerable groups (such as HIV-infected people, and those with increased antioxidant needs) is indicated.

In conclusion, it should be pointed out that "as the window for safe intakes is relatively narrow, having a clear understanding of status, by population group in relation to different dietary patterns is of great importance where interventions are indicated and any intervention requires adequate monitoring and evaluation"[48].

References

1. Gibson RS. *Principles of nutritional assessment*. Oxford: Oxford University Press, 2005: 908p.

2. Combs GF. Selenium in global food systems: review article. *Brit J Nutr* 2001; 85:517-47.

3. WHO/FAO. *Vitamin and mineral requirements in human nutrition*. Second edition. 2004.

4. McKenzie RC, Rafferty TS, Beckett GJ. Selenium: An essential element for immune function. *Immunol Today* 1998; 19:342-5.

5. Harvey LJ, Dainty JR, Beattie JH *et al*. Copper absorption from foods labeled intrinsically and extrinsically with Cu-65 stable isotope. *Eur J Clin Nutr* 2005; 59:363-8.

6. Suzuki KT. Metabolism of selenium: Se metabolites based on speciation studies. *J Health Sci* 2005; 51:107-14.

7. Rayman MP. The importance of selenium to human health. *The Lancet* 2000; 356:233-41.

8. Lowe NM, Broome CS. Evaluation of trace mineral status and bioavailability using stable isotopes (zinc, copper, selenium, molybdenum). In: Abrams SA, Wong WW. Eds. *Stable isotopes in human nutrition, laboratory methods and research applications.* Oxford: CABI Publishing 2003: 61-84.

9. Dumont E, Vanhaecke E, Cornelis R. Selenium speciation from food source to metabolites: a critical review. *Anal Bioanal Chem* 2006; 358:1304-23.

10. Thomson CD. Assessment of requirements for selenium and adequacy of selenium status: a review. *Eur J Clin Nutr* 2004; 58:391-402.

11. Zachara BA, Wardak C, Didkowski W, Maciag A, Marchaluk E. Changes in blood selenium and glutathione concentrations and glutathione peroxidase activity in human pregnancy. *Gynecol Obstet Invest* 1993; 35:12-17.

12. Rayman MP. The argument for increasing selenium intake. *Proc Nutr Soc* 2002; 61:203-215.

13. Dröge W, Breitkreutz R. Glutathione and immune function. *Proc Nutr Soc* 2000; 59:595-600.

14. Roy M, Kiremidjian-Schumacher L, Wishe HI, Cohen MW, Stotzky G. Effect of selenium on the expression of high affinity interleukin 2 receptors. *Proc Soc Exp Biol Med* 1992; 200:36-43.

15. Goyens P, Golstein J, Nsombola B, Vis H, Dumont JE. Selenium deficiency as a possible factor in the pathogenesis of myxedematous cretinism. *Acta Endocrinol* 1987; 114:497-502.

16. Vanderpas J, Contempré B, Duale M, Goossens W, Ngo B *et al*. Iodine and selenium deficiency associated with cretinism in Northern Zaire. *Am J ClinNutr* 1990; 52:1087-1090.

17. Vanderpas J. Nutritional Epidemiology and thyroid hormone metabolism. *Annu Rev Nutr* 2006; 26:293-322.

18. Burk RF, Hill KE. Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. *Annu Rev Nutr* 2005; 25:215-35.

19. Campa A, Shor-Posner G, Indacochea F *et al*. Mortality risk in selenium-deficient HIV-positive children. *J AIDS Hum Retrovirol* 1999; 20:508-13.

20. Baum MK, Shor-Posner G, Lai S *et al.* High risk of HIV-related mortality is associated with selenium deficiency. *J AIDS Hum Retrovirol* 1997; 12:370-374.

21. Rukgauer M, Klein J, Kruse-Jarres JD. Reference values for the trace elements copper, manganese, selenium and zinc in the serum/plasma of children, adolescents and adults. *J Trace Elements Med Biol* 1997; 11:92-8.

22. Stephensen CB, Marquis GS, Douglas SD, Kruzich LA, Wilson CM. Glutathione, glutathione peroxidase, and selenium status in HIV-positive and HIV-negative adolescents and young adults. *Am J Clin Nutr* 2007; 85:173-181.

23. Baum MK, Miguez-Burbano MJ, Campa A, Shor-Posner G. Selenium and interleukins in persons infected with human immunodeficiency virus type 1. *J Infect Dis* 2000; 182:69-73.

24. Baum MK, Campa A, Miguez-Burbano MJ, Burbano X, Shor-Posner G. Role of selenium in HIV/AIDS. In: Hatfield DL. (ed) *Selenium, its molecular biology and role in human health.* Kluwer Academic Publishers 2001: 247-56.

25. Arthur JR, McKenzie RC, Beckett GJ. Selenium in the immune system. *J Nutr* 2003; 133: 1475S-59S.

26. Surai P. *Selenium in nutrition and health.* Nottingham: Nottingham University Press, 2006.

27. Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. *Public Health Nutr* 2001; 4:593-599.

28. Levander OA. Evolution of human dietary standards for selenium. In: Hatfield DL, (ed). *Selenium, its molecular biology and role in human health.* Kluwer Academic Publishers 2001: 299-311.

29. Xia Y, Hill KE, Byrne DW, Xu J, Burk RF. Effectiveness of selenium supplements in a low-selenium area of China. *Am J Clin Nutr* 2005; 81:829-834.

30. Meltzer HM, Bibow K, Paulsen IT, Mundal HH, Norheim G, Golm H. Different bioavailability in humans of wheat and fish selenium as measured by blood platelet response to bioavaialbility in humans of wheat and fish selenium as measured by blood platelet response to increased dietary Se. *Biol Trace Element Res* 1993; 36:229-241.

31. Van der Torre H, Van Dokkum W, Schaafsma G, Wedel M, Ockhuizen T. Effect of various levels of selenium in wheat and meat on blood Se status indices and on Se balance in Dutch men. *Brit J Nutr* 1991; 65:69-80.

32. Levander OA, Alfthan G, Arvilommi H *et al.* Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters. *Am J Clin Nutr* 1983; 31:887-897.

33. Mutanen M, Aspila PMH. Bioavailability to rats of selenium in milk of cows fed sodium selenite or selenited barley. *Ann Nutr Metab* 1986; 30:183-188.

34. Solomons NW, Torun B, Janghorbani M, Christensen MJ, Young VR, Steinke FH. Absorption of selenium from milk protein and isolated soy protein formulas in preschool children: studies using stable isotope tracer 74Se. *J Pediatr Gastroenterol Nutr* 1986; 5:122-126.

35. Hattingh Z. *The health and nutritional status of HIV-positive women* (25-44 years) in Mangaung. Faculty of Health Sciences, Department of Human Nutrition, University of Free State, Bloemfontein, 2005.

36. Oguntibeju OO, Van den Heever WMJ, Van Schalkwijk FE. An analysis of the baseline dietary intake of HIV-positive/AIDS patients. *Med Techn SA* 2005; 19:3-9.

37. Steyn NP and Labadarios D. Dietary intake: 24-hour recall method. Labadarios D. *The National Food Consumption Survey* (NFCS).Stellenbosch, 2000.pp. 218-311

38. Donovan UM, Gibson RS, Ferguson EL, Oonpuu S, Heywood P. Selenium intakes of children from Malawi and Papua New Guinea consuming plant-based diets. *J Trace Elem Electrolytes Health Dis* 1992; 6:39-43.

39. Segal I, Gut A, Schofield D, Shiel N, Braganza JM. Micronutrient antioxidant status in black South Africans with chronic pancreatitis: opportunity for prophylaxis. *Clin Chim Acta* 1995; 239:71-79.

40. Jaskiewicz K, Marasas WF, Rossouw JE, Van Niekerk FE, Heine Tech EW. Selenium and other mineral elements in populations at risk for esophageal cancer. *Cancer* 1988; 62:2635-2639.

41. Ngo DB, Dikassa L, Okitolonda W *et al*. Selenium status in pregnant women of a rural population (Zaire) in relationship to iodine deficiency. *Trop Med Int Health* 1997; 2:572-581.

42. Van Ryssen JBJ. Geographical distribution of the selenium status of herbivores in South Africa. *SA J Anim Sci* 2001; 31:1-8.

43. Wolmarans P, Danster N, Dalton A, Rossouw K, Schonfeldt H. (eds) *Condensed Food Composition Tables for South Africa.* Medical Research Council, Parow Valley, Cape Town, 2010:1-126.

44. Walker HH, Runnels JH, Merryfield R. Determination of trace quantities of selenium in petroleum and petroleum products by atomic absorption spectrometry. *Anal Chem* 1976; 48 (4):2056-2060.

45. Sloth JJ, Larsen EH. The application of inactively coupled plasma dynamic reaction cell mass spectrometry for selenium isotopes, isotope ratios and chromatographic detection of selenoamino acids. *J Anal At Spectrom* 2000; 15:669-672.

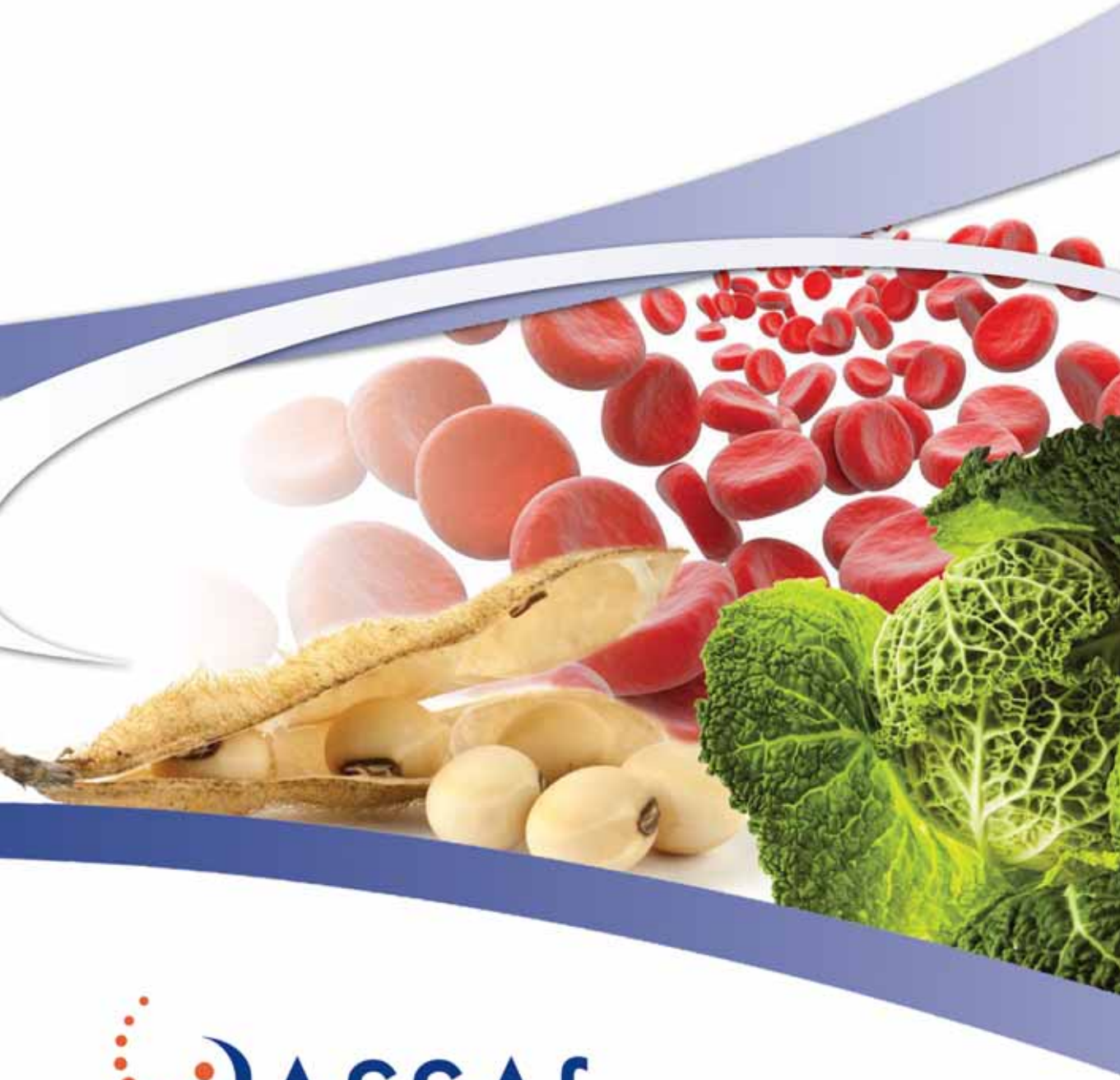
46. McKown DM, Morris JS. Rapid measurement of selenium in biological samples using instrumental neutron activation analysis. *J Radioanalytical Chem* 1978; 43:411-420.

47. Sieniawska CE, Meniskov R, Delves T. Determination of total selenium in serum, whole blood and erythrocytes by ICV-MS. *J Anal At Spectrom* 1999; 14:109-112.

48. Jackson A. Personal communication, November 2012.

Chapter 6

Iron



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Introduction

Iron deficiency, with or without anaemia, is the most prevalent micronutrient deficiency globally. It affects people in both developed and developing countries, making anaemia worldwide a significant public health problem[1]. Anaemia has its effects at all stages of life, although the severity may differ for different age groups. Young children and pregnant and lactating mothers are the most severely affected[2]. Developing countries have a higher prevalence than developed countries. A South African study[3] reported a prevalence rate of 40% for anaemia (Hb<11g/dl) and 67% for low iron stores (serum ferritin <12 µg/l) in 6 to 12-month-old infants, while the national prevalence of anaemia (Hb<11 g/dl) was estimated at 24.1%[4]. These differences probably reflect differences in prevalence in different groups of the population and also by region. The effect that anaemia and iron deficiency have in reducing productivity of individuals as well as cognitive development and performance of children, and increasing risk of morbidity and mortality, warrants the need for effective mechanisms of monitoring iron status and alleviating iron deficiency in populations.

Anaemia has remained highly prevalent despite numerous efforts in many countries to prevent iron deficiency, probably because other important contributing factors to anaemia and iron deficiency are ignored[1]. These include infectious disease, parasite infestation, interactions with other micronutrients and adiposity, which influence inflammation and iron absorption. The increasing prevalence of overweight and obesity in countries experiencing a nutrition transition necessitates that this be considered a factor that may increase the prevalence of anaemia in affected populations[5].

A World Health Organisation/Centres for Disease Control and Prevention (WHO/CDC) expert consultation group identified the lack of international agreement on how to assess the iron status of populations as a limitation to addressing anaemia[1]. They agreed on three main iron status indicators for populations, namely, *haemoglobin, ferritin* and *transferrin receptor* levels in blood and serum. It was agreed that serum ferritin was the best indicator of response to intervention, but it was recommended that haemoglobin concentration should also be measured in all programme evaluations. However, in view of the fact that ferritin is an acute-phase protein, it was agreed that it was equally important to assess if the identified indicators would be practically useful under field situations and to determine to what extent they would be subject to interference by infection for population assessment. The WHO was to coordinate a working group which would evaluate the usefulness of these indicators of iron status (ferritin, haemoglobin, transferrin receptor) in combination with acute-phase proteins, particularly C-reactive protein (CRP) and alpha-1 glycoprotein (AGP) in order to make recommendations on ways of taking inflammation into account in the determination of iron status in populations[1].

In the light of these developments, the objectives of this chapter are to:

- review iron physiology and patho-physiology in order to evaluate the usefulness of available indicators of iron status for South Africa and other African countries facing similar challenges;
- review the type and quality of information available on iron status in South Africa;
- formulate recommendations on which indicators may be most suitable at population and individual level within the South African context;
- recommend additional work that needs to be done to allow for more effective monitoring of iron status and possible gains of intervention programmes.

An Overview of Iron Physiology

Functions of Iron

Iron has several functions in the body, summarised by Gallagher[6] and MacPhail[7] as follows:

- Iron forms part of haemoglobin and myoglobin molecules. Haemoglobin is the oxygen carrier in red blood cells, and is made up of four polypeptide chains. Each polypeptide chain has a proptoporphyrin group with one iron II ion (Fe^{2+}) in the centre, forming the haemgroup. It is this structure which is central to the function of iron in oxygen transport. Oxygen binds to the iron of the haem group in a reversible reaction that allows for the oxygen to be released in tissues requiring oxygen. Iron similarly forms part of the structure of myoglobin found in muscle tissue where it plays a role as an oxygen reservoir for muscle function.
- Iron is a component of haem and non-haem containing enzymes, such as those involved in mitochondrial respiratory reactions, for example, the cytochromes and catalase, a haem-enzyme, which converts hydrogen peroxide in cells to oxygen and water.
- Iron plays an important role in cognitive development and performance. Exactly how it does this is still a subject of much research.

These functions illustrate that iron is a component of many enzymes with a complex array of possible functions in the body, involving equally complex metabolic processes. Iron deficiency would therefore affect the body at many different levels.

Requirements of iron

Iron requirements vary according to age, gender and physiological state (Table 6.1). Infants, growing children and adolescents have higher requirements because of rapid growth rates. Women of child-bearing age also have higher requirements because of increased losses of iron through menstruation and increased requirements during pregnancy and lactation to provide sufficient iron for the growing foetus and later during breastfeeding for the infant. These groups of the population with either increased requirements or increased losses become high-risk groups for iron deficiency anaemia. Any assessment of required dietary intake should also take into account the type of iron sources as this affects the bioavailability of iron. Because of the chemical structure of haeme iron in foods from animal origin, iron absorption is more

complete in these foods. The structure of non-haeme iron from plant food sources on the other hand results in partially inhibited absorption. This is one of the many reasons why exclusive breastfeeding is recommended during the first six months for all infants. Cereal and legume products both contain phytates which limit the bioavailability of the non-haem iron further. This makes it difficult to combat iron defiency and anaemia in populations that consume predominatly plant-based diets without specific interventions.

The influence of iron status on infection and the latter's role in determining iron requirements are not clear. A recent study involving Kenyan children has suggested that iron deficiency may protect against acute infection in children[8], perhaps mediated by evolutionary pressure. Another study involving postpartum HIV-infected Zimbabwean women also reported increased morbidity and mortality in the women who had higher iron stores[9]. This study, however, qualifies the results by reporting that the negative effects were observed only at serum ferritin levels above those consistent with adequate nutritional status. The fact that serum ferritin is an acute-phase protein further complicates the interpretation of the observations that were made. Nonetheless, these studies indicate that there may be an important gap of knowledge in terms of correct or prudent levels of iron requirement in situations where infection is prevalent.

Table 6.1 FAO/WHO guidelines for iron requirements and intakes

Population category	Age (years)	Requirement (absorbed Fe), µg/kg/day	Recommended intake for different levels of % dietary iron bioavailability (mg/day)*		
			Low: 5%	Moderate: 10%	High: 15%
Children	0.25 – 1	120	21	11	7
	1 – 2	56	12	6	4
	2 – 6	44	14	7	5
	6 – 12	40	23	12	8
Boys	12 – 16	34	36	18	12
Girls	12 – 16	40	40	20	13
Adult women					
Menstruating		43	48	29	16
Post-menopausal		18	19	13	6
Pregnant and lactating		24	26	13	9
Adult men		18	23	11	8

*Plant-based diets with non-haem iron would have iron of low bioavailability; animal proteins have mostly haem iron with higher bioavailability. (Adapted from MacPhail[7])

Iron Metabolism and Homeostasis

Iron exists in two oxidative states, Fe^{2+} or Fe^{3+}, and iron in these two forms readily takes part in redox reactions. Because of this reactivity which is potentially damaging to cells through oxidation, iron is generally bound to proteins in the body both for storage and transport. The level of iron in the body is tightly controlled by regulating the amount of iron absorbed from the digestive tract according to need. While the bioavailability of non-haem iron can vary from 5-10 %, that of haem iron can be 25 % or more when the need for iron is high, such as when a person is anaemic. In addition, ferrous (Fe^{2+}) (animal) iron is more bio-available than ferric iron (Fe^{3+}) (plant)[6,7].

Iron Absorption

Four phases of iron absorption are recognised[6,7]. The first is the *luminal phase* taking place in the lumen of the digestive tract. In this phase the haem group is released from haemoglobin, myoglobin and other haem containing proteins in food through digestion. Non-haem iron must be digested free from plant sources before it can be absorbed. The second phase is the *mucosal phase* which takes place at the brush border of absorbing duodenal mucosa cells. Here the haem is absorbed through the formation of endocytic vesicles into the mucosal cells. Also at the brush border, duodenal cytochrome B converts Fe^{3+} to Fe^{2+} and a different membrane transport protein, the divalent metal transporter, transports non-haem iron into the cells by facilitated diffusion. Once inside the mucosal cells in the *intracellular phase*, both iron from haem and non-haem can either be combined with apoferritin to form ferritin for storage or passed on to the basolateral membrane where in the *release phase* an active transport mechanism releases the iron into the portal circulation. This release of iron into the portal circulation is tightly controlled through the hormone hepcidin. Hepcidin is produced by the liver according to the body's need for iron. Hepcidin inhibits release of iron from mucosal cells and its production is down-regulated when the need for iron is high.

Iron Transport and Absorption into Tissue Cells for Use or Storage

Iron transport, absorption into tissue cells, its use and storage may be summarised as follows[6,7].

Once released into the portal circulation, Fe^{2+} binds to transferrin, the main transport protein for iron in circulation. Very little iron is found free in circulation. Serum transferrin saturation with iron can be used as a measure of iron status. Low serum transferrin saturation correlates with low iron status.

Tissue cells that need iron have transferrin receptors expressed on their cell membranes. Transferrin carrying iron binds to the transferrin receptors triggering absorption into the cells through a receptor mediated absorption process. When there is low iron status in the circulation the expression of trasferrin receptors is up-regulated in order to increase the efficiency of iron absorption. With an increased expression of transferrin receptors on cells, some transferrin receptors enter the circulation. The concentration of transferrin receptors in the circulation is thus inversely correlated to iron status.

The iron that is absorbed into cells is used for the various functions already mentioned. The liver is the main storage organ for excess iron where it is stored as ferritin. Other storage sites include

macrophages in the liver and spleen. Ferritin is also present in the circulation and can be measured in serum as an indicator of iron stores. A small amount of iron is also stored as haemosiderin in the liver.

More than 60% of body iron is found in haemoglobin. Iron is used in the synthesis of haemoglobin during erythropoiesis, mainly in the bone marrow, but in certain disease states also in the liver and spleen. It is incorporated into protoporphyrin forming the haem group of haemoglobin in a closely regulated process[10]. A deficiency of iron results in iron deficient erythropoiesis which may be identified either by elevated levels of protoporphyrin or the incorporation of zinc in place of iron in protoporphyrin, forming what is commonly termed zinc protoporphyrin[11]. Additonal indicators of iron deficient erythropoiesis are the production of microcytic and hypochromic red blood cells.

Iron Homeostasis

In summary, the main compartments of body iron include:

- iron in storage in mucosal cells of the digestive system awaiting release into portal circulation;
- iron in transport in the circulation;
- iron in storage in the liver and spleen;
- iron in haemoglobin of red blood cells in circulation and in bone marrow;
- iron in tissues including myoglobin, haem-enzymes and other iron containing molecules in other body tissues.

Iron homeostasis is primarily focused on conservation of iron by recycling iron from red blood cell break-down in the liver and the reticulo-endothelial system and control of absorption from the small intestine. The control of absorption from mucosal cells is mediated through hepcidin, a hormone produced by the liver. The amount of hepcidin produced is up-regulated or down-regulated according to need. A good iron balance would be a situation where there is enough dietary iron to maintain good stores of iron in the liver for use should iron intake become deficient. When iron stores are low, the amount of hepcidin produced is down-regulated to increase absorption from the digestive tract, but with high iron stores it is up-regulated to reduce absorption. An additional mechanism that has been described contributing to regulation of iron absorption involves duodenal crypt enterocytes which can take up iron from plasma for storage. As the crypt cells migrate upwards to become absorptive cells of the duodenum the increased intracellular iron concentration results in reduced iron absorption via a complex mechanism involving carrier proteins. The extent to which this process contributes to iron homeostasis is not clear. The body does not have an excretion mechanism for excess iron. Much of the iron required by the body is obtained from iron recycled from the breakdown of senescent red blood cells in the liver. The smaller balance still required is obtained from the diet. But because the body has no excretory mechanisms for excess iron, this balance is tightly controlled through adjusting the amount of iron absorbed. Significant amounts of iron can, however, be lost from the body through bleeding (as in injury, menstruation, gastro-intestinal ulceration and worm infestation) and increased loss of mucosal cells in different disease conditions[6,7,12]. Figure 6.1 summarises iron turnover and homeostasis in the human body.

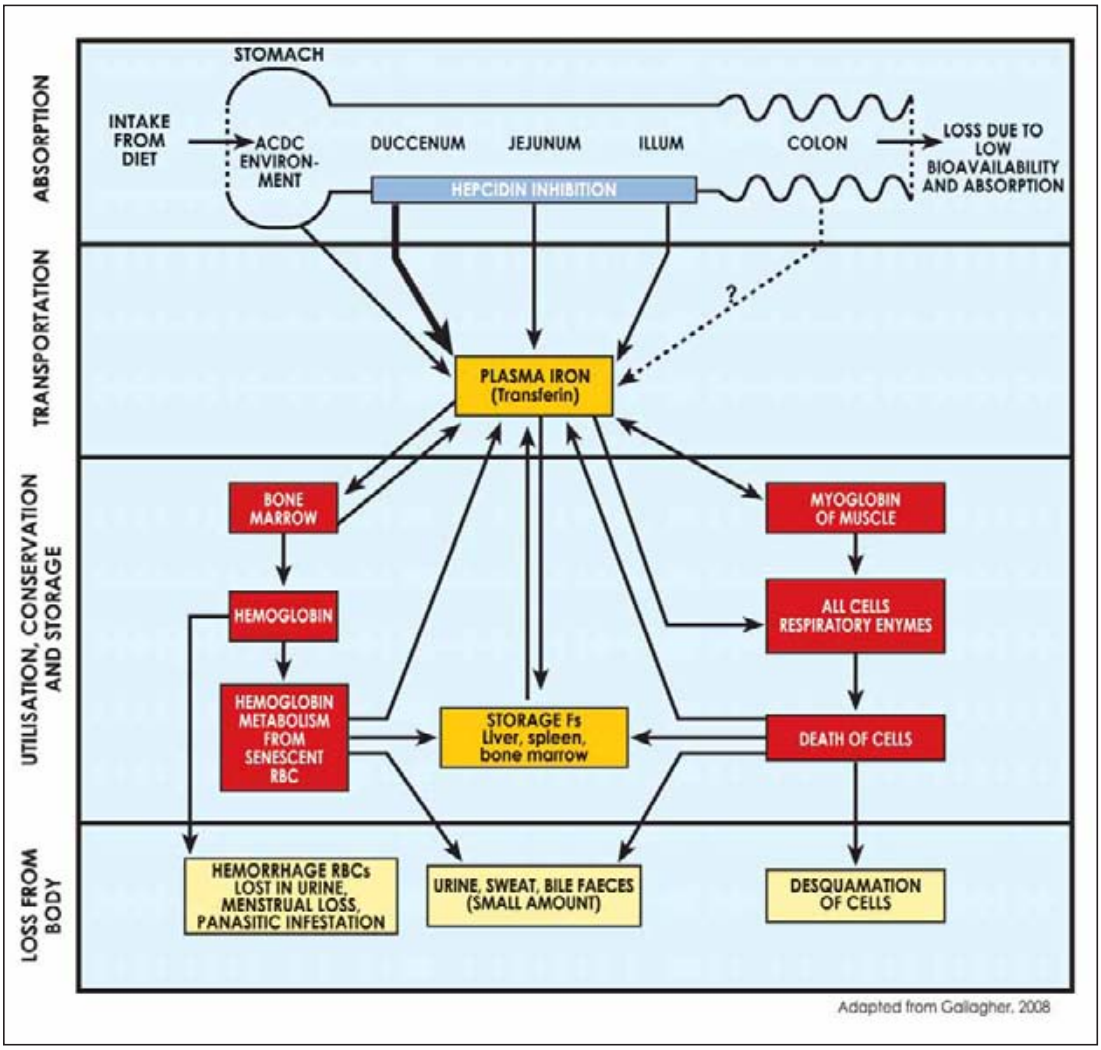

Figure 6.1 A schematic diagramme of iron turnover and homeostasis (Adapted from Gallagher, 2008)

Iron Deficiency, Iron Deficiency Anaemia and Anaemia

When the body goes into negative iron balance as a result of not enough iron being absorbed from the gut to keep up with demand, iron stores in the liver gradually become depleted. A gradual progression of iron deficiency can be observed ranging from an initial moderate depletion of iron stores with no dysfunction, severe depletion of iron stores with little or no dysfunction, severe depletion with dysfunction, and finally anaemia as indicated by the various cut-off points of haemoglobin according to age, gender and physiological state. Blood measurement of the various iron-containing biological molecules involved at different levels of iron metabolism has been used with varying levels of success under different conditions to indicate iron status. It should be mentioned that certain other micronutrient deficiencies (vitamins A, riboflavin, B_6, B_{12}, folic acid, copper, zinc, calcium and vitamin C) are associated with anaemia, emphasising the importance of assessing the status of these micronutrients together with that of iron.

Indicators of Iron Status

As indicated in the introduction, the WHO/CDC[1] evaluated the various indicators used to assess iron status. This expert committee recommended the use of haemoglobin, ferritin and transferrin receptor for iron status of populations, while serum ferritin together with haemoglobin was recommended as indicators of response to intervention. The limitation of ferritin, particularly in areas with endemic infection, was noted and additional investigation of its use in combination with acute-phase proteins such as C-reactive protein (CRP) and Alpha-1 acid glycoprotein (AGP) was recommended. Table 6.2 shows the indicators considered by the WHO/CDC working group1 including additional information based on Raiten *et al.*[13]. The three which were selected for use have been listed first in the shaded rows of the table.

Pathophysiology of Iron Metabolism

The danger of excess body iron is not common because of the tight control of absorption at the mucosal level. However, because of the lack of mechanisms to excrete excess iron it is possible to reach levels of body iron that can cause iron overload. A number of situations where iron overload can take place have been identified[6,7].

Some individuals may have a high risk of iron overload. This increased risk can be as a result of rare heritable genetic abnormalities that result in poor control of iron absorption. Excessive iron absorption in these conditions, such as hemochromatosis, leads to accumulation of iron stores in tissues. Another group at risk is people who need frequent blood transfusions as in thalassaemia. In this group, frequent transfusions may lead to accumulation of iron from breakdown of senescent red blood cells[14].

It may also be possible to get iron overload from excessive ingestion of iron as has been reported in some African settings where food and traditional beer prepared in cast-iron pots and iron drums are consumed regularly[7]. A recent South African study reported that the iron intake of individuals who consumed one litre of beer per day could reach twice the recommended intake[15]. However, traditional beer is low in alcohol concentration, thus consumption of much higher volumes are not uncommon, increasing the risk of iron overload in exposed susceptible individuals. There is now research that suggests that some of the iron overload observed in African populations, as a result of traditional beer consumption, may also be due in part to a genetic predisposition to iron overload[14].

Table 6.2 Different biological indicators of iron status and associated advantages and disadvantages

Indicator	Test sample	Units	Condition indicated	Advantage(s)	Disadvantage(s)
Haemoglobin	Whole blood; dry blood spots	g/dl	Anaemia Different cut off points by age, gender and physio-logical state	Low cost Widely used Possibility of using dry blood spots and simple field equipment	Low sensitivity and specificity Does not detect mild and moderate iron deficiency which can have functional outcomes.
Ferritin	Serum or plasma	µg/l	Iron stores Iron depletion: < 12µg/l	Good indicator of iron status especially depletion Standardized assays availlable	Increases with inflammation sub-clinical infection, some cancers No clear guidelines for populations with high disease prevalence
Transferrin receptor	Serum or plasma	µg/l	Balance between iron requirement and supply	Semi-quan-titative measure of iron deficiency even in presence of inflammation	Influenced by factors affecting erythropoiesis Inflammation effect not clear further validation and consensus needed
Red cell distribution width	Whole blood	%	Abnormal range of RBC size Small: < 11.5% Large: >14.4%	Can indicate type of anaemia	Expensive equipment May be affected by inflammation Influenced by thalassaemia
Reticulocyte Hb concen-tration	Whole blood	g/l retic-ulo-cytes	Hb concen-tration in new RBCs	18 – 36 hours old RBCs sensi-tive to recent deficiency	Expensive equipment

Indicator	Test sample	Units	Condition indicated	Advantage(s)	Disadvantage(s)
Serum/ plasma iron	EDTA free serum/ plasma	µg/dl µmol/l	Iron bound to transferrin in circulation	Measure of iron supply to tissues including bone marrow	Diurnal variation-Postprandial variationLow in chronic diseaseSusceptible to sample contamination
Erythrocyte protoporphyrin	Whole blood and dried blood spots	µg/dl whole blood or RBCs	Reduced iron supply for erythropoiesis	Useful for assessing children Advantage of using dry blood spots	Increases with inflammation and lead-poisoning
Zinc protoporphyrin (ZnPP)	Whole blood and dried blood spots	µmol /mol Hb	Reduced iron supply for erythropoiesis	Useful for assessing children Can use dry blood spots	Increases with inflammation and lead-poisoning
Total iron binding capacity (TIBC)	Serum or plasma	µg/dl µmol/l	Capacity of circulating transferrin to bind iron	Increased with iron deficiency	Large overlap of values in deficiency and normal iron status Low in inflammatory conditions
Transferrin saturation	Serum or plasma	%	Saturation of transferrin Iron deficiency erythropoiesis: < 16%	Proportion of iron bound to transferrin	Diurnal variation Postprandial variation Low in chronic disease Susceptible to sample contamination
Body iron stores	Serum or plasma	mg/kg	Iron body status	Measure of full range of iron status and has been validated by adult phlebotomy studies	Same limitations as for component parts, ie, transferrin receptor and ferritin

Indicator	Test sample	Units	Condition indicated	Advantage(s)	Disadvantage(s)
Hepcidin	Serum, plasma and urine	ng/ml	Status of regulation of iron absorption	Diminished levels when iron stores are depleted Potential for non- invasive urine measurement	still need standard methods & consensus on usefulness or interpretation affected by inflammation

(Adapted from WHO/CDC1; Raiten *et al*.[13]; RBC: Red blood cell; Hb: Haemoglobin)

Excessive alcohol consumption in itself has been shown to predispose people to iron overload. A South African study has reported that alcohol consumption doubled the number of individuals in positive iron balance for both men and women in the study[16]. Men had higher alcohol intakes and a higher percentage of individuals in positive iron balance than women. In addition, iron overload was most likely to be found in individuals with the highest alcohol consumption levels. Similar findings have been reported by others[17] and it has been suggested that regular alcohol consumption may induce a disruption of normal iron metabolism. This may lead to iron overload in about one third of alcoholic individuals[18]. In more affluent population settings, iron overload may also occur with excessive iron supplementation, particularly in individuals with an undetected genetic susceptibility to iron overloading.

The body generally counteracts iron overload by producing more hepcidin to reduce iron absorption at the mucosal level. In addition to inhibiting iron absorption, hepcidin also inhibits mobilisation of iron from liver stores and the recycling of iron by macrophages. People with hemochromatosis have been shown to produce low levels of hepcidin resulting in the excessive iron absorption observed in this condition[19].

Status of Iron Research Information in South Africa

South African studies have used a variety of available indicators, which often include ferritin, haemoglobin and transferrin receptor levels, to report on iron status. Many publications also report dietary intake data. Table 6.3 shows the spread of iron status indicators reported in selected South African publications, as well as the extent to which acute-phase proteins have been taken into account to try and control for inflammation. The latter is important, especially with the high HIV/AIDS-prevalence rates and possible parasite infestation, particularly in children. Although the number of publications given is limited it serves to indicate a need for the incorporation of acute-phase protein response markers to improve the interpretation of iron-status data. Interpretation of ferritin results is often deficient in that no attempt is being made to interpret discrepancies.

Where inflammation has been considered, the information has been used in different ways to correct for inflammation, ranging from exclusion of subjects with inflammatory markers above a certain level of inflammation[20], incorporation of information in regression analysis[21], or mention of inflammation as a possible confounding effect. Where acute-phase proteins are taken

into account, it is important to include a standardised manner of interpreting data along with any additional information of interest to the researchers, in order to facilitate comparison of data at the national level. This is necessary to develop a realistic picture of trends of iron status in relation to inflammatory effects in the population and allow for more effective monitoring of intervention programmes. Recent indications that adiposity can influence iron metabolism, possibly through its inflammatory effect[5] also need some attention in South Africa because of the increasing rates of obesity, both among children and adults, as a result of the nutrition transition that is taking place.

Against this background of variation in the manner researchers are handling iron status information, South Africa implemented a National Food Fortification Programme (NFFP) in 2003. Maize meal and wheat flour are fortified with 13 micronutrients including iron. Elecrolytic iron is used as a fortificant at the rate of 35 mg/Kg[22]. Thus far no national survey since the 2005 National Food Consumption Survey-Fortification Baseline[23] has been conducted, to monitor or evaluate the possible effect of the fortification on iron status or the other micronutrients included but some smaller studies have reported mixed trends in iron status[24,25,26]. The trends from these studies are however difficult to interpret because of the variation in the way the iron status indicators have been handled. It is therefore, necessary to have national recommendations on how the indicators should be handled for comparison across studies in the country.

South African research on micronutrient stutus in general including iron is biased in favour of low socio-economic population sub-groups. While much research is needed in these sub-groups due to the higher risks for micronutrient deficiencies, it is however noted that the entire South African population is exposed to the NFFP and in this regard it is essential that the full spectrum of the population is monitored over time.

The information presented here points toward a number of areas that require research attention in South Africa. Because a lot of the challenges that South Africa faces are also faced by other African countries, the work that may be done here will also benefit the other countries.

Assessment of Iron Status and Influencing Factors

The different methods that can be used for assessment of iron status and influencing factors are summarised in Table 6.3.

Table 6.3 Assessment of iron status

Method	Remarks
Dietary intakes: Usual intake methods plus special short questionnaires available for iron	SA food composition tables give values for iron of all food sources; computer programme give haem and non-haem iron separately. Reference intakes in Table 6.1
Haematology: Red cell distribution width	Indicates type of anaemia. Expensive equipment; for individuals
Laboratory (biochemical) methods **Haemoglobin**	Low sensitivity and specificity; cut-points available; low cost; widely used. Suitable for population assessment; does not pick up mild and moderate iron status
Laboratory (biochemical) methods **Serum ferritin**	Good indicator of iron status; cut-points available; increases during inflammation; suitable for both individuals and populations
Laboratory (biochemical) methods **Serum transferrin receptor**	Semi-quantitative measure of iron deficiency even in presence of inflammation. Indicates the balance between iron requirement and supply
Laboratory (biochemical) methods **Serum or plasma iron**	Measures iron bound to transferrin in circulation and indicates iron supply to tissues; diurnal and post-prandial variation; low in chronic disease; susceptible to sample contamination
Other methods	Often expensive equipment needed. Suitable for individuals and research
Red cell distribution width	May be affected by inflammation and thalassaemia
Reticulocyte haemoglobin concentration	Sensitive to recent deficiency; expensive equipment needed
Erythrocyte and zinc protoporphyrin	Useful in children; increased with inflammation and lead poisoning
Total iron binding capacity (TIBC)	Measures capacity of circulating transferrin to bind iron; increased with iron deficiency but values overlap with those during normal status
Transferrin saturation	Low in chronic disease; postprandial variation

Table 6.4 Iron status indicators and acute-phase proteins reported in South African research

Year of publication	Authors	Indicators	Acute-phase proteins	Correction for infection/ inflammation	Comment
2007	Faber and Benadé, (2007)[20]	Hb, serrum ferritin	CRP	Subset analysis with high CRP excluded	
2005	Labadarios *et al.*, (2005)[27]	Hb, dietary data	No	No	
1999	Faber and Benadé, (1999)[28]	Hb, serrum ferritin	No	No	WBC count as indication of infection
2004	Faber (2004)[3]	Dietary intake	No	No	
2005	Smuts *et al.*, (2005)[21]	Hb, ferritin	CRP, AGP	No	
2007	Faber (2007)[29]	Hb, dietary intake	No	No	
2007	Papathakis *et al.* (2007)[30]	Hb, ferritin	CRP, AGP	Yes analysis both with and without correction for inflammation	Did not attempt to interpret discrepancy from expected trend
2004	Vorster *et al.*, (2004)[31]	Hb, ferritin serum iron, transferrin, TIBC, hematocrit, %iron saturation	Yes	No	AST, ALT, LD measured to reflect tissue and cell damage
2002	Eley *et al.*, (2002)[32]	Hb, ferritin, MCV, MCH, MCHC, RDW, transferrin, TIBC, hematocrit, % transferrin saturation	No	No	Describes differences between HIV positive and HIV negative children, and challenges of result interpretation because of inflammatory effects
2007	Choma and Alberts, (2007)[15]	Ferritin, Hb, TIBC, % transferrin saturation	CRP	Yes	Used combinations of iron status indicators and CRP

ALT: alanine aminotransferase; AST: aspartate aminotransferase; Hb: haemoglobin; LD: lactate dehrydrogenase; MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; MCHC: mean corpuscular haemoglobin concentration; RDW: red blood cell distribution width; TIBC: total iron binding capacity; WBC: white blood cell; CRP: C-reactive protein; AGP: Alpha-1 acid glycoprotein

Recommendations for Research to Assess Iron Status in South Africa and Africa

The following recommendations emanated from the information presented. They are aimed towards effective monitoring and interpretation of iron status data in South Africa and Africa, ensuring that the entire spectrum of the population is adequately assessed for the design of suitable interventions.

- Research results that show discrepancies in trends of iron-status indicators, even after taking into account acute-phase proteins, need to be scrutinised to try and find possible reasons for such discrepancies. This type of scrutiny may in time lead to better ways of interpreting iron-status data under challenging situations.

- The manner in which the acute-phase response is taken into account when interpreting iron-status data needs to be standardised to facilitate the comparison of data across studies. This is necessary in order to form a realistic picture of trends of iron status, especially with respect to the high prevalence of HIV/AIDS and other infections within the South African and wider African context.

- The possible influence of adiposity on the prevalence of anaemia in children and adults warrants research attention, especially in view of the nutrition transition resulting in increased prevalence of obesity and non-communicable diseases.

- Research should take into account the diverse nature of the South African population and ensure that the full spectrum of the population is addressed. This is especially necessary because all population groups are affected by the NFFP – and should therefore all be monitored.

- The extent to which iron overload may be a problem and the factors that increase the risk for iron overload in the different population sub-groups need to be clearly documented. This is necessary to ensure appropriate and effective monitoring of the iron status of South Africans.

- Possible differences in the cut-off levels of iron-status variables across different ethnic groups represented in South Africa should be investigated to ensure the adoption of appropriate monitoring processes across the South African population.

- The amounts of traditional beer consumption and the circumstances under which the beer is produced, may predispose individuals to iron overload, and should be investigated to be able to provide appropriate guidelines.

- A deliberate coordination of research effort is necessary towards more effective/efficient interpretation of iron-status data within the African context.

Since many of the challenges that South Africa faces are also faced by other African countries, ways of encouraging research cooperation among these countries, towards shared solutions, should be explored. This would facilitate the accumulation of relevant research information in the region to the benefit of all.

REFERENCES

1. WHO/CDC. World Health Organisation/Centers of Disease Control and Prevention expert consultation agrees on best indicators to assess iron deficiency, a major cause of anaemia. Geneva, World Health Organisation, 2004.

2. Clark SF. Iron deficiency anaemia. *Nutrition in Clinical Practice*, 2008; 23:128-141.

3. Faber M. Complementary foods consumed by 6-12 month old rural infants in South Africa are inadequate in micronutrients. *Public Health Nutrition*. 2004; 8:373-381.

4. De Benoist B, McLean E, Egli I, Cogswell M. (eds) WHO/CDC Worldwide prevalence of anaemia 1993-2005: WHO/CDC global database on anaemia. Geneva, World Health Organisation, 2008:1-40.

5. Zimmermann M B, Zeder C, Muthayya S, Winichagoon P, Chaouki N, Aeberli I, Hurrell RF. Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and reduced response to iron fortification. *International Journal of Obesity* 2008; 32:1098-1104.

6. Gallagher M L. In: Mahan LK, Escott-Stump S. (eds) Krause's Food, *Nutrition and Diet Therapy*, Saunders Elsevier, St Louis 2008:39.

7. MacPhail P. Iron.In Mann J, Truswell AS. (eds) *Essentials of human nutrition*. 2 Oxford University Press, New York 2007:125.

8. Wander K, Shell-Duncan B, T. W. McDade TW. Evaluation of iron deficiency as a nutritional adaptation to infectious disease: an evolutionary medicine perspective. *Journal of Human Biology* 2009; 21:172-179.

9. Rawart RJ, Humphrey J, Ntozini R, Mutasa K, Iliff P, Stoltzfus R. Elevated iron stores are associated with HIV disease severity and mortality among postpartum women in Zimbabwe. *Public Health Nutrition* 2008; 12:1-9.

10. Labbé RF, Finch CA *et al*. Erythrocyte protoporphyrin/haem ratio in the assessment of iron status. *Clinical Chemistry* 1979; 25:87-92.

11. Metzgeroth G, Adelberger V, A. K. C. Dorn-Beineke AKC, Schatz M, Maywald O, Bertsch T, Wisser H, Hehlmann R, Hastka J. 2005. Soluble trasferrin receptor and zinc protoporphyrin: competitors or efficient partners? *European Journal of Hematology* 2005; 75:309-317.

12. Munoz M, Villar I, Garcia-Erce JA. An update on iron physiology. *World journal of Gastroenterol* 2009; 15(37): 4617-4626.

13. Raiten DJ, Namaste S, Brabin B, Combs Jr G, L'Abbe MR, Wasantwisut E. Excecutive summary-biomarkers of nutrition for development: building consensus. *American Journal of Clinical Nutrition* 2011; 94 (suppl): 633S-650S.

14. Lynch S. Iron metabolism. In: Kraemer K, Zimmermann MB. (eds) *Nutritional Anemia*. Sight and Life Press, Basel 2007:59.

15. Choma H, Alberts M. Effect of traditional beer consumption on the iron status of a rural South African population. *South African Journal of Clinical Nutrition* 2007; 20:62-68.

16. Gopane RE, Pisa PT, Vorster HH, Kruger A, Margetts BM. Relationships of alcohol intake withbiological health outcomes in an African population in transition: the Transition and health during Urbanisation in South Africa (THUSA) study. *South African Journal Clinical Nutrition* 2010; 23(3):S16-S21

17. Walmsley CM, Bates CJ, Prentice A, Cole TJ. Relationship between alcohol and nutrient intakes and blood status indices of older people living in the UK: further analysis of data from the National Diet and Nutrition Survey of people aged 65 years and over, 1994/5. *Public Health Nutrition* 1998; 1:157-167.

18. Berkhan L. Interpretation of an elevated serum ferritin. *NZFP* 2002; 29:45-48

19. Stopler, T. 2008. Medical nutrition therapy for anaemia. In: Mahan LK, Escott-Stump S. (eds) *Krause's food and nutrition therapy*. Saunders Elsevier, St Louis 2008: 810.

20. Faber M, Benadé AJS. Breastfeeding, complemenary feeding and nutritional status of 6-12-month old infants in rural Kwazulu-Natal. *South African Journal Clinical Nutrition* 2007; 20:16-24

21. Smuts C M, Dhansay MA, Faber M, Van Stuijvenberg MA, S. Swanevelder S, Gross R, Benadé AJS. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status and growth in South African children. *Journal of Nutrition* 2005; 235:653S-659S.

22. Department of Health (2002) Foodstuffs, cosmetics and disinfectants act, No. 54 of 1972. http:/www.doh.gov.za/docs/regulations/foodstuff/fortification.html. (Accessed Sept 2012)

23. Labadarios D, Louw R. Selected micronutrient status. Iron status. In National Food Consumption Survey-Fortification Baseline (NFCS-FB): South Africa. Labadarios D. (ed) 2007; 447-455. University of Stellenbosh, Tygerburg, South Africa

24. Van Stuijvenberg ME, Smuts CM, Lombard CJ, Dhansay MA. Fortifying brown bread with sodium iron EDTA, fumarate or electrolytic iron does not affect iron status in South African children. *Journal of Nutririton* 2008; 138: 782-786.

25. Troesh B, Stuijvenberg MA, Smut s CM, Kruger HS, Briebinger R, Hurrel HF, Baumgartner J, Zimmermann, MB. A micronutrient powder with low doses of highly absorbable iron and zinc reduces iron and zinc deficiency and improves weight-for-age z scores in South African children. *Journal of Nutririton*, 2011; 141: 237-242.

26. Baumgartner J, Smuts, CM, Kvalsvig J, Stuiuvenberg ME, Hurrel RF, Zimmermann MB. Effect of n3 faty acid supplementation alone and in combination on cognition in schoolchildren. In publication process.

27. Labadarios D, Steyn NP et al. The National Food Consumption Survey. *Public Health Nutrition* 2005; 8:533-543.

28. Faber M, Benadé AJS. Nutritional status and dietary practices of 4-24-month old children from rural a South African community. *Public Health Nutrition* 1999; 2:179-185.

29. Faber M. Dietary intake and anthropometric status differ for anaemic and non-anaemic rural south African infants. *Public Health Nutrition* 2007; 25:285-293.

30. Papathakis PC, Rollins NC, Chantry CJ, Bennish MLBKH. 2007. Micronutrient status during lactation in HIV-infected and HIV-uninfected South African women during the first 6 mo after delivery. *American Journal Clinical Nutrition* 2007; 85:182-192.

31. Vorster HH, Kruger A, Margetts BM, Venter CS, Kruger HS, Veldman FJ, MacIntryre UE. 2004. The nutritional status of assymptomatic HIV-infected Africans: directions for dietary interventions? *Public Health Nutrition* 2004; 7:1055-1064.

32. Eley BS, Sive AA, Shuttleworth M, Hussey GD. A prospective, cross-sectional study of anaemia and peripheral iron status in antiretroviral naive, HIV-I infected children in Cape Town, South Africa. *BMC Infecious Disease* 2002; 2(3): 7p. (Electronic print)

Chapter 7

Zinc

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Introduction

Zinc (Zn) is required for the activity of multiple enzymes involved in major metabolic pathways in the human body. Zinc deficiency affects functions such as normal growth and reproduction, skeletal development, immune competence and neuropsychological function[1]. High nutritional demands during periods of growth and development make children particularly vulnerable to zinc deficiency[2]. Dietary intakes of SA children indicate mostly adequate energy and protein intakes, but low micronutrient intakes include low zinc intakes[3]. At-risk groups for suboptimal zinc intakes include children aged 1 to 6 years and women throughout the reproductive years. There is evidence that low intakes of foods rich in bioavailable zinc, such as meat, and high intakes of foods rich in inhibitors of zinc absorption, such as maize, can lead to zinc deficiency[4]. Clinical manifestations of zinc deficiency are nonspecific, and it is difficult to assess marginal zinc deficiency, but severe zinc deficiency is characterised by diarrhoea, infections, delayed growth and physical development, skin lesions affecting mainly the perimucosal regions and the extremities and impaired taste acuity[5]. The zinc status of individuals, communities or populations is important for public health policy and programmes, yet there is not a single, sensitive, specific and generally affordable indicator of zinc status. Currently, plasma or serum zinc concentration is the most widely used indicator of zinc status[5].

Review of the Physiology of Zinc

Zinc is necessary for the activity of more than 300 enzymes involved in major metabolic pathways involving synthesis or degradation of macronutrients. Zinc acts as a structural component of several proteins, such as metallothionein, as well as bone and functions as an intracellular signal in brain cells. Metallothionein functions as an intracellular reservoir to donate zinc ions and may have a redox role that reduces oxidative stress. Zinc plays a role in osteoblastic activity, formation of bone enzymes and bone calcification. This trace mineral is also involved in transport, immune function and expression of genetic information. Zinc stabilises ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) structure in the nucleus and is involved in transcription and replication. Zinc deficiency therefore affects multiple functions, such as normal growth and reproduction, skeletal development, immune competence and neuropsychological function[1,6,7]. Several zinc-dependent enzyme systems are involved in haemoglobin synthesis. Aminolevulinic acid dehydrase mediates a step in haem synthesis, whereas thymidine kinase and DNA polymerase are involved in DNA synthesis[8]. Zinc-finger transcription factor, globin transcription factor 1 (GATA-1) is essential for normal erythropoesis[9]. Zinc induces increases in plasma insulin-like growth factor-1 levels, which also contributes to the stimulation of haematopoiesis[10,11].

Distribution of Zn in the Body and its Possible Regulation and Homeostasis

The human body contains about two to three grammes of zinc, mostly concentrated in the liver, kidney, pancreas, muscle and bone, but also in skin, hair and nails. Zinc functions primarily in enzymes within the cells. Zinc absorption involves a carrier mechanism, as well as passive diffusion from high to lower concentrations. Most zinc is transported by albumin in the blood, but also by transferrin and a2-macroglobulin. Most of the zinc in blood is incorporated in erythrocytes and leukocytes. Plasma zinc levels fluctuate according to a diurnal pattern and are also affected by dietary intakes. Plasma zinc concentration may decrease by up to 50% in response to injury or inflammation. Zinc is almost entirely excreted via the faeces, but in certain disease states, such as nephrosis and hepatic cirrhosis, zinc-binding amino acids are excreted in the urine and urinary zinc loss increases[7].

Pathophysiology and Consequences of Zinc Deficiency

Zinc deficiency is an important cause of morbidity in developing countries, particularly among children[12]. Human zinc deficiency was first noted in the 1970s in patients with acrodermatitis enteropathica, an inborn error of metabolism characterised by zinc malabsorption and associated with impaired growth and increased susceptibility to infections[1].

Prasad *et al*.[13] described clinical signs of zinc deficiency in young Egyptian boys with short stature, hypogonadism, mild anemia and low plasma zinc levels associated with a diet of unleavened breads with a high phytate content. In populations with a considerable prevalence of stunting and with low zinc intakes, zinc deficiency is likely to exist[14]. Zinc deficiency is associated with short stature and an increase in height and food intake occurs after zinc supplementation. Stunted children respond with a greater increase in height after zinc supplementation than non-stunted children[15]. Children on a high-phytate diet are at risk, especially if they have repeated infections and diarrhoea, due to their increased zinc requirement for growth[14]. Several studies have indicated that a low zinc:energy-ratio in the diet is associated specifically with a diminished rate of growth of lean body mass, although adipose tissue may still be deposited. There are indications that zinc supplementation is associated with an increase in lean body mass, but not in fat mass of undernourished children[16].

The mechanism for this favourable response is probably an increase in insulin-like growth factor-1 after zinc supplementation[15,17]. Although zinc supplementation may increase lean body mass in previously undernourished children, plasma zinc concentration was not associated with total body water (an indicator of lean body mass) in zinc-repleted overweight and obese preschool Chilean children from low socio-economic communities[18]. Low adult height is, however, not only an indicator of poor zinc status, but also an indicator of long-term energy deficiency in people in developing communities, because people who were poorly fed and had repeated infections grow poorly in childhood and achieve a smaller adult height. Short stature may be associated with reduced work capacity and economic productivity in these adults[19].

Clinical manifestations of zinc deficiency are nonspecific, but severe zinc deficiency is characterised by alopecia, diarrhoea, infections, anorexia, and skin lesions on the extremities[20]. Mild zinc deficiency may be associated with impaired taste (hypogeusia) and poor wound-

healing[20,21]. Suboptimal zinc status results in down-regulation of the immune system, with atrophy of the thymus, lymphopenia, reduced cell-mediated responses and increased susceptibility to infection[21]. Patients with severe zinc deficiency may die from infections and diarrhoea. Even mild zinc deficiency can reduce immune function, associated with anergy and diminished natural killer (NK) cell activity without lymphopenia[7]. Severe zinc deficiency may also cause impaired neuropsychological function and less severe deficiency impairs cognitive performance in children[17]. Repletion with zinc and other micronutrients had beneficial effects on neuropsychologic performance of Chinese children[17]. Zinc in the diet may interfere with iron absorption, but such interactions are only likely to be important at levels of intake achieved by the use of supplements[22].

Pathophysiology of Zinc Deficiency in Individuals with Tuberculosis and HIV Infection

According to a WHO Consultation on Nutrition and HIV/AIDS in Africa[23], "HIV/AIDS is affecting more people in Southern Africa than the fragile health system of the countries can treat, demoralising more children than our educational systems can inspire, creating more orphans than communities can care for, wasting families and threatening food systems". Evidence suggests that zinc deficiency may increase HIV replication, impair cellular immunity and accelerate apoptosis of cells involved in the immune response. As discussed earlier, suboptimal zinc status results in down-regulation of the immune system, with atrophy of the thymus, lymphopenia, reduced cell-mediated responses and increased susceptibility to infection. Acquired immune deficiency syndrome (AIDS) is one of the disease states associated with zinc deficiency, which may be aggravated by malabsorption due to recurrent diarrhoea[24]. Zinc deficiency is more common in South African HIV-infected breast-feeding mothers than in HIV-uninfected mothers (45% *versus* 25% respectively)[25]. There is still a lack of information on the impact of zinc status on mucosal immunology, viral defense mechanisms, acute-phase responses and incidence of infection. Further research is needed to determine the timing and extent of immune system repair with zinc supplementation. In the HIV-infected patient, diarrhoea is of particular concern, because diarrhoea causes zinc loss, but also results from zinc deficiency, creating a cycle of diarrhoea and deficiency[24].

Available data from studies in children show no association between serum zinc concentration and disease progression, and little benefit of supplementation on indicators of immunity. Excess zinc supplementation may be harmful to both children and adults[23,26]. Well-designed trials to assess zinc bioavailability in HIV-infected children and adults, the prevalence rate and consequences of zinc deficiency in HIV-infected patients and the effects of zinc supplementation on markers of the immune response critical to HIV infection need to be done[26].

Zinc Deficiency in South Africa

National data of children one to nine-years old indicate biochemical zinc deficiency in 45% of the children[27] and a large proportion of the population had low zinc intakes[3]. In secondary analysis of existing study data, the effect of fortification was evaluated by substituting fortified foods in the diet of adults for the unfortified products. Fortification of maize meal and wheat flour (bread) considerably improved mean intakes of zinc, iron and most B vitamins[28]. Despite these increased intakes from fortified staple foods, national data of children collected during

2005, two years after the start of national fortification, still indicated zinc deficiency in almost half of the children. The mean serum zinc concentration of the children remained constant with increasing age as did the proportion of children with serum zinc concentration <65µg/dL[27]. A study in rural KwaZulu-Natal among six to 12-month-old infants showed that 47% of the infants had zinc deficiency[29], whereas 32% of urban infants in the Western Cape had zinc deficiency (serum zinc concentration <65µg/dL)[30]. Low zinc intakes, associated with low intakes of animal protein foods, as well as low zinc bioavailability from the maize staple diet of the South African population may contribute to marginal zinc status. A study in preschool children in informal settlements near Bloemfontein indicated adequate protein intakes, but low zinc intakes, as well as low intakes of several other micronutrients[31]. More than 20% of the children were stunted. No association between zinc intakes and height-for-age was found, possibly due to the small sample size in this study[31].

Oldewage-Theron *et al*.[32] showed in a random sample of elderly participants in Gauteng that most participants had a diet with maize meal as staple food and more than half had Zn intakes lower than two-thirds of the RDA, which is close to the International Zinc Nutrition Consultative Group (IZINcG)[5] estimated average requirement (EAR). More than three-quarters of the participants had a serum zinc concentration <70µg/dL. These results may be typical of the zinc status of the elderly in low socio-economic status urban areas in South Africa[32].

Assessment of Zinc Deficiency

The zinc status of communities or populations is important for public health policy and programmes while individual zinc status is important for the clinical management of the individual. There is currently not a single, sensitive, specific and low-cost indicator of zinc status.

Biochemical Assays of Zinc Defiency

Rationale for Using Serum/Plasma Zinc Concentration as an Indicator of Zinc Status

Plasma or serum zinc concentration is currently the best and most widely used indicator of zinc status in populations and has been jointly recommended by the World Health Organisation (WHO), United Nations Childen's Fund (UNICEF), the International Atomic Energy Association (IAEA) and IZiNCG[33]. Serum zinc concentration declines within days or weeks after restriction of dietary zinc intake and rises when zinc supplements are taken[34]. However, serum Zn concentrations change during infection, inflammation, chronic disease, liver disease, pregnancy and malnutrition, or other stress conditions, unrelated to zinc status. It is, however, still recommended to measure serum/plasma zinc concentration of people in communities in developing countries[20,35] as part of national nutrition studies.

Reference Data for Serum Zinc Concentration

Gibson[36] recommends a cut-off point of plasma or serum zinc concentration of <10.71 µmol/L (<70µg/dL) for fasting blood samples and <9.95µmol/L (<65µg/dL) for non-fasting blood samples as an indication of zinc deficiency, together with measurement of dietary zinc intakes. The IZiNCG[5,37] suggested lower cut-off points for serum zinc concentration by age group, sex,

time of blood collection and time since the last meal (Table 7.1). Zinc concentration can be measured in plasma or serum, but for simplicity IZiNCG technical briefs[37] use serum zinc to refer to both plasma or serum. When the prevalence of serum zinc concentration below these cut-off points is >20% in a community or population, the risk of zinc deficiency is elevated and of public health concern[37].

Table 7.1 Suggested lower cut-offs for serum zinc concentration (µg/dl)* by age group, sex, time of blood collection and fasting status[37]

Time of blood collection and fasting status	Time of blood collection and fasting status Suggested lower cut-offs for serum zinc concentration (µg/dL)		
	< 10 years	>/= 10 years	
	Male and female	Male	Non-pregnant female
Morning, fasting for at least 8 hours	Not available	70	74
Morning, non-fasting	65	66	70
Afternoon, non-fasting	57	59	61

**Divide by 6.54 to convert to µmol*

Serum zinc data of subjects free from infection on the day of blood collection in the NHANES II study have been used to derive these cut-offs for low serum zinc concentrations and to identify potential confounders on serum zinc concentration. The four major confounding variables were age, sex, time of day of blood sampling and fasting state of the subjects. During childhood, up to the age of 10 years, boys had lower serum zinc concentrations than girls[38]. Garenne *et al*.[39] also reported that boys and girls, aged six to 30 months, responded differently to zinc supplementation, with a faster linear growth velocity in girls who received zinc supplementation than in control girls, but not in boys. A lower cut-off for serum zinc concentration of 65µg/dL (9.94µmol/L) for morning non-fasting blood samples was proposed for children (boys and girls) younger than ten years. The lower cut-offs of fasting morning serum zinc concentration for males older than ten years are 74µg/dL (11.3µmol/L) and for females, 70µg/dL (10.7µmol/L). Data on fasting blood samples were not available for children younger than 10 years. Factors associated with significantly lower serum zinc concentrations, but independent of zinc status included sex (boys < girls), serum albumin concentration (positive association), pregnancy, oral contraceptives and current diarrhoea (lower serum zinc in boys only). Morning serum zinc concentration is used as a marker of zinc status, but the factors mentioned should be taken into account and treated as confounding variables[38].

Systemic infections that produce an acute-phase response caused decreased plasma zinc concentrations[40]. Serum zinc concentration may thus not be a useful indicator of zinc status in populations with a high prevalence of infections. However, in community-based cross-sec-

tional studies of children no association between the presence of infection and plasma zinc concentration was found[41], possibly because the infections in the children were less severe than in the adults studied. Despite a high prevalence of childhood infections in low-income countries, plasma zinc concentration remains a useful indicator of population zinc status for these children[35]. Droke *et al*.[42], however, found lower plasma zinc levels in low-income children with infection, indicated by elevated C-reactive protein and leukocyte counts.

The appropriateness of serum zinc as an indicator of zinc status is confirmed by the response of initial serum zinc concentrations to severe dietary restriction and zinc supplementation. Serum zinc concentrations reflect dietary zinc intake and also predict functional responses to zinc interventions, but may not be a reliable indicator of individual zinc status[34,43]. Reference data are also available for most age and sex groups.

Technical Issues Regarding Blood Sample Collection and Laboratory Analysis

Several important technical issues must be considered regarding sample collection, laboratory analysis and interpretation of serum zinc concentrations. The concentration of zinc in serum is very low and can be dramatically altered by any contamination.

IZiNCG[37] describes strict step-by-step procedures to collect, prepare and store samples, as well as technical advice on sample analysis and necessary precautions to avoid zinc contamination of the samples. These include *inter alia* the following:

- All needles, syringes, collection and centrifugation tubes, pipettes and storage vials must be zinc-free. The IZiNCG[37] protocol describes the necessary steps to avoid contamination of the samples with external zinc, also from air and water or contact with the analyst. Haemolysis of samples must be avoided.

- A strict protocol should be followed at blood collection. Information on time of the day, age, sex, time since last meal, presence of infection, and other possible confounding factors must be recorded. Measurement of some acute-phase proteins is recommended.

- Blood samples should be stored in a cool box or refrigerator until centrifugation.

- Serum or plasma may be stored in screw-top vials under refrigeration for a few days, or frozen for longer periods until analysis.

- Zinc concentration can be measured by flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry and neutron activation analysis, depending on availability and level of precision desired[37].

Interpretation of the Results: Reference Values and Cut-offs for Serum Zinc Concentration

For population or community-based studies, serum zinc results should be presented as means, range and % subjects below the appropriate cut-off values for the population and for selected sub-groups. The serum zinc concentration of an individual can be compared to appropriate reference data for age, sex, time of blood collection and fasting state (Table 7.1). Ideally, C-reactive protein (CRP) or a-1 acid glycoprotein (AGP) should be measured as acute-phase proteins indicative of infection or underlying inflammation, which reduces serum zinc concentration. When elevated acute-phase protein levels are found, serum zinc values may be adjusted statistically. If values for all subjects with increased CRP or AGP are excluded from analysis, selection bias may be introduced[37].

Other Biochemical Indicators of Zinc Status

Due to fluctuation in serum zinc concentrations during the day and after meals, alternatives, such as enzymes, zinc-binding proteins (metallothionein), and hair analysis have been investigated, but a convenient reliable assessment tool has not been identified[43,44]. Metallothonein is the most abundant, non-enzymatic zinc-containing protein, thought to have a role in zinc absorption. Reverse transcriptase polymerase chain reaction (PCR) to measure metallothionein synthesis and function, as well as exchangable zinc pools, holds promise as a future zinc biomarker. Currently serum zinc is the only biochemical indicator of zinc status that meets the criteria of reflecting dietary intake, responding to supplementation and having available reference data[37].

Dietary Zinc Intakes and Sources

Estimated Average Requirements for Zinc

Although there are limitations to nutritional assessment using dietary intakes, assessment of dietary zinc intakes remains an important component of the assessment of zinc status of populations. The prevalence of inadequate zinc intakes can indicate the risk of zinc deficiency in the population. Dietary deficiency is the most likely cause of zinc deficiency. The IZiNcG[37] revised the estimated average requirements (EARs) for zinc and developed EARs appropriate for international use (Table 7.2). The estimated physiological requirement for adults adequately predicts zinc status as determined by serum zinc and/or zinc balance studies. The reported prevalence of low serum zinc concentrations and the estimated prevalence of inadequate zinc intakes predict similar levels of risk of zinc deficiency, especially in women (pregnant or non-pregnant). There is less conformity between the two indicators for children. If the prevalence of inadequate zinc intakes in a population is greater than 25%, the risk of zinc deficiency is elevated in that population[45].

Table 7.2 IZiNCG[36] estimated average requirement (EAR) for zinc by life-stage and diet

Age/life-stage	Sex	Reference body weight (kg)	IZiNCG EAR for zinc (mg/d)	
			Mixed/refined plant-based diets	Unrefined plant-based diets
6 – 11 months	M+F	9	3	4
1 – 3 years	M+F	12	2	2
4 – 8 years	M+F	21	3	4
9 – 13 years	M+F	38	5	7
14 – 18 years	M	64	8	11
14 – 18 years	F	56	7	9
Pregnancy	F	-	9	12
Lactation	F	-	8	9
> 19 years	M	65	10	15
> 19 years	F	55	6	7
Pregnancy	F	-	8	10
Lactation	F	-	7	8

Bioavailability of Zinc

Endogenous factors influencing zinc bioavailability include the efficiency of digestion and absorption of food, gut transit time and the presence of gastrointestinal disorders. In developing countries plant-based diets have a low zinc content and a high content of phytate that significantly inhibits zinc absorption. About 60-75% of a zinc supplement taken with water on an empty stomach may be absorbed, whereas only 30% of the supplement will be absorbed if it is taken with a typical Western diet. The percentage absorption will be even lower with a diet high in phytate content, such as a maize staple diet[46]. Phytic acid (inositol hexaphosphate) is a normal constituent of unrefined cereals and interferes with intestinal zinc absorption[47]. Iron and zinc are found in the same food sources, with animal protein foods the best sources of both. Dietary factors associated with low-serum zinc concentration in children from low-income households are high intakes of sweetened beverages, whereas consumption of more than 15g meat per day is positively associated with serum zinc concentration[48]. The EAR cut-point method was used in a study among pregnant women from subsistence households in Ethiopia and showed a 100% prevalence of dietary zinc inadequacy, using the IZiNCG EAR. The women had low animal protein intakes and subsisted on staple foods high in phytates[49].

Maize and bread, the staple foods in South Africa, have high phytate contents. Maize samples tested in a Guatemalan study[46] contained 763mg phytate per 100g dry maize meal. The study showed that eating low-phytate maize for 10 weeks was not associated with significantly better zinc absorption than from high-phytate maize in children. However, if variables could be controlled better, it may be possible to demonstrate differences between zinc absorption in groups with different phytate intakes.

Steps to Assess the Adequacy of Zinc Intake in a Population

Determine the Survey Design

Design the study to estimate the prevalence of inadequate zinc intake in a population or to estimate the mean zinc intake in a population. The distribution of usual zinc intakes by a population should be described to estimate the probability of inadequate zinc intakes in the population[37].

Select a Representative Population Sample

The sample size for the dietary survey must be large enough to detect inadequate zinc intakes with the desired degree of precision. If data on zinc intakes are not available, an indirect estimate derived from food balance sheet data can be used[5].

Select a Food Intake Measurement Method

Measure food intake by weighed food records, 24-hour recalls or food frequency questionnaire. Choose the method that would be most appropriate for the specific circumstances.

Assess the Intake of Absorbable Zinc in the Diet

Intakes of both zinc and phytate can be calculated from food intake data. The next stage is to calculate the phytate:zinc molar ratio of the diets of each individual to provide an estimate of zinc absorption using the formula: (mg phytate per day/660)/(mg zinc per day/65.4). This ratio may be used to classify diets as having low or average zinc bioavailability. Choose the most appropriate EAR to assess the prevalence of inadequate zinc intakes by life-stage and diet type as shown in Table 7.2.

Estimating the Prevalence of Inadequate Zinc Intakes

The process will depend on the survey design chosen. Gibson[50] and Gregory *et al*.[51] have descibed the process in detail.

Stable Isotopes and Zinc Bioavailability

Analysis of zinc-stable isotope kinetics can provide answers about bioavailability of zinc from foods. There are five naturally occurring stable isotopes of zinc of which three have a natural abundance of less than 20%. Simultaneous oral and intravenous administration of two different isotope tracers can be done to study zinc kinetics in human subjects. Stable isotope studies are labour intensive and expensive and can only be done in studies with small sample size[44].

A dual-isotope tracer ratio technique that uses urine enrichment with zinc-stable isotopes administered intravenously and orally was used successfully to measure fractional absorption of zinc during the third trimester of pregnancy in low-income pregnant women in Ethiopia[52].

Functional Indicators of Zinc Status

Stunting

Height-for-age is the easiest technique to measure adverse outcome of zinc deficiency in populations. A prevalence of stunting among children younger than five years in a community or population >20% indicates a public health concern[33]. The WHO child growth standards[53] should be used to assess the growth of children, because these standards adopt a prescriptive approach to describe how children should grow.

Zinc deficiency is, however, not the only factor influencing a child's growth. Dietary zinc intake and serum zinc levels can be used to confirm the prevalence of zinc deficiency in high-risk populations[33]. The change in population mean serum zinc concentration before and after intervention can be used as an indicator of the successful delivery of zinc supplements in children[15].

Motor Development

Motor development may be delayed in zinc deficient infants and children. However, zinc supplements given daily for a year did not improve the growth or motor development of 5-11 month old infants in Zanzibar[54].

Interpretation of Zinc Assessment Data of Zinc in Populations

When the prevalence of low serum/plasma zinc concentrations, and/or rate of stunting among children younger than five years in a community is >20% and/or the prevalence of inadequate zinc intakes is >25%, zinc deficiency is a public health concern in such a community. Inadequate intake is defined as a usual intake below the EAR[5]. Ideally, all three types of indicators should be used together to obtain the best estimate of the risk of population zinc deficiency. The indicators can also be used to identify specific subgroups with increased risk and to indicate the need for zinc interventions. The impact of interventions may be evaluated by measuring changes in the prevalence of low serum zinc and inadequate zinc intakes[12].

Recommendations for Assessment of Zinc Status in South Africa

- Although ideally all three types of indicators should be used together to obtain the best estimate of the risk of population zinc deficiency, it may not be practical and affordable. Because drawing, processing and analysis of blood samples place a burden on limited human, financial and physical resources, generally a combination of rate of stunting among children younger than five years and prevalence of inadequate dietary zinc intakes may be appropriate for assessment of zinc status in South Africa, provided that all the precautions already mentioned are taken into account. All of these make it difficult to include serum zinc concentration in national studies in developing countries, but serum zinc may be measured in subsamples of the population under rigorously controlled conditions as described.

- Among pregnant and non-pregnant women the estimated prevalence of inadequate zinc intakes predicts similar levels of risk of zinc deficiency to the reported prevalence of low serum zinc concentrations[45], although in some studies intake data overestimated a biochemical deficiency[46].

- Conformity between intake and serum zinc data is inconsistent in children. It is therefore recommended that serum zinc concentrations of children should be measured[45].

- Assessment of zinc status should as far as possible be included in existing public health programmes. The benefits of increased zinc intakes on child growth and overall morbidity in populations with low zinc status have been proven, indicating that action is warranted when high prevalence of zinc deficiency is confirmed.

- Population assessment of zinc status should be repeated periodically to monitor changes in the risk of zinc deficiency and response to intervention programmes, such as fortification of staple foods[37].

References

1. Moynahan EJ. Acrodermatitis enteropathica: a lethal inherited human zinc deficiency disorder. *The Lancet* 1974; 2:399-400.

2. Grantham-McGregor SM, Ani CC. The role of micronutrients in psychomotor and cognitive development. *Brit Med Bull* 1999; 55(3) :511-527.

3. Labadarios D. (ed) National Food Composition Survey – Fortification Baseline (NFCS-FB): South Africa, 2005. Tygerberg: *NFCS*, 2007.

4. Sandstead HH. Causes of iron and zinc deficiencies and their effects on brain. *J Nutr* 2000; 130:347S-349S.

5. International Zinc Nutrition Consultative Group (IZiNcG). Assessment of the risk of zinc deficiency in populations and options for its control. Hotz C, Brown KH, Eds. *Food Nutr Bull* 2004; 25:S91-S204.

6. Samman S. Zinc. In : Mann JI, Truswell AS. (eds) *Essentials of human nutrition*. Third Edition. Oxford, Oxford University Press 2007: 138-142.

7. Gallagher ML. The nutrients and their metabolism. In: Mahan LK and Escott-Stump S, (eds). *Krause's Food Nutrition and Diet Therapy*, St Louis MI, Saunders, 2008:120-125.

8. Garnica AD. Trace metals and haemoglobin metabolism. *Ann Clin Lab* Sci 1981; 11:220-228.

9. Labbaye C, Valtiere M, Barberi T, Meccia E, Pelosi B, Condorelli GL. Differential expression and functional role of GATA-2, NF-E2, and GATA-1 in normal adult hematopoiesis. *J Clin Invest* 1995; 95:2346-2358.

10. Dash S, Brewer GJ, Oelshlegel FJ. Effect of zinc on haemoglobin binding by red blood cell membranes. *Nature* 1974; 250:251-252.

11. Nishiyama S, Kiwaki K, Miyazaki Y, Hasuda T. Zinc and IGF-1 concentrations in pregnant women with anemia before and after supplementation with iron and/or zinc. *J Am Coll Nutr* 1999; 18:261-267.

12. De Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. *Food Nutr Bull* 2007; 28:S480-484.

13. Prasad AS, Miale A Jr, Farid Z, Sandstead HH, Schulert AR. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism and hypogonadism. *J Lab Clin Med* 1963; 61:537-549.

14. Martorell R. Benefits of zinc supplementation for child growth. Am *J Clin Nutr* 2002; 75: 957-958.

15. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2002; 75:1062-1071.

16. Golden BE, Golden MHN. Plasma zinc, rate of weight gain, and the energy cost of tissue deposition in children recovering from severe malnutrition on a cow's milk or soya protein-based diet. *Am J Clin Nutr* 1981; 34:892-899.

17. Sandstead HH, Penland JG, Alcock NW, Dayal HH, Chen XC, Li JS, Zhao F, Yang JJ. Effects of repletion with zinc and other micronutrients on neuro psychologic performance and growth of Chinese children. *Am J Clin Nutr* 1998; 68:470S-475S.

18. Weisstraub G, Hertrampf E, Lopez de Romana D, Salazar G, Bugueno C, Castillo-Duran C. Plasma zinc concentration, body composition and physical activity in obese preschool children. *Biol Trace Elem Res* 2007; 118:167-174.

19. Shetty PS, James WPT. Body mass index: a measure of chronic energy deficiency in adults. *FAO Food and Nutrition Paper*. Rome: FAO, 1994, 56.

20. Shrimpton R. Zinc Deficiency. In: Semba RD, Bloem MW Eds. *Nutrition and Health in Developing Countries*. Totowa, NJ. Humana Press, 2001:307-326.

21. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr* 1998; 68(suppl):447S-479S.

22. Fairweather-Tait SJ. From absorption to excretion of minerals, to the importance of bioavailability and adaptation. *Br J Nutr* 1997; 78(suppl):S95-S100.

23. WHO. Participants' statement. WHO Consultation on Nutrition and HIV/AIDS in Africa. Durban, South Africa, 10 – 13 April 2005.

24. Costello RB, Grumstrup-Scott J. Zinc: what role might supplements play? *J Am Dietet Assoc 2000; 100:371-375.*

25. Papathakis PC, Rollins NC, Chantry CJ, Bennish ML, Brown KH. Micronutrient status during lactation in HIV-infected and HIV-uninfected South African women during the first 6 months after delivery. *Am J Clin Nutr* 2007; 85:182-192.

26. Coovadia HM, Bobat R. Zinc deficiency and supplementation in HIV/AIDS. *Nutr Res* 2002 22:179-191.

27. Dhansay MA, Marais CD, Labadarios D. Selected micronutrient status: zinc status. In: Labadarios D, Ed. *National Food Composition Survey* – Fortification Baseline (NFCS-FB): South Africa, 2005. Tygerberg: NFCS, 2007.

28. Steyn NP, Wolmarans P, Nel JH, Bourne LT. National fortification of staple foods can make a significant contribution to micronutrient intake of South African adults. *Publ Health Nutr* 2008; 11:307-13.

29. Smuts CM, Dhansay MA, Faber M, van Stuijvenberg ME, Swanevelder S, Gross R, Benade AJ. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in South African infants. *J Nutr* 2005; 135:653S-659S.

30. Oelofse A, Van Raaij JM, Benade AJ, Dhansay MA, Tolboom JJ, Hautvast JG. Disadvantaged black and coloured infants in two urban communities in the Western Cape, South Africa differ in micronutrient status. *Publ Health Nutr* 2002; 5:289-294.

31. Dannhauser A, Bester C, Joubert G, Badenhorst P, Slabber M, Badenhorst A, Du Toit E, Barnard H, Botha P, Nogabe L. Nutritional status of preschool children in informal settlements areas near Bloemfontein, South Africa. *Publ Health Nutr* 2000; 3:303-312.

32. Oldewage-Theron WH, Samuel FO, Venter CS. Zinc deficiency among the elderly attending a care centre in Sharpville, South Africa. *J Hum Nutr Diet* 2008; 21:566-574.

33. WHO/UNICEF/IAEA/IZiNCG. Executive summary. Recommendations for indicators of population zinc status. Report of WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. *Food Nutr Bull*, 2007; 28:S399-S400.

34. Hess SY, Peerson JM, King JC, Brown KH. Use of serum zinc concentration as an indicator of population zinc status. *Food Nutr Bull* 2007; 28:S403-429.

35. Brown KH. Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries. *Am J Clin Nutr* 1998; 68:425S-429S.

36. Gibson R. Zinc: The missing link in combating micronutrient malnutrition in developing countries. *Proc Nutr Soc* 2006; 65: 51-60.

37. International Zinc Nutrition Consultative Group (IZiNcG). Quantifying the risk of zinc deficiency: *Recommended indicators*. Technical brief no 01-03, Davis, CA. 2007.

38. King JC. Assessment of zinc status. *J Nutr* 1990; 120:1474-1479.

39. Garenne M, Becher H, Ye Y, Kouvate B, Muller O. Sex-specific responses to zinc supplementation in Nouna, Burkina Faso. *J Pediatr Gastroenterol Nutr* 2007; 44:619-628.

40. Falchuk KH. Effect of acute disease and ACTH on serum zinc proteins. *N Engl J Med* 1977; 296:1129-1134.

41. Brown KH, Lanata CF, Yuen ML, Peerson JM, Butron B, Lonnerdal B. Potential magnitude of the misclassification of a population's trace element status due to infection: example from a survey of young Peruvian children. *Am J Clin Nutr* 1993; 58:549-554.

42. Droke EA, Kennedy TS, Hubbs-Tait L. Potential for misclassification of micronutrient status in children participating in a Head Start programme. *J Am Diet Assoc* 2006; 106:376-382.

43. Gibson R, Hess SY, Hotz C, Brown KH. Indicators of zinc status at the population level: a review of the evidence. *Br J Nutr* 2008; 99:S14-23.

44. Lowe NM. In search of a reliable marker of zinc status – are we nearly there yet? *Nutr* 2005;21:883-884.

45. Hotz C, Peerson JM, Brown KH. Suggested lower cut-offs of serum zinc concentrations fr assessing zinc status: reanalysis of the second National Health and Nutrition Examination Sutvey data (1976-1980). *Am J Clin Nutr* 2003;78:756-764.

46. Mazariegos M, Hambidge KM, Krebs NF et al. Zinc absorption in Guatemalan schoolchildren fed normal or low-phytate maize. *Am J Clin Nutr* 2006;83:59-64.

47. Turnland JR, King JC, Keyes WR, Gong B, Michel MC. A stable isotope study of zinc absorption in young men: effects of phytate and a-cellulose. *Am J Clin Nutr* 1984;40:1071-1077.

48. Schneider JM, Fujii ML, Lamp CL, Lonnerdal B, Zidenberg-Cherr S. The prevalence of low serum zinc and copper levels and dietary habits associated with serum zinc and copper in 12-36 month-old children from low-income families at risk for iron deficiency. *J Am Diet* Assoc 2007;107:1924-1929.

49. Abebe Y, Bogale A, Hambidge KM, Stoecker BJ, Arbide I, Teshome A, Krebs NF, Westcott JE, Bailey KB, Gibson RS. Inadequate intakes of dietary zinc among pregnant women from subsistence households in Sidama, Southern Ethiopia. *Public Health Nutr* 2008;11:379-386.

50. Gibson R. *Nutritional Assessment: A Laboratory Manual.* New York: Oxford University Press, 1993.

51. Gregory J, Lowe S, Bates CJ *et al*. National Diet and Nutrition Survey. Young people aged 4 to 18 years. Volume 1: *Report of the diet and nutrition survey*. London: The Stationary Office, 2000.

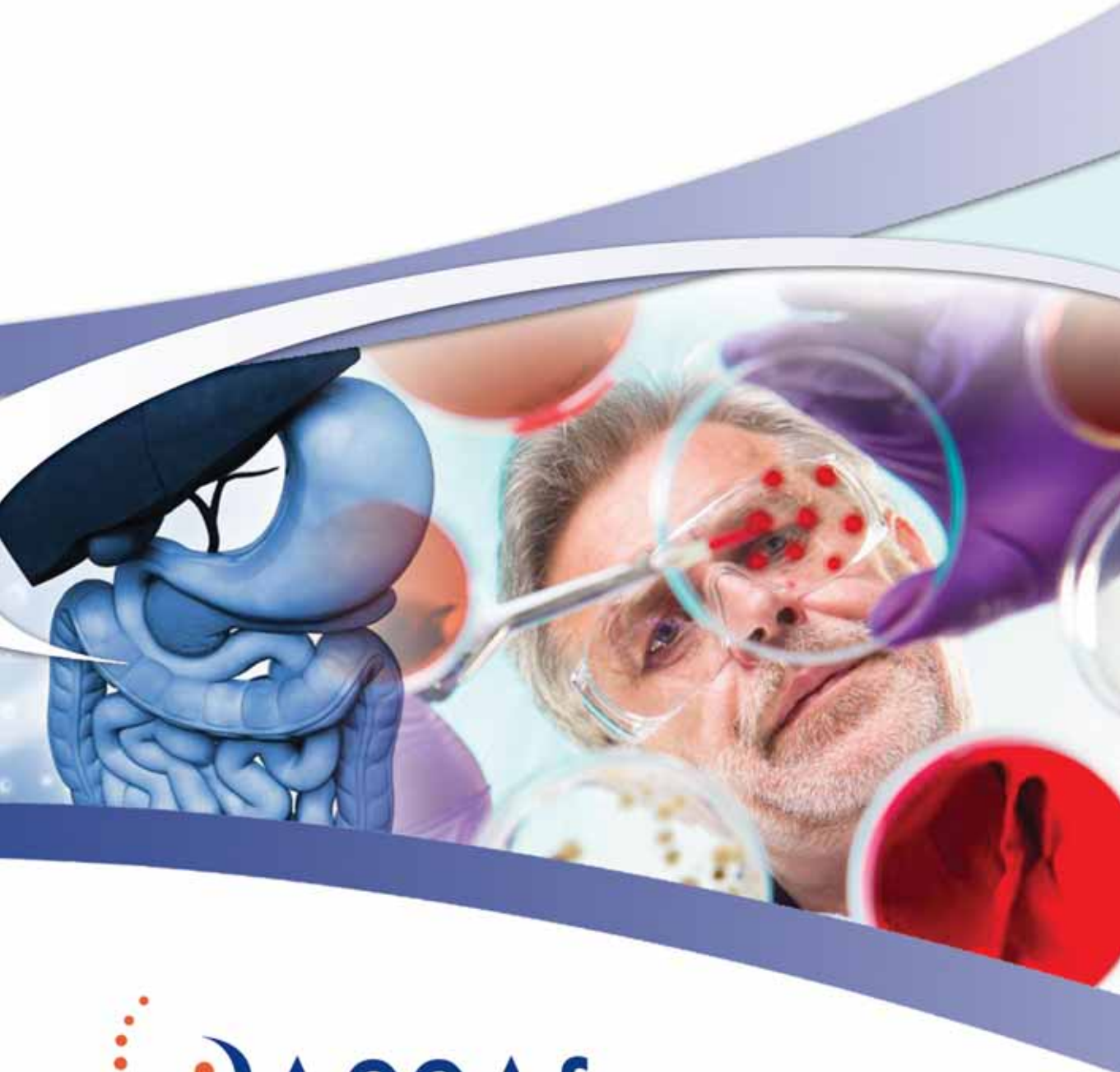
52. Hambidge KM, Abebe Y, Gibson RS, Westcott JE, Miller LV, Lei S, Stoecker BJ, Arbide I, Teshome A, Bailey KB, Krebs NF. Zinc absorption during late pregnancy in rural southern Ethiopia. *Am J Clin Nutr* 2006; 84:1102-1106.

53. De Onis M, Onyango AW, Borgi E, Garza C, Yang H. Comparison of the World Health Organisation (WHO) growth standards and the National Centre for Health Statistics/WHO international growth reference: implications for child health programmes. *Publ Health Nutr* 2006; 9:942-947.

54. Olney DK, Pollitt E, Kariger PK, Khalfan SS, Ali NS, Tielsch JM, Sazawal S, Black R, Allen LH, Stolzfus RJ. Combined iron and folic acid supplementation with or without zinc reduces time to walking unassisted among Zanzibari infants 5-11-month old. *J Nutr* 2006; 136:2427-2434.

Chapter 8

The Bowel Microbiota (Microflora) in Relation to Nutritional Assessment



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Parasitic infestations and their influences on particular micronutrients are mentioned in the relevant chapters. The focus of this chapter is on new information regading the beneficial microbiotica effects on micronutrient status

Introduction

An issue in nutritional assessment is the contribution of bowel microorganisms to the nutrition of the human host, and particularly whether that contribution is in essence a kind of constant that can be ignored or discounted in clinical terms, or whether it varies sufficiently in individuals at different times or in different circumstances, or between people, to become a factor that needs evaluation or quantitation. This topic has become a matter of increasing interest in recent times, as methods to characterise the component organisms of the microflora improve and their previously vaguely presumed roles in digestion, chemical transformations and immune functioning become better understood and quantified. The purpose of this chapter is to evaluate the contribution of the bowel microflora to micronutrient status in humans, with special reference to nutritional assessment and responses to supplementation regimens.

Physiology

The canonical view of gut physiology in relation to nutrition and diet is that the small bowel predominates in highly efficient, liquid-phase digestive and absorptive functions. The large bowel, by contrast, absorbs mainly water from indigestible and unabsorbable residual contents, in a progressively more solid-phase medium resulting in the formation of semi-solid or near-solid faeces. In addition, there is a large mass of living and dead microorganisms in the large intestine (between 1.5 and 2 kg in an adult human) which are involved in the biotransformation of many biologically active, unabsorbed endogenous and exogenous compounds in the intestinal contents. Most but not all of these resulting in inactivation or detoxification, as well as the fermentation of residual carbohydrates that escape digestion in the small intestine, including resistant starch, dietary fibre, fructo-oligo-saccharides, inulin and galacto-oligosaccharides. Fermentation of the carbohydrates is associated with inter alia the generation of short-chain fatty acids (acetic, butyric and propionic acids)[1].

From the point of view of micronutrition of the host, there have been vague ideas for many years about absorption of small but possibly significant amounts of provitamins and/or vitamins in the large intestine, either through reflux of digestive residua into the ileum from the caecum, or through poorly defined processes in the colonic mucosa itself. Vitamins K and B_{12} have been most prominent in these models, often based mainly on clinical observations[2]. For the rest, the

notion that in a species which has often been under severe micronutrient nutritional pressure, an extremely large potential source of most vitamins (released by dead microorganisms and not yet reabsorbed by their living compatriots) is regularly present in the healthy human large bowel, just beyond the reach of the absorption systems in the small bowel, has simply presented to thoughtful observers further evidence of the 'missed opportunities' of evolution.

Recent work on the microbiota colonising human intestines has begun to point to much greater complexity in the intestinal digestive and absorptive systems[3]. The gut microbiome comprises 100 times more cells than there are somatic and germ cells in the host body, and probably 100 times more genes coding for protein products. It represents a kind of self-sustaining 'organ' symbiotically added to those of the host, with different cell types able in complex ways to communicate with each other and the host, consuming and generating energy, and carrying out vast numbers of chemical transformations, some of which generate products which enter and are further metabolised by host tissues and may even be excreted in the urine[4]. The previous view that these microorganisms are 'commensals' (benefiting by their location but not benefiting their host) has now shifted to one of 'mutualism' (both partners benefiting). The large bowel contents are now seen as a specialised bioreactor, largely anaerobic, that degrades large quantities of host-indigestible polysaccharides, including plant-derived cellulose, hemicellulose, pectin and resistant starches[1].

Many kinds of microorganisms are present in the bowel contents, including eukaryotes like fungi and protozoa, as well as viruses that are only now being investigated[5]. Bacteria vastly predominate and achieve very high cell densities, amongst the highest known in any ecosystem[3]. They are nearly all drawn from only two of the 55 bacterial divisions, compatible with highly selective colonisation mechanisms. The genus called *Bacteroides* and the genera called *Clostridium* and *Eubacterium* constitute the majority of the organisms. There appear altogether to be at least 7 000 strains of bacteria, many representing closely similar relatives. Because many of the individual bacterial species cannot be cultured and isolated and characterised *in vitro*, information about them has come mostly from 'brute force' methods like 'next-generation' sequencing of short DNA sequences from mixed intestinal samples, called metagenomic sequences[6], and more recently as putative reference genomes of about 180 specific bacterial species, most from the gut[7].

Important preliminary generalisations can already be made about the microbiota of the human large bowel. Newborns have virtually sterile intestines, colonisation begins soon after birth, and adult patterns are attained by the age of two years. The organismal composition differs between individuals, and groups of individuals[4]; it varies from time to time in the same individual, adapting to different dietary regimens; and it is subject to change imposed by certain external interventions, such as the administration of broad-spectrum antibiotics. The intestinal mucosa exerts subtle controls on gut microorganisms through a variety of compounds released into its adhering mucus layer[8], and the bacterial populations in turn affect mucosal immune processes[9]. An average of about 10% of food energy is generated through large-bowel fermentations, mostly in the form of short-chain fatty acids, on which the colonic mucosa is wholly dependent for its continued vitality. This value varies considerably, however, contributing to the notion that the *de facto* caloric densities of ingested foods are not necessarily the same in different people.

The subject of interest to this report is the extent and significance of the absorption of micronutrients generated by microorganisms in their intestinal bioreactors. Estimates suggest that the small intestine, especially the ileum, contains a diverse selection of the typical bowel microorganisms, at a density of about $1X10^4$ to $1X10^8$ per ml, while the colon has 1X1012 organisms per ml as indicated by Gibson and Rastall[10]. The transit times affecting these densities in these two locations generally differ by ten-fold, 2-3 hours in the small intestine, and 24-72 hours in the large intestine. The organisms use both food constituents and mucus as feedstocks. These data clearly indicate that microbiota in the distal small intestine can generate small-molecule products, amongst which can be vitamers (provitamins, vitamins and co-enzymes), that can be absorbed by specific carrier-mediated processes in the mucosal walls. There is no need for reflux of large-bowel organisms from the caecum to achieve this host 'benefit', although it may also occur and may also be significant.

Extensive work on the bacterial genomes present in human intestines has revealed specific enrichment of clusters if genes involved in the biosynthesis of deoxyxylulose 5'phosphate (DXP) and isopentenyl pyrophosphate (IPP)[11]. These pathways lead to the formation of a number of vitamins, and their enrichment may signify evolutionary pressure to supply vitamins or their precursors to their hosts, as well as to their own rapidly dividing progeny.

Recent findings extend this picture by identifying and characterising specific transporters in the large intestinal mucosa for a variety of water-soluble micronutrients, including thiamine, folate, riboflavin, biotin, and pantothenic acid[12]. Transcriptional control mechanisms exist, pointing to physiological control of the processes concerned. Clinically significant mutations have been observed in experimental animals. The authors conclude that "water-soluble vitamin absorption occurs in the large intestine, involving regulated and specific mechanisms, interference with which can lead to deficiency. The large intestine is capable of absorbing water-soluble vitamins that are synthesised by the normal microflora". It should be noted that the mucosa of the large intestine is a rather large 'organ' by mass in its own right, and it is possible that some of the absorbed nutrients (as in the case of butyrate) are utilised by the epithelial cells and not translocated to the portal circulation for whole-body use.

Deficiencies or Depletion States

The assumption has often been made in clinical practice that deliberate partial sterilisation of the bowel (before certain types of abdominal operations) or as a significant side-effect of the administration of broad-spectrum antibiotics for infections elsewhere, influences the micronutrient status of the patient concerned. Vitamin K or multi-vitamin preparations are often given to such patients as a precaution. Re-population of the bowel with orally administered 'desirable' microorganisms ('probiotics') after depletion is also widely practised, sometimes accompanied or replaced by nutrients that are supposed to support the growth of desirable bacteria ('prebiotics'). A massive retail industry has also grown up around the supposed health benefits of 'probiotics' and 'prebiotics' in the absence of any prior depletion of microbial flora[10]. All of this has been done in the virtually complete absence of any quantitative information about the mass or nature of the actual microflora in the situation concerned.

It has become possible to compare metagenomic sequences of bowel organisms isolated from faecal samples obtained from different people, as mentioned above. The availability of

reference genomes of about one-fifth of the bacterial species commonly found as commensals in human intestines (See above) may also soon permit more specific characterisations to be performed. This may also permit the proper testing of the outcomes of interventions involving 'probiotics' and 'prebiotics' in clinical practice and in private health promotion.

Recent studies by members of the Metagenomics of the Human Intestinal Tract (MetaHIT) Consortium of the European Union found evidence of three stable clusters, each with a main 'driver' genus, Bacteroides (Enterotype 1), Prevotella (Enterotype 2) and Ruminococcus (Enterotype 3)[6]. The dynamic nature of these clusters has not been established, but it is clear that advanced age, vegetarianism, administration of antibiotics, and obesity are associated with marked changes in at least some members of the microbiota[13].

A detailed study of faecal samples from individuals varying in age and geographic/cultural locations (USA, Venezuelan Amazon jungle and Malawi) revealed similar developmental features in early childhood in all groups, including age-associated increases in the bacterial systems for vitamin synthesis and metabolism[14]. Both the childhood and adult microbiomes of the US group could be clearly distinguished from those of the other geographic groups, however. Particularly relevant were differences in genes representing different vitamin-related processes at different ages in the different groups, some of them possibly correlating with nutritional status of the groups concerned.

What future possibilities offer that are relevant to nutritional assessment will mainly lie in biomarkers and measurable parameters that accurately reflect the activities of bowel microorganisms in relation to nutrient metabolism. Li *et al*.[4] have shown through an approach based on 'functional metabolomics' that certain ultimate end-products of microbiotal metabolism may be readily detectable and measurable in the urine and related to the presence in the gut of particular organisms. This may lead to ways of assessing the contribution of the microbiota to host metabolism in individual people, including micronutrient supply and distribution. A variant approach would be to assess the impact of various degrees of micronutrient or 'probiotic'/'prebiotic' supplementation on the patterns of excreted compounds.

Recommendations

1. We recommend that the local biomedical community carefully monitors the growing literature on intestinal microorganisms, particularly that which deals with the measurement of a variety of bacterial metabolites in the urine, and most particularly that which will permit the assessment of bacterial cell mass in the small and large intestines, qualitative aspects of the populations present, and quantitative aspects of the metabolic contribution of the microflora to the nutritional status of their hosts.

2. We also recommend that deliberate attempts should be made to develop and extend local expertise in the field of the human microbiome and its importance in health and disease.

References

1. Cummings JH, Mann JI. Carbohydrates. In: Mann JI, Truswell AS. (eds) Essentials of human nutrition. 3rd edition. Oxford, Oxford University Press. 2007:9-32.

2. Ball GFM. *Vitamins: their Role in the Human Body*. Blackwells Publishing, Oxford, UK, 2004.

3. Baeckhed, F, Ley, R E, Sonnenburg, J L, Peterson, D A and Gordon, J I. *Science* 2005; 307: 1915-1919.

4. Li M, Wang B, Zhang M *et al*. Symbiotic gut microtypes modulate human metabolic phenotypes. *Proc Acad Nat Sci* 2008;105: 2117-2122.

5. Cadwell K, Patel KK, Maloney NS *et al*. (2010) Virus-plus-susceptibility gene interaction determines Crohn's Disease gene Atg16L1 phenotypes in intestine. *Cell* 2010; 141:1135-1145.

6. Qin J, Li R, Raes J, *et al.* A human gut microbial gene catalogue established by metagenomic sequencing sequencing. *Nature* 2010; 464 : 59-65.

7. Nelson KE, Weinstock GM, Highlander SK, *et al.* A catalogue of reference genomes from the human microbiome. *Science* 2010; 328:994-999.

8. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria: direct expression of an intestinal bacterial lectin. *Science* 2006; 313:1126-1130.

9. Hayashi T. Breaking the barrier between commensalism and pathogenicity. *Science* 2006; 313: 772-773.

10. Gibson GR, Rastall RA. (2004) When we eat, which bacteria should we be feeding? *ASM News* 2004; 70: 224-231.

11. Gill SR, Pop M, DeBoy RT, *et al*. Metagenomic analysis of the human distal gut microbiome. *Science* 2006; 312: 1355-1359.

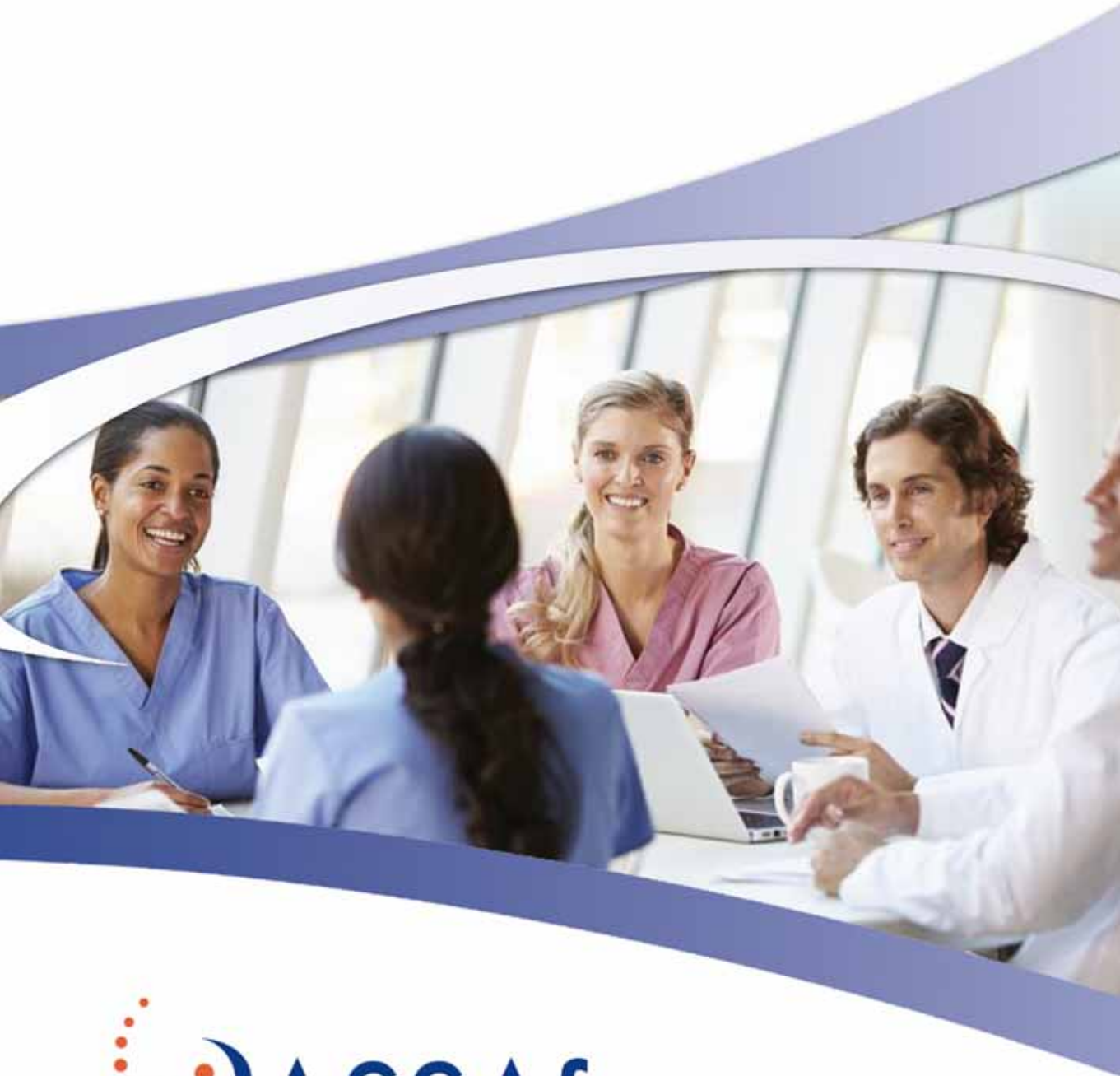
12. Said HM, Mohammed, ZM. Intestinal absorption of water-soluble vitamins: an update. *Curr. Opin. Gastroenterol* 2006; 22: 140-146.

13. Thomas, LV & Ockjuizen, T. New insights into the impact of the intestinal microbiota on health and disease. *Brit J Nutr* 2012; 107: S1-S13.

14. Yatsunenko, T, Rey, F E, Manary, MJ *et al.* Human gut nicrobiome viewed across age and geography. *Nature* 2012; 486: 222-227

Chapter 9

Findings, Discussion and Conclusions



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Introduction

The objectives of this consensus study on six selected micronutrients were to examine:

- how modern developments in our understanding of the physiology and pathophysiology of these micronutrients have influenced developments to improve assessment of their status;
- which of the methods recommended will give maximum information on nutritional and actual depletion/repletion status;
- which methods are sufficiently reliable and affordable as to have the ability to be used in different settings and mass application; and
- which factors will influence the application of the methods and interpretation of the information they produce.

These objectives were formulated to assist in the development of recommendations that will improve the assessment of micronutrient malnutrition in individuals and populations. The six micronutrients chosen were shown in a previous consensus study[1] to contribute to the malnutrition epidemic in South Africa, with a potential to impact on the HIV/AIDS and TB pandemics.

In this chapter, the main findings of the previous chapters will be integrated, highlighting the methods recommended, as well as the gaps in our knowledge and understanding of deficiency states.

The general findings and principles that collectively emerged from the different chapters will be integrated first. This is followed by a discussion on micronutrient 'balance', based on a model of a whole-body systems approach and response to vitamin intake. In conclusion, the specific methods recommended for each of the six micronutrients will be summarised to support the recommendations that are made in Chapter 10.

General Findings and Principles regarding the Six Micronutrients

Micronutrient Deficiency in South Africa

The first important finding is that despite the limited information available, there are indications that the micronutrients chosen may be deficient in many groups of South Africans. This micronutrient malnutrition is possibly not confined to the previously perceived vulnerable groups (infants, children, women of reproductive age and the elderly), but for various reasons may also be prevalent in apparently healthy and often overweight and obese South Africans. The reasons include poverty, food and nutrition insecurity, rapidly changing lifestyles and behaviours (the nutrition transition), as well as increased availability and affordability of energy-dense, micronutrient-poor foods.

Roles in HIV/AIDS and TB

The second important observation is that as shown in the previous consensus study[1] these micronutrients may be directly or indirectly involved in the susceptibility to and/or development of HIV/AIDS and TB, probably as a result of effects on the immune system. The precise role of micronutrients in helping to maintain the immune system, and in helping the body to respond adequately to environmental stressors to maintain homeostasis, is still not clear, however. In some instances, for example iron, depletion of the micronutrient is associated with susceptibility to HIV infection and progression of the disease, but the mechanisms and relationships of cause and effect are not understood.

Despite our lack of knowledge about the mechanisms, it is clear that malnutrition in respect of these micronutrients impacts on the development of HIV/AIDS and TB, and therefore has a potentially important role in prevention and treatment. This emphasises the need to accurately assess the status of these micronutrients in the population, as well as in affected individuals.

Non-classical or 'New' Functions of the Selected Micronutrients

A third general observation was that in addition to their well-established functions, related to specific clinical symptoms and signs arising in depletion states, and their effects on immune responses mentioned above, the selected micronutrients may also have a protective role against the development of non-communicable diseases (NCDs), as well as in their treatment.

The mechanisms through which these micronutrients may protect against the development of many NCDs, including diabetes, cardiovascular disease and some diet-related cancers, are not clear. It could be related to anti-oxidant functions or to effects on the immune system. The NCDs are all multifactorial in aetiology: a large variety of interacting environmental and behavioural exposures collectively influence their development over long periods in genetically susceptible individuals. This makes it difficult to design studies in which the contribution of a specific nutrient can be determined.

The new perception that micronutrients have additional benefits at intakes higher than those required to prevent classical deficiency syndromes, has already impacted on micronutrient recommendations[2].

The Use of a Combination of Methods

The fourth general observation was that it is often necessary to use more than one method to assess micronutrient status. In some instances, for example in the case of vitamin D, one laboratory or biochemical method may in fact be sufficient to determine the status with respect to a particular micronutrient. A combination of at least two or more types of methods is, however, recommended in the case of most micronutrients. As background for the recommendations in Chapter 10, a summary of the present state of the classes of nutritional assessment methodology[3-5] is given in Box 9.1.

Box 9.1 Summary of General Nutritional Assessment Methods	
Nutritional assessment methods	**Notes regarding use, interpretation**
Nutritional assessment methods	
Dietary and nutrient intake measurement	
Using validated questionnaires, interviews and recording methods to assess dietary patterns, food and nutrient intake over specific times (habitually or shorter): 24-hour recalls; weighed food records, diet histories, qualitative and quantitative food frequency questionnaires (QFFQ), as well as short questionnaires for specific foods or groups of food. For calculation of nutrient intake from reported food data, acceptable food composition tables must be available. Assessment of sunlight exposure in the case of vitamin D status assessment	The most non-invasive method Low-cost, high through-put Suitable in all settings Have many limitations: under and over-reporting, impaired memory, etc Values of recommended intakes for inter-pretation and comparisons must be available Reported intakes can be verified by using biomarkers: metabolomic finger-printing of the food metabolome, using spot urine samples, may identify new biomarkers in the future* Validated instruments and good food composition tables available in SA
Anthropometry	
Measurement of physical dimensions and gross body composition; usually weight and height (for age), body circumferences (waist, hip, mid-arm, etc) as well as 7 skin folds (to calculate fat %). Several indices and ratios are calculated from these measurements	Non-invasive and reliable Cut-points and standards available Low-cost, high through-put Suitable in all settings, especially to monitor growth of children and assess hospital patients Indicative of past nutritional history Standardised equipment should be used
Laboratory methods	
Static and functional tests, using body fluids (capillary blood, dried blood spots, venous blood, serum, plasma, spot urine, 24-hour urine or saliva). Tests can be behavioural, physiological, biochemical, nutrient challenge tests, or using genomics and/or a	Provide "unbiased" scientific data May be too invasive For some nutrients, cut-points and normal ranges available But may be non-specific (other contributing and confounding factors influence results)

metabolomics approach* to identify dietary intake markers and/or markers of nutritional status	May be difficult to interpret because of a lack of standards and cut-points* Often expensive equipment and 'sophisticated' expertise needed
Clinical methods	
Medical history and physical (clinical) examinations looking for well-established signs and symptoms of micronutrient deficiencies (skin, hair, tongue, eyes, etc.)	Suitable for all settings Health professionals must be specially trained to recognise symptoms
Ecological methods	
Documenting all other factors known to influence nutritional status	Non-invasive Suitable for all settings Non-specific

*Proof-of-principle has been provided that metabolomic technologies are successful in identifying markers for food intake, by using either a 'targeted top-down' or 'untargeted bottom-up' approach to examine the food metabolome in spot urine samples[5]. Equipment to do metabolite fingerprinting is expensive (flow infusion electro spray mass spectrometry and gas chromatography-time-of-flight-mass spectrometry). Further developments that are necessary before use of this method can be recommended include validation of the biomarkers concerned, establishment of cut-off values, development of rapid and inexpensive assays, as well as testing their utility in different settings (cohort studies and dietary surveys)[5].

Present Advantages and Limitations of Laboratory Tests

The fifth general observation was that laboratory tests have become preferred for reasons of affordability, feasibility, reliability, relevance and conclusiveness. These tests have usually been derived from a detailed understanding of the physiological phenomena underlying the absorption, intravascular transport, organ metabolism and pre-excretory biotransformation of the micronutrient concerned. In clinical settings, these tests should be used by physicians to optimise diagnosis and treatment of patients.

As our knowledge increases, the tests available will undoubtedly become better and more informative, and will include genetic assessments now in their infancy. In general, there is still at present a lack of useful markers for the detailed micronutrient status of individuals. In general, complex and expensive tests that demonstrate functional deficits directly correctable with vitamin administration or addition, are superior to simpler and cheaper 'snapshot' measurements of the levels in blood, plasma and/or urine of micronutrients or their metabolites.

There is no doubt that the ready availability of tests that are both accurate, informative, affordable and usable in field settings, is a high priority for South Africa, a country where 'hidden hunger' (equivalent to functional deficiencies of one or more micronutrients) is very common. Moreover, clinical research relies heavily on establishing valid inclusion criteria, baselines and outcomes for nutritional interventions in chronic infectious diseases like HIV infection and clinical tuberculosis.

Different Levels of Depletion/Repletion

The sixth general observation was that for each of these micronutrients, there are different levels or stages of depletion and repletion. The physiology that underlies these stages is discussed in the next section of this chapter. Of importance here is that these stages may need different methodologies to assess the particular stage. Blood values of a micronutrient will not necessarily reflect intakes or status. This is *inter alia* related to the fact that the body has well-co-ordinated, tightly controlled regulatory systems in place to protect blood levels of nutrients within narrow ranges, as well as different storage mechanisms for different nutrients.

The stages of depletion and repletion and the suggested assessment methods are briefly summarised in Box 9.2.

Box 9.2 Stages of Depletion/Repletion of Nutrient Status and Recommended Type of Assessment Methods for Each (Adapted from Gibson[3])

Depletion/repletion stage

1. Dietary inadequacy
2. Decreased level in reserve tissue store
3. Decreased level in body fluids
4. Decreased functional level in tissues
5. Decreased concentrations and activity of nutrient-dependent enzymes or mRNA for relevant protein synthesis
6. Functional change
7. Clinical symptoms
8. Anatomical signs

Suggested assessment method for stages 1-8

1. Dietary intake
2. Laboratory: biochemical
3. Laboratory: biochemical
4. Anthropometry and/or biochemical
5. Laboratory: biochemical and molecular techniques (static and/or metabolic functional challenge tests)
6. Physiological and behavioural
7. Clinical (e.g. anthropometry for growth)
8. Clinical (e.g. anthropometry for growth)

Assessment of Individuals versus Populations

The seventh general observation was that assessment in individuals and populations requires different approaches. Individuals are assessed for diagnosis to prescribe treatment, e.g. in hospital and clinical settings, where established protocols or algorithms are used. Populations are assessed for screening as a basis for policy and large-scale interventions. The choice of an assessment method will therefore be determined by the purpose. The results of applying specific methodologies should feed into and inform nutritional assessment systems[3], such as surveys, surveillance, screening, interventions and in clinical settings. The emphasis should be on selection of methodologies that are low-cost with high through-puts.

The interpretation of the measured variable may also differ for individuals and populations. For example, to evaluate the adequacy of the diet of individuals, intakes should be compared to the Individual Nutrient Level (INL_{98}), previously known as the recommended dietary allowance (RDA)[6]. To evaluate adequate intakes of the population or specific groups, also calculating the proportion of people below or above recommended intakes, the average nutrient requirement (ANR)[6] should be used. The ANR (as well as the upper nutrient level (UNL)) are derived from estimates of amounts needed for a specific physiological criterion such as tissue stores, metabolic balance, or a biochemical function. The INLx is derived from the distribution of the ANR, where x is a percentile chosen which indicates the likelihood of meeting an individual's nutrient requirement, historically 98%. If two standard deviations of the requirement are added to the ANR, the likelihood of meeting an individual's needs is 98%, meaning that the risk of inadequacy is 2%[6].

Interpretation of Test Results: Possible Confounders

The last (and eighth) general observation that emerged from the individual chapters is related to the many possible confounders that should be considered when interpreting test results. These include, for example, the presence of inflammation and catabolic states, which have been discussed in detail in the respective chapters. The interpretation of measurement data should be based on an understanding of the way the human body 'handles' micronutrients. In the discussion secton below, this is explained, using vitamins as an example how conceptual thinking should influence the interpretation of nutrient assessment data.

Discussion

In this section, our understanding of the paradigm of micronutrient turnover and how the body responds to different levels of intake is described to serve as background for the interpretation of measurement results. A living human body is a 'system' in respect of any given micronutrient:

- At any one time, the body has a **total content** or 'whole body pool' of the vitamin and all its vitamers (defined as all compounds capable of being converted into physiologically active forms of the vitamin).

- **Additions** are continually made to that 'whole body pool' by the intake of **food** containing the vitamin and/or its vitamers, AND/OR as **supplements or therapies** taken either orally or parenterally, AND/OR as **biosyntheses** effected in a few cases by the body itself body (Vit D, B_3) AND/OR by microbiota mainly located in the intestines.

- Losses from the 'whole body pool' take place mainly in **faeces**, either as unabsorbed vitamins and vitamers originally derived from food (very little), or as inactive metabolic breakdown products, or as microbiotal residues in the faeces (very substantial); AND/OR in the **urine** as vitamin/vitamer 'surpluses' (usually as a result of oral supplementation), or as inactive metabolic breakdown products (varying quantities).

Shifts in location of parts of the 'whole body pool' take place continuously:

(i) from the intestines through the portal blood to the liver and then to other organs and tissues; AND/OR

(ii) from extra-intestinal synthesis sites (Vit D) or parenteral administration sites through the peripheral circulation to all organs and tissues;

(iii) from 'stores' in tissues capable of holding 'surplus' vitamin o vitamer (e.g. liver in respect of Vit A, B_{12}) through the blood to other organs and tissues; AND/OR

(iv) from organs or tissues (all with individually varying steady-state content) through the blood to other organs.

- A body is in **balance** in respect of a vitamin if the additions are equal to the losses over a period of time, e.g. a day, a week or a month.

- The response of the body to a **surplus of additions** of a vitamin over a period of time is:

(i) uptake and physiological 'storage' (non-toxic) in one or more organs if this option is available; AND/OR

(ii) increased catabolism AND/OR excretion from the body; AND/OR

(iii) in some cases, uptake and progressively toxic accumulation in one or more organs and tissues.

- The response of the body to a shortfall in vitamin balance over a period of time is:

(i) decreased catabolism AND/OR excretion from the body; AND/OR

(ii) decreases in stored vitamin/vitamers in physiological 'storage' organ(s) or tissue(s); AND/OR

(iii) a fall in the blood/plasma content of the vitamin/vitamers, further lowering catabolism and lowering tissue utilisation if these are gradient-driven; AND/OR

(iv) progressively depleting cells/tissues/organs of their vitamin/vitamer content, depending on the relative 'tenacity' of each cell-type in retaining its content despite lowered supply/concentration in the immediate environment, which overall 'tenacity' also reflects internal competition between binding components.

- Generally, detectable functional disorder arising from negative whole body balance will first affect cells, tissues or organs with low relative 'tenacity', and in terms of intracellular components with low affinity for the vitamin/vitamer concerned. This will be the mildest and earliest level of clinical symptoms and signs, or there may be no detectable disorder (sub or pre-clinical levels of deficiency).

- Worsening of the imbalance will progressively affect cells, tissues and organs with higher relative 'tenacity' for retention of the vitamin or vitamer, and inside them the components with higher intracellular affinity for these compounds – this will be a more advanced (moderate) level of deficiency in terms of clinical symptoms and signs, but may still be sub or pre-clinical.

- In both of the preceding (respectively **mild and moderate**) **deficiency** levels, there may already be a reduction in urinary and/or faecal excretion of the vitamin/vitamer or their metabolites, and, if blood/plasma levels are not maintained from 'storage' sites and/or from net tissue losses associated with the deteriorating condition of the subject, lowered circulating levels of these compounds also; some tissues may have reduced mildly or moderately content as measured by biopsy analysis.

- **Serious deficiency** (heavily negative body balance of intake *versus* excretion) will be reflected by a frankly clinical, full spectrum of clinical symptoms and signs, perhaps dominated by some florid organ or tissue failures over others, as well as diminished urinary and/or faecal excretion of vitamin or vitamer or metabolites, lowered blood/plasma levels of any or all of these compounds, and also severely reduced tissue levels as measured by biopsy analysis.

- In each of the above progressively worse deficiency states, the metabolic dysfunction associated with a shortfall in vitamin/vitamer (co-enzyme) supply may result in a measurably significant increase or decrease in the concentration of a blood/plasma component, and/or a urinary or faecal excretory component (which equals a 'windfall' phenomenon in terms of a **deficiency biomarker**).

- In some cases, there may be another kind of 'windfall' phenomenon in the form of an administrable **'challenge' test** that reveals a particular state of deficiency with respect to a particular vitamin.

- It must be borne in mind that the **microbiota** (typically 1-2 kg in mass) are NOT part of the 'whole body balances' described above, excepting for their role in providing part of the biosynthetic input to the body content or in (sometimes) creating catabolised compounds in the faeces.

This general model for sequential stages of a negative whole body balance of a vitamin or mineral can also be extended to progressively more **positive balances**, but this will not be done here. However, in the individual chapters, possible positive balance (e.g. of iron) and toxic intake levels (e.g. vitamin A) are mentioned.

The above model for increasing levels of vitamin deficiency can be used to map out the kinds of detectable clinical symptoms and signs, and measurable parameters in urine/faeces, blood/plasma and tissue biopsy, that would be associated with a refined capacity to characterise the precise status of any subject with respect to a particular vitamin. It would be particularly helpful in mapping the so-called sub or pre-clinical levels of deficiency. The model may also guide the search for new tests or measures to probe these prevalent states, in terms of **secondary susceptibilities** (e.g. to infection of the airways or intestines) or other clinical significant epi-phenomena.

An important background consideration is the unsatisfactory status of the working definition of 'health'. It has been argued that the WHO concept of 'health equals well-being and the absence of disease' should be modified in favour of a more dynamic concept of 'health = ability to adapt to internal and external stimuli in order to limit the loss of homeostasis'[7]. The main implication is that challenge-type tests are more indicative than snap-shots at any one time. The above model can be adapted to accommodate this approach, and in fact agrees with the notion that sub and pre-clinical vitamin deficiency states are best defined under challenge conditions.

Conclusions: Summary of Recommended Methodologies to Assess Status of Each Nutrient

In conclusion, the reported findings regarding the assessment of the status of the six selected micronutrients are briefly summarised in Tables 9.1 – 9.6 to serve as basis for recommendations.

Table 9.1 Assessment of Vitamin A Status

Methods	Remarks
Dietary intakes: 24-hour recalls; food frequency questionnaires; short questionnaires specially for vitamin A and also other interacting nutrients	Reference intakes given in Table 2.1; SA food composition tables use appropriate conversion factors to calculate vitamin A from carotenoids in foods
Laboratory (biochemical) methods: retinol, RBP, TTR, RBP:TTR ratio, in plasma, serum, dried blood spots where overt deficiency is absent; RBP measured with HPLC (or HPCE for dried blood spots); most common method in absence of infection	Cut-points for normal ranges and for mild, moderate and low status (overt deficiencies) given in Table 2.2; RBP also a negative acute-phase protein and values must be interpreted with care; validation of the use of dried blood spots ongoing
Retinol in breast milk: retinol palmitate using 3,4-didehydroretinyl acetate as internal marker	Levels < 27.9 micro mol/g fat indicative of individual and population risk of vitamin A deficiency
Retinoic acid: Limited use in routine measurements	Levels 1.25% of that of retinol
Clinical methods: Impression cytology and dark adaptation	Used to assess overt vitamin A deficiency; Special training of observers needed
Functional dose-response methods: Relative dose response and modified dose response methods available	Indirect methods to assess liver reserves; Response >20% indicate deficiency
Stable isotope methods: Assess total body pool size	Time consuming and costly; appropriate in research settings

Table 9.2 Assessment of Vitamin D Status

Methods	Remarks
Dietary intakes: not appropriate because of endogenous synthesis	Only few SA foods fortified with vitamin D; South Africans dependent on sunlight exposure to maintain status. Reference intakes given in Table 3.1
Laboratory (biochemical) methods: serum or plasma 25(OH)D with ELISA or other methods; serum 1,25$(OH)_2$D also possible but less informative of vitamin D status	Values of 25(OH)D < 25 nmol/L indicates deficiency; 25-50 nmol/L: low or borderline status; > 50 nmol/L indicates normal vitamin D status

Table 9.3 Assessment of Folate Status

Methods	Remarks
Dietary intakes: Usual methods applicable	SA food composition tables give folate values of SA foods
Haematology	Robust, affordable, reliable (in good hands), poor sensitivity and specificity, for individuals only
Biochemical: Serum folate	Robust, affordable, reliable, index of folate intake, suitable for individuals and population. Low in acute and chronic inflammation
Biochemical: Red cell folate	Reasonable robust and reliable, affordable, index of folate stores, suitable for individuals and population. Low in chronic inflammation
Biochemical: Homocysteine	Fasting blood sample required; reasonable reliable, expensive, non-specific to exclude deficiency, individual only

Table 9.4 Assessment of Selenium Status

Methods	Remarks
Dietary intakes: Usual methods suitable. Recommended intakes established (see Chapter 5)	The SA food composition tables incomplete regarding Se values of some foods: therefore at present not suitable to measure intake in SA. Differences in soil Se content can influence values
Laboratory (biochemical) methods: Whole blood selenium Plasma selenium Functional markers of SE status: Blood GSHPx and selenoprotein P Urinary Se Hair and toenail Se	Plasma Se most often used; reflects short-term changes in Se intake; reference values not established. Choice of marker depends on Se function being investigated. Hair and toenail Se reflect long-term status, but Se-containing shampoos may be a confounding factor in hair analyses

Table 9.5 Assessment of Iron Status

Methods	Remarks
Dietary intakes: Usual intake methods plus special short questionnaires available for iron	SA food composition tables give values for iron of all food sources; computer programme give haem and non-haem iron separately. Reference intakes in Table 6.1
Haematology: Red cell distribution width	Indicates type of anaemia. Expensive equipment; for individuals
Laboratory (biochemical) methods **Haemoglobin**	Low sensitivity and specificity; cut-points available; low cost; widely used. Suitable for population assessment; does not pick up mild and moderate iron status
Laboratory (biochemical) methods **Serum ferritin**	Good indicator of iron status; cut-points available; increases during inflammation; suitable for both individuals and populations
Laboratory (biochemical) methods **Serum transferrin receptor**	Semi-quantitative measure of iron deficiency even in presence of inflammation. Indicates the balance between iron requirement and supply
Laboratory (biochemical) methods **Serum or plasma iron**	Measures iron bound to transferrin in circulation and indicates iron supply to tissues; diurnal and postprandial variation; low in chronic disease; susceptible to sample contamination

Table 9.4 Assessment of Selenium Status

Methods	Remarks
Other methods	Often expensive equipment needed. Suitable for individuals and research
Red cell distribution width	May be affected by inflammation and thalassaemia
Reticulocyte haemoglobin concentration	Sensitive to recent deficiency; expensive equipment needed
Erythrocyte and zinc protoporphyrin	Useful in children; increased with inflammation and lead poisoning
Total iron binding capacity (TIBC)	Measures capacity of circulating transferrin to bind iron; increased with iron deficiency but values overlap with those during normal status
Transferrin saturation	Low in chronic disease; postprandial variation

Table 9.6 Assessment of Zinc Status

Methods	Remarks
Dietary intake: Usual methods applicable. Dietary patterns also need to assess bioavailability of zinc, because of inhibitors of absorption in many plant foods	Dietary deficiency is the most likely cause of zinc deficiency. SA food composition tables give zinc content of foods. The IZiNCG EARs of zinc requirements are shown in Table 7.2
Laboratory (biochemical) methods: Serum or plasma zinc	Serum or plasma zinc a good indicator of zinc status. Reference values given in Table 7.1 and the many technical issues for sample collection and also interpretation of data given in Chapter 7 and should be followed
Functional indicators: Growth of children	Non-specific; Stunting has many causes, of which one is a dietary deficieny of zinc

Concluding Remarks

The summary and integration of the information from Chapters 1 to 8, confirmed the presence of micronutrient deficiencies in South Africa, in particular deficiencies of the six selected micronutrients. From these chapters it can be concluded that, although not always optimal, there are suitable methods available to assess the status of these micronutrients. It is also clear that in many cases more precise methods are needed. The increased knowledge about the metabolism and physiology of these micronutrients, especially their role in maintaining the immune system and protecting the body against both infectious and non-communicable diseases, should help to motivate and stimulate further research in the development of appropriate new methods. Recent progress in using genetic, proteomic and metabolomic technologies to identify suitable markers to assess intake, as well as status at all levels of depletion/repletion of micronutrient nutrition are promising.

To effectively address the malnutrition-poverty cycle in sub-Saharan Africa, Prof Alan Jackson[8] pleads for "a balanced approach to food and farming policy and social protection policy. For example, any food fortification policy has the potential for widespread direct and indirect effects that may be both positive and negative. The ability to anticipate, recognise and address these outcomes in a timely way is potentially of very great importance, but the assurance of effective integration of policies and opportunities represents a high level responsibility that may not always be adequately recognised and protected. The penetration and impact of policies in practice should be the subject of ongoing monitoring and evaluation. The special role played by women in food systems should be explicitly acknowledged to ensure that they are adequately enabled, empowered and appropriately educated. This has obvious implications for their health during pregnancy, the promotion of exclusive breastfeeding, reliable infant and young child feeding practices. The problems of chronic disease related to early child growth and the impact of early interventions which have been found to be effective should be seen as direct benefits of the ability to assess nutritional status better".

References

1 Academy of Science of South Africa (ASSAf). *HIV/AIDS, TB and Nutrition: Scientific enquiry into the nutritional influences on human immunity with special reference to HIV infection and active TB in South Africa,* 2007.

2 Pijls L, Ashwell M, Lambert J. EURAKA – A network of excellence to align European micronutrient recommendations. *Food Chem* 2009; 113(3):748-763.

3 Gibson RS. *Principles of nutritional assessment. (2nd edition)* Oxford, Oxford University Press. 2005:1-908.

4 Thomas LV, Ockhuizen T. New insights into the impact of the intestinal microbiotica on health and disease: a symposium report. *Brit J Nutr* 2012; 107(Suppl 1):S1-S13.

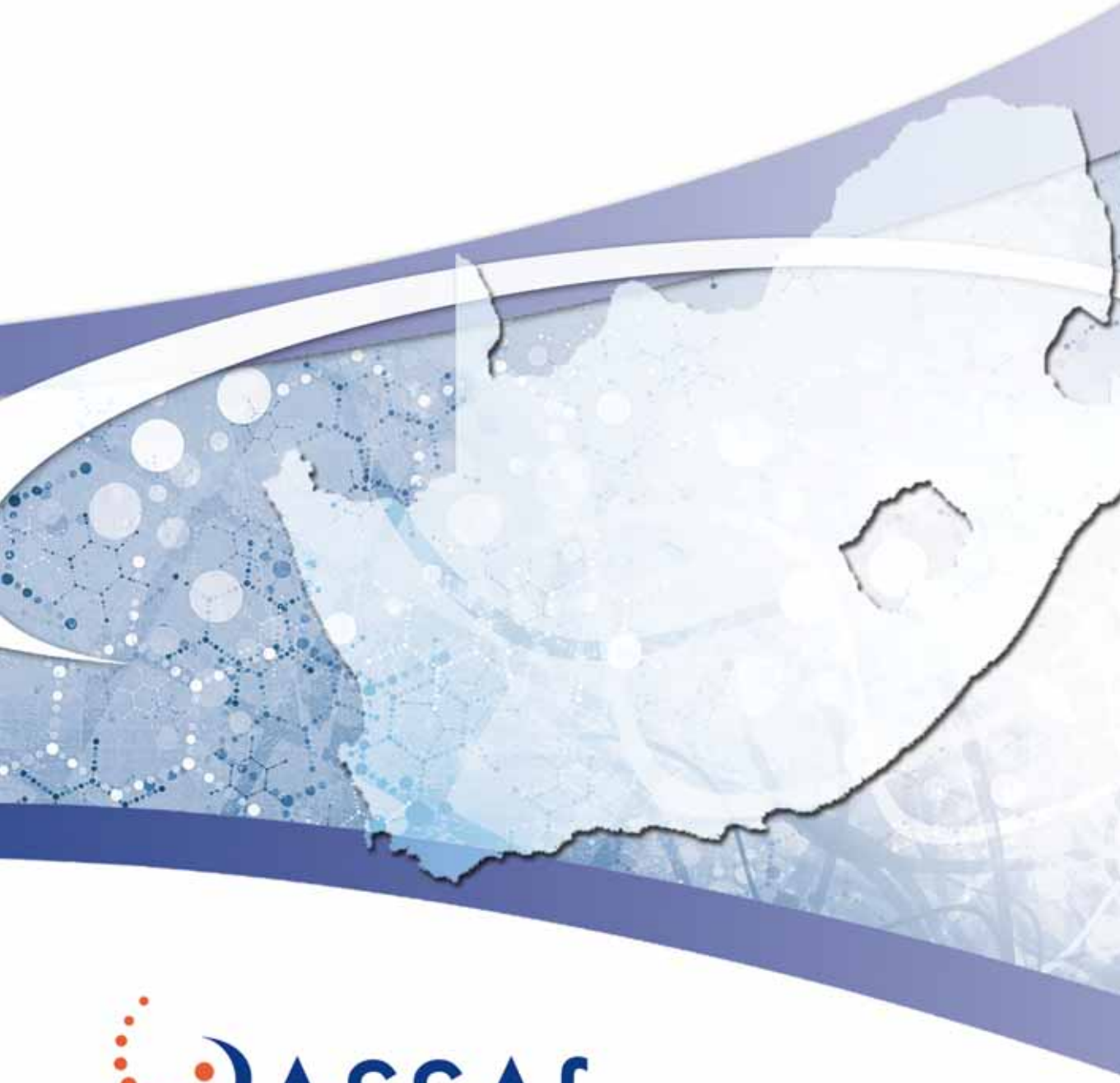
5 Primrose S, Draper J, Elsom R, Kirkpatric V, Mathers JC, Seal S. *et al*. Metabolomics and human nutrition. Workshop report. *Brit J Nutr* 2011; 105(8):1277-1283.

6 King JC, Garza C. Harmonisation of nutrient intake values. *Food and Nutrition Bulletin* 2007; 28(1):S3-S12.

7 Van Ommen B, Keijer J, Kleeman R, Elliot R, Drevon CA, McArdle H, Gibney M, Muller M. The challenge for molecular nutrition research 2: quantification of the nutrition phenotype. *Genes Nutr.* 2008; 3(2):51-59.

8 Jackson A. Personal communication, November 2012.

Chapter 10
Recommendations



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In this chapter policy recommendations regarding the assessment of micronutrient status are made. This is followed by general and specific recommendations for further research to refine and develop the methodology for this assessment. An overarching recommendation is that the findings and recommendations of this report are distributed widely to impact on health policy and practice in South Africa.

Policy Recommendations for Assessment of Micronutrient Status

Recommendation 1: Implementation of a regular nutritional status surveillance system

Noting the limited information on micronutrient status of the South African population, and that the selected six micronutrients reviewed have, *inter alia* through their effects on the immune system, important roles in optimising nutritional status and therefore in childhood development and in human resistance to diseases, including HIV/AIDS, TB, micronutrient deficiency diseases, as well as chronic non-communicable diseases:

- South Africa should **implement a nutritional surveillance system** in which the nutritional status of the South African population is regularly (at least every five to ten years) monitored and evaluated,

    - the results of the surveillance should be used **to formulate appropriate interventions** and to adapt existing programmes where necessary. As an example, evaluation of the outcomes of the present mandatory food fortification programme as part of the surveillance is needed to ensure that upper limits of intake of specific micronutrients, such as vitamin A and folate, are not exceeded by some individuals or groups.

- A **Task Team** should be appointed to assist government in planning a nutritional surveillance system.

- The **biochemical methods** (laboratory tests) that are used to assess nutritional status as shown in this report should be used in tandem with dietary intake and anthropometry measurements (especially the growth of children), where applicable, to assess micronutrient status as part of the surveillance.

- A **data bank** of information about changes in nutritional status over time should be established and shared with other countries in Africa (meaning that all surveillance and research data should be properly dated and centrally stored).

Recommendation 2: Routine assessment of micronutrient status

Noting the importance of these six micronutrients in the treatment of both infectious and non-communicable diseases in individuals, coupled to the different potential levels of deficiencies ranging from pre-clinical, sub-clinical, and to overt deficiency, as well as the complexity of interpretation of test results:

- The status of these six micronutrients should be **assessed routinely** in medical settings in patients suspected of being **nutritionally compromised**.

- **Laboratory methods** as given in this report can be employed to assess status, since they have been shown to be **affordable, robust, reliable**, capable of decentralised operation and of mass application for individuals and populations.

- **Standard procedures, protocols and algorithms** should be developed to assist health personnel to assess different levels of deficiency and to interpret test results in situations of infections and disease.

- Medical personnel should be **better trained, informed and educated** on how to assess nutritional status, and how to design appropriate nutritional interventions that will lead to better recovery and further prevention of disease,

    - this training should include a food-based approach as first priority but also the development of expertise to address potential problems associated with overdosage with supplements and fortified foods.

Recommendation 3: Improving the training of health personnel in the assessment of micronutrient status and improving awareness of the importance of good nutritional status

Noting the far from optimal micronutrient status of South Africans (as could be judged from the limited information available), the important role that nutrition plays in alleviating poverty, and in addressing the HIV/AIDS and TB epidemics in our country:

- A central, government-appointed authoritative body responsible for nutrition should be formed to improve the **awareness of the importance of good nutritional status for a better quality of life** for all South Africans,

    - such a body could be the already mentioned **Task Team or a Committee of Experts** and could consist of members from the Nutrition Directorate of the Department of Health (ND DoH), the Nutrition Society of South Africa (NNSA), the South African Medical Research Council (MRC), the Association for Dietetics in South Africa (ADSA) and the Academy of Science of South Africa (ASSAf).

- The Task Team or Committee of Experts should **promote improved nutrition training** of all health and medical personnel, as well as other nutrition professionals and should advocate for training and employment of more public health nutritionists within all communities.

- The Task Team could take the responsibility for making **evidence-based recommendations** regarding healthy eating and prevention of disease.

Recommendation 4: Developing expertise

Noting the growing perception that the microflora in the gut contribute to micronutrient status, and noting the existing changes in traditional diets in South Africa, as well as the increased usage of prebiotica and probiotica supplementation by sections of the South African population:

- **Expertise** to examine the contribution of the micobiotica to nutritional status should be developed,

    - this can be part of research development and/or re-training of personnel in the **National Health Laboratory Services (NHLS).**

Recommendation 5: Investing more in local nutrition research

Noting that most of the basic research quoted in this report comes from studies outside South Africa, and noting the development in our knowledge about the impotant role of micronutrients in health and disease, as well as the recent developments in nutrigenomic, proteomic and metabolomic methodologies to look for better markers of nutritional status:

- South Africa should **invest more resources in research regarding nutrition** in general and specifically in the selected six micronutrients and the role of the bowel microflora in contributing to nutritional health.

- Local South African **expertise in using modern technology** to assess micronutrient status should be encouraged and developed.

- It is accepted that many laboratories in South Africa have the available instrumentation and expertise, but nutritional assessment should become a priority.

A corollary of these recommendations is that the NHLS should be in the vanguard of establishing best practice in the assessment of micronutrient status, and should seek to offer the most informative tests as widely as possible and at the lowest possible cost.

Recommendations for Further Research

Recommendations for further research are made in the individual chapters of this report. In this section, the most important **identified gaps** in our knowledge that dictate specific research efforts are reiterated:

1. All the policy recommendations made above regarding public policy and individual care should be supported by more research. This would include **epidemiological, clinical and basic laboratory research** to improve data collection and interpretation on the micronutrient status of all South Africans. For example, the South African food composition tables should be extended to include reliable data on the selenium content of all South African foods (taking into account that Se content may differ depending on the soil Se) and also on the bioavailability of zinc in food sources. It would furthermore imply that nutrition research results from the tertiary institutions that train nutritionists should become part of the South African database for regular surveillance and assessment of nutritional status.

2. For most of these micronutrients, **better biological markers** of intake, and better markers of the different levels of status should be identified. Expertise in the use of the rapid developing technologies of nutrignomics, proteomics and metabolomics should be locally developed to identify relevant markers of both intake and status. These markers should also be able to assess human heterogeinty in responses to dietary and other medical interventions, should be applicable in field, clinical and research settings, and provide results to inform design and measurement of outcomes of individual and poplutaion programmes that aim to improve nutritional status. There is thus a need to develop more reliable, more informative, robust and low-invasive, low-cost, high-throughput laboratory assessment methods.

3. One area that has not been covered in detail in this study is the issue of **uncontrolled multivitamin supplementation** and the possible disadvantages of this widespread mode of preventive practice. We need to establish the precise physiology and (possibly competitive) pharmacokinetics of food-derived *versus* synthetic vitamin and/or mineral intakes/supplements, the latter singly or as multi-component preparations. There is a natural and well-tried suspicion in medicine of poly-pharmacy and 'shotgun' approaches to therapy, yet many manufacturers are marketing 'multivitamins' that contain an enormous variety of organic and inorganic nutrients and other supplements, purportedly restoring lost energy, lost years, and lost immune function, as the claim may be. The panel is of the opinion that the widespread use of these convenient but expensive supplements justifies investigations aimed at detailed scientific understanding of the consequences of their regular ingestion. To quote from the 2007 ASSAf report:

   "Bioavailability is determined by comparing the effectiveness, in terms of a selected measurable parameter(s), of vitamins present in different foodstuffs with synthetic/pure compounds administered (usually singly) in the same amounts. Multiple-vitamin supplementation is complicated by the fact that the bioavailabilities of the component substances may not equate to those determined individually; some may be lower because of competition for carriers, and others may be higher, for reasons of synergism in absorptive mechanism, for example. In addition, some supplements require additional components for absorption (e.g. bulk fat for fat-soluble vitamins), and natural foods typically containing uncharacterised compounds that may also be nutritionally beneficial in as yet unknown way."
   The panel thus believes that attention has urgently to be given to finding out in some detail

how synthetic vitamins are handled by human bodies in different situations and in different contexts. This applies especially to the many components in commonly available (and strongly promoted) multivitamins preparations. Are the consequences of self-administration of multivitamin preparations properly understood in terms of interactions between constituent compounds and body constituents? Are the individual bioavailability patterns affected by bulk ingestion? Do different preparations differ in their effects/effectiveness? Are measurable parameters of immune function altered when multivitamin preparations are taken by uninfected persons? What about HIV-infected persons?

Dosage safety is a key consideration in many guidelines for nutritional supplementation, especially in HIV-infected persons, and it is not easy to see how an injunction to take not more than "one recommended dietary allowance or individual nutrient level (INL)[98]" of an entire set of micronutrients can be followed by individuals also taking in the same materials as mixes of vitamers in naturally occurring foods. Such persons may also be partaking of a variety of drugs with which the metabolism of the vitamins concerned may intersect (by becoming involved in drug interactions that attenuate the efficacy of therapies or that enhance them by competing for shared disposal pathways and prolonging half-lives in the body). Some persons may be showing signs of body 'inflammation' involving pro-inflammatory cytokines. In addition, the pre-history of an individual subject may include a massive loss of tissue mass, altering the dynamics of micronutrient utilisation.

Consumption of multiple INLs of vitamins appears to be highly problematic in terms of what our review of the available evidence has revealed. Everything mentioned above becomes more cogent at higher and less well-controlled dosages, with enhanced risks of deleterious hypervitaminoses and/or toxic states of trace element excess.

We need to understand all these possible risks much better, through appropriate research efforts. We also need to understand the relationship between soil health and nutrient content of foods and utilisation. But most importantly, we need to turn the knowledge thus gained into optimised public policy and more effective individual care.

List of Panel Members

(in Alphabetical Order)

1 Professor Tola Atinmo, University of Ibadan, Nigeria *(PhD)*

He is the Head of the Department of Human Nutrition, College of Medicine, University of Ibadan in Nigeria, and also President of the Federation of African Nutrition Societies (FANUS) and in this capacity also member of the Executive Committee of the (nternational Union of Nutritional Sciences (IUNS). He is also a member of the International Council for Science (ICSU). He is a public health nutritionist with an interest in undernutrition in mothers and children, and with a good understanding of policies to prevent undernutrition.

2 Dr Namukolo Covic, North-West University *(MSc, PhD [Nutrition])*

She is a Senior Lecturer of Nutrition at the Centre of Excellence for Nutrition at the North-West University. She is actively involved in research involving micronutrients and cognition in children. Her past work experience include being a lecturer and laboratory demonstrator at the University of Botswana and the Pan African Institute for Development – East and Southern Africa. She has also done work for the United Nations Industrial Development Organisation (UNIDO) in Rwanda, the Gambia, Tanzania and Zambia.

3 Professor Ali Dhansay, Medical Research Council *(MBChB, DCH, MMed [Paed], FCPaed)*

He was Vice-President: Research and Acting President of the SA Medical Research Council. He has reverted to his position as Director of the MRC's Nutritional Intervention Research Unit (NIRU). He was awarded a two-year MRC post-specialisation scholarship with the National Research Institute for Nutritional Diseases (NRIND), investigating lipid metabolism in kwashiorkor. He has served or is currently serving on advisory committees for the SA provincial and national Departments of Health such as: Paediatric Case Management Guidelines; Paediatric Food-Based Dietary Guidelines; Nutrition Advisory Committee; Vitamin A Supplementation Task Team; Maternal, Child and Women's Health Advisory Board; Deworming Task Team; and on the Council of the Nutrition Society of SA. He is a member of the SA Paediatric Association, the SA HIV Clinicians Society and the American Academy Of Pediatrics. He was on the Editorial Board of the *SA Journal of Clinical Nutrition* and was Chair of the SA Committee of International

Union of Nutritional Sciences, Member of the Advisory Board of South African Cochrane Centre, Trustee and President of the International Life Sciences Institute in South Africa. He is a founding member of the World Public Health Nutrition Association.

4 Emeritus Professor Wieland Gevers, University of Cape Town *(MBChB, MA, DPhil, DSc Hons Caus, FCP SA Ed Eundem, MASSAf)*

He is an Emeritus Professor at the University of Cape Town. He was the Executive Officer of the Academy of Science of South Africa. He was Senior Deputy Vice-Chancellor at the University of Cape Town, and Professor of Medical Biochemistry. He was (founder) President of the South African Biochemical Society, President of the Royal Society of South Africa, and President of the Academy of Science of South Africa from 1998-2004. He also directed MRC Research Units at both the University of Stellenbosch and Cape Town using biochemical, cell-biological and molecular genetics approaches to heart contractility, intracellular protein turnover and cholesterol metabolism. He was awarded two honorary doctorates, the Wellcome Gold Medal for Medical Research, and the Gold Medals of the South African Society for Biochemistry and Molecular Biology and the South African MRC. He is an elected Fellow of the Academy of Sciences of the Developing World (TWAS) and a Life Fellow of the University of Cape Town.

5 Professor Salome Kruger, North-West University *(MSc [Diet], MPharm, PhD [Nutrition])*

She is a full Professor and Programme Leader at the Centre of Excellence for Nutrition at the North-West University. She is an expert in child nutrition and has published in the field of obesity, nutrition, body composition and physical activity. To date she has published 82 papers in peer-reviewed journals, is a NRF-rated scientist, and has delivered 31 research Masters' students and 8 PhD graduates. She has received awards for her research, including the John M Kinney award for a publication in *Pediatric Nutrition: the International Journal of Applied and Basic Nutritional Sciences* in 2005.

6 Professor Xikombiso Mbhenyane, University of Venda *(MSc [Dietetics], PhD [Nutrition])*

She is the Deputy Vice-Chancellor: Academic at the University of Venda. She was previously the Deputy Dean of the School of Health Sciences and the Head of the Department of Nutrition. She is an elected Member/Chairperson of the Professional Board for Dietetics. Her involvement in human nutrition research has been at various levels: supervision of Master's and doctoral research at the University of Venda and externally. Her research area of interest is indigenous foods, nutrition and prevention of disease and poverty, nutrition and hunger. She has published 33 papers in accredited journals over a ten-year period. She was named the 2009 Businesswoman of the Year in the professional category by the South African Council for Businesswomen.

7 Professor Barry Mendelow, University of Witwatersrand/ NHLS *(MBBCh, PhD, FCPath [Haem], FRSSAf, MASSAf)*

He was previously Professor and Head of Pathology at the Chris Hani Baragwanath Hospital and founding Professor and Head of the Department of Molecular Medicine and Haematology. His other previous appointments include those of Assistant Dean (Research) at the Faculty of Health Sciences and Executive Director (Research) of the University of the Witwatersrand.

8 Emeritus Professor Jack Metz, Royal Melbourne Hospital, Australia *(MB, BCh, MD, DSc[Med], FRCPath, FCAP, FRCPA, FRS[SA], DSc Med Hon Caus)*

Emeritus Professor Jack Metz is life-long Honorary Consultant Haematologist with the Royal Melbourne Hospital and consultant Haematologist for Dorevitch Pathology. He graduated from the University of Witwatersrand, and his career highlights included Professor of Haematological Pathology, University of Witwatersrand, Chairman of the School of Pathology, Director of The South African Institute for Medical Research and after moving to Australia, Director of Haematology, Royal Women's Hospital, Director of Haematology Royal Melbourne Hospital and Professorial Fellow University of Melbourne. His special areas of interest include nutritional anaemia, flow cytometry, appropriate use of blood products, and folate food fortification. He has published over 200 articles and has written over 20 book chapters. He has also received numerous international awards for his work.

9 Emeritus Prof John Pettifor (Chairperson), University of Witwatersrand/ Chis Hani Baragwanath Hospital/MRC Unit *(MBBCh, PhD[Med], FCPaed[SA], MASSAf)*

He is Professor Emeritus in the Department of Paediatrics and Honorary Professorial Researcher in the MRC/Wits Developmental Pathways for Health Research Unit of the University of the Witwatersrand. He is an NRF A-rated scientist. His research interests are: paediatric bone disease, vitamin D and calcium homeostasis. He is past President of the Nutrition Society of South Africa and the College of Paediatricians of South Africa. He was awarded the Dr Charles Slemenda Award for his contribution to children's bone health by the International Conference on Children's Bone Health held in Sheffield, UK in 2002. He is on the Editorial Boards of the *Journal of Bone and Mineral Research, Bone, Calcified Tissue International* and the *European Journal of Pediatrics*. He has published 180 research articles and 30 chapters in books.

10 Professor Esté (HH) Vorster, North-West University *(DSc [Physiology], MASSAf)*

She is a Professor of Nutrition and previous Director of the Centre of Excellence for Nutrition at the North-West University. She started the Nutrition Research Group at this institution. She has served as President of the Nutrition Society of South Africa (NSSA) and on the National Board of the International Union of Nutritional Sciences (IUNS) and ICSU. She is a Member of the IUNS

Task Force for the Nutrition Transition. She has served as invited expert and Chair of several WHO/FAO expert consultation groups, including the WHO Nutrition Guidance Expert Advisory Group (NUGAG) group that advises on dietary recommendations. She has conceptualised nutrition research as a transdisciplinary but integrated action "from molecules to society" and has brought together a group of scientists who are studying health outcomes as a consequence of how individuals, groups, communities and populations respond and adapt to changing environments. She has published more than 300 research outputs. She is the first woman who received the NSSA award for Outstanding Contributions to Nutrition Research (in 1996.) Her group was awarded the five-yearly Nestlé Nutrition Institute Africa (NNIA) award for sustainable contributions to Nutrition Research in Africa in 2005 and she received the Havenga Medal for medical research outputs from the *SA Akademie vir Wetenskap en Kuns* in 2007 and the Nevin Scrimshaw award for services to nutrition from the African Nutrition Society in 2012.

11 Professor Michael Zimmerman, ETH Zurich, Switzerland *(MD, MSc)*

He is Professor and Head of the Laboratory of Human Nutrition at the Swiss Federal Institute of Technology in Zurich. His research includes interactions of micronutrient deficiencies. He was awarded the American Society of Nutritional Sciences 2004 Mead Johnson Award and the 2005 Pfizer International Award for published clinical research in the *Journal of Clinical Endocrinology and Metabolism (JCEM)*.

ASSAf Consensus Study Methodology

- The value of Academy-type studies is the provision of authoritative, objective and independent advice based on a particular methodology that is unique to academies. The consensus study methodology involves the assembling of expert panels that deliberate on a topic and reach consensus on a set of strong recommendations based on the evidence assembled. It yields a substantial consensus report which aims to influence policy and to have a long-term impact.

- The panel of experts is chaired by an Academy of Science of South Africa (ASSAf) Member appointed by the ASSAf Council. Panel members are appointed on the basis of expertise – ensuring a balance of perspectives, gender and race and absence (or clearly defined and admitted in advance of the study) of a conflict of interest.

- Their main task is to review available evidence, conduct further research (should there be a need) and seek agreement (consensus) on the major questions or concerns in the area of inquiry. To reach consensus, a panel should have opportunities to gather information in public and to deliberate in private. Consensus is usually expressed as findings, conclusions, and/or recommendations in publicly released statements, reports, or brief advisory documents.

- Before the consensus study report is approved by the ASSAf Council and released, it is peer reviewed by independent and external reviewers who are experts in their respective fields. The peer-review panel of three to four members consists of international and national experts.

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