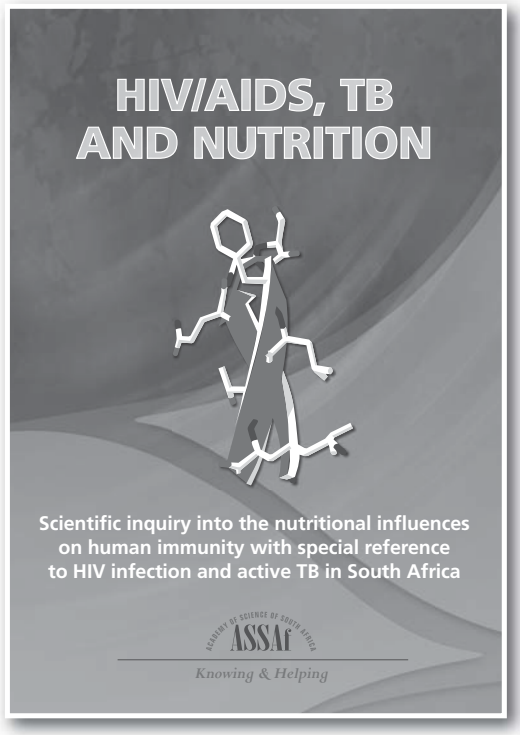


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*The Academy of Science of South Africa (ASSAf) was inaugurated in May 1996 in the presence of then President Nelson Mandela, the Patron of the launch of the Academy. It was formed in response to the need for an Academy of Science consonant with the 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, and the Act 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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# Opening section

# Foreword

This report; the second of its type published by the Academy; considers the influence of nutrition on two of the major disease epidemics currently affecting South Africa – tuberculosis and HIV infection. The research in this report highlights the starting recognition that the country is currently facing 'three concurrent epidemics'. Two of these epidemics are caused by disease organisms – the human immunodeficiency virus and the bacterium *Mycobacterium tuberculosis* – while the third, malnutrition, is the result of socia, historical and political factors. In order to manage a response to these linked epidemics, the Study Panel was commissioned to review the scientific evidence that could provide an understanding of the dynamics of this tripartite interaction. The particular focus of the report has been on examining the influences of nutrition on human immunity in this specific context.

Based on the understanding that they gained of each of these epidemics and their interaction, the Study Panel has made recommendations as to the best way to proceed in terms of policy, intervention and development. A matter of great potential interest and importance is the accumulating evidence that one, perhaps the major site at which HIV infects and hence depletes $CD4^+$ T cells is the gut, raising the possibility of links between diet and HIV infection quite different from the usual pre-occupations of traditional nutritional theory and practice. Immune deficiency also exacerbates susceptibility to TB infections; which fuels the vicious concurrent cycle of the three epidemics that are the subject of this study.

The thorough review undertaken by the members of the Study Panel has led them to make the following statement:

> "The Study Panel is frankly appalled by the dearth of reliable and truly informative studies of the nutritional influences/interventions on the course and outcomes of the pandemic chronic diseases addressed in this report."

This conclusion has led them to make a series of important recommendations about the urgent need for well-designed research studies to deal with an unacceptable situation.

Furthermore, they make a number of recommendations in relation to nutritional policy and practice in relation to both HIV and TB infections. The Council of the Academy hopes that this report will provide the basis for productive debate and subsequent interventions that will foster solutions to the three epidemics currently having such a profound impact on South Africa.

This report was prepared by a consensus Study Panel and the methodology followed is based on that of consensus panels of the US National Academies, with some modifications to accommodate our unique circumstances. The report has been reviewed by three independent reviewers all of whom recommended that it be published. The Council of the Academy reviewed the report and considered both the reviewers' comments and the way in which the Study Panel had addressed the comments made by the latter. After thorough consideration of the full report and of the reviewers' comments and recommendations, the Council approved the report for publication.

The proposers of the study, Prof. Wieland Gevers and Prof. Jimmy Volmink, are thanked for convincing the Council that this was a suitable and urgently needed subject for a formal consensus study to be undertaken by the Academy. The Chair of the Study Panel, Prof. Barry Mendelow, and all the panel members are thanked for agreeing to participate in the study, and for bringing it to a successful conclusion. The staff of the Academy, Ms Boitumelo Mabina (Study Director), Ms Rudzani Ramaite and Dr Xola Mati (Projects Director) are recognised for their crucial support to the Study Panel.

*R M Crewe*
*President*
*July 2007*



# Preface

The core function of the **Academy of Science of South Africa** is to harness the highest levels of scholarly achievement and excellence in the application of scientific thinking for the benefit of society. The activities of the Academy are defined, resourced and monitored by the **ASSAf Council**. The constitution of specific expert panels and the prescription of their tasks is one of a variety of instruments which the Council of the Academy may apply in the attainment of its mission. The ASSAf Council, in October of 2005, resolved to constitute its first expert panel to address one of the most highly profiled issues confronting South African Society today, that of the role, if any, of nutritional intervention in the integrated management of the epidemics of HIV/AIDS and tuberculosis which are currently ravaging South African society.

In defining the **brief for the Consensus Study Panel on Nutrition, HIV/AIDS and TB**, the Council identified three foci for in-depth study. These were, firstly, to review critically the literature pertaining to the intersection of nutrition and nutritional status with fundamental immune functions, with special reference to immune functions in the context of HIV/AIDS and *Mycobacterium tuberculosis* infections; secondly, to review critically what has been reported concerning the impact of these infections on nutritional status, and thirdly, to compile a comprehensive evidence base concerning the effects of nutritional interventions on the course of those infectious disorders.

The Council approved the constitution of a **Study Panel,** the 15 members of which comprised experienced nutritionists, immunologists, biochemists, infectious disease physicians and paediatricians, policy experts, epidemiologists and generalists. It was further decided that the Study Panel should be chaired by a non-specialist in the field, specifically to minimise the risk that the activities of the Study Panel might be unintentionally influenced by any particular research agenda, and also to serve as a *de facto* interface between the Study Panel and the general public and other intended users of the Study Panel's final report, including politicians and policy makers.

After the first meeting of the Study Panel, the chairperson met individually with each panelist to solicit individual opinions as to optimal ways to proceed with the project.

The Study Panel met subsequently five more times over the course of the following 16 months, interspersed with many smaller-group meetings and discussions between the chairperson and individual panelists or ad hoc working subgroups. Initial tasks were allocated to appropriate specific experts whose preliminary reports constituted the material for general discussions at subsequent meetings.

The final report represents the consensus views of the Study Panel members on the topics defined by the Council, and is arranged with a slight departure from the format of the original brief. This was necessitated by the early realisation during the Study Panel's schedule of activities that while there was abundant evidence establishing linkage between malnutrition and adverse outcomes of the infectious diseases under study, it was not always clear which was cause and which was effect. It was therefore impossible to segregate observational studies categorically into effects of infectious diseases on nutritional status, as opposed to effects of nutrition on infectious disease outcomes. The report has nevertheless attempted where possible to determine the appropriate cause-and-effect relationships of the observations discussed in the relevant chapters, addressing the brief indirectly, while the group of chapters devoted to clinical evidence has clearly identified and analyzed the published effects of nutritional interventions on HIV/AIDS and tuberculosis, addressing the brief directly.

In addition to these activities, the Study Panel also availed itself of two other instruments available to the Academy in the pursuance of its mission – these being the constitution of workshops to discuss specific topics directly or tangentially related to the mainstream task, and the commissioning of outside experts to supplement the acquisition of knowledge on specific items outside the immediate ambit of the panelists, or where specific external international authorities were readily identifiable and available to address or advise on particular points. Two workshops were convened during the period of tenure of the Study Panel. These were a general academy **symposium on evidence-based decision-making** in March 2006[1], and a Study Panel-directed **workshop on HIV and the gastrointestinal tract** in November 2006. Key insights gleaned from these activities and external commissions have been incorporated into the appropriate chapters of the final report.

Supplementary to the critical review of the evidence mandated by Council is a set of **policy recommendations** that concludes the body of this report. This chapter includes recommendations for practice, recommendations for further research that will be required to address as yet unanswered questions, and a collation of the Study Panel's views on three **existing sets of guidelines** concerning nutritional advice for persons affected by HIV/AIDS and/or TB. These recommendations have respectively emanated recently from the World Health Organization, from the Southern African HIV/AIDS Clinicians Society, and the South African National Department of Health. In this task,





the Study Panel sought to identify commonalities and/or divergences of opinion on the part of three organisations with distinct and separate perspectives on the same topic.

A concluding section of the report amongst other things addresses some of the issues we have not been able to investigate, namely the new field of appropriate and optimal nutritional support for persons receiving antiretroviral treatment, and the as yet largely undocumented field of biologically active phytochemicals that may or may not have effects on the health of infected persons.

The pre-final draft of the report was circulated to all panelists, and edited by Professor Dan Ncayiyana, prior to submission for external review by the three reviewers selected by the ASSAf Council.

Finally, it is noteworthy that this report is a **consensus document** of the panelists. Although its individual components were initially drafted by individual Study Panel members, the final document should be regarded as having been authored jointly by the entire Study Panel.

# Acknowledgements

**This report is the joint work of a 15-member Study Panel appointed by the Council of the Academy of Science of South Africa (ASSAf). Each panelist has agreed to the specific formulation of the report and to its conclusions and recommendations**. (The Academy's guidelines permit dissenting viewpoints to be included in the report as authored footnotes on the pages where material is presented with which one or more of the Study Panel members disagree). The Study Panel members were Barry Mendelow (BM) – chairperson, Peter Cegielski (PC), Muhammad Ali Dhansay (MD), Wieland Gevers (WG), Clive Gray (CG), Glenda Gray (GG), Liesl Grobler (LG), Gregory Hussey (GH), David McMurray (DM), Gernard Msamanga (GM), Dan Ncayiyana (DN), Helen Rees (HR), Francois Venter (FV), Jimmy Volmink (JV), and Este Vorster (EV).

The Study Panel found it practical to request members (or, on occasion, outside experts) to provide initial drafts of parts of the report that were designed to deal with particular topics. These drafts were discussed at Study Panel meetings after prior circulation, and stock was taken of problems such as critical omissions, controversies, overlaps and contradictions. Further drafting then took place, often by several panelists other than the original author. In one instance, the Study Panel decided to arrange a public workshop, convened by WG and CG, to review and discuss highly topical recent advances in the inter-disciplinary field of HIV immunology in the gut, inflammatory bowel disease and the burgeoning area of intestinal microbiota; the insights obtained from this exercise were taken up in various sections of the consolidated draft report. A short paper on the possible programming of the human immune system as a result of fetal insults of various kinds, including maternal under- and malnutrition, was commissioned from Dr Andrew Prentice and Sophie Moore, of the London School of Hygiene and Tropical Medicine, UK. A special policy workshop was attended by a subset of panelists convened for this purpose by HR. The Study Panel met face-to-face as a whole on six occasions during late 2005 until early 2007.

The pre-final consolidated version of the report was edited by DN, assisted by Ms Emma Buchanan. The independent peer reviewers were Prof Martin Bloem, Prof Zulfiqar Bhutta and Prof John Pettifor who are warmly thanked for their considerable contributions to finalising the approved text of this ASSAf report.

## Individual sections

### Introduction and background

The **brief from the ASSAf Council** was initially drafted by WG and JV, and revised after approval by the Council. BM drafted the **conceptual overview**, taking into consideration information from the relevant chapter of the entire report. Parts of the Report dealing with the **three South African epidemics** were initially drafted by GG (Nutritional Status of South African children), while EV and MD provided initial drafts on the nutritional status of South African (Epidemiology of Malnutrition). GG and FV created the first drafts on the epidemiology of HIV/AIDS, while the first drafts on the epidemiology of TB in South Africa were developed by FV with contributions from GH. The various contributions were collated by BM. The first drafts on **evidence-based practice and recommendations** were drafted by EV.

### Relevant Physiology and Pathophysiology

The parts of the Report dealing with **the physiology of human nutrition** were initially drafted by WG; who also provided the first drafts of the parts dealing with relevant aspects of **human immunity.** Prof Jack Metz* is acknowledged for his advice on folate. The first drafts on **pathogenesis of *Mycobacterium tuberculosis* infection in humans** were provided by DM, while those on the **pathogenesis of HIV infection – moving from older to newer thinking** were drafted by CG and WG. The parts on **special considerations of infancy and childhood** were initially drafted by GG, assisted by WG and BM. Prof Haroon Saloojee* and Prof Shabir Madhi* are acknowledged for making invaluable contributions to the paediatric components of HIV, nutrition and TB on this topic.

### Clinical Evidence

LG provided a first draft on **clinical evidence concerning the effects of nutritional intervention in people with HIV/AIDS: macronutrients** assisted and supported by JV. The parts on **clinical evidence concerning the effects of nutritional intervention in people with HIV/AIDS: micronutrients** were initially drafted by GH and MD; James Irlam* contributed significantly to the finalization of this chapter. PC and DM initially drafted parts of the Report dealing with **evidence concerning the influence on nutrition on the risk and outcomes of tuberculosis**.

### Recommendations for Policy and Practice, and for Research

HR provided an initial draft of the **prioritised recommendations for policy and practice,** building on the outcomes of a special workshop, and using parts drafted by others that were about relevant to policy and/or practice. The **prioritised recommendations for research** were initially drafted by WG, taking into consideration relevant parts from other chapter. The **collation of the existing guidelines from the World Health Organisation (WHO), the Southern African HIV Clinicians Society (SAHCS) and the National Department of Health (NDoH)** were initially drafted by EV. The final part on **the way forward – concluding remarks** was initially drafted by WG.

**Acronyms and abbreviations** were prepared by Ms Boitumelo Mabina, as well as the other appendices.





The Study Panel wishes to thank the **Study Director, Ms Boitumelo Mabina**, for her unstinting hard work, as well as her substitute during leave from mid-2006, Ms Rudzani Maila, and the ASSAf Projects Director, Dr Xola Mati, for his support.

Dr Agatha Masemola, (who resigned from the Study Panel activities due to an excessive personal work load) is acknowledged for participating as a Study Panel member from 2005 – 2006.

The Study Panel is deeply indebted to the **United States National Academies** for both their financial and their mentoring contributions. In particular, Dr Enriqueta Bond, chairperson of the Board for the African Science Academies Development Initiative (ASADI); Dr Patrick Kelley, director of the ASADI board; Dr Barney Cohen the Programme Officer of the US National Academies who was responsible for the development and the necessary mentoring of the ASSAf staff, and for helpful comments on the pre-final draft of the report; and Jim Banihashemi, also from the US National Academies, for his assistance with financial matters.

The Study Panel, in its unremitting efforts to make this report as innovative, recent and evidence-based as possible, arranged a workshop in November 2006 on "**Diet and Nutrition in relation to the functioning of the gastrointestinal tract in HIV-infected individuals**". The following people (listed in alphabetical order) are acknowledged for their participation in the workshop as speakers: Dr Linda-Gail Bekker, Dr Wendy Burgers, Dr Paul Kelly, Dr Roy Kennedy, Dr Satish Keshav and Dr Ron Veazey. Key elucidations gleaned from the workshop have been built in into the appropriate chapter of the final report.

* Not a Study Panel member of this study.

# Executive summary

The availability of highly effective drugs to control HIV/AIDS and cure tuberculosis has tended to downplay the potential benefits that may accompany appropriate supportive measures, designed to complement or precede pharmacotherapy, to arrest progression from infection to disease (TB), or to delay the rate of progression of the earlier phases of the illness, before specific medicinal interventions are indicated (HIV). Nutritional support is one such potentially valuable measure. Clearly the clinical efficacy of nutritional intervention is likely to be dependent on the extent to which individual infected subjects suffer from functionally significant nutritional deficiencies prior to nutritional intervention. The same argument is generally pertinent to interventions at population level – populations of infected subjects with a high prevalence of nutritional deficiency, such as those in developing countries, are more likely to benefit from health policies aimed at eradicating or diminishing nutritional deficiencies than are populations in developed countries, from where much of the reported research has emanated.

It is important to bear in mind that the pre-antibiotic phase of dealing with TB was characterized by intense concentration on strengthening the immune defenses of infected subjects with diets, improved and altered environmental conditions and every other conceivably helpful measure. After the discovery of effective drugs, this aspect of TB therapy quickly became secondary and largely uncontroversial. Because HIV infection cannot be cured but only controlled, with drugs being applied at particular, serious stages of progressive disease (according to current guidelines, at least) the emphasis in the management of infected people during the phases prior to drug administration is still on general, non-pharmacological support, especially as for many reasons it is highly desirable to postpone the introduction of specific antiretroviral therapy for as long as possible.

This background has motivated the current study, which has analyzed the relevant scientific literature, concentrating on regionally relevant studies, with a view to providing the best possible, evidence-based advice for South African policymakers. The over-arching conclusion from this analysis is that there is a lamentable paucity of

relevant, solid data on which to base sound policies for this country. Recognizing the dire need to deal optimally, right now, with an existing health crisis, this study has evaluated the current nutritional guidelines from the World Health Organization, the national Department of Health, and the Southern African HIV/AIDS Clinicians Society, in the light of what scientific evidence is available. Broadly, these guidelines are endorsed in the current analysis as being generally close to the best possible in the prevailing circumstances. The study, having identified serious gaps in our knowledge, has compiled a set of critical research questions, guided by evolving understanding of the relevant basic science. The panel urges that the answering of these questions should be given high priority by research policy makers in the hope that the insights thereby gained will provide the kind of solid evidence on which refined policies for the practice of health care can safely and effectively be based in future.

## The Study Panel concludes from its studies that:

1. South Africa is in the grip of **three concurrent epidemics**: malnutrition, brought about by a conglomeration of socio-economic factors; HIV/AIDS, caused by the human immunodeficiency virus; and active TB, caused by progressive infection with *Mycobacterium tuberculosis*. Although caused by separate factors, there is evidence that each epidemic acts synergistically to aggravate the other two.

2. An understanding of the **basic science of nutrition**, **immunity** and **infectious disease pathogenesis** is necessary for the rational identification of appropriate therapeutic interventions. Recent studies investigating the pathogenesis of **HIV infection** have provided the exciting novel conceptual understanding that the **gastrointestinal tract is a major anatomical front line of the disease**, and that lymphocyte activation is a key step in the $CD4^+$ T cell depletion that defines AIDS. Together, these insights have major implications for our dawning understanding of the intersection between nutrition and HIV/AIDS, both in terms of the potential impact of HIV infection on nutritional status, and in redefining our conceptions of how nutritional intervention might impact on HIV/AIDS pathogenesis.

3. While **observational clinical studies** may provide further, often empirical clues concerning possibly effective interventions, direct proofs usually necessitate suitably rigorous **prospective, randomized, controlled trials.**

4. Systematic review of the clinical literature has identified the following **macronutrient-related issues of importance in relation to HIV infection:**





- In people infected with HIV, weight loss and loss of body cell mass are strong predictors of poor prognosis. These features are associated with increased resting energy expenditure, accelerated protein turnover, decreased energy intake, diarrhoea and malabsorption.
- Macronutrient supplementation may be of benefit in HIV-infected individuals. Targeted interventions with balanced nutritional supplements seem to increase energy and protein intake. There is also preliminary evidence that specific dietary supplements, such as amino acid mixtures, increase body weight and reduce HIV viral load.
- Balanced supplementation increases body weight, and supplementation with medium-chain triglycerides is more effective than long-chain triglycerides in reducing HIV-associated intestinal dysfunction and fat malabsorption. Supplementation with a whole-protein diet has been found to increase body weight and fat-free mass in HIV-infected adults. Specific amino acids and polyunsaturated fatty acids may increase energy intake, body weight and fat-free mass. Finally, ready-to-use-therapeutic food is effective in reversing the poor nutritional status found in severely malnourished HIV-infected and non-infected African children.
- Evidence-based advice on the use of macronutrient supplementation in HIV-infected individuals in developing countries is constrained by the fact that the few randomised trials that exist have mainly been conducted in high-income countries where most patients are well nourished and have access to life-prolonging antiretroviral therapy.
- Existing trials have focused on intermediate endpoints (such as actual energy and or protein intake) and most have been too small to assess important clinical outcomes (such as death and morbidity). There are also substantial variations in the nutritional composition of the experimental and control interventions, the use of dietary counseling, disease stage and treatment status of the participants across studies.

5. Systematic review of the clinical literature has identified the following **micronutrient-related issues of importance in relation to HIV infection:**
   - Dietary intakes studies show that micronutrient intakes are sub-optimal in many South Africans. Although the existing evidence is not conclusive, such deficient intakes if they lead to functional impairment may hasten disease progression, increase mortality, and facilitate mother-to-child transmission (MTCT) of HIV.
   - Observational studies have shown a direct correlation between micronutrient intake (especially vitamins A and B, multivitamins, zinc and selenium) and

favourable clinical outcomes in patients with HIV infection. Observational studies however, mostly lack valid markers of micronutrient status, and the effects of micronutrient deficiencies are prone to confounding by other factors, including micronutrient interaction.

- Supplementation with vitamin A/beta-carotene does not seem to have any significant beneficial or adverse clinical effects in HIV-infected non-pregnant adults. In HIV-infected children receiving vitamin supplements, reductions in morbidity and mortality have been reported, but these conclusions are based either on small trials or subgroup analyses. Vitamin A supplementation possibly increases the risk of vertical transmission in HIV-infected pregnant and lactating women, and the risk of mortality in infants of supplemented mother-infant pairs.
- There is sound evidence that multivitamin supplementation (excluding vitamin A) in HIV-infected pregnant women reduces the risk of disease progression, AIDS-related mortality and adverse pregnancy outcomes.
- Results derived from a few small trials indicate that zinc supplements given to HIV-infected children are safe and effective in reducing morbidity, but zinc supplementation in HIV-infected pregnant women seems to have no benefit and may be harmful to the women.

6. Systematic review of the clinical literature has identified the following nutritional issues of importance in relation to **active *Mycobacterium tuberculosis* infection:**
   - Nutritional support of undernourished populations at high risk of TB (e.g. young children, household contacts of TB patients, health care workers, institutionalised populations, the elderly) may reduce the incidence of TB.
   - Although the risk of TB in severe malnutrition may be higher than in mild or moderate malnutrition, severe malnutrition occurs in a very small fraction of the population, except in famine, war, or natural disaster-type situations. Mild to moderate protein energy malnutrition or micronutrient deficiencies may affect large fractions of the population at risk for TB so that prevention efforts will not be highly successful if they target only severely undernourished groups.
   - Supplementation with particular macronutrients (to meet energy and protein needs) and specific micronutrients (such as vitamin D, arginine and protein) during conventional anti-TB therapy may be of value, especially in patients who are demonstrably deficient in those nutrients. Some RCTs have shown clear improvements in nutritional and general health status in nutrient-supplemented patients receiving appropriate TB chemotherapy; Studies are also urgently needed to test a biologically plausible role of vitamin D supplementation in certain susceptible populations in preventing progressive infection by *M.tb.*





   - Scarce resources in TB-endemic countries must be focused where they are needed most, or under circumstances where they will have the greatest impact. Nutritional support to undernourished contacts of TB patients and for patients with multi-drug resistant (MDR)-TB and extensively drug-resistant (XDR)-TB seems warranted.

7. Scrutiny and collation of the recent **guidelines from three external bodies**, the World Health Organization, the Southern African HIV Clinicians Society and the South African National Department of Health, revealed a number of principles to be considered in formulating recommendations for nutrition policy in relation to HIV/AIDS and tuberculosis.
   - Optimum nutrition at the population (public health) level is necessary as part of a set of a general measures for reducing the spread of HIV/AIDS and TB, and at the level of individuals to improve health, quality of life and response to drug treatment, but it cannot directly prevent transmission of these infections or cure them or supervening infections.
   - Nutrition recommendations should do no harm.
   - Nutritional interventions to address the HIV/AIDS and TB pandemics should be part of a holistic, comprehensive, integrated approach, including both public health and therapeutic nutrition strategies and actions.
   - Nutritional care of people infected with HIV and/or with active TB should focus on diversified diets including available, affordable and traditional foods. On the other, the fact sub-optimal intakes are endemic to South Africa, the characteristic wasting of infected persons, and the known effects of the infections on food intake and nutrient turnover (absorption, metabolism and losses), however, dictate the use of fortified foods, as well as macro- and micronutrient supplements at safe levels.
   - Established, well-described steps and protocols should be followed in public health nutrition interventions and in the therapeutic nutritional care of patients
   - HIV-infected pregnant women, lactating mothers and their babies need special advice and nutritional care to ensure best possible outcomes.

8. In light of the findings in the present study, and in accordance with the principles established from scrutiny of outside bodies, the following **recommendations for practice** are proposed for South Africa:
   - The implementation of the existing integrated nutrition programme of the Department of Health is should be evaluated and adequately resourced for implementation to address undernutrition in all vulnerable groups, but especially in women and very young children.

- Resources should be directed to ensure food security based on locally available, affordable and traditional foods to vulnerable populations.
- The nutritional care of people infected with HIV should focus on diversified diets including locally available, affordable and traditional foods, and should be complemented by appropriate, locally acceptable macronutrient supplements.
- Everything possible should be done to promote and support adequate dietary intake of micronutrients at recommended (that is, INL98) levels, while recognising that this may not be sufficient to correct nutritional deficiencies in all HIV-infected individuals. In situations where micronutrient deficiencies are endemic, these nutrients should be provided through food fortification or micronutrient supplements where available that contain at least 1-2 INL98s.
- HIV-infected women should be offered multivitamin supplementation at INL98 levels.
- The nutritional care of individuals infected with TB should focus on adequate diversified diets including locally available, affordable and traditional foods. In addition, the use of appropriate, locally acceptable macronutrient supplements is recommended, especially for those patients who are demonstrably deficient in these nutrients. Nutritional interventions for patients with TB should extend to their close contacts and families.
- The existing legislation and regulations should be enforced for all products claiming medicinal benefits with respect to HIV infection or active TB.
- Government should identify accessible, scientifically valid ways to accelerate the investigation of promising traditional or herbal products.
- An urgent national expert consultation should be convened to develop national guidelines for HIV-infected infant feeding. This should be aligned with the Paediatric Food-Based Dietary Guidelines for South Africa by the SA Nutrition Society.
- More nutritionists and dietitians should be trained, employed and utilised in all programmes addressing HIV/AIDS and tuberculosis, and the nutritional knowledge of all health care workers in community, clinic and hospital settings should be improved and extended.

9. The existing literature is woefully inadequate to answer many of the pressing questions facing policymakers in South Africa. Accordingly, the following **research priorities** have been identified for those tasked with promoting and funding research activities:
   - Well-designed and informative clinical and epidemiological studies are urgently needed to generate and test hypotheses in relation to nutritional support for HIV-infected subjects (both those who are not being treated with antiretrovirals, and those who are), and patients exposed to, or suffering from, active tuberculosis. Pragmatic, high-powered approaches should be developed in which explanatory designs are embedded as far as is possible. The studies should include adults and children, pregnant women, infants and children.
   - The indicators of both vitamin and inorganic micronutrient depletion and repletion in individuals and populations need to be much better defined; in particular, the dynamic impact of the widespread HIV and TB epidemics on the overall nutritional status of the population must be assessed with finer-grain tools than were applied before.
   - Reliable and accessible biomarkers are urgently needed to assess immune function in nutritional studies and in interventions in human subjects.
   - A major effort must be made to integrate and deepen our understanding of the functional interactions between HIV infection, gut immune mechanisms and the intestinal microbiota.
   - There is a need to determine 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.
   - A better understanding is needed of the significance of lifelong programming of the human immune system arising from fetal "insults".
   - Recent developments in the understanding of some micronutrients are so important that they merit thorough study and new kinds of interventional trials.
   - There is a need to factor into population-directed studies the genetically determined differential susceptibility of different members of the population to HIV and TB infection.

10. The implementation of the above recommendations will require a concerted and well-coordinated series of actions by different role-players in the system. We believe that making this happen should be regarded as a national priority, and that focusing resources in this direction will contribute greatly to addressing effectively three concurrent epidemics that threaten the future viability, let alone prosperity, of our country.

# The brief from the ASSAf Council

## Concise description of the Brief

The brief of the Study Panel appointed by ASSAf was to examine the most relevant and reliable evidence that has a bearing on the following issues, and to make recommendations based on that evidence that are the most appropriate and feasible :

i. Nutritional modulation of the normal human immune system (innate and adaptive, at different ages, in both sexes, over short or long periods) with respect both to general undernutrition (macronutrients) and to specific deficiencies of micronutrients;
ii. Modulation of human nutritional status in states of infection, both acute and chronic, with special emphasis on active infection with *Mycobacterium tuberculosis (M.tb.)* and the human immunodeficiency virus (HIV);
iii. Effects of nutritional interventions on morbidity and mortality in adults and children infected with HIV or suffering from clinical tuberculosis, or both.

## Why should this topic be addressed?

The issues concerning nutritional influences on human immunity and response to major pandemic infections, such as *M.tb.* and HIV infections, have been among the most controversial in South Africa in the last half-decade. These issues have given rise to serious differences in the approach to public policy in addressing the ravages of these diseases. There has been a belief in some quarters that poverty and under/malnutrition may themselves be the main aetiologic agents of acquired human immunodeficiency syndrome (AIDS), with the HIV infection being a non-contributory or trivial supervening circumstance. Others consider nutritional deficiencies to be an, or even the, appropriate target of primary therapy of "HIV infection and AIDS". The majority view, however, is that nutritional support of persons infected with either *M.tb.* or HIV, or both, is a necessary and helpful part of a therapeutic approach that primarily concentrates on the eradication or, at least, control of the infected state in each affected person. In addition,

there is a plethora of products available over the counter in the country that purportedly favourably "modulate" the immune system to prevent or ameliorate HIV and/or active *M.tb.* infections ; these products range from "nutritional factors" present in certain foods to processed "drugs" that are probably outside the realm of nutrition.

These controversial opinions and products threaten to dissipate the national will to address the serious problem presented by these out-of-control pandemics. It is therefore essential that a thorough review of all the evidence bearing on the issues listed above be conducted by an independent, impartial, multi-disciplinary and authoritative body such as the Academy, to move the relevant national policy framework and public understanding in a productive, effective and efficacious direction. The outcome of the Study should provide clear guidance on the key issues, and a set of recommendations that are based on the best evidence and the most integrated understanding of the ways in which nutrition affects people suffering from infections such as tuberculosis and HIV-caused immunodeficiency states.

## Why should the topic be addressed by the Academy and not by another body?

The Academy is committed to providing advice to the government and the nation on key issues requiring scientific enquiry and analysis. Other government or state-appointed bodies also have well-defined roles in this domain, such as the Medical Research Council (MRC), the National Health Research Committee, and the Cabinet-appointed Group that made recommendations on the National Antiretroviral Programme now being implemented across the country. Internationally, the World Health Organisation (WHO) is committed to providing developing countries with information and support through a variety of channels (see below). The date the efforts of these bodies have failed to date to resolve the present controversial and publicly confusing situation in respect of the role of nutritional factors in enhancing or impairing immunity and thereby affecting morbidity and mortality from pandemic diseases such as tuberculosis and HIV infection leading to AIDS. It is accordingly considered that South Africa's independent national science academy, ASSAf, has a responsibility to provide much-needed, authoritative advice in respect of the proposed topic, in the national interest.

## Particularly significant background factors

South Africa has recently seen remarkable growth in the capacity to perform "meta-analysis" of large tracts of available data in public health fields where statistical analyses in many varying kinds of studies yield conflicting conclusions; the Cochrane Centre





located in the MRC has been instrumental in much of this growth. ASSAf will have access to this capacity in setting up and supporting the Study Panel to review the full range of evidence in the field concerned.

A preliminary survey of the review literature related to the topic has revealed a paucity of comprehensive, relevant and recent reviews of the topic in the mainstream literature of the relevant disciplines, and a relative absence of focus in the field. For example, amongst approximately 500 review articles in the last 10 years published in the authoritative Annual Reviews of Nutrition, Immunology, Microbiology, Medicine and Physiology respectively, not a single article was devoted even indirectly to the topic in question. There are a small number of monographs that cover nutritional aspects of human immunity in a systematic way but any search of the literature using appropriate key-words generates large numbers of papers that do not provide a compelling, conceptually integrated picture in relation to the above-listed brief.

## Previous efforts and their outcomes

As mentioned above, despite conferences being held on related aspects of the topic, such as the International Conference on HIV/AIDS and Food and Nutrition Security held in Durban, South Africa in April 2005 ( see the full web record at www.ifpri.org/events/conferences/2005/20050414HIVAIDS.htm), and despite the publication of the dedicated monographs mentioned in the previous chapter, the published, peer-reviewed literature relating to nutrition and human immunity has not been systematically reviewed in a nationally contextualised and integrated way, with a specific (but not exclusive) focus on the linked pandemics of HIV infection and tuberculosis (but see WHO activities mentioned in the next paragraph). In contrast, reams of newsprint and large numbers of isolated papers have been devoted to the topic without resolving the main issues to any useful extent.

## International comparisons and similar studies done outside South Africa

The WHO constituted a Technical Advisory Group on Nutrition and HIV/AIDS in 2003 to draw up several technical reports on different aspects of nutrition and HIV infection, involving a number of South African participants and contributors; the report of a "Technical Consultation" conducted in 2003 is at www.who.int/nut/documents/hivaids_nut_require.pdf, a more recent fact sheet is at www.who.int/3by5/mediacentre/fsFood/en, and the final reports on six sub-topics were posted on the WHO website at the end of October, 2005. A variety of presentations made by delegates from many countries at

the 18th Congress of the International Union of Nutritional Sciences (IUNS) also usefully dealt with aspects of the topic of the study.

The ASSAf Consensus Study must obviously not duplicate the above work or add to community confusion in the area. The following are distinctive features of the kind of report the Academy seeks to produce, and the unique benefits it should provide:

i. The study is being carried out independently by a selected group of eminent scholars who will bring a variety of disciplinary insights and conceptual strengths to the topics to be examined;
ii. Both tuberculosis and HIV infections are being studied, separately and as conditions that frequently coexist, against a background of a general, evidence-based understanding of the functional inter-relationships between infection and nutrition;
iii. More attention is given to the functioning of the immune system in relation to nutritional factors than has been the case in other reviews and reports mentioned above;
iv. Specificity related to genetic and individually applicable developmental factors is being thoroughly explored and interpreted in relation to generally applicable human responses;
v. The study is rooted in the national context; and
vi. The report on the study, while strong in reviewing and evaluating all the available, reliable scientific evidence, is written in very clear, non-technical language so that it will be useful to a broad range of users (the reporting model established by the U S National Academies for its extensive set of consensus studies is prototypical, based as it is on a very impressive track record of effective intervention on topics of national importance in that country and beyond.)

## How is the study to be approached?

### Methodology

The Study Panel guided by its Chairperson selects from a range of methodologies in order to meet the brief provided by the ASSAf Council, as described in the relevant "Guidelines" mentioned above. These include:

i. hiring researchers to address sub-topics and help provide draft chapter of the report;
ii. holding public workshops with invited speakers and/or panel discussions;
iii. holding Study Panel workshops to debate and resolve particular questions and issues;
iv. delegating initial analysis of topics of the study to individual members or sub-groups of the Study Panel; and





v. any other ways of working towards a proper understanding of the evidence and information that can help to complete the study.

## Design and lay-out

### Introductory aspects

Nutrition and malnutrition are operationally defined in the context of the above-described brief of the study, as are the macro- and micronutrients in human diets, in an integrated and modern conceptual framework of whole-body functioning in respect of individuals and populations.

Malnutrition in the world, sub-Saharan Africa and South Africa is reviewed, and the focus of the Study defined as reflecting primarily malnutrition as a result of poverty/socioeconomic background/circumstances, including the supervention of nutritionally altered states such as those caused by HIV and active or clinical *M.tb.* infection.

A brief systematic summary of the literature relating nutrition and human immunity is presented, and the primary objectives and the expected outcomes of the review described.

- **First topic:** Nutritional modulation of the normal human immune system, innate and adaptive, at different ages, in both sexes, over short or long periods, with respect both to general undernutrition (macronutrients), and to specific deficiencies of micronutrients.
- **Second topic:** Broad overview of immunity and its components, and of how the human immune system responds differently to different pathogens

  Current understandings of the cellular and humoral components of the innate and adaptive arms of the immune system of humans are contextually reviewed, including the checks and balances within the immune system, and recent insights into genetic differences between individuals and populations that affect immune responses.

  The measurable components of immune function and their proxies are systematically evaluated: detectable antibodies and their properties; cytokine assays in body fluids; in vitro immune cell marker analysis, reactivity to antigen and other challenges, and other functional assays; skin tests; histological and histochemical tissue analysis; genomic analyses; etc.
- **Third topic:** "Immunonutrition"

  The apparent nutritional requirements of humans of different ages are assessed in the light of the need to ensure the maintenance of a healthy immune system ("immunonutrition"); this includes prenatal stages and the "physiological"

supervening state of pregnancy. The analysis takes into account differences between individuals and groups associated with specific genotypes interacting with environmental circumstances at different stages of the life-cycle, with transient and sometimes long-lasting residual effects ("nutrigenomics" and "programming").

The involvement of specific nutrients in different components of immune function is reviewed, and reliable evidence of the effects of specific and mixed nutritional deficiencies as well as general malnutrition on the functioning of the human immune system described (animal studies are only included when these have a clear bearing on human functioning). Particular attention is given to micro-and macronutrients believed to affect immune system function, such as vitamin A and the pro-vitamin, beta carotene; vitamin B group (especially niacin, pyridoxal and riboflavin), vitamins C and E; vitamin D; folic acid; iron, copper, zinc and selenium; amino acids such as L-arginine, L-glutamine and sulphur-containing amino acids; and polyunsaturated fatty acids.

- **Fourth topic:** Modulation of human nutritional status in states of chronic infection, with special emphasis on infection with the human immunodeficiency virus and ***Mycobacterium tuberculosis***.

  Essentially this topic includes the examination of the evidence for systematically altered nutritional status and dietary requirements of individuals in infected states, especially those caused by infection with HIV and *M.tb.,* including the effects of pre-existing malnutrition of various kinds, as well as the occurrence of other preceding or supervening infections and/or infestations. The objective is to define the general and specific minimum and optimum nutritional requirements of HIV-infected individuals suffering from clinical tuberculosis, both males and females and at all ages, in terms of assisting the immune system to deal with the underlying infection.

  Heterogeneity of immunomodulation is explored by conducting the following subgroup analyses on published studies:
  - the degree of immuno-activation and -suppression in the infected participants;
  - the severity of the main illness; and
  - the presence and degree of other (opportunistic) infections.

  The rationale for reliably assessing nutritional status in different ways and by different proxies is assessed, for example, body mass changes; loss of muscle and fat mass; nitrogen and sulphur balance, micro-and macronutrient levels in different body compartments or excretae; etc.
- **Fifth topic:** Effects of nutritional interventions on morbidity and mortality in adults and children infected with HIV or suffering from clinical tuberculosis, or both
  - **Randomised controlled trials** that have evaluated the effects of various nutritional interventions on at least one of the primary outcomes listed below are included in the review; the participants are males or females of all ages with confirmed HIV infection, clinical tuberculosis, or both conditions.
  - **Interventions** that are assessed (taking into account whether nutrients are provided as supplements or as actual food) include:
    - provision of additional micronutrients, such as vitamins and pro-vitamins and trace elements;
    - provision of additional macronutrients, such as proteins, carbohydrates, lipids/fats;
    - (if possible) combinations of nutritional intervention with or without drugs, primarily antiretroviral (ARV) and/or anti-TB treatment.
  - The **systematic reviews** (and, where possible) **meta-analysis**, aim to compare single or multiple nutritional interventions versus no nutritional intervention, with or without ARV and/or anti-tuberculosis treatment, as well as alternative nutritional regimens with or without ARV and/or TB treatment
  - **Outcomes** of interest in the **primary category** include morbidity (e.g. frequency of hospitalisation, days spent in hospital, type, frequency and duration of opportunistic infections, degree of weight loss, and progression to AIDS); and mortality (all-cause and specific).
  - Outcomes in the **secondary category** include correlates of morbidity, nutrition or immune function (including nutritional status/markers, markers of changed immune function, effectiveness of nutrient absorption, viral load, CD4 count, etc), recorded and contextually used in the analysis of the primary outcomes.
  - **Heterogeneity of effects** are evaluated by conducting the following subgroup analyses:
    - Underlying or prior nutritional status
    - Severity of illness at time of inclusion into study (CD4 count, clinical stage of infection).

## Likely outputs, outcomes and impacts of the proposed study

The main tangible output of the proposed study is an authoritative ASSAf Report published in the public domain but presented in an appropriate way (with contacts and briefing sessions prior to the public release of the report), to the Ministries/Departments of Science and Technology and of Health, the National Health Research Committee, the Medical Research Council and the various Provincial health departments; the main conclusions and recommendations are summarised in a readable and useful form. The document is one that can also be made available to teaching and training institutions to

influence the content and approach of their programmes and to the media in order to reach the broad mass of the public to help in preventive and promotive health.

The ASSAf report should influence public policy in the area of prevention, support and therapy of the pandemic infections now current, such as those with *M.tb.* and HIV, to achieve the anticipated benefits through provision of clear guidelines inter alia for:

i. the conduct of trials to assess the efficacy of nutritional interventions in HIV- and/or *M.tb.*-infected individuals;
ii. the use of general and specific nutritional interventions in preventing, controlling and ameliorating the effects of infections on their hosts;
iii. cost-effective planning and resourcing of appropriate support measures for infected individuals and communities in meeting their nutritional requirements;
iv. programmes of public health education, and partnerships with industry in employee support; and
v. the production/processing of foods and nutritional supplements.

The report is written to maximise its impact of the resolution of controversy in this area, of improved health service planning and purposeful resourcing, and the general promotion of national cohesion in addressing the concurrent pandemic infections. It aims to be decisive in achieving a much-needed turnaround of a critical national success factor, the health of our youth and of our economically active people, and the preservation of families as the social core of the nation.



# Introduction and Background

CHAPTER 1

# Conceptual overview

Animals, plants and micro-organisms have evolved through biological history to create the enormous biodiversity of the planet today. Because they inhabit the available spaces together, much of what has happened along the way has been co-evolution, characterized by beneficial inter-dependence and reciprocal competition.

Few things illustrate this better than the relationship between humans and their by now ancient enemy, the bacterium that causes tuberculosis. Humans, as large, unspecialized and highly adaptable mammals with big brains and intricate social systems, have spread to almost every available niche and habitat on the globe; *Mycobacterium tuberculosis*, a tiny, invisible, but very tough enemy, has achieved an almost identical distribution, living in as many as one third of all humans and spreading through frequent flare-ups, highly injurious to their host, constituting the "white death" of our recorded history[1].

HIV, by contrast, jumped within the last century from its long-term host, populations of particular great apes, to vastly numerous human populations, and now lives in some 50 million people in most parts of the globe. It spreads relentlessly during a slowly progressive disease process. Even this short period of interaction between very big humans and very tiny retroviruses is full of co-evolutionary phenomena[2].

Humans have highly evolved immune systems (many of the most striking differences between the genomes of mammals such as rodents and us involved proliferation of defense mechanisms against microorganisms). We have gone beyond our formidable biological weapons to acquire pharmaceuticals (often, ironically, "borrowed" from harmless micro-organisms) to give us decisively effective additional advantages and back-ups in our ceaseless fight against the harmful microbes. Thus highly effective drugs have been developed specifically to counter *Mycobacterium tuberculosis* and HIV, in the first case curatively through the use of multi-drug combinations, and in the second case suppressively, also through multi-drug combinations.

**It is important to bear in mind that the pre-antibiotic phase of dealing with TB was characterized by intense concentration on strengthening the immune defenses**

**of infected subjects with diets, improved and altered environmental conditions and every other conceivably helpful measure. After the discovery of effective drugs, this aspect of TB therapy quickly became secondary and largely uncontroversial. (With the more recent emergence of multi-drug resistant and extensively drug-resistant TB strains especially, these older measures may again have to be looked at as potentially valuable supportive measures in our total arsenal).**

**Because HIV infection cannot be cured but only controlled, with drugs being applied at particular, serious stages of progressive disease (according to current guidelines, at least) the emphasis in the management of infected people during the phases prior to drug administration is still on general, non-pharmacological support, especially as for many reasons it is highly desirable to postpone the introduction of specific antiretroviral therapy for as long as possible.**

This study has examined the role nutritional support can play, in individuals and populations, at this time and in this place, against these two pandemic microbial enemies of *Homo sapiens*, in the light of the above macro-considerations.

## Basic science of immunonutrition – basic concepts

Nutrition is conventionally recognised as being essential for two main purposes. These are firstly, the generation of energy to power the needs of the body, and secondly the provision of building blocks for growth, replacement and repair of cells and tissues. The fact that growth is the cardinal characteristic of infancy and childhood as opposed to adulthood is largely why adequate nutrition is particularly critical in early life. Not only is nutrition a critical requirement for the survival of the infant or child until the attainment of adulthood, but it is also emerging that failure to receive adequate nutrition during early life may have lifelong adverse consequence for immune functions in adult life. This ominous concept is briefly explored in a later chapter.

Chief among the energy sources are the carbohydrates and fats, while the amino acids derived from proteins constitute the building blocks needed for growth and cell or tissue maintenance. Carbohydrates, fats and proteins are traditionally characterised as the macronutrients, because they are required in large quantities relative to the micronutrients – vitamins and trace elements. Broadly, the function of the micronutrients is to facilitate or enable the safe and effective utilisation of the macronutrients. A developing, perhaps more realistic trend, as the specificity of nutritional chemistry and molecular biology unfolds, is to regard each of the 21 individual amino acids more as micronutrients rather than as constituents of a single macronutrient, but for the purposes of this report the traditional classification of macronutrients and micronutrients is retained.





The complexity of the immune system has been notoriously difficult to convey to students of human physiology and pathophysiology, and teachers have made frequent use of military analogies to help to explain some of the basic principles. Thus, pathogenic organisms are depicted as foreign invaders, with the cells and molecules of the immune cells cast in the role of "home-army soldiers" and their "weapons", respectively. In terms of these analogies, and with special relevance to the intersection of the science of immunology with the science of nutrition, the destruction of foreign invaders, such as the *M. tuberculosis* organism, is critically dependent on effective deployment of one of the most effective weapons in the immunological arsenal – the "**flamethrower**". This analogy is representative of the respiratory burst, whose central role in the intracellular killing of pathogenic organisms is well established. Moreover, it has emerged that strains of *M. tuberculosis* that have effectively evolved strategies to evade oxidant damage (effectively dousing the "fire") are associated with particular virulence in causing severe disease in the human host[3]. The "flamethrower" is thus the weapon that burns (oxidises) foreign organisms to death. It is self-evident that such a weapon is rendered ineffective if not kept supplied with adequate energy, explaining the nutritional need for energy fuels such as carbohydrates and fats. The complete burning, or oxidation within cells of these fuels to generate the energy needed to power "flamethrowers" is accomplished as they are metabolised through the Krebs cycle. Vitamins $B_1$ (thiamine), $B_2$ (riboflavin) and $B_3$ (niacin), are essential precursors of components of the electron transport chain, and are critical requirements for this process.

Not surprisingly, such dangerous weapons as "flamethrowers", when deployed to fight foreign microorganisms, are prone to cause significant collateral damage to friendly forces and structures. Accordingly, a critical nutritional component in the functioning of the immune system concerns the generation of chemical energy in a form that can be safely directed at foreign organisms. The risk of damage to host structures, cells and tissues is minimised by antioxidants, whose functions are thus analogous to "f**ire extinguishers**". Many of the micronutrients function in this antioxidant capacity. While B-group vitamins have a number of critical roles in cellular metabolism, including rate-limiting functions in cell growth and regeneration (a vital process in the production of sufficient white cells to combat infectious microorganisms), indirectly contributing towards intracellular antioxidant activity is one such role. Similarly, many of the trace elements are metals, each of whose available electron orbitals is precisely tailored to absorbing or releasing a specific energy quantum; the full complement of trace metals such as iron, zinc, magnesium and copper thus provides a range of quanta to cover the equivalent range of biological energy transfer oxidation-reduction reactions. Sulphur and selenium are, like oxygen, chalcogens (Group VIa elements). They are thus similar chemically to each other and to oxygen, but with subtly different electron affinities and

electronegativities. Sulphur is a component of the amino acids methionine and cysteine, the latter being the central active component of the archetypal intracellular antioxidant "fire extinguisher", the tripeptide, glutathione. Its other constituents are the amino acids glycine and glutamic acid, the latter derived from dietary glutamine, another amino acid. Selenium is a component of the 21st amino acid, selenocysteine. The role of selenocysteine containing proteins, such as glutathione peroxidase, is to regulate a number of critical oxidation-reduction reactions.

Cell membranes are composed largely of fats, and in the metaphoric sense being used here, highly "inflammable". Vitamin E (tocopherol) is one of the fat-soluble vitamins whose prime function is to act as a fire extinguisher for deployment in cell membranes. Collateral oxidation damage resulting from the deployment of intracellular "flamethrowers" is not necessarily contained within cells, because cells may die and release oxidising chemicals. The role of vitamin C (ascorbic acid) is to mop up potentially dangerous extracellular oxidants, and its prime function is therefore also largely that of a "fire extinguisher".

To summarise the role of nutrition in immune function, a key strategy is the use of chemical energy to "burn" or oxidise foreign microorganisms. Much of the nutritional demand of the immune system concerns the safe provision of chemical energy for this purpose. The macronutrients, carbohydrates and fats, are the raw fuels for this energy, and the prime function of many of the micronutrients, including most of the B-group vitamins, vitamin C, vitamin E, many of the trace elements and some of the amino acids, is to provide the machinery for this energy to be extracted and deployed safely, minimising the risk of collateral damage to host cells and tissues. The dietary vitamin A precursors, the carotenoids are also antioxidants, while vitamin A itself has other immunologically relevant functions, including gene regulation and the preservation of mucous membrane integrity. With regard to the second generic need for nutrition, the growth, replacement and repair of cells and tissues, virtually all the other nutrients not covered above in this brief conceptual overview of immunonutrition, are largely devoted to this purpose in immune cells. The B-group vitamins, folic acid and vitamin $B_{12}$ are primarily concerned with the process of DNA synthesis, which is needed especially for the replacement of immune cells undergoing rapid turnover during infectious episodes. (Soldiers have a high attrition rate in wartime). Vitamins A and D are critical requirements in the process of regulating the production of many of the immunologically important proteins such as antibodies, cytokines, etc (more weapons in the military arsenal, other than the "flamethrowers"). The amino acids needed to construct these weapons are derived from the macronutrient, dietary protein.

The assessment of **subclinical nutritional deficiency** is notoriously difficult. This factor confounds the identification of early phases of nutrient depletion, which may well impact on immune function. Simple measurements of blood or tissue fluid levels of





specific nutrients frequently fail to reveal even quite advanced depletion states. Nutritional depletion states, leading in time to overt deficiencies, represent a spectrum of accumulated negative balance between whole-body intake and loss. With the typical elegance that is characteristic of biological systems, there are mechanisms to protect vital functions, and decreasing urinary excretion, or curtailing it completely, is one of the first of the strategies the body deploys to preserve its vital nutritional resources. This is accompanied by the scaling up of intestinal absorption efficiency, but eventually these devices may fail and only in very advanced deficiency states do blood concentrations of many micronutrients begin to fall. Functional impairment to cell or tissue systems follows, with the inevitable development of the clinical symptoms and signs of a deficiency state.

Different cells and tissues vary in their position within the hierarchy of claimants to available resources, reflecting variations in the affinities and turnover rates of tissue specific vitamin-derived coenzymes. Where the immune system lies within this hierarchy is currently poorly understood, partly because the correlates of immune deficiency and its restitution are generally ill defined, and in any event these correlates are probably themselves highly variable between immune responses to differing microbial pathogenic organisms. Nevertheless, certain markers to measure putative dietary immunomodulation are recommended in a general immunological setting[4]. These include vaccine-specific serum antibody synthesis, total and vaccine antigen-specific salivary IgA, delayed hypersensitivity reactions, responses to attenuated pathogens, natural killer cell cytoxicity, lymphocyte transformation assays, cytokine production by activated immune cells, and phagocyte respiratory burst activity. Which of these assays are of value in the setting of widespread HIV and TB prevalence is not yet clear, and few reported studies have applied these markers. Despite this, the word "immunomodulation" is widely used to describe activities of foods, often in the complete absence of evidence to support such claims.

## Immunopathogenesis of HIV/AIDS and TB – essential concepts

Humans, like all biological organisms, exist in either a state of harmony, neutrality or conflict with the other organisms occupying the biosphere. Epithelial tissues, comprising the skin on the outside, or the mucous membranes (mucosae) internally, constitute the major contact zone between us and our environment. For this reason, skin and even more so, the mucosae, are particularly richly endowed with cells and molecules of the immune system. Although these cells comprise a variety of types, most are lymphocytes, and therefore these tissues are generically designated the Mucosa Associated Lymphoid Tissue (MALT). Of these tissues, those of the gastrointestinal tract are designated Gut

Associated Lymphoid Tissue (GALT). In efforts to reconcile the primary absorptive functions of the respiratory and gastrointestinal tracts with the need to exclude harmful microorganisms, these mucosae rely additionally on symbiotic flora, which protect their host partly by competing for nutrients and space with potentially harmful organisms, just as grasses compete with weeds for nutrients and space on the lawn. These flora are initially ingested in the diet as natural probiotics, which then colonise the mucosae and depend subsequently on ingested but undigested oligosaccharides (prebiotics) for their subsequent stable symbiosis with their human host.

It is against this background that the recent demonstrations that the GALT constitutes a major primary battleground between HIV and its human host are of particular interest. To what extent this battleground activity affects the absorption of nutrients, and the prospect of gastrointestinal opportunistic infections arising and their nutritional consequences are just two of the new questions arising from these insights. Another concerns the possibility that constituents of foods may influence HIV/AIDS pathogenesis by food allergy-type immune activation. Moreover, the extent to which the related flora of the gastrointestinal and reproductive tracts participate in defence against HIV, TB and opportunistic infections due to other organisms, and the effect of microbicides on such flora, may prove to affect the efficacy or otherwise of microbicides as HIV-preventive strategies.

The main cellular sentries protecting mucosal surfaces from invasion are the **macrophages** (big eaters) and the $CD4^+$ T **lymphocytes**, both of which are targets for the human immunodeficiency virus. These cells collaborate in the destruction of potentially pathogenic microorganisms such as *Mycobacterium tuberculosis*, whose survival strategies involve escaping to the relative sanctuary they find inside macrophages, away from the extracellular domain, where an abundance of antibodies, complement, and other immune related molecules represent a major threat to their survival. Generally it is the organisms utilising this survival strategy of living and growing within macrophages, which constitute the major opportunist threats to HIV-compromised patients. In normal individuals, TB organisms and the like can be identified within these intracellular sanctuaries by a group of molecules known as **MHC** class II proteins. These proteins belong to the same family as antibodies, and function in a not dissimilar fashion by binding to target peptide fragments characteristic of the organism. These peptide-MHC complexes are exported to the surface of the infected macrophage, where they attract the molecular attention of suitably configured $CD4^+$ T lymphocytes, initiating a molecular "conversation" which culminates in the deployment of the intracellular "flame thrower" referred to above, and the destruction of the TB organisms within. HIV infection, whether of T cells or macrophages, causes a failure of this process. Undoubtedly the major reason for this failure is the result of the loss of $CD4^+$ T cells as part of the pathogenesis of HIV infection. What is not so clear is the reason for this destruction of $CD4^+$ T lymphocytes.





One explanation suggests HIV-infected cells simply die. An alternative explanation posits the indirect destruction of uninfected T cells that are inappropriately activated, possibly during abortive immune activation attempts by HIV-infected or HIV-laden macrophages. Recent insights obtained from knowledge of the structural biology of the CD4-HIVgp120 and CD4-MHC II molecular interactions[5] reveal a precise molecular mechanism for such abortive immune activation attempts.

Whatever the mechanism of T cell destruction, nutritional impairment could impact adversely on a number of events during this process, leading to a vicious cycle of escalating HIV and TB pathology and aggravated malnutrition. Moreover, the new focus on HIV infection of the GALT affords a scenario for abortive immune activation as at least contributory to the immune deficiency state of HIV-infected individuals. To what extent this scenario is supported by evidence, and the prospect of applying this insight in the development of new therapeutic strategies, nutritional or otherwise, was explored more fully during the ASSAf workshop devoted to this topic.

The main reason for the inclusion of the basic science of immunonutrition, and the pathogenesis of HIV/AIDS and tuberculosis within the scope of the consensus panel's activities, is to provide a rational, as opposed to an empirical, background to some of the observational studies that have been conducted to explore the nature of the relationship between malnutrition and infectious diseases. It also serves the purpose of complementing clinical observational studies in identifying hypothetical nutritional interventions that might be tested formally, before asserting that the benefits ascribable to such interventions are indeed evidence based.

## Interpreting Clinical evidence

The major objective of the introductory chapter on **Evidence-Based Practice and Recommendations** is to show how observational and intervention studies that examined the role of nutrition in the prevention and treatment of TB, HIV and AIDS should be evaluated and used to grade the quality of evidence to form a basis for recommendations for nutrition policy and practice to help to prevent and treat these diseases. Evidence-based health policy and practices are defined, the different types of nutritional studies discussed, and guidelines (checklists) to evaluate these given with special reference to the complexities and potential pitfalls of nutrition studies.

The scientific process is characteristically divisible into two distinct cognitive processes. These are induction, during which generalisations are made from observations of particular instances (evidence) of what is being studied, and deduction, which is the process whereby a particular prediction is inferred logically from a general principle, often in the absence of direct evidence. Within the context of clinical science, basic

science and clinical observation constitute the general principles, from which deductions of predictable clinical events may sometimes be inferred.

Together with empirical and other observational clinical trials, an understanding of the basic science of nutrition and immunity may thus be useful in formulating hypotheses concerning possible therapeutic nutritional interventions that might be implemented with beneficial effect, but clinical science is increasingly aware of the need for direct evidence to support therapeutic intervention policy. The complexity of the field of nutrition is, however such that direct evidence to support generalisations is often fraught with confounding circumstances. For example, a policy to supplement diet with specific nutrients may have been based on study of a population that happened to be deficient in those nutrients, and may be entirely inappropriate for another population in which the prevalence of deficiency is different from that of the study population. An obvious population variable is life stage – for which reason this report considers adults children separately, but even within matched life stage populations, there are major heterogeneities in different geographical regions. Stemming from this is the difference between **relative risk** and **attributable risk**. In an individual with a documented, specific nutritional deficiency, say protein deficiency, the relative risk of an adverse outcome from say, tuberculosis, might be very high. On the other hand, if the prevalence of protein deficiency is low in the local population, then the risk of adverse outcome of tuberculosis attributable to protein deficiency will be proportionately lowered in that population. Clearly these issues are relevant to the development of locally or regionally appropriate policy guidelines based on scientific evidence.

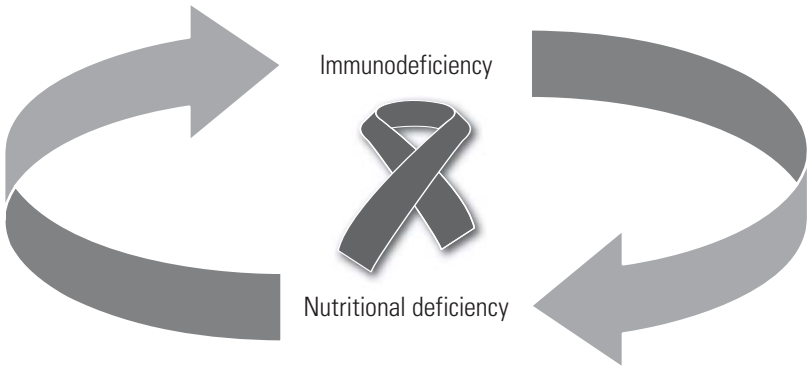
For the purposes of this consensus study, the population is defined as African, and particularly, South African, and it is acknowledged that even within this narrow definition, there may be significant variation. A consequence of too narrow a definition of population is that the evidence base inevitably becomes proportionately restricted, which necessitates application of the deduction process from the basic science-generated hypothesis in many instances.

Another trap for the unwary in this field concerns the difference between evidence of association and evidence of causation. Observational studies frequently reveal associations between identifiable clinical states. These may be causally related, in which case it is not always clear which is cause and which is effect, or both states may have been caused independently by some third, unidentified factor. In resolving the exact nature of cause and effect relationships, and in thereby determining optimal therapy, there is no real substitute for the prospective, randomised controlled intervention trial, and this is particularly relevant to the field of nutrition, and even more so in the context of HIV, because nutritional deficiency and immune deficiency, whether caused by HIV infection or not, constitute a vicious cycle of mutually reinforcing abnormalities (**Figure 1**).





***Figure 1. Conflating cause and effect***

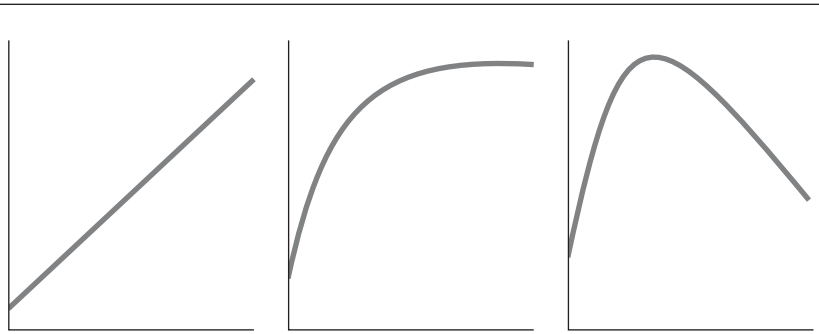



Infections with various microorganisms constitute the major burden of disease in the developing world, which also happens to be plagued by inadequate food security and epidemics of under-nutrition. Undoubtedly there has long been evidence of association between infectious disease and nutritional deficiency states, and this is compounded by the synergies between immune deficiency and nutritional deficiency. HIV is detonating this vicious cycle on an unprecedented scale in the developing world particularly in sub-Saharan Africa.

Another important consideration has been the recognition that nutrients, including essential nutrients, may be potentially harmful in excess. Three patterns of health benefit/dose response are recognised (**Figure 2**).

It appears unlikely that any substance, even water or oxygen, can be beneficial in unending proportionality to the administered dosage, and most essential nutrients would

***Figure 2. Theoretical nutrient dose response curves***



be expected to exert a saturable response, viz. beneficial up to a certain level in correcting overt or sub clinical deficiency states, with diminishing returns thereafter. Some (such as vitamin A) may even be toxic at high doses. These are important considerations because they expose the flawed reasoning of those advocating unconsidered supplementation (of multi vitamins for example) as necessarily harmless.

## Formulating policy for research and practice

It is a prime function of ASSAf to serve as an interface between scientists and policymakers. With regard to the current study, policymakers include those involved in health care delivery, and also those concerned with setting research policy. In other words, it has been the task of this panel not only to identify policy for best practice in relation to nutrition HIV/AIDS and TB, but also to define gaps in the evidence base as priority areas for further research.

One of the criticisms of a radical, purist evidence-based approach to medical decision-making and policy formulation is that professional caregivers and clinicians have expressed reservations that many years of hard-earned practical experience can potentially be discounted and undervalued in the evidence-based medicine (EBM). Similarly, the National Department of Health might argue that they are accountable for the practical, real world (i.e. not the ivory tower) situation, and have special knowledge and skills relating to what can and cannot be done on a national level. The response of the panel to this has been to look at all the opinions, whether based on firm scientific evidence, or on clinical experience, or from the vantage point of a National Department of Health charged with the responsibility of forming and implementing policy on a national scale. The panel has looked at these 3 perspectives to identify commonalities or divergences of opinion, in the light of its own process and policy recommendations. The source material for each perspective is as follows:

1. Evidence-based guidelines from the consensus panel's own studies and the 2005 recommendations from the World Health Organization.
2. The 2006 guidelines from the Southern African HIV Clinicians Society.
3. The 2006 guidelines from the National Department of Health.

## Scope of the Consensus Study

Food contains a vast and bewildering array of different substances, both characterised and uncharacterised, and for the purposes of this study, this complexity has necessarily restricted the scope of investigation to include the immunological effects of elements and compounds universally recognised as macronutrients or micronutrients, of which





some 40 or so are considered to be essential constituents of a healthy balanced diet (13 vitamins, 17 elements, nine essential amino acids, and essential fatty acids and carbohydrates).

The complex field of **phytochemicals and other food constituents**, which may well exert profound effects on immune functions, has not been addressed. Both western pharmaceutical agents, (whose parent molecules may have originally been discovered as food or plant constituents), and traditional pharmacologically active compounds have been excluded in their entirety, not because they are considered unimportant or without potential value, but because in the view of the panel they fall outside the scope of nutrition. On the question of the immunologically beneficial effects or otherwise of traditional remedies and their constituents, the ethical societal rules pertaining to any potential or actual pharmaceutical product would appear to apply. Claims made in respect of such remedies should be subject to rigorous review before the scientific community can endorse them, and they must proceed through the same channels of description, characterization and chemical identification, and animal and human safety and efficacy trials as are necessary for any pharmaceutical product. The South African Medical Research Council has a policy and a dedicated research unit devoted to the study of Indigenous Knowledge Systems (IKS). A significant segment of the work of the South African MRC IKS Research Unit is focused on the process to be followed in respect of HIV interventions claiming benefits of one form or another.

**The Study Panel unanimously endorses the view that specific antiretroviral agents are the only established direct weapon in the treatment of HIV infection itself; unfortunately, unlike the drugs used to treat active tuberculosis, and for a variety of complex reasons, they cannot (yet) be used immediately after diagnosis, creating the situation where indirect support and care must sustain infected subjects until (as well as after) antiretroviral drugs are applied.**

The important intersection between **nutrition and antiretroviral therapy (ART)** has not been addressed in the current Academy of Science of South African consensus study because it has been reviewed comprehensively recently and was included in the recent World Health Organization Reports on Nutrition and HIV infection.

Microbial safety of foods, food preservation and/or preparation has not been addressed in this report, but much attention has been paid to food safety in the revised National Guidelines referred to in the chapter on policy guidelines. Similarly, examination of the social science evidence underpinning recommendations concerning food security have not been addressed in this study.

CHAPTER 2

# Three South African epidemics

This chapter reviews the South African epidemics of malnutrition, HIV infection and tuberculosis as separate entities. The prevalence of each epidemic is sufficiently high in South Africa to ensure significant overlap purely on mathematical grounds – in other words, even if there were no synergies between them. In fact, there is considerable prima facie evidence from observational studies to suggest that each epidemic reinforces the other two.

## The epidemiology of nutrition in South Africa

### Nutritional status of South African children

Malnutrition is the world's most serious health problem. It is responsible directly or indirectly for nearly 60% of the 10.9 million annual deaths of children under five[1]. Although nutrition has steadily improved in most regions of the developing world between 1980 and 2006, in sub-Saharan Africa malnutrition is on the rise[2]. In southern Africa, this increase is strongly linked to the HIV/AIDS pandemic.

In Africa, 50% of children with severe nutrition die during hospital treatment due to inappropriate care, and one quarter of preschool children suffer from undernutrition, which may affect mental and physical development[3]. Inappropriate feeding of infants and children is responsible for one-third of the causes of malnutrition[3]. The most common forms of malnutrition include **micronutrient malnutrition** (MNM) and **protein energy malnutrition** (PEM).

Micronutrient malnutrition is caused by poor quality diets, characterized by high intakes of staple but low consumption of animal and fish products, fruits, legumes, and vegetables, which are rich sources of bioavailable minerals and vitamins. Most of the malnourished are those who cannot obtain these foods to substitute in an impoverished subsistency situation from their own production. 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 in terms of lives lost and quality of life are staggering[4].

**Protein energy malnutrition** (PEM) refers to inadequate availability or absorption of energy and proteins in the body. **Kwashiorkor** and **marasmus** are some of the more severe forms of PEM. Kwashiorkor (meaning the disease of the displaced child in the language of Ga/Kwa) was first defined in the 1930s in Ghana as primarily a protein deficiency while marasmus is characterised by both protein and energy deficiency. Marasmus is frequently found in infants less than 6 months of age and it is been seen more frequently now in older infants and young children with the increasing prevalence of HIV in that group. In South Africa, kwashiorkor occurs predominantly in the 6-month to 2-year-old age group. Established risk factors vary widely in different settings with few consistent epidemiological patterns emerging globally. Frequently described risk factors include:

- poor family dynamics (e.g. mother pregnant or poorly educated, father unemployed or separated, parental illness)
- sub-optimal nutrition (e.g. lower breastfeeding rates or earlier introduction of complementary feeds, lesser dietary diversity)
- lower socio-economic status (e.g. inadequate housing, absence of water and sanitation)
- less frequent use of health services (e.g. preference for traditional healers)[5].

Marasmic malnutrition is known to be commoner than kwashiorkor in HIV-infected children. HIV infection has become an important contributor to severe malnutrition, with over 80% of severely malnourished children at some South African hospitals being infected[5]. At the King Edward VIII Hospital, Durban, 68% of children admitted with diarrhoea were classified as HIV-infected and 61% were classified as malnourished, with 53% having evidence of both[6]. In the Bohlabela district of rural Limpopo, statistically significant risk factors for severe malnutrition included suspicion of HIV infection in the family (parents or children) (Odds Ratio (OR) 217.7), poor weaning practices (OR 3.0), parental death (OR 38.0), male sex (OR 2.7) and higher birth order (third child or higher) (OR 2.3); protective factors included a diverse food intake (OR 0.53) and receipt of a state child support grant (OR 0.44)[5].

There is insufficient evidence from national studies to indicate whether the HIV/AIDS epidemic has caused an increase in under-nutrition in South Africa. One group has integrated and summarised the available literature on the largely unchanged nutritional status of South Africans from 1975 to 1996[7]; later studies and publications have also not shown definitely evolving differences. Wide variations between areas, population groups, etc are the norm, however. It is likely that improvements in nutritional status because of better food security have, on balance, been negated by the impact of widespread HIV/AIDS. This view is concordant with changes in key health indicators over time: under-5 mortality rates have in fact increased from 59.4 in 1998 to 100.0 in 2002[8]. A thorough





re-assessment of the current situation with respect to population nutritional status in the light of the HIV/AIDS and TB epidemics is urgently necessary.

National food consumption surveys published in 2000 and 2005 found that **stunting** remained the most common nutritional disorder, affecting between 21.6% to 19.3% of children aged 1–9 years of age[9, 10]. The highest prevalence of stunting was found in 1–3 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 [11]. 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 4 to 6 months of life, largely associated with successful breast-feeding. Thereafter, the proportion of children falling below the 3rd weight-for-age centile (underweight) increases. The prevalence of underweight-for-age and stunting increases rapidly after 6 to 12 months of age (at the time of the introduction of complementary foods into the diet of the breast-fed infant). The WHO recommends a Z-score cut-off point of <-2 standard deviations (SD) to classify low weight-for-age (underweight), low height-for-age (stunting) and low weight-for-height (wasting) as moderate malnutrition; and <-3 SD to define severe malnutrition. The cut-off point of >+2 SD classifies high weight-for-height as overweight in children[12].

In South Africa, stunting (low height-for-age) is thus the major form of undernutrition. The relative rarity of wasting and the high prevalence of stunting in South Africa suggest that the main problem is chronic socioeconomic underdevelopment. Most stunting occurs before the age of 3 years, and stunted children usually become stunted 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 insecure, with 38% of African households often or sometimes going hungry[8]. There are currently insufficient anthropometrical data for South Africa; **Table I** reflects the country's 1999 statistics[9, 13].

The health and nutritional status of mothers greatly influences the growth and development of their babies during pregnancy and infancy. If mothers are undernourished, of 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 effects[14]. About 16%of

*Table I. Estimated percentage prevalence of malnutrition among children (0-5 years)*[9, 13]

| Setting | Underweight | Stunting | Wasting | Overweight |
|---|---|---|---|---|
| Global | 21 | 24 | | |
| All developing countries | 23 | 27 | 8 | 3.4 |
| Africa | 25 | 35 | 10 | 5.2 |
| South Africa (all) | 9 | 23 | 3 | 6.7 |
| Urban South Africa | 7 | 16 | | |
| Rural South Africa | 11 | 27 | | |

infants globally are born with a low birth weight (LBW) (<2500g), although LBW rates vary considerably from country to country (from about 5 to 32 percent)[7]. While there are no reliable national data for South Africa, the LBW rate is estimated to be between 10 and 15%[8]. Rural areas generally have lower rates of LBW than urban areas[7]. Among the possible causes of LBW are deficiency of iron and vitamin A.

**Iodine deficiency** disorders refer to a range of impairments resulting from primary iodine deficiency and can include foetal loss, stillbirth, congenital anomalies, and hearing impairment. The vast majority of iodine-deficient individuals experience mild mental retardation. **Iron deficiency** is the commonest cause of anaemia in South Africa although other causes (such as malaria, HIV, worm infestation or folate deficiency) contribute variably. Iron deficiency can result from insufficient intake and/ or absorption of iron or from excess loss of iron. In a 1994 survey, 10% of children in the 6 to 71 months age group were iron deficient, while goitres were noted in only 1% of children in 1994[10]. The national prevalence rate for iron-deficiency anaemia in the 6 to 71 months age group was 5%. Provincial rates ranged from 5% in the Eastern Cape to 16% in the Western Cape[10]. The same survey found the prevalence of anaemia in children less than 6 years of age living in South Africa to be 21.4%[13]. Children most affected by maternal deficiency of iron were those aged 6 to 23 months, of whom 33% were found to be iron deficient. Prevalence rates ranged from 2% in the Eastern Cape to 9% in the Limpopo province[10].

**Vitamin A deficiency** can result in night blindness, various forms of eye damage (collectively known as xerophthalmia) and, ultimately, blindness that is preventable. These clinical forms are, however, now becoming less frequent. The detection of sub-clinical forms through measurement of serum retinol is thus gaining more importance. Sub-clinical or overt vitamin A deficiency was reported in a third of South African





children in 1994[10]. Limpopo province had the highest rate of vitamin A deficiency, with the Northern Cape showing the lowest rate. Based on these findings, vitamin A deficiency is a serious public health problem in South Africa[14].

There are also new dimensions to the malnutrition problem. The established **obesity** and diet-related **non-communicable diseases (NCDs**) pandemic in developed countries is spreading to the developing world. 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 TB). Childhood obesity is rapidly emerging as a global epidemic that will have profound public health consequences as overweight children become overweight adults. In South Africa in 1998, 29% of men and 56% of women were overweight or obese[11]. Trends in overweight among children under age five are alarming for all developing countries and particularly for those in Africa, where rates seem to be increasing at a far greater rate (58% increase) than in the developing world as a whole (17% increase)[11]. The "globesity" epidemic is well established in SA, with 6% of 1–9-year-olds classified as overweight or obese, with a higher prevalence among children of well-educated mothers. The situation worsens in adolescence with 5% of males and 18% of females classified as overweight, and 2% and 6% obese[11]. More recent data published in the South African Youth Risk Assessment Survey 2002 by the South African Medical Research Council show the prevalence of overweight (including obesity) among young people aged 13–19 years to be 17% overall, and affecting more girls (25%) than boys (7%). Prevalence was highest (over 20% for boys and girls combined) in white and Indian population groups[15].

## Nutritional status of South African adults

Research on the nutritional status of South Africans during the past 10 years has focused on children and to a certain extent on the **nutrition transition** associated with urbanisation of black South Africans. Other than in children, no national study has been done to assess nutritional status in adults. Nevertheless, a number of *ad hoc* cross-sectional epidemiological surveys by academics in the different provinces of South Africa as well as the 1998 South African demographic and health survey have provided data on nutritional status variables, markers, and associated risk factors in adults. Research results from these studies have been collated by Vorster *et al.*[16] and more recently by Steyn[17]. The more recent report[17], which included studies done after 1996, confirmed observations and predictions of future trends of the earlier report[16]. Both these reports indicate that on a population level, the nutritional status of South Africans is far from optimal. The major problems related to **malnutrition**, of which the causes and consequences have to be addressed by public health policy are:

- 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 chronic diseases of lifestyle 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 (westernisation).

Ideally, nutritional status is assessed by integrating and evaluating information on nutrient intakes, nutritional anthropometry, and biochemical markers of nutritional status and clinical signs of malnutrition or nutritional deficiencies.

In the following section, nutrient intakes of adult South Africans and prevalences of overweight and obesity will be briefly discussed to illustrate the above-mentioned nutritional problems. The risk factors for malnutrition will be mentioned to show the implications of the nutritional problems for the HIV, AIDS and TB epidemics.

## Nutrient intakes of South Africans adult – Macronutrients

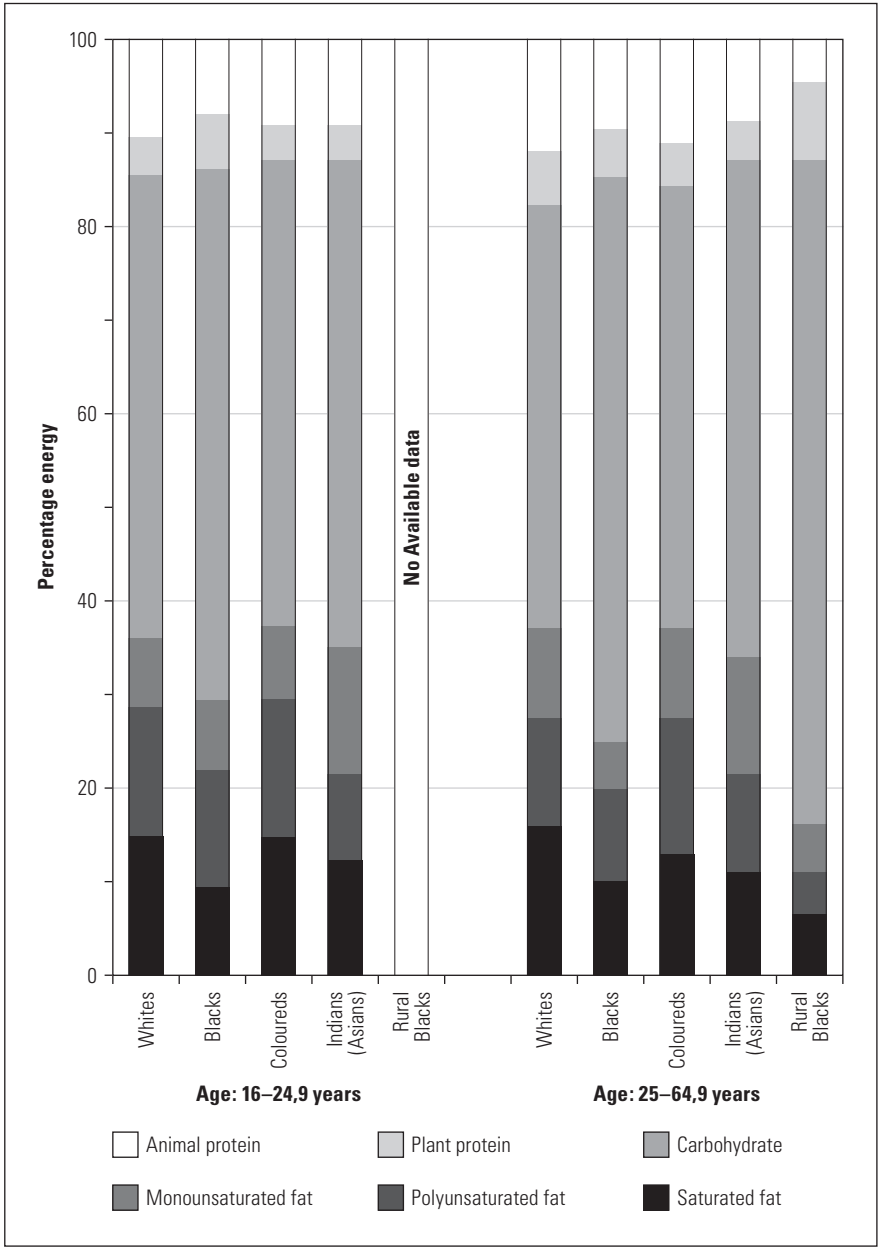
The macronutrients protein, fat and carbohydrate provide energy; therefore, to assess the macronutrient adequacy and quality of diets, total energy (TE) as well as the contribution of different types of proteins, fats and carbohydrates to total energy should be evaluated. Anthropometric measurements of weight and height, and calculation of body mass index (BMI), are also used to assess adequate energy intake. To evaluate if diets contain sufficient minimally processed (unrefined) plant foods, dietary fibre intake (also a macro-carbohydrate) should be assessed.

**Figure 1** compares the energy distribution of the diets of rural and urban black women with that of Indian, white and coloured (mixed race) women. The energy distribution observed in white, coloured and Indian women is typically that associated with a western diet: more animal than plant protein; high in total fat (> 30% of TE) and saturated fat (>10% of TE); and relatively low in carbohydrate (< 55% of TE). The mean dietary fibre intakes of these groups were 13.7, 14.6, 10.7, 13.1 and 37 g/day, respectively for white, urban black, coloured, Indian and rural black women. The typical "western" diet followed by white, coloured and Indian groups is associated with a higher risk of chronic diseases of lifestyle, such as insulin resistance, diabetes mellitus, coronary heart disease and certain forms of cancer[16].





*Figure 1. Energy distribution of the diet of South African women[16]*



The rural African women had a much more "prudent" energy distribution, with more plant than animal protein, less fat (< 20% of TE) and much more carbohydrate (> 60% of TE). Their fibre intake was 37 g/day. This distribution of energy, reflecting a "prudent" diet, is associated with a low risk of the above-mentioned chronic diseases of lifestyle.

The urban black women reported nutrient intakes with an energy distribution between that of rural black women and that of white, Indian and coloured women, illustrating that, as they become more urbanized, Africans eat more animal protein foods, more fatty foods and less minimally processed cereals and grains or carbohydrate-rich foods. These changes in nutrient intakes during urbanisation of Africans have been confirmed in the THUSA-study[18] in both men and women. It is therefore not surprising that urbanisation of Africans is also associated with an increase in the prevalence of many of the chronic diseases of lifestyle.

### Nutrient intakes of South Africans adult – Micronutrients

Analyses of the nutrient intakes of South Africans[16] show that most population groups do not reach their requirements (dietary reference values) for calcium, iron (especially black girls and women) zinc (most groups), riboflavin (most groups), vitamin $B_6$ (most groups), folate (Indian and black women) and vitamin C (Indian, coloured and black groups).

*Table II. Selected mean (SD) micronutrient intakes of African women in transition[18]*

| Nutrient (and recommended dietary allowance) | Deep rural n=290 | Farm dwellers (rural) n=148 | Informal settlements (peri urban) n=172 | Urban "middle class" n=292 | Urban "upper class" n=106 |
|---|---|---|---|---|---|
| Calcium (mg) (800) | 384 (14) | 418 (20) | 387 (18) | 405 (14) | 512 (23) |
| Zinc (mg) (12) | 7.6 (0.2) | 7.1 (0.3) | 7.6 (0.3) | 8.2 (0.2) | 10.6 (0.3) |
| Iron (mg) (15) | 8.4 (0.2) | 7.5 (0.3) | 8.3 (0.3) | 8.8 (0.2) | 10.4 (0.4) |
| Vitamin A (RE) (800) | 573 (40) | 533 (56) | 773 (52) | 892 (40) | 1246 (66) |
| Vitamin C (mg) (60) | 30 (2) | 25 (3) | 32 (3) | 43 (2) | 83 (4) |
| Folic acid (mg) (180) | 181 (5) | 177 (6) | 182 (6) | 209 (5) | 225 (8) |





The THUSA-study[18] compared micronutrient intakes of Africans in different stages of the nutrition transition. **Table II** compares micronutrient intakes of urban[18] and rural African women with recommended intakes, illustrating that urbanisation was accompanied by increases in micronutrient intakes, although mean intakes of calcium, iron and zinc of all groups did not attain recommended intakes. This study showed that more than half of all subjects (men and women) had intakes of vitamin A, folate, ascorbic acid (vitamin C), zinc, iron and calcium that were less than 67% of the recommended dietary allowance[18].

Therefore, it seems that at present 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.

## Under- and over-nutrition in South Africans adults: anthropometric data

Anthropometric measurements of height and weight and calculation of body mass index (BMI as $kg/m^2$) can be used to assess under- and overweight and therefore the adequacy of energy intake.

The cutpoints[17] recommended by the World Health Organization usually are:

| Weight | BMI |
|---|---|
| Underweight | < 18.5 $kg/m^2$ |
| Normal weight | 18.5 – 24.9 $kg/m^2$ |
| Overweight | 25.0 – 30.0 $kg/m^2$ |
| Obese | > 30.0 $kg/m^2$ |

Puoane *et al.*[19] used these cut-points on the South African demographic and health survey data of 1998 and showed that of 5390 men, 12.2% were underweight, 58.6% had normal weight, 21.7% overweight and 7.5% obese. In the 7717 women, the corresponding figures were 5.6% underweight, 37.8% normal weight, 26.6% overweight and 30.0% obese.

**Table III** shows that there are marked differences between the population groups. The white groups had lower prevalences of underweight; white men and African women had the highest prevalences of overweight plus obesity (54.5 and 58.5%, respectively). The Table shows that although the prevalences of overweight and obesity were much higher than those of underweight in all population groups, a substantial percentage (> 10%) of African, coloured and Indian men, and coloured and Indian women were underweight.

**Table III. Percentage prevalences of weight status of adult South Africans[19]**

| Population Group | Men | | | | | Women | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Under-weight | Normal weight | Over-weight | Obesity | Over-weight plus obesity | Under-weight | Normal weight | Over-weight | Obesity | Over-weight plus obesity |
| **BMI** | **<18.5** | **18.5-25** | **25-30** | **>30** | **>25** | **<18.5** | **18.5-25** | **25-30** | **>30** | **>25** |
| African (Black) | 12.9 | 61.7 | 19.4 | 6.0 | 25.4 | 4.8 | 36.7 | 26.7 | 31.8 | 58.5 |
| Coloured (mixed) | 12.1 | 57.1 | 23.1 | 7.7 | 30.8 | 10.5 | 37.3 | 25.9 | 26.3 | 52.2 |
| Indian | 16.9 | 50.3 | 24.0 | 8.7 | 32.7 | 14.9 | 36.1 | 27.8 | 21.1 | 48.9 |
| White | 5.0 | 40.4 | 36.6 | 18.2 | 54.5 | 3.1 | 47.8 | 26.5 | 22.7 | 49.2 |

This co-existence of under- and overweight has been described for other populations of the developing world that are experiencing the nutrition transition[20].

It is not known what percentage of overweight and obese men and women suffer from micronutrient deficiencies (hidden hunger). Given the low intakes of calcium, iron, zinc, riboflavin, vitamins $B_6$ and C, however, it is estimated that substantial numbers of South Africans that meet or exceed their energy needs, do not meet their micronutrient requirements. **Table III** also shows that only 40.4% of white men and just over a third of black, coloured and Indian women have normal BMIs, while about two thirds of African men and about half of coloured and Indian men and white women have BMIs in the normal range.

Therefore, using 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. Furthermore, the THUSA-study has confirmed that urbanisation and "modernisation" of black South Africans improve but do not optimise micronutrient status, indicating that large percentages of blacks (as well as other population groups) still have micronutrient deficiencies[18].

## Determinants of nutritional status for malnutrition in South Africans adults

The importance of an optimal nutritional status for health and normal functioning of the immune system has already been mentioned repeatedly in this text. To improve



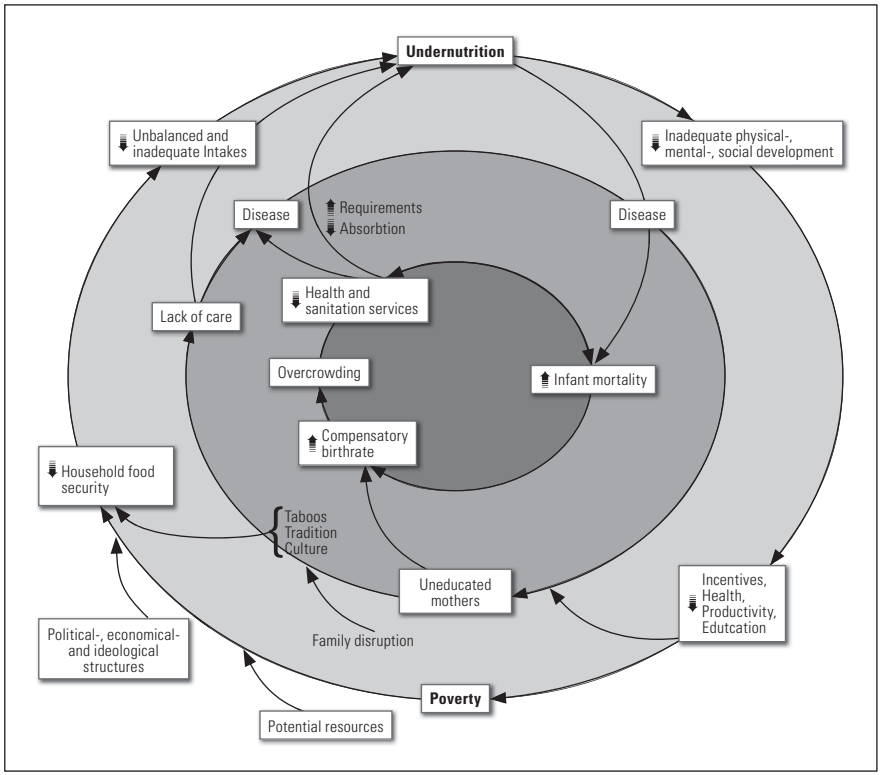


the nutritional status of both children and adults in South Africa, the multiple, inter-related factors associated with the development of malnutrition should be addressed by concerted efforts in multidisciplinary and integrated policies and programmes from all of the involved sectors: health, agriculture, housing, economics, etc. Some of these risk factors and their inter-relationships will now be briefly discussed:

### Poverty and underdevelopment

The multifactorial and inter-related causes of malnutrition are illustrated in **Figure 2**, showing that the consequences of undernutrition often aggravate the causes. It is difficult for undernourished individuals to escape this vicious cycle: they have low energy levels and micronutrient deficiencies, associated with an inability to optimally benefit from education and development programmes to sufficiently create a healthy socio-economic environment for their families to prevent undernutrition. Poverty is a fundamental or

*Figure 2. The vicious cycle of undernutrition causes and consequences[16]*



root cause of undernutrition, because it is associated with unemployment, inability to pay for food, health care and basic services, disintegration of family life, inability to care for children, vulnerability, homelessness and despair. **Figure 2** further illustrates that to address undernutrition these interrelated factors should be mitigated in community-based, intersectorial programmes, with a focus on alleviating poverty and development of "human capital". Undernutrition increases the sk of infectious diseases. It is clear how the HIV/AIDS pandemic contribute to this vicious cycle of undernutrition and poverty.

### Food and nutrition insecurity

Lack of access to adequate, affordable, safe and nutritious food is a major determinant of malnutrition. Although South Africa is food secure on a national basis, and is 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 head of households and main "breadwinners", also contributes to food insecurity.

### Increased vulnerability for obesity: the double burden within individuals

There is an increasing awareness that maternal malnutrition, foetal undernutrition (low birth weight) as well as infant and childhood undernutrition (stunting) may be related to an increased risk of obesity and other chronic diseases of lifestyle in adulthood. Levitt *et al*[21] recently reviewed South African studies that examined this hypothesis (or phenomenon) and concluded that an association between adverse early life exposures and propensity to obesity has been observed in several of these studies, which could explain the high prevalence of obesity, especially amongst black South African women. The quality of diets which consist of cheaper energy-dense, but micronutrient-deficient foods, probably contributes to adult obesity combined with "hidden hunger" in poor households. The mandatory micronutrient fortification of maize meal and bread flour in South Africa aims to address this problem.

### The nutrition transition

As mentioned earlier, urbanisation, modernisation, acculturation or westernisation of black South Africans is characterised with changing dietary intakes that increase the risk of overweight and obesity without rectifying micronutrient undernutrition. In all population groups, increased exposure to cheaper energy-dense, high-fat and sweet foods, are leading to food choices that contribute to overnutrition in respect of macronutrients, and undernutrition in respect of micronutrients in many individuals. 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 food security, education regarding healthy food choices, and creating an environment where these choices are available and affordable. Cooperation of the media and industry is essential for attaining this environment. Promotion of traditional and indigenous foods, emphasising their value in healthy diets, can also help to improve nutritional status and decrease disease risk.

### Physical environment

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. Much has been done during the past years to improve housing conditions in South Africa. Many South Africans in transition, however, still reside in informal settlements in conditions not conducive to optimal nutrition[18].

### Family unity and cohesion

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[19]. In the past, migrant workers were probably a main contributor to this situation. At present, the HIV/AIDS pandemic is one of the main reasons for the disruption of family life with resultant malnutrition.

### Pregnancy, lactation and weaning

Repeated pregnancies may jeopardise the nutritional status of both mother and child. Pregnant women have a high risk of developing iron-deficiency anaemia. Urban women are known to breastfeed for shorter periods (if at all). Several South African studies have indicated that one of the major reasons for childhood undernutrition is inappropriate weaning practices[16], with possible long-term consequences in adulthood[21].

### Alcohol consumption

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. Alcohol consumption may also influence sexual behaviour and risk of HIV transmission. The World Health Organization recently published data which showed that although South Africans drink less than 43 other populations, those that do drink consume very large amounts, and binge drinking is a serious problem[22]. Policies that address malnutrition should also include strategies that will promote healthy drinking behaviours.

## The epidemiology of HIV/AIDS in South Africa

### Adults

One senior South African epidemiologist characterized HIV/AIDS as "the biggest natural event in the history of our species for the last 500 years" [23]. HIV/AIDS currently accounts for almost 50% of all South African deaths. The virus has proven surprisingly difficult to prevent, and management consumes a large proportion of health care resources directly, while indirect costs are a further drain because the disease affects a significant fraction of the economically active population[24, 25].

HIV infection causes a steady deterioration in immune function, and without treatment, opportunistic infections and other complications are inexorable and progressive. A variety of complex factors determine how quickly this process occurs, with some patients progressing to death very rapidly in only a few years, while others survive decades. The advanced form of the viral infection, where illness is severe, is referred to as the **Acquired Immune Deficiency Syndrome** (AIDS). In Southern Africa, the great majority of infections are acquired sexually or, in the case of children, from their mothers before, during and after birth. Other forms of transmission, such as acquisition from shared needles during intravenous drug use, blood supply contamination and other mechanisms, appear to be uncommon[25]. Interestingly, sexual acquisition is a relatively infrequent event per contact, even during unprotected intercourse, and much recent energy has been focused on the co-factors behind transmission, in attempts to explain why Southern Africa accounts for the vast majority of the global HIV infection load[26]. The protracted, potentially infectious but asymptomatic early course of the infection, and the near certainty of progression to death if no treatment is taken, makes the transmission dynamics very complex. A range of factors affects sexual exposure in uninfected populations. Recent research has demonstrated that the risk of transmission from a newly infected person is many-fold greater than from one with well-established infection, which has made prevention intervention planning even more difficult[27, 28].

South Africa has one of the best **epidemiological records** regarding the tracking of HIV infection of any developing country, with excellent data from several different sources. The Department of Health has invested significant resources in its annual HIV and syphilis infection antenatal clinic sentinel survey since 1991, and complex statistical models have extrapolated HIV infection rates amongst pregnant women to the general community. The trend of HIV infection rates over time has been particularly useful to track, and has demonstrated that the incidence rate is only now beginning to stabilize in certain age groups[29].

Subsequently, other research groups have further modelled the epidemic using other sources of data[24, 30-33]. The Actuarial Society of South Africa (ASSA) has developed a





complex and sophisticated **statistical model** that predicts the impact of HIV infection based on a series of information sources. A recent report models the impact of this disease over the next 10 years[24]. Over the last 5 years, the Human Sciences Research Council (HSRC) has undertaken two very large household surveys, which have been remarkable in their approximate agreement in estimating the overall number of infections[31, 32]. A further study of the youth presented both infection data and attitudes to HIV[33].

**Death certificate data**, i.e. gender and whether the death is 'natural' or not (and hence an autopsy is required) are relatively accurately recorded and collected. While death certificate data do not allow for specific analysis of the causes of death, the steady increase in deaths in people in the 20 to 50 age group over the last decade is very striking. The classic peak seen after the age of 60, due to chronic disease, is now equaled by an earlier peak in the 20 to 50 age group. This peak is correlated strongly with the increased HIV seroprevalence observed in the antenatal surveys. Deaths in this group of young people have traditionally been due to trauma (so-called 'unnatural deaths' and more commonly affecting men), while the new peak comprises largely 'natural' deaths, and seems to affect largely women. It is probable that HIV infection is causing this new peak[30, 34].

HIV infection was first noted in South Africa in 1982, with the death of a flight attendant who died of pneumocystis pneumonia[35]. The epidemiology followed that of the US, with the virus infecting predominantly homosexual men and haemophiliacs. Haemophiliacs, their treatment revolutionised by the wider availability of pooled clotting factor for treatment, were further exposed to a product imported from San Francisco in the United States during a countrywide shortage. This product, pooled from a large number of donors was contaminated with HIV, resulting in the infection of a large number of South African patients. In other southern African countries the epidemic appears to have been spread hetereosexually from the beginning.

The virus appears to have spread rapidly to the heterosexual community in the late 1980s. It was firmly established as a generalized epidemic by 1991, when the first Department of Health – African National Congress survey demonstrated infection of 1% of pregnant women attending antenatal clinics, thus defining the epidemic as being "generalized"[36]. Prevention strategies at this time focused largely on information-based media campaigns.

The epidemic continued to grow unabated over the next 15 years, although in a geographically inconsistent way. KwaZulu-Natal suffered explosive growth in the epidemic in the 1990s, for reasons ascribed to social disruption due to the political conflict in the province. Other factors included migration, lack of access to condoms, low circumcision rate, poverty resulting in sex work and female economic reliance on men, and gender violence. Other provinces such as Mpumalanga, Free State, Gauteng

and North-West province also demonstrated high prevalences, while Limpopo and the Eastern Cape, both very poor provinces, demonstrated significantly lower HIV infections. It appears, however, that many of these lower infection provinces are now rapidly approaching the levels in the other provinces, with the exception of the Western Cape, where the epidemic is predicted to stabilize at a lower level[24].

Currently, the Department of Health estimates that 5.54 million South Africans (approximately 10.8% of the population) have been infected with HIV. The estimate by ASSA is of 5.4 million people infected, out of a population of 48 million, giving a prevalence rate of 11%, an incidence rate of 1.2%, and 600 000 people living with AIDS, in mid-2006. The HSRC estimates from its household survey a prevalence of 4.8 million in those over the age of 2 years, Statistics South Africa estimates 4.5 millions, and UNAIDS, using its specific model, between 5.3 and 5.5 million. This level of concordance is remarkable, considering that the information sources in the case of the HSRC are completely independently collated, while the others rely on overlapping data sources[24, 30–33].

The ASSA model suggest that prevalence rates are attaining a plateau across all the provinces, albeit at different levels, with KwaZulu-Natal estimated to have the highest prevalence, and the Western Cape the lowest. This plateau refers to the situation where death rates approximate new infection rates. Deaths from AIDS complications in SA are expected to peak in 2015. **Life expectancy** in South Africa in 2006 was estimated to be 49 in men and 53 in women, 13 years lower than they would have been without AIDS. HIV infection further impacts on demographics by removing many women of child-bearing potential from the population pool, as well as causing a steady decrease in fertility in women who have HIV infection. The model suggests that after 2011, population growth will settle at 0.5% annually, although a UN report actually suggests that the country would enter negative population growth in 2005[36]. The proportion of deaths directly attributable to the virus varies from province to province according to prevalence. While 47% of overall deaths in the country in 2006 were HIV-related, this was as low as 30% in the Western Cape, while approaching 60% in higher prevalence (and more highly populated) provinces such as KwaZulu Natal, Mpumalanga and Gauteng[24].

Race was measured in the HSRC survey, and despite sampling problems, has allowed fairly accurate predictions of racial vulnerability, with black Africans at highest risk, but with all race groups being heavily affected. Again, both biological and social issues have been advanced as reasons for this, and the likelihood is that complex interplay of the two accounts for the differences[31, 32].

**Gender and age dynamics** play a large role in the spread of HIV infection. Surveys have indicated that young women, especially in the 15 to 24 age groups, are several-fold more vulnerable to HIV infection than their age-matched male counterparts (16.9% vs. 3.7%)[24, 33]. Deaths of women aged 20–49 years increased 150% between 1998 and 2003





according to analysis of death certificates, despite adjusting for population growth and possible improvement in registration[30, 34]. The vulnerability of women to HIV infection may aggravate other areas of gender inequality. For instance, illness due to HIV infection may make entry into the already difficult labour pool even more complex. Both social and biological theories to account for this vulnerability have been advanced, and prevention strategies targeting the youth have been a major focus of government programmes. This group is of epidemiological interest, as it represents the most accurate pool of new infections (i.e. incidence), and a decrease in this number may indicate a decrease or delay in the age of infection. This was one of the significant changes that were noted in Uganda, which is often cited as a success in the prevention of HIV infection, where age at sexual debut was significantly elevated amongst women. Disappointingly, however, the HIV infection rate in this group in SA has remained relatively stable in the last few years, probably demonstrating a plateau in the number of new infections[24, 30].

**Gender violence** undoubtedly plays a role in the transmission of HIV. Women who are exposed to violence at home have a higher HIV infection prevalence than those who do not. Sexual assault is associated with a high risk of HIV exposure, with 50% of rapists estimated to be HIV-infected in one provincial police survey. Poor coverage with post-exposure prophylaxis has meant that this seemingly effective measure is only available to a small number of assaulted people[37, 38].

The South African government has committed significant resources to **educational HIV prevention and support programmes** over the last decade, and surveys indicate that knowledge about the transmission of the virus is relatively sophisticated, although perceptions of risk are disappointingly low[31–33]. This failure to effect significant behaviour change has been a major challenge. A further frustration has been the improvement in free male condom distribution throughout the country, again with seemingly little impact on the epidemic[24].

**Circumcision** is a major protective factor in the epidemic, and there is strong epidemiological evidence that it plays a role in SA, and has been used to partially explain the provincial differences noted in HIV prevalence, specifically between KwaZulu Natal and the Eastern Cape[26, 39].

Finally, there would be an estimated 505 000 deaths annually from AIDS complications by 2010, in the absence of antiretroviral therapy[24]. However, the increased accessibility of this highly effective but complex intervention has made this assumption more complex.

The implementation of the **National Comprehensive Plan** of the Department of Health (now strongly reinforced by the **HIV/AIDS and Sexually Transmitted Infections Strategic Plan for South Africa, 2007 to 2011**) is intended to be focused on provision of antiretroviral therapy (ART), which is highly effective in reversing the immunological deficiency induced by HIV infection. This will annually defer death in approximately

100 000 people with advanced HIV. While the lifespan in people on ART is not yet apparent, mathematical models (based on cohort data that have been available now for over 12 years of ART treatment) suggest average lifespans of over 20 years from the time of initiating medication. This presupposes an uninterrupted drug supply, however, and consistent coverage and adequate health facilities. The original model assumed that over 200 000 people would be on ART by the middle of 2006, and that just over 700 000 were in need of ART. It further suggested that without the provision of widespread ART, life expectancy would drop 19 years by 2015, while with ART it would drop by 16 years[24, 40–42]. New mass interventions being considered and researched include mass circumcision programmes, HIV vaccines and vaginal microbicides; the last two interventions are years away from demonstrating efficacy, while the first will be complex and costly, although undoubtedly effective. More targeted interventions such as chemical prophylaxis for 'high risk' groups are also being explored.

## Children

In 2004, the antenatal HIV infection prevalence amongst pregnant women attending government antenatal clinics was 29.5%[43]. **Table IV** summarizes HIV prevalence by province and year indicating the lack of apparent leveling-off of the epidemic in reproductively active women. Of great concern is the increase in prevalence in adolescent and young pregnant women, with rates as high as 16% in women less than 20 years of age (**Table V**).

***Table IV. HIV infection antenatal prevalence by province 2002–2004[43]***

| Province | HIV prevalence (95% CI) | | |
|---|---|---|---|
| | 2002 | 2003 | 2004 |
| KwaZulu-Natal | 36.5 (33.8–39.2) | 37.5 (35.2–39.8) | 40.7 (38.8–42.7) |
| Gauteng | 31.6 (29.7–33.6) | 29.6 (27.8–31.5) | 33.1 (31.0–35.3) |
| Mpumalanga | 28.6 (25.3–31.8) | 32.6 (28.5–36.6) | 30.8 (27.4–34.2) |
| Free State | 28.8 (26.3–31.2) | 30.1 (26.9–33.3) | 29.5 (26.1–32.9) |
| Eastern Cape | 21.7 (19.0–24.4) | 23.6 (21.1–26.1) | 28.0 (25.0–31.0) |
| North West | 26.2 (23.1–29.4) | 29.9 (26.8–33.1) | 26.7 (23.9–29.6) |
| Limpopo | 15.6 (13.2–17.9) | 17.5 (14.9–20.0) | 19.3 (16.8–21.9) |
| Northern Cape | 15.1 (11.7–18.6) | 16.7 (11.9–21.5) | 17.6 (13.0–22.2) |
| Western Cape | 12.4 (8.8–15.9) | 13.1 (8.5–17.7) | 15.4 (12.5–18.2) |
| South Africa | 26.5 (25.5–27.6) | 27.9 (26.8–28.9) | 29.5 (28.5–30.5) |





CI: confidence interval. The true value is estimated to fall within the two confidence limits.

***Table V. HIV infection prevalence by age group among antenatal clinic attendees, South Africa: 2002–2004[43]***

| Age group (Years) | HIV prevalence (95% CI) | | |
|---|---|---|---|
| | 2002 | 2003 | 2004 |
| < 20 | 14.8 (13.4–16.1) | 15.8 (14.3–17.2) | 16.1 (14.7–17.5) |
| 20–24 | 29.1 (27.5–30.6) | 30.3 (28.8–31.8) | 30.8 (29.3–32.3) |
| 25–29 | 34.5 (32.6–36.4) | 35.4 (33.6–37.2) | 38.5 (36.8–40.3) |
| 30–34 | 29.5 (27.4–31.6) | 30.9 (28.9–32.9) | 34.4 (32.2–36.6) |
| 35–39 | 19.8 (17.5–22.0) | 23.4 (20.9–25.9) | 24.5 (21.9–27.2) |
| 40+ | 17.2 (13.5–20.9) | 15.8 (12.3–19.3) | 17.5 (14.0–21.0) |

The first national population-based survey conducted in South Africa in 2002 showed that the prevalence of HIV infection in children aged 2–14 years was 5,6% (**Table VI**)[43]. A second national survey conducted in 2004 showed reported rates of HIV infection in children aged two to four years to be 4.9 % in boys compared with 5.3 % in girls[24]. There are no national surveillance data available for the prevalence of HIV infection in infants. Based on the prevalence of HIV among pregnant women, however, and assuming varying access to interventions to prevent transmission, it is estimated that between 45 000 to 60 000 children are newly infected annually in South Africa.

***Table VI. HIV infection prevalence by age group, South Africa, 2002[43]***

| Age (Years) | N | HIV-infected (%) | 95% CI |
|---|---|---|---|
| Children (2–14) | 2 348 | 5.6 | 3.7–7.4 |
| Youths (15–24) | 2 099 | 9.3 | 7.3–11.2 |
| Adults(>25) | 3 981 | 15.5 | 13.5–17.5 |
| Total | 8 428 | 11.4 | 10.0–12.7 |

Perinatally acquired HIV infection or HIV transmitted from mother to child accounts for the majority of paediatric HIV infections occurring in South Africa[32]. Mother-to-child

transmission (MTCT) of HIV infection can occur in utero, during labour and delivery or through breastmilk, with the bulk of transmission occurring in the intra-partum period[44]. Transmission rates vary from less than 2% in the developed world due to the use of highly active antiretroviral therapy (HAART), elective caesarean section and safe replacement feeding, to more than 30% in the developing world without access to antiretroviral prophylaxis and with prolonged breast-feeding[45]. MTCT rates vary in South Africa from 2% to 30% depending on use of interventions to prevent MTCT and the duration and method of breast-feeding[46]. Factors that are associated with MTCT transmission are as follows:

### Maternal risk factors

These include advanced maternal disease or surrogate markers thereof, including viral load and $CD4^+$ count, viral phenotype and genotype, smoking or other substance abuse, lack of ARV therapy or ARV resistance, sexually transmitted infections (STIs) or other co-infections and sexual behaviour.

### Obstetric risk factors

These include vaginal or pre-term delivery, prolonged rupture of membranes, placental disruption or abruption, chorio-amnionitis, invasive fetal monitoring, and episiotomy or use of forceps.

### Neonatal risk factors

Major factors include prematurity, oral thrush, gender and exposure to infected breastmilk.

## The epidemiology of TB in South Africa

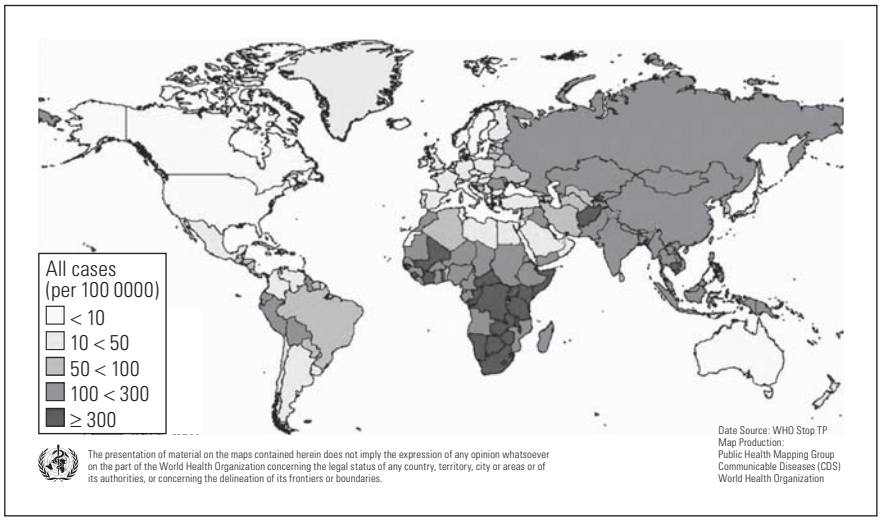
TB continues to be a disease of major public health importance globally, with over 90% of cases occurring in developing countries[47, 48]. As TB is one of the diseases associated with resource-poor countries, it has historically attracted limited attention and investment into the development of novel and improved drugs, vaccines and diagnostics, all of which are desperately needed. It has often been termed a "neglected disease", along with HIV infection, malaria and other infections common in developing countries. As a result, the prevention and treatment of TB has lagged behind most other diseases that are or were common in developed countries.

TB cases occur worldwide, but predominantly in poor countries (**Figure 3**). This is due to socio-economic factors, such as crowding, poverty, unemployment, malnutrition, HIV infection, poor health standards affecting diagnosis and poor treatment intervention. TB has infected approximately 2 billion individuals worldwide, about a third of the





*Figure 3. Estimated tuberculosis incidence – 2003*[49]




*Source: World Health Organization, September 2003*

world's population. Over 8 million new TB cases occur annually and 60% of cases occur in only 10 countries (see **Table VII**). In addition close to 2 million TB-related deaths occur annually; it is responsible for more deaths than any other curable infectious disease. TB is a leading killer of young adults in their most productive years. The WHO estimates that infected adults lose an average 3–4 months of work while recuperating from the disease, while society loses an average of 15 years of economic activity from each adult TB death. The WHO has declared TB to be a global emergency and has called for urgent and extraordinary action[49].

The global burden of TB is increasing, largely due to the spread of HIV/AIDS. HIV-infected subjects are far more susceptible to TB, are more difficult to diagnose, and in addition, are also more difficult to treat. TB lifetime risk in HIV-uninfected residents of southern Africa is approximately 10%; in HIV-infected people, this approaches 10% per annum. Furthermore, HIV-infected people have a much higher mortality in the period following TB treatment, with 30% dying within a year of diagnosis and treatment. In addition, smear-negative TB is far more common in HIV-infected individuals (estimated to be as high as 50%), requiring sophisticated imaging and laboratory techniques to make the diagnosis[50].

With the HIV epidemic continuing to spread at alarming rates both nationally and globally, it is anticipated that this will contribute to the increasing numbers of TB cases.

*Table VII. Top 10 high TB burden countries*[49]

| Country | Population | All TB cases | TB cases per |
|---|---|---|---|
| | 1000's | 1000's | 100 000 pop |
| 1. India | 1 087 124 | 1 824 | 168 |
| 2. China | 1 307 989 | 1 325 | 101 |
| 3. Indonesia | 220 077 | 539 | 245 |
| 4. Nigeria | 128 709 | 374 | 290 |
| 5. South Africa | 47 208 | 339 | 718 |
| 6. Bangladesh | 139 215 | 319 | 229 |
| 7. Pakistan | 154 794 | 281 | 181 |
| 8. Ethiopia | 75 600 | 267 | 353 |
| 9. Phillipines | 81 617 | 239 | 293 |
| 10. Kenya | 33 467 | 207 | 619 |

*Source: WHO report 2006: Global Tuberculosis Control*

In addition, the **diagnosis of TB** continues to be problematic, particularly in infants and young children. Diagnostic assays for TB have not developed at the pace of diagnostic tests for other disease, and significant investment is urgently needed to improve their sophistication.

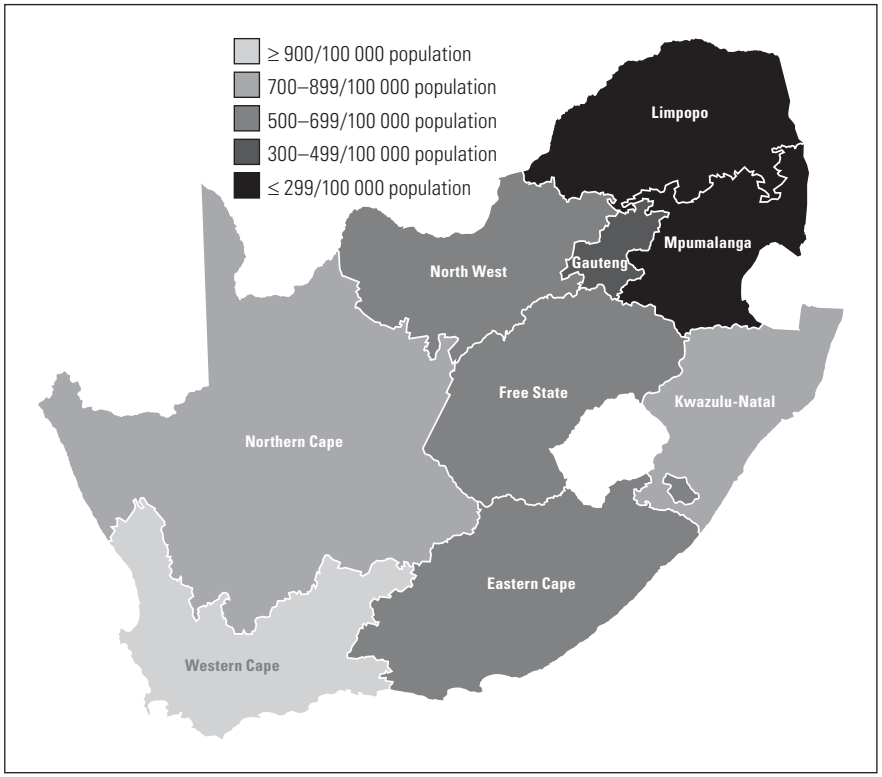
The escalation of the TB epidemic worldwide has occurred despite the availability and widespread use of Bacillus Calmette-Guérin **(BCG) vaccine** and the use of directly observed therapy short course (DOTS) for persons diagnosed with active disease. BCG is currently the only licensed vaccine. BCG affords ~80% protection against tuberculous meningitis and miliary tuberculosis in infants and in young children, but protection against lung disease, at all ages, and has been variable. DOTS, the global control strategy aimed at controlling TB transmission through prompt diagnosis of symptomatic smear-positive disease, has failed to prevent rising tuberculosis incidence rates in many parts of the world[49].

Annually, over 250 000 new cases of tuberculosis (TB) occur in South Africa. The estimated annual incidence rate of TB is in excess of 700/100 000 population and in some areas the rate exceeds 1 000/100 000 (1%). In SA, rates vary between provinces with the Western Cape, which accounts for only 10% of the SA population, bearing the brunt of the epidemic, with 25% of the case load (**Figure 4**). Children account for 15–20% of the TB case load. The high burden of childhood TB reflects recent transmission within the





*Figure 4. TB incidence in 2004*




population and is representative of the failure of TB control measures. Children contribute little to the maintenance of the TB epidemic, but they suffer severe consequences such as miliary or disseminated disease and meningitis. TB is currently one of the leading causes of death in adults and children in South Africa and the case fatality rate has increased from 3% in 1993 to 7.4% in 2003. A major reason for the escalation of the TB epidemic in South Africa is the evolution of the AIDS epidemic. It is estimated that approximately 60% of adult TB cases aged 15–49 years are HIV co-infected[51].

In the 1970s, after confirmation that short-course (6 months) TB therapy was highly effective, the prospect of TB eradication led to renewed vigour in tackling TB as a public health issue. Despite the availability of effective therapy, and the implementation of improved directly observed therapy short course (DOTS) programmes in the mid-1990s, southern Africa continues to have a TB problem that is growing, fuelled by a growing HIV epidemic. The WHO advocates treatment of infectious (smear-positive) TB as the

most cost-effective method in tackling TB control. The DOTS programme emphasises five key components, including:

- political commitment
- access to improved sputum microscopy
- access to standardised treatment using quality assured drugs in a monitored environment
- uninterrupted drug supply, and
- improved programme monitoring.

Even in countries with a large commitment to DOTS, such as Botswana, the impact of DOTS on TB numbers has been disappointing. In Peru and China, DOTS appears to have had significant impact, but neither country has a generalised HIV epidemic as is present in southern Africa. The DOTS approach itself is contested, with three trials assessing the effect of the approach showing conflicting results (two showing no effect[52, 53], including one done in SA, the third in Thailand[54] showing significant effect). In developed countries, a modified DOTS approach that includes aggressive patient support and follow-up of defaulters has yielded impressive results, especially in the United States[54].

Effective treatment of TB is available, but cure rates remain unacceptably low in South Africa, despite clear guidelines and **standardised drug formulations**, including fixed dose formulations. Not a single South African province has attained the 85% cure target set by the WHO[55]. Diagnosis remains challenging, with many areas still relying on unreliable and laborious microscopy techniques developed in the 19th century. The time from onset of symptoms to initiation of treatment in African countries is estimated to be 3–4 months in patients with smear-positive TB. Finally, treatment of TB is estimated by the WHO to be over $700/patient in SA, significantly more than in other high-TB burden countries, largely owing to the high cost of labour, the cost determines who can provide drug treatment[55]. The indirect economic consequences described above are likely to be far in excess of this figure.

In resource-poor areas, a reliance on the clinical diagnosis of TB leads to confused and inconsistent treatment protocols, and may contribute to the development of **multidrug-resistant (MDR)** TB. The rate of MDR TB, a further problem, made famous by the 1991 outbreak in New York is estimated to be at 1.7% in South Africa (6.6% of retreatment cases), with over 6000 cases annually. Due to South Africa's large TB caseload, this translates into the highest absolute number of MDR TB cases in the world. MDR TB is defined as resistance to at least two of the most effective anti-TB drugs, rifampicin and isoniazid. MDR TB identification requires relatively sophisticated and expensive laboratory facilities. MDR TB treatment is complex, prolonged and very expensive, with a significant failure rate, resulting in further TB mortality. In the case





of HIV, MDR TB appears to be particularly aggressive, and usually progresses rapidly to death, in the absence of antiretrovirals[56–59].

A recent outbreak of **extensively drug-resistant** (XDR), (generally known inappropriately as extremely drug resistant) TB has been described in several provinces within South Africa, mainly amongst HIV-infected patients[59]. In addition to resistance to rifampicin and isoniazid, XDR TB is defined as resistance to at least three of the six classes of the drugs available to treat MDR TB. The outbreak was associated with a very high mortality, despite the availability of antiretrovirals in many cases: 52/53 patients in the original report died, with an average time to death of 16 days from sputum collection. While XDR cases have been described previously in South Africa, the sheer number of cases and the high mortality, including deaths of health care workers, attracted significant attention. In the cohort studied, 39% of patients had MDR TB, it associated with significant mortality in both HIV-uninfected and in particular HIV-infected individuals[56, 60].

A report on progress of the epidemic revealed successful completion of treatment in only 65% of patients, cure in only half, and a defaulter rate of 11.5 % in 2003. Interestingly, KwaZulu-Natal (completion rate of 55% and cure rate of 35%) and Mpumalanga (50% and 32%), the sites where MDR and XDR have been identified as severe problems, fared worst; both provinces have very high prevalence of drug resistance. Treatment of MDR TB in terms of drug costs alone is estimated at R28 000 per patient[56, 60].

Both MDR and XDR TB have at their root poor management of the systems providing treatment to people with TB. In particular, in South Africa poor prescribing practices, interrupted drug supply and poor support to patients attending the clinics, along with poor infection control measures, have fuelled the resistant TB epidemic. The TB programme has often been divorced from the HIV control programme, although it is clear that HIV is a major driver of the epidemic. International efforts to achieve patient-centred case management have had limited success, despite claiming improved coverage of the country with the DOTS approach. The XDR outbreak has brought renewed calls for expansion of laboratory techniques for diagnosis of resistant TB to be made more widely available. A recent editorial in the *Lancet* has by contrast called for a "back-to-basics" approach that emphasizes strengthening of the public health strengthening at a primary care level, rather than a focus on solely improving access to second-line TB drugs and diagnostics[60].

CHAPTER 3

# Evidence-based practice and recommendations

The objectives of this chapter, a narrative review, are firstly to define the concept of scientific evidence-based policy and practice (evidence-based medicine, nutrition and/or health practice); secondly, to review briefly the different types of nutritional studies that could and should be considered and used in gathering the available evidence or standard of proof, indicating both strengths and weaknesses of such studies; and thirdly, to show how the quality of evidence from different types of sources can be evaluated, judged and graded to determine the strength of recommendations for formulation of policy and practice.

## Introduction

TB and HIV/AIDS are preventable diseases. In addition to avoiding exposure to the infectious agents, nutrition may play a role in the prevention, reducing the risk of infection, slow progression and in the treatment of these diseases.

The objective of this ASSAf report is to examine the nutritional influences on human immunity to inform policy and practice in South Africa so that existing scientific evidence is used in policies aimed at the prevention and nutritional treatment of TB and HIV/AIDS.

This chapter is motivated by the realisation that nutrition recommendations to the public can often be a drastic intervention in lifestyle and behavioural choices. Also, if food and nutrition aid or assistance forms part of health policy and practice, it is important that resources are spent cost-effectively. These recommendations should therefore be reliable, credible, applicable, and responsible, do no harm, and should be effective. The advice should be ethical, should take the complexity of situations into account, and if followed, should lead to beneficial changes in health. To ensure that nutrition recommendations comply with these criteria, they should be systematically developed, based on the best available evidence or standard of proof.

## Evidence-based health policy and practice – definitions

According to Miller and Miller[1] (2005), "*Evidence is information – it supports or undermines a proposition, whether a hypothesis in science, a diagnosis in medicine or a fact or point in question in a legal investigation. In medicine, physicians marshal evidence to make decisions on how to best prevent, diagnose and treat disease and to improve health*". The quality of evidence indicates the extent of confidence that an estimate of an effect is correct[2].

**Evidence-based medicine** is defined by Sackett *et al.*[3] (1996) as "*the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients. The practice of evidence based medicine means integrating individual clinical expertise with the best available external clinical evidence from systematic research. By individual clinical expertise we mean the proficiency and judgement that individual clinicians acquire through clinical experience and clinical practice. Increased expertise is reflected in many ways, but especially in more effective and efficient diagnosis and in the more thoughtful identification and compassionate use of individual patients' predicaments, rights, and preferences in making clinical decisions about their care. By best available external clinical evidence we mean clinically relevant research, often from the basic sciences of medicine, but especially from patient centred clinical research into the accuracy and precision of diagnostic tests (including the clinical examination), the power of prognostic markers, and the efficacy and safety of therapeutic, rehabilitative, and preventive regimens. External clinical evidence both invalidate previously accepted diagnostic tests and treatments and replaces them with new ones that are more powerful, more accurate, more efficacious, and safer*".

Evidence-based nutrition has been defined[4] as "*the application of the best available systematically assembled evidence in setting nutrition policy and practice. It provides an objective framework in which to gather and review all available evidence to help inform policy and clinical practice*".

Therefore, evidence-based recommendations should be based on the outcomes of a **systematic process of seeking, evaluating and grading contemporaneous research findings.** Because few clinicians (practitioners) will have time to base individual treatment on such a lengthy process, panels or working groups are often formed that follow the process to develop guidelines and recommendations to inform policy and practice. Many sources[2, 5, 6] agree that the following basic steps (with many sub-steps) are needed for a successful process to develop evidence-based guidelines and recommendations:

- **Step 1**: To prioritise problems, formulate clear questions and to get agreements within the panel or group of all steps of the process.
- **Step 2:** To follow all sub-steps of constructing a systematic review of the relevant literature searches to grouping studies, evaluating the quality and importance of individual studies, defining the critical outcomes, and profiling the evidence for the defined outcomes.
- **Step 3:** To evaluate (critically appraise) the evidence for each outcome for quality, validity, importance, and balance of benefits and potential harm: in other words to rate or grade the evidence for specific recommendations.
- **Step 4**: To formulate recommendations, describe the strength of the recommendations, implement and evaluate the recommendations. The strength of a recommendation will depend on the balance between benefit and harm, the quality of the evidence, the translation of the evidence into specific circumstances, the certainty of baseline risk and the collective costs of implementation[2].





Applied to the objectives of this ASSAf consensus study on nutritional influences on human immunity, these four steps are as follows:

- **Step 1**: A working group has been formed, the process agreed upon and the main questions formulated: What is the role of nutrition in the prevention and treatment of TB and HIV/AIDS in South Africa? The population is all South Africans at risk, the exposure is nutrition (dietary patterns, nutrient intakes, food security) and the outcomes are resistance to infections and improvement of clinical profiles (health) of infected persons.
- **Steps 2 and 3**: How to search and evaluate or judge the available literature, and how to grade the quality of the evidence, will be discussed in the remaining part of this chapter.
- **Step 4:** The implementation of this evidence in policy and practice recommendations, is the topic of the final chapter of this document.

## Reviewing the literature

After formulating the question (What is the role of nutrition in the prevention and treatment of TB and HIV/AIDS in South Africa?), the relevant literature has to be gathered, using a structured search strategy, not restricted by language. It should include unpublished literature where possible. Clearly and explicitly defined and transparent criteria should be used to differentiate between different types and qualities of studies. **Tables I and II** shows checklists of questions and appropriate steps to follow when reviewing papers and intervention studies. These checklists are in agreement with the recommendations of the GRADE Working Group[2] namely that individual study *design* and *quality* should be evaluated, combined with an evaluation of *consistency* (similarities of effects across studies) and *directness* (the extent to which people [experimental subjects], exposures [interventions] and outcome measures are similar to those of interest). It is important to realise that the "quality" of individual studies for selection

to use, as well as a collection of studies for evaluation and grading of the total body of existing evidence are needed.

*Table I. General checklist for reviewing papers*[4].

| Questions | Steps |
|---|---|
| 1. Is the paper of interest? | Read the abstract; if yes, proceed. |
| 2. What was done? | Read the introduction; does it justify why the study was worth doing? |
| 3. What was the purpose of the study? | Are there clear research aims, question and/or hypotheses? |
| 4. How was the research question(s) addressed? | Is the study design appropriate for the question?<br>Is the subject recruitment described fully? (e.g. sampling, frame, inclusion and exclusion criteria, response rate, size and power)<br>Is data collection methods described fully, appropriate methods and measures, validity, quality control, etc?<br>Is there bias in subject recruitment and information?<br>Are the statistical methods appropriate? |
| 5. What are the results? | Read the result section: are results clearly presented? |
| 6. Discussion | Read the discussion: does it reflect the results, the literature and is it self critical? |
| 7. Conclusions, recommendations | Read the conclusions: are they justified?<br>Are recommendations practical, feasible? |

Some sources add funding or sponsorship as a final item to these checklists because of the possibility that funding or sponsorship of a particular study may introduce bias, which should be considered in evaluating studies. The review process to evaluate available evidence may be of other reviews or of original research papers and reports. Reviews may include narrative reviews and systematic reviews. The latter includes meta-analyses.

- **Narrative reviews:** these may be or are often commissioned papers written by experts, giving their own (potentially biased) viewpoints of a particular topic, quoting only literature supporting their viewpoints.
- **Systematic reviews:** these are review papers in which a detailed search strategy was used to include all relevant research on the topic (and not only those that suit the writer's point of view), differentiating between different types of studies and bringing together all available evidence on a particular topic so that informed judgements can be made.
- **Meta-analyses:** these are reviews in which data from different studies are pooled and re-analysed statistically[7]. The objective is to combine two or more studies to decrease confidence intervals and increase statistical power. A meta-analysis may be based on all available studies collected systematically, or it may not, depending on the comparability of studies and data collected. It is often difficult to pool data from nutritional studies because exposure and outcome measures of different studies may not be the same[4]. Nevertheless, meta-analyses have specific advantages for policy makers, providing a single number summary of the overall estimate of an effect. Moreover, a test of heterogeneity may reveal variations between studies that could point to important interactions.





The different types of studies to be used in evaluating evidence about the relationships between nutrition and TB, HIV/AIDS are discussed in the next chapter.

## Types of nutritional studies

Corroborative findings from many different kinds of nutritional studies can and should be used to gather sufficient evidence that nutrition is related to a disease[8]. For the purpose of this discussion, nutritional studies examining the relationships between past, present and future dietary or nutrient intake exposures to health outcomes related to TB, HIV/AIDS, will be grouped into either *observational* or *intervention* studies. The *observational* or descriptive studies includes ecological, cross-sectional, case-control and cohort studies and the *intervention* studies, include all experimental studies in which the scientist controls the system by making measured interventions in humans, animals, tissues or cells and compares the outcomes of these interventions to reference, control or placebo situations[8].

### Observational or descriptive studies

The descriptive nutritional epidemiology of TB, HIV/AIDS will include data on the relationships between dietary exposures and incidence, mortality and risk. The different types of descriptive or observational studies that could provide data to be evaluated for policy decisions are as follows:

### Ecological or correlation studies

In these studies, population or group indices of dietary intake or nutritional status (exposure) are related to population or group indices of health status (outcome). The unit of analysis is not an individual but a group defined by time (e.g. calendar year, birth cohort), geography (e.g. country, province or city), or sociodemographic characteristics (e.g. ethnicity, religion or socioeconomic status)[8]. An example could be plotting the intake of a specific food, nutrient or marker of nutritional status (such as body mass index) against the incidence or severity (mortality) of TB, HIV or AIDS for each country in Africa.

Ecological studies are helpful when within-group (country or region) variation in exposure as small compared to between-group variation. Ecological studies are ideal to explore newly proposed hypotheses, serving as a basis to develop follow-up studies. They are useful for monitoring national trends in health indicators and the wider environmental (social, cultural and economic) factors that influence health.

The limitations or disadvantages of ecological studies are that they only show associations between exposures and outcomes on group level (never on an individual level). They do not prove causal links. In fact, observed relationships or associations between a dietary exposure and health outcome may be with a different, diet-associated confounding factor (such as a lack of hygienic environment or lack of care). Furthermore, a lack of an association between an aspect of diet and disease may disguise an actual relationship because of possible varying genetic predispositions in different populations[9].

### Cross-sectional studies

Cross-sectional studies measure exposures and outcomes in the present, and at the same time in individuals. These individuals are sampled from the population in such a way as to reflect the population characteristics for both exposure and outcome. If information on other population characteristics are collected (such as age, gender, income, education, etc.), the effects of these factors on the exposure-outcome relationship can also be assessed.

Some characteristics to note of cross-sectional studies to ensure that their results are reliable and usable[8] are selection bias, information bias, sample size and power. If the source population and sampling frame are not clearly described in the methods, the results may not reflect the source population and may not be generalisable. If a particular sector of the source population is excluded (e.g. individuals too ill to participate), the prevalence estimate and reported associations may be misleading. The sample size must be adequate to provide a reliable estimate of the population prevalence. Sample size must, therefore, be calculated with available formulas and should be reported as part of the methods. There must be sufficient information reported in the methods to evaluate if the exposure, outcome and other variables were measured with the required level of accuracy to answer the question. Therefore, validation and standardisation of methods employed must be reported.

The main disadvantage of cross-sectional studies is that the exposures are not measured before the onset of the outcome: it is therefore not possible to disentangle cause from effect. It could be for example that the illness (HIV infection) changes dietary patterns and nutrient intakes. A measured relationship between dietary patterns and HIV infection will therefore not necessarily reflect any causal relationship between malnutrition and risk of infection.





### Case-control (case-referent) studies

In these studies, patients with a disease are compared to controls without the disease. These studies recruit subjects or participants on the basis of their outcome status and then explore past exposure measures. Case-control studies are especially efficient where the outcome is rare and all available cases can be recruited from the population of interest. If the cases are self-selected, for example from people attending clinics, there may be selection bias because some patients, for various reasons, may not attend clinics. Therefore, the paper must report recruitment procedures clearly to be of use in gathering evidence. Controls should be recruited at random from the same population as the cases. After random selection, controls could be matched to cases on certain characteristics (e.g. age, gender, etc.) that are known to influence outcome but of themselves are of no direct interest in the study. Because case-control studies usually rely on past exposure, information bias is possible, especially when past dietary intakes are related to present outcomes. It is therefore important when reviewing these studies to assess if past exposure is reported with the same accuracy and precision in cases and controls. The impact of exposure on outcome in case-control nutritional studies is usually expressed as an odds ratio. Participants are ranked and intake grouped into quartiles or thirds of the distribution (e.g. high, medium and low) and risk of outcome assessed according to this distribution. The absolute intake is therefore not necessary. Provided that the ranking of intake is consistent between cases and controls, the estimate of risk will reflect the underlying risk of exposure on outcome[8].

Case-control studies are suitable to study rare outcomes. They are restricted to assessing one outcome (or at the most subsets of related outcomes) but may be able to assess many different exposures. The biggest problem and disadvantage of case-control studies is the potential of information bias in past exposure dietary recalls[8].

### Cohort (prospective) studies

Cohort studies measure exposure in the present and outcome is assessed at some point in the future. A cohort study can therefore be used to draw causal inferences about the effect of the exposure on outcome because the exposure is measured before the outcome is known. In evaluating cohort studies, it should be noted that the sample is not always selected to represent the distribution within the whole population – it may be weighted to maximise the heterogeneity of exposure, or it might be selected to minimize loss to follow-up. These factors may be considered to be of more importance than representativeness of the sample. The sample size is important: it should be large enough and subjects should be followed up over a sufficient length of time to have sufficient disease endpoints to calculate an estimate of the risk of disease.

The biggest disadvantage or concern of cohort studies is loss to follow-up, particularly when this may be differential by level of exposure. Therefore, when reviewing and evaluating cohort studies, one must ensure that the authors have described the drop-out rate and the reasons for loss of follow-up. It is possible that those who were lost to follow-up differed in important ways to those who were not lost. Other disadvantages of cohort studies are that they are often large, take many years to conduct and are expensive.

The biggest advantage of cohort studies is that they provide strong evidence for a causal relationship between exposures and outcomes, because it is unlikely that the measure of exposure is biased, being measured before the outcome is known.

### Intervention (experimental, biological) studies

These are studies where the researcher controls or manipulates the exposure. They include human clinical trials (therapeutic, secondary or tertiary prevention), field trials (primary prevention) or field intervention studies. The best known clinical trial design, often used to examine effects of pharmaceutical agents, is the double-blind, placebo-controlled, cross-over, randomised clinical trial. This design is also used to examine effects of dietary interventions in healthy subjects or patients. Intervention studies include experimental studies using animal models or biological material. Human intervention trials provide the most robust test of a causal hypothesis. Human intervention trials are most often conducted at the individual level, but are sometimes conducted at population level. Experimental studies in animal models or other biological material are necessary to provide information on underlying biological mechanisms that could explain relationships between exposure and outcome in humans. It is not always possible, safe nor ethical to test some dietary interventions in humans, especially if the exposure is suspected of causing or increasing risk, or aggravating a disease. There are some general principles that are relevant to all types of intervention studies. **Table II** gives a checklist based on these principles that should be followed when reviewing intervention studies.

Gray and Gray[10] matched the different types of studies to the types of questions or actions in health (dietetic) practice as follows:

- Cross-sectional study – diagnosis;
- Randomised controlled trials – treatment;
- Cohort study – prognosis;
- Cohort study and case-control study – etiology or harm.

This is a rather simplified model (as will be seen in the next chapter), but it does indicate the type of studies needed for evidence to formulate diagnostic and treatment guidelines, to assess etiology and prognosis and whether an intervention may be harmful.





## Evaluation of dietary (nutrient) intake reports

When evaluating the literature to gather evidence that nutrition plays a role in immunity, TB and HIV/AIDS, the complexity of human nutrient intake and inter-related relationships between nutrients and between nutrition and health should be considered. The quality of any nutrition study will depend on the quality of information on food and nutrient intakes of participants in both descriptive and intervention studies. The reader is referred to Gibson[11] for a detailed description of nutritional assessment methodology. Following is a listing of some of the potential pitfalls and issues that should be kept in mind.

- The foods and drinks we consume daily contain thousands of chemicals. Some are well-known and classified as nutrients (see **Table III**). Some are identified with known effects on human physiology and disease risk, such as the phytochemicals, but not classified as nutrients; some are little known and unmeasured.
- Dietary intakes are variable: within and between individuals, from day-to-day, during different seasons and through the life cycle.
- There are associations and interactions between the different components in foods, between foods in the diet, and between diets and other behavioural characteristics (e.g. vegetarians often are non-smokers and non-drinkers). Effects will also depend on the duration of any intervention, and compliance to interventions.
- Changing a dietary component in intervention studies, invariably lead to other dietary changes which are not always possible to control for. For example, reducing fat in the diet, one of the other macro-nutrients must be increased to keep energy intake constant; increasing fibre in intervention diets introduce not only the fibre component, but many fibre-associated compounds as well as a dilution and volume factor, which may influence nutrient density, food intake (appetite), gastrointestinal responses to foods, etc.
- Food processing and cooking methods influence the composition of foods and should be reported.
- Illness, especially the infectious diseases under consideration, often change dietary behaviours, appetite, absorption and metabolism of nutrients, as well as nutrient and energy requirements.
- The measurement of dietary patterns, food consumption and nutrient intakes are prone to imprecision and systematic biases. There are different methodologies to measure intakes: each with specific advantages and disadvantages. In evaluating reports on intake, it should be assessed if a suitable and appropriate methodology for a specific study design was followed, whether the measurements were validated and whether appropriate chemical methods or food composition tables were used for nutrient analyses.

- In addition to heterogeneity in dietary interventions, studies may differ extensively because of heterogeneity in participating subjects and the endpoints (outcomes) measured.

*Table II. Checklist for reviewing intervention studies[8].*

| General principles | Details |
|---|---|
| Selection of the study population | Needs to be relevant to the study question; exclusion rules may apply, but these should be clear. |
| Allocation of treatment regimens | Randomisation is essential; a comparison group is essential (either placebo or other treatment). If a community population level experiment, ideally communities should also be randomised. |
| Length of observation | The study needs to be long enough for the effect of the exposure on outcome to occur, if it is going to. |
| Observer effects | Ideally should be blinded. |
| Participant effects | Ideally should be blinded. |
| Compliance | Should be described to make sure participants did (or did not) get the treatment. In some studies the treatment may be inadvertently shared with the controls, so they may also "benefit" from the treatment and the results would look as though there is no difference between treatment and control, and therefore that the treatment did not work (even though it really did!). |
| Ascertainment of exposure and outcome | Both need to be measured at baseline and follow-up with required accuracy. |
| Study size/statistical power | The study needs to be big enough to reduce the potential of a chance null effect. The number required depends on the effect expected and the accuracy of the methods used. If this is not described, beware! |
| Analysis and interpretation | Should be responsible. |

## Grading (rating) evidence

There are various systems available and in use to rate the quality of scientific evidence[2, 5, 12]. The quality and grading of evidence will determine the strength of recommendations for policy and practice, and therefore the confidence that adherence to the recommendation will do more good than harm[2]. The American College of Cardiology together with the American Heart Association's Task Force on Practice Guidelines[13] promote a system of Classification of Recommendations (see **Table IV**) combined with three levels of evidence (see **Table V**) for clinical practice. Probably the most experienced





body to grade evidence from nutritional studies is the panel responsible for the global report on Food, Nutrition and the Prevention of Cancer. In grading the scientific evidence "as a basis for dietary recommendations designed to prevent cancer" the panel used uniform methods and terminology. In their first report the panel used the terms *convincing*, *probable*, *possible* and *insufficient* to summarise a body of evidence. The panel further rated the relative strengths of associations between diet and cancer in individual

*Table III. Chemical substances in foods and drinks to illustrate possible heterogeneity in dietary interventions[11].*

| Class | Nutrient group | Nutrient/substance |
|---|---|---|
| **Nutrients** | | |
| Macronutrients (Provide energy) | Protein | 21 amino acids<br>*saccharides, starch, non-starch, polysaccharides (dietary fibre) |
| | Carbohydrate | |
| | Fat | glycerol, cholesterol, fatty acids (different chain lengths, saturation)# |
| Micronutrients | Water-soluble vitamins | C and B-group |
| | Fat-soluble vitamins | A, D, E and K |
| | Minerals | Ca, Fe, Zn, Na, Mg, K, I etc. |
| | Trace elements | Cu, Se, Mn etc. |
| Water | Water | Water |
| **Non-nutrients** | | |
| Phytochemicals (Thousands of different compounds) | Phytoestrogens | Genistein, daidzein |
| | Lignans | |
| | Indoles | Dithiothiones etc. |
| | Phenolic acids | |
| | Resveratrol | |
| | Tannins etc. | |
| Other non-nutrients | Pigments | |
| | Alcohol | |
| | Additives etc. | |

* Mono-, di-, oligo- and polysaccarides (sugars and starch); non-starch polysaccharides (dietary fibre)
\# omega-3, -6 and -9 fatty acids (with chain lengths from 12 to 22)

*Table IV. Classification of recommendations for evidence-based clinical treatment*[13]

| Level | Description |
|---|---|
| Class I | Conditions for which there is evidence and/or general agreement that a given procedure or treatment is useful and effective. |
| Class II | Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a procedure or treatment. |
| Class IIa | The weight of the evidence/opinion is in favour of usefulness/efficacy. |
| Class IIb | The usefulness/efficacy is less well established by evidence/opinion. |
| Class III | Conditions for which there is evidence and/or general agreement that the procedure/treatment is not useful/effective, and in some cases, may be harmful. |

studies as *strong* (relative risk or odds ratio >2.0 or <0.5 and statistically significant), *moderate* (relative risk or odds ratio >2.0 or <0.5 but not statistically significant, or else 1.5–2.0 or 0.5–0.75 and statistically significant) and *weak* (relative risk or odds ratio is 1.5–2.0 or 0.5–0.75 but not statistically significant)[9].

In their more recent report[9] the panel added grade 1 to convincing evidence, grade 2 to probable, grade 3 to possible and grade 4 to insufficient evidence. The panel described the requirement for evidence to fall into these grades as follows:

### Grade 1 – Convincing evidence

A "convincing" relationship should be robust enough to be extremely unlikely to change over time, as new evidence accrues. The panel required the following characteristics:

- Evidence from more than one study type (epidemiological, experimental or clinical trial), with more than one prospective cohort study of sufficient duration;
- Presence of a plausible biological gradient ("dose response") in the association (such a gradient need not be linear or even in the same direction across the range of exposure);
- Evidence from experimental studies demonstrating one or more plausible mechanisms actually operating in humans;
- No significant qualitative heterogeneity between studies of different types, or in different populations or regions that could not be reasonably explained;
- Good quality studies to exclude with confidence the possibility that the observed association results from residual confounding;
- All reasonable alternate explanations for observed associations to be excluded (that is the relationship found to be specific to the particular exposure and outcome).





All other levels of evidence were defined according to the degree to which the evidence did not meet the above standards. It has not been possible to define precisely the specific deficits which might lead to one or another grading. Failure to achieve a higher grade might result from the accumulation of several small deficits against a number of standards, or from a major lack in one particular aspect of evidence. The definitions below give illustrations of the types of deficit that might lead to an association being judged less likely to be causal.

### Grade 2 – Probable evidence

- There must be evidence from more than one study type, but there may be only one prospective cohort study;
- There may be unexplained heterogeneity between study types;
- There may be absence of one of a number of characteristics, e.g. a plausible dose response relationship, or evidence of a plausible mechanism operating in humans;
- There may simply not be a large enough body of good quality evidence to be able to exclude the possibility of residual confounding or other alternate explanations.

### Grade 3 – Possible evidence

- There may be evidence from only one type of study, or from different study types but no prospective data;
- There is unexplained heterogeneity especially within but also between study types;
- The quality of studies may be inadequate;
- There may be absence of evidence for a plausible mechanism operating in humans, or there may be evidence for a mechanism unsupported by observational data;
- A dose response may not be observed;
- The body of evidence is inadequate in size or quality to exclude confounding, or other reasons for the observed association.

### Grade 4 – Insufficient evidence

- There is insufficient evidence from any study type to draw firm conclusions;
- A plausible mechanism has not been demonstrated to operate in humans;
- Features characteristic of a causal relationship (e.g. dose response, homogeneity, specificity of association) are absent;
- Confounding or other reasons for the observed association remain likely.

This system has been developed to evaluate evidence of relationships between dietary exposures and health outcomes. It seems largely suitable for judging the evidence or the role of nutrition in preventing and treating TB, HIV/AIDS.

Gray and Gray[10] suggested a hierarchy of evidence with 10 subclasses.

*Table V. Level of evidence (for recommendations)*[13]

| Level | Description |
|---|---|
| A | Data derived from multiple randomised clinical trials. |
| B | Data derived from a single randomised trial, or non-randomised studies. |
| C | Consensus opinion of experts (no research studies to support) |

Atkins and co-workers[5], part of the GRADE Working Group, concluded that all of these systems had important shortcomings. The GRADE Working Group[2] proposed an alternative, simplified system for grading the quality of evidence, namely:

- ***High:*** further research is ***unlikely*** to change our confidence in the estimate of effect.
- ***Moderate***: further research is ***likely*** to have an important impact on our confidence in the estimate of effect and ***may*** change the estimate.
- ***Low***: further research is ***very likely*** to have an important impact on our confidence in the estimate of effect and is ***likely*** to change the estimate.
- ***Very low***: Any estimate of effect is very uncertain.

## Discussion, conclusions and recommendations

As mentioned at the beginning of the this chapter, the objective of this ASSAf report is to make strong recommendations for policy and practice, based on the existing evidence regarding the role of nutrition in prevention and treatment of TB, HIV/AIDS. This chapter indicated that there is agreement on how the quality of the different observational and intervention nutritional studies can be evaluated for use in an evidence-based approach. There are different systems available and in use to grade scientific evidence. Two of the systems presented here have the potential for application in this report: that of the WCRF and AICR[9] (1997; 2007) and that of the GRADE Working Group[2, 5]. The first system[9] has been developed to examine and grade the evidence of the relationships between diet (nutrients) and a disease (cancer). There is a huge body of literature on these relationships, and the system seems the preferred one if sufficient studies have been done. The second[2, 5] is a "simplified" system, developed to be applied across a wide range of interventions and contexts. It has been favourably tested against other systems, and seems to be particularly useful when only limited evidence is available. The simplicity of the system favours communication of recommendations. The GRADE system therefore seems to be the system of choice to use in the area of nutritional influences on TB, HIV/AIDS, because at present relative limited information is available. The simplicity of the system should furthermore ease communication of the important recommendations for policy emanating from this report.





*Table VI. Hierarchy of evidence*[11].

| Quality | Type of evidence (study designs) |
|---|---|
| 1a (best) | Systematic review of randomised controlled trials |
| 1b | Individual randomised controlled trials with narrow confidence interval |
| 1c | All or none case series (when all patients died before a new therapy was introduced, but patients receiving the new therapy now survive) |
| 2a | Systematic review of cohort studies |
| 2b | Individual cohort study or randomised controlled trials with <80% follow-up |
| 2c | Outcomes research; ecological studies |
| 3a | Systematic review of case-control studies |
| 3b | Individual case-control studies |
| 4 | Case series |
| 5 (worst) | Expert opinion |

# Physiology and pathophysiology of nutrition, immunity, HIV infection and active tuberculosis

CHAPTER 4

# Human Nutrition

This chapter of the report provides important background information necessary for the contextualisation of following chapters describing nutritional interventions in persons infected with HIV or affected by clinical tuberculosis. Because much of the information is the result over many years of a vast array of studies and conceptual contributions, one or a small number of general references is cited at the level of the title for each part or section of the chapter, rather than assembling an enormous number of original citations. Where specific, usually recent studies are involved, the citations concerned are individually provided.

## Food consumption patterns[1 - 5]

Humans mostly eat to live but they also, to a lesser or greater extent, live to eat. The first is because a living body that is moving around and doing things needs a supply of fuel matched to the activity level and the "idling" consumption, and must replace irreversible losses of body constituents that happen all the time but vary in their individual rates; the second is because humans are social beings for whom the usually pleasurable act of eating and drinking foods is an important ritual in both the social and personal domains. Which foods and drinks, and how much of them, are used by individuals over longer steady-state periods (which is what is almost always the factor that is nutritionally significant) is determined inter-relatedly by culture and by prevailing personal, sometimes even physiological or pathological circumstances. The total consumption can systematically exceed fuel and/or body-replacement requirements, or it can chronically fall short in one or both of these categories.

For purposes of this report, the importance of medium to long-term food consumption, quantitatively and qualitatively, lies in its role as a primary determinant of the nutritional status of the body of each individual in a population. People who are in negative nutritional balance with respect to either fuels and/or replacement nutrients are malnourished in degrees that range from marginal to sub-clinical to overtly clinical; their

bodies respond to this problem in evolved compensatory ways that become progressively more ineffective as the duration and extent of the shortfall in nourishment increases. Supervening incidents such as acute or chronic infections may perturb the body systems of both well-nourished and malnourished people in complex ways (see below).

It is obviously necessary to emphasise that nutritional deprivation can result over time not only from an inadequate intake (itself variably caused by absolute food shortage, the availability only of food of poor nutrient value, and/or poor appetite) but also from poor food retention (e.g. in bulimia), or from poor digestion in, and/or poor absorption from, the gastrointestinal tract. In addition, many disease processes can be associated with increased breakdown of body constituents not compensated for by increased resynthesis, leading to net losses of materials in excretions or secretions.

## Digestion and absorption of the macro-constituents of foods

The processes of digestion of simple and complex carbohydrates, fats and proteins are reasonably well-understood, even if many details remain to be discovered. In essence, bulk macromolecular **carbohydrates** such as starches and glycogen (polysaccharides) as well as more complex carbohydrates such as glycoproteins, proteoglycans and mucins, are hydrolysed to the level of free monosaccharides, mostly glucose, partly in the mouth but mostly in the upper small intestine. Some of the indigestible oligosaccharides are broken down in the large intestine by fermentative bacterial action, forming short-chain fatty acids that are used a body fuels (usually about 10% of daily energy intake). Monosaccharides are absorbed through the small intestinal wall by facilitated transport processes, and passed through the portal circulation to the liver, to be stored there as glycogen or passed onto other organs through the systemic circulation, for use as a general fuel or (only in muscles) as a second store of glycogen, this time strictly for local use during physical activity. Small amounts of the carbohydrates reaching body cells eventually serve as replacement molecules for tissue constituents lost during the functional turnover mentioned above. Liver glycogen releases fuel glucose to other organs as soon as the last meal has been fully handled. The brain and the red cell mass in the blood depend completely on the glucose supply for fuel; other organs have a choice of fuel type (see below).

**Fats** in foods are nearly all in the form of triglycerides, but phospholipids, cholesterol and plant sterols are also present. Digestion occurs after thorough emulsification of the fats in the upper small intestine with the help of bile secreted by the liver; as in the case of carbohydrates, enzymes secreted by the pancreas into this segment of the intestines perform partial hydrolysis so that free fatty acids and residual monoglycerides can be





absorbed in bulk through the intestinal wall and parcelled up in chylomicrons on their way to the liver and from there to the systemic circulation.

The uptake of the circulating dietary fats by different organs, catalysed by lipoprotein lipase, leads to their immediate use as energy source or alternatively to their deposition as stored fuel, mostly in adipose tissue but also in organs such as cardiac and red skeletal muscle. Small amounts of the fats are used in rebuilding tissue constituents such as membrane phospholipids lost during body turnover. Following the handling of the last meal, adipose tissue releases fatty acids into the blood as a primary fuel for many organs and tissues. Dietary cholesterol is absorbed with the triglycerides and utilised at macro-level in membranes as structural constituents, and at micro-level for biosynthesis of steroids in a variety of endocrine and other tissues. Plant sterols are not absorbed by normal human intestines.

**Proteins** (polymers of a basic set of 21 high-prevalence amino acids) present in foods are initially digested in the churning acid contents of the stomach, after which complete hydrolysis to the level of the constituent amino acids occurs in the upper small intestine, catalysed by enzymes secreted into the bowel lumen by the pancreas. The amino acids are absorbed across the intestinal wall in facilitated fashion by a limited number of carrier molecules, and are then despatched to the liver via the portal circulation. The 20 amino acids undergo a large number of specific and general biotransformations in the liver and in other organs, which they reach through the systemic circulation. Some amino acids or their products are used as organ or tissue fuels, others serve extensively as body replacement molecules, and yet others have a wide variety of specific functions in the cells in which they end up. In this sense, proteins are both macro- as well as micronutrients, because their constituent **amino acids** vary in amount in different foods sources, and some play physiological roles, either as precursors of other compounds or in their own right, that are similar in principle to those played by the recognised micronutrients such as vitamins and trace metals. In fact, at least one "rare" amino acid is a pro-vitamin (tryptophan can be converted to vitamin $B_3$ when sufficient amounts are available to the enzyme pathway concerned), while others are precursors of glutathione, a key intracellular anti-oxidant (see below), and yet another (arginine) is the direct precursor of the widespread signalling molecule, nitric oxide. Some amino acids are, or give rise to, important neurotransmitters.

It will be evident from the above account that all the macro food constituents serve as fuels for the working body, but they are also stored for later use, as a fuel supply between meals, so to speak, and used as materials to replace lost body constituents arising from the endless turnover of many of the components of cells and tissues. (There are highly complicated neuro-endocrine mechanisms regulating the processes concerned, reviewed in[6–8]).

## Plasticity of the intestinal tract in macro-nutritional deprivation

The **small intestine** has a huge absorptive area in that the mucosa is thrown up into many tall folds, and each fold is literally covered by a population of absorptive cells, functionally maturing towards the tip; each mucosal (epithelial) cell also has a microscopic "brush border" consisting of tightly packed microvilli, through which the actual absorption takes place. When nutritional demands are generally increased, such as in pregnancy or sustained exercise or cold stress, the intestinal folds become taller and the total number of absorptive cells increases, making the intestine more efficient per unit of its length. This means that there does not have to be a linear increase in food intake as the body demand increases[9].

When food deprivation is prolonged, the intestine responds adaptively by slowing cell division and the migration of immature new cells up the absorptive folds, effectively shortening the folds but increasing the percentage of actively absorbing mucosal cells; the result is conservation of energy and materials, and an intestine that is still functional in handling such macronutrient loads as are offered to it[9].

## Contribution of (large) intestinal bacterial populations to host macro-nutrition

Healthy adult humans constantly harbour about 1.5 kilograms of bacteria (about 10–100 trillion) in their intestines, some in the small intestine but almost all in the large intestine or colon; the bacteria are established soon after birth, have reached a unique compositional mix by 2 years of age, and are maintained thereafter in what is probably a highly individual pattern of steady-state colonisation[11]. The gut flora ("microbiota") are highly specialised in that 99% of them are drawn from only two of the 70 divisions of bacteria found in Nature, and only one of the 13 divisions of archaeobacteria. Other organisms, including protozoa and fungi exist in much smaller numbers, and occasionally pathogenic microbes succeed in establishing themselves in the bowel; there are effective mechanisms for detecting these and responding to them (see Chapter 7).

The **gut microbiota** assist in the intestinal processing of fibrous food residues; detoxify (through biotranformations) many residual xenobiotics but may also generate their own toxins; convert hydrogen gas formed by fermentations into methane; and release a number of micronutrients for use by the host human body[11]. They may also serve other useful symbiotic functions, including supporting normal immune function. The bacterial mass contributes only an estimated 10% of nutrients absorbed from the gut as a whole, even in starvation, but diminution or alteration of the population





can lead to disturbances in gastrointestinal function and metabolic status. There are indications that both local (gut) and systemic immunity can be affected by interactions between "normal" bacteria (non-pathogenic commensals) and the gut epithelium, but experimental evidence is so far mostly restricted to certain probiotics (exogenously introduced beneficial bacteria) and prebiotics ( food components that selectively support the outgrowth of beneficial bacteria), both of which appear to be able to modulate immune signalling in the mucosal wall, as well as immune interactions between the gut and the distributed general immune system[12, 13] (see Chapter 7).

## Common disturbances of gastrointestinal function associated with nutritional perturbations

Consumption of available food can be affected when various pathophysiological disturbances occur as a result of one or the other disease. **Loss of appetite (anorexia**) is the simplest of these, even though the phenomenon may vary from mild and temporary to severe and long-lasting, depending on the precipitating factor(s). Loss of appetite commonly accompanies "inflammation", defined as the systemic response of the body (mediated by cytokines and hormones) to an infection (or, sometimes, another "foreign stimulus "such as an auto-antigen or a cancer), provoking activation of innate and/or adaptive immunity. The cytokine most often responsible for anorexia in inflammatory states is the form of tumour necrosis factor called TNF-$\alpha$. Appetite, counter-intuitively, is a very imperfect stimulus of a need for nutritional repletion; it can be habitually "abused" by over-eaters (of both the "binging" and the "grazing" type), while starved humans adaptively learn to keep it at bay; it is highly dependent on social cues and/or the state of the psyche; it is influenced by smell and taste factors that are themselves determined by habit and by temporary local disturbances like nasal colds or mouth/tooth/throat pathology; it is severely depressed when people feel nauseous (see below); it is ultimately a mostly subjective feeling that is nevertheless usually the single most important determinant of food intake over time in situations where food supply itself is not the determining factor.

**Dyspepsia**, or discomfort experienced while, or just after, eating food is distressing but not usually a cause of under- or malnutrition unless it is complicated by other phenomena (see below) or adaptively depresses the appetite. **Nausea** is a very uncomfortable subjective state with objective upper gastrointestinal as well as general body symptoms, which prevents or minimises the consumption of food over a short period, or chronically (for example, in patients taking chemotherapy for advanced cancers) . Intense nausea often leads to v**omiting,** which is a severely distressing event accompanied by extensive autonomic nerve system activity, and made possible by a

combination of concerted abdominal muscle contraction and relaxation of the region of the gastro-oesophageal sphincter. Food that has been taken into the stomach and upper intestine is lost before it can be digested. Vomiting that is short-lived does little harm, and is part of an evolutionarily preserved reaction to potentially (or actually) poisonous food, or to prevent food being added to a stomach or intestine which is temporarily undergoing pathological processes and needs to have time to deal with these. Vomiting that is prolonged, on the other hand, rapidly runs down the body in nutritional terms as it usually forms part of a serious disturbance in which abnormal amounts of hormones and cytokines are circulating in the body, the autonomic nervous system is in disarray, and water and electrolyte balance are both abnormal.

**Diarrhoea**, like vomiting, can be short-lived and mild, or it can become life-threatening in diseases such as toxic gastroenteritis, cholera and various dysenteries. Massive loss of water and accompanying electrolytes, in frequently and urgently passed watery stools, is usually associated with severe loss of appetite, and often with nausea and vomiting; nutrition essentially comes to an end as all or most of the food previously ingested is lost with its partially digested products as a result of severe "intestinal hurry", dysfunctional absorption mechanisms, and temporary cessation of new intake. Mild, intermittent but chronic diarrhoea is found in sub-acute disorders of the small and large bowel, as well as the liver and pancreas, and also in HIV infections (see Chapter 7). This may be accompanied by **malabsorption** of nutrients (usually fats in the case of liver or pancreatic disease), or micronutrients. Fat malabsorption readily leads to deficient absorption of fat-soluble vitamins, while malabsorption arising from primary intestinal disease of medium or long-term duration may additionally affect the uptake by the body of dietary water-soluble vitamins and essential trace metals particularly zinc (see below). The effects of acute and chronic "immunitis" (immune activation in terms of various kinds of cells and release of certain cytokines, and/or depletion of specific lymphocyte populations) on nutrient absorption over time, of obvious importance in HIV-infected persons, has not been adequately studied to date (see Chapter 7).

## Whole-body energy metabolism – energy balance

A balance between energy intake and expenditure is achieved when an adult maintains a stable body weight; effective energy intake (that part of all the food ingested which is actually absorbed from the gut) must equal total energy expenditure. Malnutrition, either under- or over-nutrition, results when one side of the equation "outweighs" the other over a substantial period of time. The amount of energy the body needs in order to function appropriately is determined by the basal metabolic rate; the energy expended due to dietary thermogenesis (heat generated by the body when digesting and absorbing





the food), and for the maintenance of a constant body temperature; the energy needed to deposit or replace cells and tissues; and the energy expended during physical activity.

The different aspects of energy metabolism are tightly regulated by the **neuroendocrine system**[6–8]. Electrochemical messages from the nervous system are translated into hormonal messages, delivered to target cells, tissues or organs; in turn, circulating hormones and cytokines act back on neural systems. The **hypothalamus**, a key component of the neuro-endocrine system, is directly and indirectly involved in energy metabolism. Through direct innervation of the adrenal medulla, it can stimulate the general release and distribution of noradrenaline, resulting in an increased metabolic rate (mobilisation of glucose and fatty acids from tissues; increased heart rate and force). Cortisol may be released into the circulation from the adrenal cortex through the chain effect of the release of corticotropic-releasing hormone (CRH) from the hypothalamus, acting on the pituitary gland causing the release of adrenocorticotropic hormone (ACTH). Cortisol also acts to mobilise fuels, predominately via the breakdown of muscle protein into amino acids and their sunsequent conversion into glucose. Stress and infection are powerful stimuli for the release of cortisol via this neuroendocrine pathway. An example of a stimulus that originates in a peripheral tissue is the release of leptin from adipose tissue in response to an increase in the amount of triglycerides present in the fat cells; leptin's action on the hypothalamus results in decreased food intake and lowered metabolic rate.

### Factors affecting energy balance

**Appetite** is controlled by a complex system of sensations (taste, smell, fullness and satiety) as well as neural and humoral factors. Central control of appetite resides primarily in the hypothalamus and the brain stem. Peripheral control of appetite occurs by means of hormones and peptides released by the gut (ghrelin, cholecystokinin, pancreatic polypeptide and others) and other endocrine tissues (insulin and leptin). During episodes of illness and stress, appetite is decreased (see below). **Digestion and absorption** (see above) affect the actual delivery to the body of ingested food components, and any disruption of the architecture, function and immunity of the intestines (as is the case with HIV infection), is likely to result in intestinal inflammation and/or secondary gastrointestinal infections and consequent malabsorption and diarrhoea, severely reducing the amount of energy available to the body for its functions (see also Chapter 7).

The **basal metabolic rate (BMR)** accounts for 60–75% of total energy expenditure in the absence of (immediate) past, as well as present, physical activity. Measured upon waking after a 12-hour, overnight fast and prior to the ingestion of any food (i.e.in the post-absorptive state), it is indicative of the energy needed to sustain the metabolic activity of cells and tissues and to maintain blood circulation and respiration in the resting but awake state. **Resting metabolic expenditure (REE)** is typically measured

only 3–4 hours after a meal at any time of the day and prior physical activity is not controlled for. While **sleeping metabolic rate** is approximately 5–10% lower than basal metabolic rate, resting metabolic rate is about 10–20% higher than basal metabolic rate.

Basal metabolic rate is dependent on age, gender, body composition, nutritional and health status. An individual's fat-free mass (bones, muscles etc) is composed of the most metabolically active components of the body and is for this reason the major predictor of basal metabolic rate; the decline in BMR that occurs with aging (approximately 1–2% every 10 years in weight-stable individuals) is most likely due to the progressive decrease in fat-free mass and the increase in fat mass that occurs over time.

**Thermogenesis** is the use by the body of increased metabolic oxidations to generate heat. The conversion of energy from food into the high-energy biochemical compounds that can be used as "chemical energy currency" by the body for various metabolic processes is normally an inefficient process, so that roughly 50% of the ingested potential energy is lost to heat production. The **dietary thermogenic response** to protein consumption (20–30% increase in energy expenditure above BMR) is far greater than the effect caused by the consumption of carbohydrate (5–10% increase in energy expenditure above BMR) and fat (5% increase in energy expenditure above BMR). The ingestion, digestion and absorption of a typical mixed meal elicit an increase in energy expenditure equivalent to approximately 10% of the kilojoules consumed. Ingestion of caffeine, a sympathetic nervous system stimulant, can increase metabolic rate by 10–30% above baseline for up to 3 hours post ingestion; on a daily basis, a typical caffeine intake can cause up to 3% increase in total energy expenditure.

**Growth, pregnancy and lactation** are special body states in which energy metabolism adapts upwards to meet the particular extra needs. Infants and children require energy to synthesise and deposit tissue so that their bodies can grow; in the first months of a person's life, the energy required for **growth** accounts for approximately 35% of the total energy required by the body. This energy cost of growth decreases to about 3% of the total energy required by the body after 12 months of life and remains low until puberty, when the energy cost of growth again increases to 4% of the total energy required. During **pregnancy** the basal metabolic rate increases, the energy cost of physical activity due to increased weight-bearing increases by approximately 20% (lessening in advanced pregnancy), and extra energy is required for the deposition of maternal and foetal tissue. The synthesis of breast milk and the process of **lactation** accounts for a 4–5% increase in basal metabolic rate.

Typical amounts of **physical activity** on a daily basis accounts for approximately 20–30% of the total energy expended by an individual, with "fidgeting" making up a surprisingly large fraction of the expenditure. Increasing levels of physical activity, in terms of both intensity and duration, require appropriately increasing amounts of energy to execute. The body will also continue to require energy once the activity is ceased, proportional to the degree of effort **(post-exercise thermogenesis**). During prolonged, intense exercise the body heat content will rise, mainly due to inability to lose heat quickly enough. During low intensity exercise, fat stores (muscle and adipose triglycerides) are more likely to be utilised, whereas during more intense exercise carbohydrate stores (muscle glycogen and glucose) are utilised.

## Activation of the immune system and inflammation: important determinants of energy balance

The body defends itself against the invasion of bacteria or viruses by activating its immune system in ways that entail mobilisation of stored energy-yielding fuels (see below) accompanied by a downward modulation of food intake (anorexia).

The neural, endocrine and immune systems are interlinked in that they produce and respond to a number of common regulatory molecules including hormones, neuropeptides, cytokines and neurotransmitters, an interaction well illustrated in the relationship between inflammation and appetite control. During infection, **pro-inflammatory cytokines** such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) are released by active immune cells (see below); amongst other effects, these cytokines increase the release of **leptin** from adipose tissue and decrease the release of **ghrelin** from the stomach[14, 15]. Leptin acts as a pro-inflammatory cytokine and enhances Th 1-dependent T-cell functions (especially in $CD4^+$ cells, see below) and increased energy expenditure. Decreased ghrelin levels result in a loss of appetite and decreased anti-inflammatory effects of this hormone. Loss of appetite and increased energy expenditure result in negative energy balance in the medium to long term. **Fever and shivering** often accompany inflammation: polymorphonuclear neutrophils release an endogenous pyrogen whose action on the hypothalamus, via prostaglandins, results in the resetting of the body's thermostat.

The hypothalamus senses that the pre-infection temperature is too low and initiates shivering (involuntary means of increase body temperature through muscle contraction and movement) and vasoconstriction of blood vessels to the skin, both of which effects increase body temperature.

## Energy balance when food intake falls

In the face of starvation or prolonged fasting, the various stores of fat, protein and glycogen are mobilised and oxidised in order to meet the body's energy requirements. Varying proportions of these stores can be lost without serious health implications; of 9

kg of body fat, not more than 1 kg is essential, but loss of more than 25% of 10 kg of body protein and 1 kg of carbohydrate results in impairment of body function. Mortality rates increase dramatically with a decrease of 30% of body weight. The primary adaptation of the body to starvation is the preferred utilisation of ketone bodies (a by-product of rapid and energy-intense fatty acid oxidation) instead of glucose; this adaptation halves the rate of loss of body protein.

## Energy balance in chronic infections

Prolonged HIV and/or *Mycobacterium tuberculosis* (TB) infections, associated with negative energy balance, result in the loss of body protein stores as well as adipose tissue[16, 17]. Weight loss and wasting, for example, are independent contributing factors to increased morbidity and mortality in HIV infections, but wasting is not an inevitable consequence, as there appears to be a high degree of variability in the extent of weight loss and wasting in HIV-infected individuals and, in most cases, acute weight-loss episodes are associated with secondary infections. Following recovery from secondary infections, HIV-infected individuals tend to gain weight and remain relatively weight-stable [18]. Instances of chronic weight loss in HIV infection are normally associated with gastrointestinal inflammation and/or infection, and associated malabsorption. A very different picture is seen with active tuberculosis, where rates of whole-body protein synthesis and breakdown are less accelerated than in HIV-infected individuals. In addition to this, upon re-feeding the mediators of the immune system appear to block anabolic processes in TB patients, preventing them from regaining weight even when provided with sufficient food[16, 17].

The reasons for **HIV-associated weight loss and wasting** are multifactorial. Negative energy balance may be due to an inability of HIV-infected individuals to increase food intake sufficiently to balance the increased resting energy expenditure and/or the decreased absorption of ingested food components due to gastrointestinal disease or virus-related disruption of the intestinal mucosa. **Food security and availability,** particularly in the developing world, is an important aspect of HIV-associated weight loss and wasting; it is usually further complicated by the fact that individuals who are ill are not able to go to work to earn enough money to buy food, nor can they work in their fields which may be the only source of food. **Decreased appetite**, a common complaint of many HIV-infected individuals, is most profound in the later stages of HIV infection, probably due to the anorexic effect that the pro-inflammatory cytokines exert on the body (see below). **Malabsorption** of food is another important contributor to the decreases energy availability in HIV-infected individuals; disruption of the architecture and function of the intestines and consequent malabsorption and diarrhoea are common





features of HIV infection, and HIV-infected individuals may decrease their food intake to minimize the episodes and severity of the diarrhoea.

Resting energy expenditure (see above) is indicative of the basal metabolic activity of the body. In HIV infections, resting energy expenditure is expected to be elevated due to increased cytokines linked to on-going viral replicative activity, as well as increased protein turnover and de novo hepatic lipogenesis. Increased REE is usually the result of increased levels of thyroid hormones, catecholamines and cortisol, but in HIV-infected individuals, the levels of these hormones appear to be normal. The findings of a recent meta-analysis investigating resting energy expenditure in HIV-infected individuals were that **resting energy expenditure**, expressed per kg fat-free mass (REE/FFM), was significantly higher (~11%) in HIV-infected individuals than in healthy HIV-negative controls[19]. Sub-group analyses showed that the REE/FFM of symptomatic HIV-infected individuals tended to be higher than that of asymptomatic, weight-stable HIV-infected individuals, although not significantly so. There is also evidence to suggest that the type of secondary infection complicating HIV infection might influence changes in the resting energy expenditure. A number of studies have shown that HIV-infected individuals are not hypermetabolic in terms of total energy expenditure (TEE), even though one would expect them to display an increase in TEE as a result of the increased resting energy expenditure, this was not the case. In fact, TEE was significantly reduced in HIV-infected, rapidly weight-losing men, apparently because they significantly reduced their **activity-related energy expenditure.** Since total energy input (after digestion and absorption of food) and total energy expenditure are the major determinants of energy balance, net weight loss in HIV infections is associated with greater losses of inputs than of outputs in terms of energy.

## A possible reason to look again at obesity

Many obese persons may live on diets that contain inadequate amounts of certain micronutrient; in some cases these may have functionally significant repercussions (see Chapter: Brief from Council). Macrophages in the plentiful fat tissue of such persons are now known to release pro-inflammatory cytokines, primarily but not only TNF-$\alpha$; this may be part of the pathophysiology of what is called the “metabolic syndrome” typical of obese individuals[8] . The may well be immunological defecits under these conditions that intersect with HIV associated changes, but little evidence to date has become available. A possibly serious problem for HIV-infected obese individual may be activated transcription of HIV in infected cells (see Chapters 6 and 7) through the NF-$\kappa$B pathway especially if there are simultaneous deficiencies in anti-oxidant vitamins and minerals.

## Micronutrients: Vitamins[20]

Vitamins are organic micronutrients which have been shown to be required in medium to long-term human diets for optimum health and to avoid asymptomatic deficiency states or full-blown diseases. Most of them are precursors of some of the **coenzymes** 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, multi-cellular, 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 coenzymes. 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 coenzyme biosynthesis, to increase the "catchment" of vitamin materials by enabling food consumers to use a number of prevalent precursors rather than only the finished coenzyme itself; coenzyme 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 coenzymes 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. (An example well-known in pellagra prevention is the binding of vitamin $B_3$ 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 (see below) 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 (see above), 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 coenzymes 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 coenzymes 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 coenzyme 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" (now called the "Average Nutrient Requirement", see below) 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 (see following chapter).

**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 coenzymes and other vitamins or vitamin-derived compounds in different body situations, such as heavy physical activity, pregnancy, lactation, rapid growth and – crucially for our topic – 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, inter-cell 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 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 things: 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 thirteen 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 ($B_6$), vitamin C, biotin, folate, and cobalamin ($B_{12}$); 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 (pale) skin, and vitamin K made available from intestinal bacteria (niacin can be biosynthesised from the essential amino acid tryptophan provided this is itself provided 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 it is eaten is important. Storage losses are proportional to temperature, pH and moisture content (enzymatic decomposition), by light exposure (riboflavin, folate) or 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.

## Vitamin requirements

Because absolute bioavailability is never determinable in any given person for any given diet (for the many reasons already given above), it is very difficult to specify how much of a particular vitamin must be consumed by that person per day, averaged over a period of time. Nutrient-based dietary recommendations used and applied to assess diets and plan nutrition policy, programmes and interventions of individuals and groups, are standards (reference values) proposed for all healthy people. They are based on scientific knowledge of human nutrient requirements (and all known factors influencing these). Recently, the United Nations University, supported by the International Union of Nutritional Sciences (IUNS), the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO), convened a group of international experts to harmonize nutrient-based dietary recommendations[21]. The group inter alia agreed that:

- The umbrella term to describe these recommendations should be **Nutrient Intake Values (NIVs)**; this should replace the USA/Canadian term of Dietary Reference Intakes (DRIs) and the British term of Dietary Reference Values (DRVs);
- The average or mean nutrient requirement, estimated from a statistical distribution of requirements for a specific criterion (e.g. to prevent a deficiency disease or to maintain body stores) for a specific life-stage and gender group (age and sex), should be termed the **Average Nutrient Requirement (ANR)**. (In the USA, Canada and Britain this is known as the Estimated Average Requirement (EAR) and in the European Communities as the Average Requirement Intake (ARI));
- The "Recommended Dietary Allowance" or RDA (USA and Canadian term) known in Britain as the Reference Nutrient Intake (RNI) and in Europe as the Population Reference Intake (PRI), and usually set at two standard deviations above the ANR, should be known as the **Individual Nutrient Intake Level (INLx)**. If the INL is set at two standard deviations above mean intake, it will be shown as INL98. This opens the door for populations to set a lower value if their circumstances or policies do not permit achieving an intake to the 98$^{th}$ percentile for the total population.

These definitions, while operationally useful in nutrition and the food industry, are obviously highly problematic when dealing with an individual who is not "apparently" healthy (for example, in being infected with HIV and/or M.tuberculosis), who may or may not be subject to causes of deficiency other than primary dietary insufficiency, who may or may not have asymptomatic deficiency/ies as described above, and/or who may differ genetically from the majority members of the population.

## Assessment of vitamin deficiency status of individuals and populations[20, 22]

Because **dietary history combined with food tables**, while important, is not a wholly reliable guide to the nutritional status of individuals, cl**inical 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 features** to be looked for include various anthropometric measures, like body weight, body-mass indices and standardised skin-fold thickness, especially valuable if observed over time; 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** that have become preferred for reasons of affordability, feasibility, reliability, relevance and conclusiveness 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 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 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 micronutrients) is very common[23] (see Chapter 10) 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 re-direction 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**", C-Reactive Protein (CRP), serum amyloid A (SAA), fibrinogen and alpha-antitrypsin. Recognised "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 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[24]. 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 concentration of negative APPs were not consistently reduced and the overall patterns were similar to those of other chronic viral infections[25] (see Chapter 5).

A second confounder of laboratory measurement for nutritional assessment in respect of many vitamins is the fact that vitamin-derived coenzymes 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.

## Food-derived vitamins versus synthetic (single or multiple) supplements

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 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 (see the conceptual framework concerning dosage effects, Chapter 1) ,

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.

## Micronutrients: inorganic elements[26]

**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. Asubset of these inorganic micronutrients have been shown to be especially relevant to human immunity and resistance to infections, and will be briefly discussed here. In general, they resemble vitamins in being present in **particular food sources** in notable amounts; in varying in **bioavailability** because of differential binding to other food constituents, either in the native food (e.g. phytic acid) and/or 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 reflecting 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; 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 measurement [27]. 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.

## Specific effects of some vitamins and inorganic micronutrients on human immunity

**Vitamin A[28, 29]:** This fat-soluble vitamin exemplifies the vitamer concept in that both the pre-formed natural retinoids (retinol/retinaldehyde and retinoic acid) and the pro-vitamin A **carotenoids**, which can be bio-converted into **retinoids** in the intestinal wall, are nutritionally active as vitamin A but differ significantly in their bioavailability, not only intrinsically but also dependent on factors such as whether the food containing them is cooked or not. The absorption of these compounds depends on dietary fat being present and is complex, but in essence leads to the packaging of retinol esters and some unchanged carotenoids into lipid-rich particles called chylomicrons which are distributed to the body tissues via the portal and systemic circulations, leading to loss of their triglyceride fat and the formation in the plasma of residual particles called remnants. These are cleared by the liver, and their retinol content mostly ends up in special stellate cells representing the considerable liver store of the vitamin. The store is drawn on demand by release of free retinol bound to a liver-produced protein, **retinol-binding protein (RBP**); this circulates in the plasma in association with another protein. The retinol is taken up by retinol-requiring cells through a receptor, and bound to intracellular proteins, some of which convert it to **retinoic acid** (a form of vitamin A that acts on transcription in cell nucleus like a steroid hormone) while others have special functional roles in different cell types.

The most useful test of vitamin A status in humans uses the storage/supply role of the liver in maintaining a virtually constant plasma retinol level; free RBP accumulates in the livers of vitamin A-deficient subjects, and is released with the newly available vitamin after vitamin A is administered in a standardised way and absorbed. The **relative dose response (RDR)** test is calibrated at +50% indicative of severe depletion, +20-50% marginal depletion, and lower values are normal. RBP measurements are unreliably low in

the "acute phase" of systemic "inflammation". Nutritional factors that may cause or worsen vitamin A depletion are low-fat diets, and severe protein, zinc and vitamin E deficiency.

The effects of vitamin A deficiency on the human immune system are varied, amounting to significantly **depressed immunity**, especially in children[28, 29]. Both cell-mediated and humoral mechanisms mediated primarily by lymphocytes are impaired, natural killer (NK) cells are reduced in number, neutrophils show functional defects, and macrophages become less effective in various ways. The barrier and immune defence functions of mucosal surfaces in the airways, intestines and genital tracts are negatively affected by the replacement of secretory cells in epithelia by keratin-producing cells. Circulating carotenoids have important anti-oxidant activity, which may promote certain immune mechanisms (see below for other anti-oxidants).

**Vitamin D[30]:** This is a fat-soluble vitamin that can be supplied from animal-derived foods (especially fish oils) in a form known as **$D_3$ (cholecalciferol**) and from plants as **$D_2$ (ergocalciferol**); vitamin $D_3$ can also be synthesised in the body from endogenous precursors through the action of ultraviolet rays in sunlight on the skin.

Only few foods in South Africa are fortified. Infant formulas are vitamin D fortified as are some powder milk preparations and margarines, but in general few of our normal dietary constituents contain more than marginal amounts of vitamin D. Absorption of the equipotent dietary vitamins $D_2$ and $D_3$ requires the co-presence of dietary fat, and involves their packaging in chylomicrons, transport to the liver where 25-cholecalciferol is formed, and systemic circulation in the course of which the active form, **1,25-cholecalciferol**, really a hormone, is formed by the kidney. Both of these forms circulate in the blood bound to a specific protein, **D-binding protein (DBP**). The active vitamin (hormone) D affects nuclear transcription rates of specific genes after binding to the **vitamin D receptor (VDR**), an important cellular protein, the amount of which is itself under complex regulation, including positively by the active vitamin D itself. The liver inactivates vitamin D to water-soluble forms lost in the faeces via the bile.

The actions of active vitamin D on the human immune system include enhancement of innate immune reactions through promotion of **macrophage** differentiation; activation of resident macrophages and chemotactic recruitment of monocytes[31]. By contrast, adaptive immunity is suppressed through complex effects on lymphocytes. Of particular interest is that 1,25-cholecalciferol synthesis and VDR induction followed activation of the Toll-like receptors (TLR) of macrophages infected with M.tuberculosis (Mtb), associated causally with the generation of an antibiotic peptide called cathelicidin and **enhanced microbial killing**[32]. Dark-skinned persons who are less able to synthesise their own vitamin D were found to have systematically lowered serum 25-cholecalciferol levels, compared with matched pale-skinned persons, and their sera were much less active in supporting Mtb killing in vitro[32].





**Vitamin E[22, 33, 34]**: The third fat-soluble vitamin with effects on human immunity is a mixture of tocopherols and tocotrienols which are especially plentiful in the oils of cereal grains and nuts. While not the most plentiful vitamer in foods, **alpha- tocopherol** has the highest relative potency in the group and is predominant in plasma and the tissues. Absorption during digestion requires the presence of bulk fats, and diets high in polyunsaturated fats increase the dietary requirements. While there is no specialised storage depot for vitamin E, most of the body's content is dissolved in the triglyceride droplets in adipose tissue. From a functional point of view, the presence of vitamin E molecules lodged in **cellular membranes and plasma lipoproteins** is critically important ("fire-extinguisher role") to protect the constituent unsaturated fatty acids from damage caused by peroxidative "flame-thrower" chain reactions, and thus to keep these lipid-rich structures in "working order" insofar as many of the mechanisms involved in innate and adaptive immunity depend on cell-cell interactions or on complex membrane processes. Work on the possible role of vitamin E in immune reactions in laboratory animals[22, 32] has revealed a multitude of promotive roles for the vitamin, as would be expected from a membrane-stabilising agent, but low serum vitamin E levels have consistently been found in HIV-infected human populations, and an inverse correlation existed between disease severity and serum levels of vitamin E[35].

**Vitamin C[36]**: This water-soluble vitamin is present (at high bioavailability) in food sources such as vegetables and fruit, and in human plasma and tissues, in its reduced (ascorbic acid) and oxidised forms (dehydroascorbic acid, DHA). The vitamin is absorbed from the gut and transported in the blood to body tissues which have a

highly variable uptake and retention capacity, so that some cell types (adrenal cortex, macrophages, neutrophils, lymphocytes) concentrate the vitamin to a much larger degree than do others, for evolved functional reasons. The redox system of circulating water-soluble vitamin C is a front-line defence for plasma lipoproteins and cellular membranes attacked by aqueous-phase oxidants, complementing the role of vitamin E already described. Vitamin C as it turns over is mainly excreted in the form of soluble catabolic products in the urine; this is also the route of disposal of surplus vitamin.

The roles of vitamin C in human immunity probably reside mainly in its specialised functions in phagocytic cells (in which it becomes even more heavily concentrated during activation than in resting stages); they may possibly also exert redox effects limiting inflammatory cytokine production and even the replication of retroviruses such as HIV (see below).

**Vitamin $B_6$[37]:** Unlike vitamins $B_1$, $B_2$, $B_3$ and $B_{12}$ (which while very important in nutrition are not associated with specific roles in immunity, hence their omission together with biotin and pantothenic acid and fat-soluble vitamin K from this chapter), vitamin $B_6$ nutrition may well be very significant for effective human immunity against pathogens.

The six equally bioavailable low- molecular weight **vitamers** are pyridoxine, pyridoxal and pyridoxamine, and their respective phosphorylated derivatives, but the main food sources such as whole-grain cereals and vegetables contain mainly carbohydrate-conjugated compounds with much diminished bioavailability. Absorption of dietary $B_6$ in the intestine leads to intravascular transport to the liver and other organs, mostly as pyridoxal phosphate bound to albumin; tissue uptake requires dephosphorylation and subsequent trapping of free pyridoxal by intercellular phosphorylation. Excess vitamin is oxidised and excreted mainly as 4-pyridoxic acid in the urine. Intracellular pools of vitamin $B_6$ are highly distributed through differential affinities between a host of enzymes using the compound as co-catalyst, and a number of proteins to which pyridoxal phosphate is bound for their structural stability, particularly glycogen phosphorylase in skeletal muscle (which tissue normally contains about 80% of the body content of the vitamin, mostly in this form.) The evolved pathways for compensatory redistribution of vitamin $B_6$ in human bodies that are undergoing progressive depletion of the vitamin are necessarily highly complex and variable, and the pathophysiological consequences unpredictable, yet they may be very significant. **Laboratory assessment** of vitamin $B_6$ status is directly by plasma levels, and indirectly by urinary 4-pyridoxic acid assays, tissue enzyme activity measurements, and a number of metabolic load tests.

The effects of $B_6$ deficiency on the immune systems of elderly humans have been focused mainly on **T lymphocyte maturation** and cytokine production. A pyridoxal phosphate-dependent enzyme has recently been shown in mice to be involved in creating a sphingosine 1-phosphate gradient across lymph nodes required for the egress of antigen-directed lymphocytes that have homed into the nodes after immune stimulation; $B_6$ antagonists had a similar action to that of a common food additive which suppressed immunity in this way[39]. $B_6$ is needed to maintain cellular anti-oxidant defences through its rate-limiting function in cysteine production, hence glutathione biosynthesis (see below).

**Folate[40] (and vitamin $B_{12}$[41])**: The vitamers of folate are all derivatives of pteroic acid that display folate activity in humans. Food sources like green vegetables, legumes and nuts contain mainly the dihydro- or tetrahydro reduced forms of the vitamin, which apart from various functionally important one-carbon substituents are conjugated to 5-7 residues of glutamic acid (poly-gamma-glutamyl chains), which are the typical intracellular and/or storage forms of the vitamin; synthetic (supplemental) folate is in the mono-glutamyl form, which is the form in which in the human body the vitamin circulates in the blood and traverse membranes. Digestion requires enzymatic removal of the polyglutamate chain in the intestine (chronic excessive use of ethanol inhibits dietary folate utilisation by lowering the deconjugase activity) and this is followed by absorption using specific carriers. The **bioavailability** of food folate is lower (by about 50%) than





that of the synthetic folate because of the need for digestive removal of the polyglutamate side-chains and physical entrapment of folate in fibrous plant materials. Humans obtain folate both from their diets (small intestine) and from intestinal bacteria (colon). The liver plays a major role in folate disposal and distribution, containing about half the total body store, continuously undergoing rapid metabolic turnover for endogenous purposes and for release of folate to the receptor-rich tissues most requiring it in particular circumstances (e.g. rapid cell division).

Folate is pervasively involved in body metabolism (nucleic acids, nucleotide coenzymes, amino acids) as coenzyme for a very large number of **one-carbon transfer reactions**, such as methylation and formylation. No special effects of folate deficiency on the mechanisms of human immunity have been described, but HIV infection involves the enhanced daily turnover of huge numbers of T-lymphocytes eventually resulting in severe ($CD4^+$) T-cell-lymphopenia, which may put special pressure on folate balance in the body (see below)

Although the biochemical pathways were not fully elucidated at the time, it is of interest that the crucial role of folate in biology was first established during the development of the first effective antimicrobial therapeutic agent, sulphanilamide, which inhibited bacterial growth by competing with para-amino benzoic acid for incorporation into the pteroin precursor of folate, a vital step in folate synthesis in bacteria.

Of all the reactions involving folate as coenzyme, perhaps the most relevant for clinical medicine are those involving purine and pyrimidine synthesis. In particular, the synthesis of deoxythymidine monophosphate from its precursor deoxyuridine monophosphate is a rate-limiting factor in *de novo* (as opposed to salvage) pathways of DNA synthesis. Because this reaction is critically dependant on the presence of 5, 10 methylene tetrahydrofolate polyglutamate, folate in this form is thus the major quantitative determinant of adequate rates of DNA synthesis in conditions characterized by high cell turnover. The role of vitamin $B_{12}$ is to demethylate methyl tetrahydrofolate, the form of folate taken up by cells from plasma. Deficiency of vitamin $B_{12}$ thus becomes the other major rate limiting nutritional factor in DNA synthesis under such conditions.

With regard to eukaryotic, and particularly, human, cell biology, deficiencies of folate and $B_{12}$ are common causes of the haematological cytopenias known as the megaloblastic anaemias. Patients are frequently not just anaemic, but exhibit reductions in numbers of white cells and platelets as well, emphasizing the generic role of these vitamins in DNA synthesis, especially when the deficiency is severe. Indeed, the consequences of folate or vitamin $B_{12}$ deficiencies are not restricted to cells of the haemopoietic system, but are also encountered by epithelial cells of the gastrointestinal and genitourinary tracts as well, these tissues also comprising cells with high natural turnover rates[42]. While the natural rate of lymphocyte turnover is not generally at this level, HIV is well known to escalate

lymphocyte turnover to a point at which folate or vitamin $B_{12}$ deficiencies might become rate-limiting, perhaps acting synergistically with other causes of lymphocyte destruction.

There is abundant precedent for the emergence of relative folate deficiency in patients experiencing escalated cell turnover rates, such as those occurring in inherited haemolytic anaemias, such as sickle cell anaemia, or in pregnancy, where the development of folate deficiency is so common that it is standard policy to supplement folate in pregnant women. Turning to lymphoid cell turnover, folate inhibition by methotrexate is a standard mechanism of therapeutic immunosuppression, and the mechanism of action of methotrexate is to inhibit dihydrofalate reductase. Moreover, the prototype condition characterized by enhanced lymphocyte proliferation is acute lymphoblastic leukaemia, against which the first effective therapy was aminopterin, also a dihydrofolate reductase inhibitor similar to methotrexate. Finally, lymphocytes cultured from folate-deficient patients show that the addition of folate enhances the incorporation of deoxyuridine into DNA, which implies that folate deficiency is a limiting factor in the *de novo* synthesis of DNA in lymphocytes from such patients. It is thus highly likely that folate deficiency would be limiting for lymphocyte regeneration in states of enhanced lymphocyte turnover, of the kind required to maintain lymphocyte counts in patients with HIV/AIDS.

**Iron**[43]**:** This is an important micronutrient for many bodily functions, but the focus here is on iron's still poorly understood roles in human immunity. Amongst other documented effects in humans are diminished intracellular killing of bacteria in neutrophils and variably altered T-cell functions. Subjects suffering from **iron-deficiciency anaemia** are much more vulnerable to infections of all kinds than are normal individuals, but the exact reasons for this has not been elucidated to date.

**Zinc**[22, 44]**:** A central clinical feature of zinc deficiency in humans, already detectable on marginal regular daily uptakes that are less than the recommended 5–7 mg per day (especially if cereal-rich diets are taken that are rich in phytic acid), is **increased susceptibility to infectious diseases**, through impairment of multiple mediators of immunity ranging from the skin to both cell-mediated and humoral immunity. Apoptosis of T-cells is enhanced, associated with thymic atrophy; plasma thymulin necessary for T-cell maturation and other functions is also inactive. Almost all cell types that contribute to human immunity are negatively affected in zinc deficiency, in both number and activity. Progressively lowered serum zinc levels have in fact been documented in HIV-infected individuals[45]. Zinc administration reverses most or all of the described effects in both laboratory animals and humans, but excessive doses are counter-productive.

**Selenium**[46]**:** The physiological role of this trace element (Se) is to become part of a number of important enzymes in the form of active site-located selenocysteine residues that are necessary for function; these enzymes include glutathione reductase and thioredoxin reductase. The incorporation of these particular residues occurs through a specific process and is coded in the mRNA for the enzyme concerned. Many body proteins also contain selenium in the form of both selenomethionine and selenocysteine, which have been incorporated at methionine- and cysteine-coded sites by simple, concentration-dependent competition with these unselenated residues; these selenoamino acids, being more plentiful, are the main natural source of selenium in the diet.After digestion, some of this selenium is made available (in a highly regulated demand/supply mechanism) for the functionally important selenocysteine incorporation into the enzymes mentioned above. Se is essential for both cell-mediated and humoral immunity**,** mainly through **anti-inflammatory and/or anti-oxidant effects.** Bacterial killing in neutrophils and other phagocytes is decreased through diminished action of glutathione peroxidase caused by Se deficiency. Various cytokine receptors necessary for immune signalling are upregulated by Se, as are B-cell antibody-producing processes. Selenoenzymes are important for maintaining an anti-oxidant environment in HIV-infected cells that limits transcriptive generation of new virus genomes and particles, mainly through effects on the pro-inflammatory NF-kappa B system. Excess dietary Se (over 400 ug per day) is toxic to humans and is associated with immunosuppression.

## Some additional relevant topics in human immunonutrition

**Fatty Acids**[47]**:** The fatty acid mix contained in a daily intake of macronutrient fat varies considerably depending on the type of food consumed; the potentially immuno-modulatory long-chain polyunsaturated fatty acids (PUFA) are present in quantities that are in the micronutrient range (50–300 mg per day). Only artificially high dietary levels of the longer-chain PUFA, however, exert potent anti-inflammatory effects in

humans, reducing phagocyte chemotaxis and the production of pro-inflammatory cytokines. Eicosanoids (prostaglandins, leukotrienes and thromboxanes) are highly bioactive mediators of which the series generated from arachidonic acid (20:4n-6) is generally significantly more active that that generated from the n-3 (also called omega-3) PUFAs (20:5n-3 and 22:6n-3); this may mean that diets may strongly influence the scope and nature of immune-related mechanisms involving eicosanoid release.

**Amino acids**: The catabolic state promoted by the pro-inflammatory cytokines during infections is associated with enhanced proteolysis in muscles and elsewhere, generating a pool of amino acids needed as fuel by immune cells and for making various bioactive products. **Arginine**, an essential amino acid that is plentiful in most dietary proteins, now becomes a large-scale precursor of nitric oxide generated for pathogen killing in phagocytes (it has also proved useful in surgical immuno-nutrition for the

treatment of septicaemia)[39, 48]. **Glutamine**, a non-essential amino acid that represents about 50% of the free amino acid pool in the body and is the most important transport form of amino-nitrogen in varied states of protein turnover, is next to glucose the main energy source of lymphocytes (about 35%) and becomes operationally an essential nutrient in severe catabolic stress, preventing functional immuno-suppression from impairment of lymphocyte functions caused by lack of an adequate energy supply.. The nutritionally essential, sulphur-containing amino acids **methionine** and **cysteine/cystine** released by muscle protein breakdown, play modulating roles in concerted anti-oxidant defences following infection, partly through their roles in the formation of the principal intracellular reducing agent, **glutathione**[49]. The loss in the urine of sulphur arising from intensive metabolism of these amino acids is increased in infected persons in states of systemic "inflammation", but less so than that of nitrogen, as they are conserved for their important roles in various defensive mechanisms. Even in asymptomatic HIV-infected subjects, the daily loss of sulphur was found to be 3–4 times that of healthy individuals[50]. Glutathione tempers the potentially destructive effects of systemic "inflammation" by reacting with the oxidants generated inside immune-activated macrophages and lymphocytes, amongst other things limiting the pro-inflammatory transcriptional activation brought about by NF-Kappa B, which may include enhanced transcription of HIV genomes incorporated into the host chromosomes, generating new retroviruses. Working in concert with ascorbic acid and vitamin E, glutathione controls oxidative chains harmful to membranes and many macromolecules. Various **therapeutic strategies** have been devised to increase the steady-state content of glutathione in leucocytes and other cells needed to fight infections; some of these have been used to improve the clinical status of HIV-infected subjects[50].



CHAPTER 5

# Human immunity

## Overall design of the defences against pathogenic organisms[1–4]

The ability of human bodies to avoid becoming infected by pathogens, and to overcome infections if they do happen, is associated with a number of **barrier physical and chemical functions**, on the one hand, and with the **immune system**, on the other. In the first instance (to be described no further in this chapter), bacteria and fungi, as well as parasites and viruses, are kept out or killed by the tough keratinised integument provided by the skin, supplemented by secretions and sweat containing compounds with microcidal action; by the intense acidity in the stomach encountered by all foods; by tightly contiguous epithelia that do not easily "leak" and by sticky mucus in the various passages opening to the exterior, sometimes (as in the airways) combined with ciliary action carrying particles outwards. In the second case, teams of specialised cells provide non-specific, immediate **innate immunity** through a variety of complementary local and systemic mechanisms; later, members of the same cellular teams, working in conjunction with other types of cells, develop two slower but more powerful supplementary killing systems that both also have the unique quality of being able to "remember" the infection after it has been dealt with, in order to prevent or strongly attenuate second infections by the same pathogens – **adaptive immunity.**

All cells of the immune system develop from just one cell type in the bone marrow, haemopoietic stem cells. **Lymphocytes** (and perhaps natural killer **(NK**) cells) constitute one series, and all the others are from the myeloid series – mononuclear phagocytes, like **monocytes, macrophages and dendritic cells**; neutrophilic, eosinophilic and basophilic **polymorphonuclear phagocytes**; **mast cells**; and megakaryocytes (precursors of **platelets**).

Pathogenic microorganisms that despite the natural physical and chemical barriers have succeeded in gaining access to, and thereby infecting human bodies, multiply and establish themselves in different ways: some (most, but not all bacteria and some parasites)

**divide rapidly in body or tissue fluids**, outside the host cells which they can damage or kill, but within host tissues that they can disrupt and destroy through the agency of secreted enzymes or toxins. To overcome these pathogens, the body must ensure that they are killed en masse, the resulting residues removed, and the affected tissue architecture restored through healing. Other organisms (all viruses, a minority of bacteria and some parasites) **enter target host cells to replicate inside them**, in the process often also causing tissue disruption and destruction through host response processes; in these cases, the infection can only be overcome if the infected cells are identified and killed together with their pathogen cargo, or persisting infected cells are sequestered in a complex lesion called a granuloma (as in mycobacterial infections like tuberculosis).

## Innate immune responses[4]

Innate immunity is very old in evolutionary terms, is present in humans from birth, and is ready quickly to address every kind of invading pathogen, whatever and wherever it may be. Amongst the cells that take part are the **motile phagocytes**, **polymorphonuclear neutrophils (PMNs), eosinophils and basophils**, (collectively called granulocytes), which respond to chemical signals caused **chemokines**, engulf pathogenic organisms that have been pre-coated with targeting molecules called **opsonins** (see below), internalise them in phagosomes, and kill them in a two-stage process in which they first release **lethal oxidants** through the "respiratory burst" ("flamethrower action", see Chapter 1: Conceptual Overview), and then expose the bacteria to **powerful hydrolytic enzymes** by fusing their lysosomes with the phagosomes to form phagolysosomes, finally to destroy/digest them. The oxidants produced by phagocytes from oxygen are **superoxide**, part of which dismutates to **hydrogen peroxide**; in the presence of iron and other free transition metal ions, the highly reactive microbicidal **hydroxyl radical** is formed, while in the case of polymorphonuclear phagocytes, an enzyme called myeloperoxidase is present that forms **hypochlorous acid**, another very destructive microbicidal agent. **Nitric oxide,** formed enzymatically from the essential amino acid arginine, is a product of all phagocytes and can severely damage molecules in invading microorganisms, further enhancing the killing rate[5]. It is important to note that all phagocytes are protected from self-destruction to some extent by their own reducing systems ("fire-extinguisher action", see Chapter 1: Conceptual Overview) involving **catalase** and **zinc-dependent glutathione peroxidase**, working in conjunction with other **anti-oxidants such as vitamins C and E** (ascorbic acid, very plentiful in all phagocytes but especially in macrophages, and alpha-tocopherol, respectively). Despite these resources, polymorphonuclear phagocytes ultimately die in their interactions with invading microorganisms, while macrophages mostly survive.





**Macrophages** are derived from circulating monocytes and reside in a number of organs and tissues in quiescent mode (**resident macrophages**), mostly under the lining of body cavities and blood capillaries; each such population displays special distinguishing features, but they all share the property of responding rapidly to specific stimuli (through a set of receptors called "**Toll-like receptors**"(TLRs[6]) which function like barcodes for different kinds of typical microbial components) by becoming **inflammatory macrophages**, which express additional sets of receptors and secrete many bioactive substances (see below), in addition to taking part in the direct phagocytic killing of microbes as already described. (They may later become activated macrophages as part of adaptive immune responses, see below). Large, non-phagocytic **natural killer (NK)** cells specifically recognise cells that harbour viruses and other internally replicating pathogens, and kill them (and the pathogens) directly by chemically puncturing their cell membranes. **Mast cells** are full of membrane-bound granules that upon activation release mainly vasodilatory agents to increase blood flow and increase the ability of proteins and cells to reach infection sites. **Platelets** can also release vasoactive substances and cytokines (see below).

Humoral contributions to innate immunity are complement, interferons and acute phase proteins. **Complement** involves over 20 plasma proteins that can become a polyfunctional system on activation[7], in this case (the "**alternative pathway**", see below for the "classical pathway" involved in adaptive immune mechanisms) by bacterial cell walls and other foreign surfaces. A controlled cascade of activated proteinase reactions forms bioactive proteins such as C3b (which is an **opsonin**, coating phagocytes to facilitate phagocytosis, see above), C5a which promotes **chemotaxis** of phagocytes to sites of inflammation, and C3a which with C5a produces mast cell degranulation. Coating of certain bacteria (mostly of the so-called Gram-negative variety) with C3b also unleashes the complement **membrane-attack complex** (C5b and C6–9) which causes lysis of the walls and death of the bacteria concerned**. Interferons** are cytokines secreted by virus-infected cells which confer resistance to viral infection on bystander cells, i.e. uninfected cells in the vicinity. **Acute phase proteins (APP)**[8, 9] are produced by the liver during infections in such quantities that their concentrations can rise by three orders of magnitude, usually for short periods; the stimulus for the liver to re-direct its usual pattern of protein synthesis are mainly the cytokines, interleukin-1 (IL-1) and interleukin 6 (IL-6), produced by macrophages encountering particular microbial proteins. One of the APP is **C-reactive protein (CRP**), which is an efficient initiator of the alternative complement activation pathway described briefly above, and which appears also to be present in increased concentrations in chronic viral infections such as HIV[10].

The main processes of **local inflammation** provoked by localised microbial invasion of human tissues thus involve migration of phagocytes (though the epithelium of

venules), egress of plasma proteins through hyperpermeabilised venules, and dilatation of arterioles to increase blood flow, together giving rise to redness, warmth, swelling and pain. Mast cells and platelets contribute to these very early changes at the site, as described above; amongst the agents acting at various times are histamine, various prostaglandins and leukotrienes. Resident macrophages become motile inflammatory macrophages, and are soon joined by large numbers of **polymorphonuclear neutrophils (PMNs**); both are guided to the site by chemotactic agents **(chemokines)**[11]. These cells engage with invading microbes, and release many cytokines, as described above. Specific cytokines that act both locally and systemically include interleukins-1 and -6 (IL-1 and IL-6), as well as tumour necrosis factor-alpha (TNF-$\alpha$); these exert powerful effects on the brain (fever and anorexia), liver (production of acute phase proteins), muscles and adipose tissue (catabolic mechanisms to break down proteins and stored fats, and release fuels for the now-active cells of the immune system). This is the state of **systemic inflammation**, which will subside after local inflammation has been successful in overcoming the infection, or the resistant pathogens have been walled off in a granulomatous focus (see below).

## Adaptive (acquired) immunity

Adaptive immunity makes use of specific parts of pathogens, their **antigens**, to direct both **humoral (antibodies**) and/or **cell-mediated attacks (cytolytic lymphocytes**) on invading pathogens that have survived, but are still under attack by, the mechanisms of innate immunity. It is both a powerful second line of defence and a way of priming the body to deal effectively with repeated insults from a particular pathogen through **immunological memory**.

The key cells of adaptive immunity are $CD3^+$ **lymphocytes**, motile, non-phagocytic cells that circulate constantly through the body although they spend most of their time in **lymphoid tissues** including the spleen, gut-associated lymphoid tissue (GALT), lymph nodes and the thymus. Lymphocytes do not use the handful of about ten generic barcode-type receptors (TLRs) of innate immunity already described, , but have receptors on their surface membranes that are completely (or almost completely) specific to a particular antigen (or, more correctly, its **epitope**, the business part of an antigen) that is unique out of literally millions of similar antigens/epitopes. Like quiescent macrophages, lymphocytes are in an inactive, resting state until the specific subsets of these cells which are capable of recognising the antigens present on an invading pathogen are activated during an adaptive immune response when they become significantly larger, as **lymphoblasts**. After a successful response, with elimination of the pathogen concerned, the cells either die or revert in stages (**"recently activated" lymphocytes**) to the resting





state. Some in the latter group will retain the memory of the specific invasion, as **memory cells**, and will be capable of mounting a rapid and powerful adaptive immune response if the same pathogen is again encountered.

There are two types of lymphocytes: **B lymphocytes (B-cells**) that develop and mature in the bone marrow before they enter the blood and lymphoid tissues, and **T lymphocytes (T-cells**), which develop up to a point in bone marrow but require a key maturational passage through the **thymus gland** before traversing the blood en route to lymphoid tissues (see below). B-cells are each genetically programmed to make **antibody** molecules with specificity for only one antigen (out of millions); an antibody molecule integrated in the B-cell membrane serves as the receptor for the particular antigen when the pathogen bearing has entered the body, and this leads to multiplication of the B cell concerned, differentiation of the resulting cells into **plasma cells**, and production by the latter of large numbers of antibody molecules (in forms variously called **immunoglobulin M (IgM**, early response), immunoglobulin A (IgA, secreted through mucous membranes), and **immunoglobulin G (IgG**, later response), all specific for the same antigen. It is important to remember that most pathogens will present many antigens to the immune machinery, some of which will be **immunodominant** in that they are the ones that generate the largest number of antibodies; the pattern of differential antibody generation in different people is partly determined genetically, by "**restriction**" through the inherited histocompatibility genes working through the T-cell helper function (see below). Some antibodies have high, others a low affinity for their particular epitopes; some are capable of directly neutralising bacteria or viruses by forming highly cross-linked complexes (neutralising antibodies) while others act as specific "tell-tale agents" for other cells to complete the destruction of the pathogens concerned.

## Anatomical arrangement of the inductive immune system

The specialised lymphoid tissues of the immune system serve as primary and secondary depots of cells involved in the immune response. Cells of the immune system migrate from lymphoid tissue to the blood and lymph circulation and back to lymphoid tissue. Bone marrow is the primary lymphoid organ, where the precursors to all mature immunocompetent cells are generated as pluripotential progenitor cells.

T-cells develop into mature immunocompetent cells in the **thymus**, a gland that is very active in newborns and is responsible for the selection and inductive "education" of T-cells in the body that can provide protection against a multitude of foreign antigens/pathogens during life. The thymus eliminates most, but not all T-cells that are directed against the antigens of host tissues. The remaining T-cells that leave the thymus are 'naïve' cells and have yet to encounter invading antigens/pathogens. Cells specific

for each of all the possible individual antigens that could be encountered exist in small numbers only, and each population will need to be massively expanded (clonal expansion) to be effective when particular antigens/pathogens enter the body . During life, the thymus gland becomes smaller and atrophies and until recently it was thought that it became inactive during the third decade. Recent evidence, however, has shown that the thymus is probably active throughout life.

The **lymph nodes** serve as platforms for the elaboration of the proliferative antigen/pathogen-specific immune responses and are structurally arranged into discrete anatomical compartments of tissue and cells. Cells arrive in a lymph node from the peripheral or lymphatic circulation through the afferent vessels; B-cells develop and encounter antigens within the germinal centres, while T-cells encounter them in the paracortical region. The microenvironment of the lymph node allows B and T-cells to be presented with either whole or pieces of invading pathogens (for presentation mechanisms involving antigen-presenting cells see below), ensuring that the inductive phase of immune responses can occur, and active, specifically targeted effector B and T-cells of both the $CD4^+$ and $CD8^+$ series generated. Some of the cells are also provided with "homing signals" that will ensure their congregation in particular tissues, such as the lamina propria in the lining of the bowel (see below) .Disruption of the architecture of the lymphoid tissue (as progressively occurs, for example, in HIV-infected individuals), affects the way in which B and T-cells can be primed to deal with new invading pathogens.

Some T $CD4^+$ cells are endowed with regulatory functions and are known as $CD25^+$ $\mathbf{T_{regs}}$. ("Peacekeeper cells") These are an important minority of the T-cell populations, antigen–specific like the others, but capable of limiting immune activation and inflammation in certain locations[12]. Mast cells appear to cooperate with $T_{regs}$ in controlling immune "attacks" in this way[13]. $T_{regs}$ may be particularly active in controlling immune activation in the bowel wall (see below for the significance of this in HIV-infected persons).

## Effector functions of immune cells

T-cells thus occur in several types which have effector and /or regulatory functions. **T helper lymphocytes ($CD3^+$, $CD4^+$ cells**) are central to the immune system and can be thought of as the "conductors" of the immune orchestra; any microbe that impedes or destroys $CD3^+$, $CD4^+$ T lymphocytes will cause the immune system to lose order and eventually degenerate. Helper T-cells are key "organisers" of both humoral and cell-mediated adaptive immunity, acting mainly through release of a range of **cytokines,** and inter alia enabling cytotoxic T-cells to do their work, B-cells to differentiate and develop





into plasma cells, and macrophages to destroy pathogens they have ingested through phagocytosis. **Cytotoxic T Lymphocytes (CTL; $CD3^+$ $CD8^+$ cells**) specifically kill target cells identified in a complex way (see below) as containing an organism bearing a specific antigen. They do this by perforating the target cell membranes by secretion of perforin and cytolysins, and by releasing other cytotoxic substances directly into the dying target cells. In healthy humans, $CD3^+CD8^+$ lymphocytes make up the smaller proportion of $CD3^+$ T-cells, but a homeostatic balance exists between $CD4^+$ and $CD8^+$ T-cells, in that the size of the total pool of T-cells (as marked by CD3) is apparently maintained by a variety of mechanisms. As mentioned above, **Regulatory T-cells ($T_{regs}$,** characterised by the surface marker CD25 and previously known as suppressor T-cells) usually but not always limit the extent and may change the nature of certain immune responses, partly through cytokine release, partly through competitive effects and partly through direct cell-cell interactions[12].

The ability of the adaptive immune system to respond to invasion of the body by a pathogen depends critically on events that link the antigens exposed on the surface of the pathogen concerned with the production within a few days of both soluble antibodies (produced by vastly expanded clones of plasma cells derived from activated B-cells) and cytotoxic T-cells (generated likewise by clonal expansion of a subset of T-cells) directed specifically at the foreign antigen set. Some of these events are part of the innate immune response already described, especially those involving the phagocytic action of macrophages; others are associated with the special capabilities of "sentinel cells" called **dendritic cells** located strategically in parts of the body where infections are likely to occur, in mucous membranes of the airways, the gut, genitourinary tract, etc. Both macrophages and dendritic cells are **"antigen-presenting cells" (APC**) in that they digest complex pathogen-derived macromolecules to fragments, some of which will be the actual antigens or, more correctly, epitopes, to which small subsets of the vast numbers of B and T lymphocytes have specificity. (Sometimes, the presenting cells are coated with the foreign antigenic pathogen, as in the case of HIV – see below). When pathogens have multiplied outside body cells and been ingested by phagocytosis, as previously described, their digestion in the phagolysosomes is the route of antigenic fragment generation, and the processed fragments are bound tightly and stably to the specific peptide-binding cleft found in proteins of the **major histocompatibility complex (MHC) class II**, and lodged in the cell membrane to act as the "presented antigen". When pathogens have gained access to target cells and are replicating inside them, it is the intracellular processing or turnover of their antigenic molecules by the infected cells that generates the epitope-bearing processed fragments which become bound in this case to **MHC class I** molecules, which after membrane localisation present the specific foreign antigens to the external environment as a tell-tale signal of what is lurking within.

The peptide clefts on MHC proteins of both classes available for binding antigenic fragments, while each possessed of binding rules, are promiscuous enough to make it likely that at least one or more antigens will in fact be presented on the cell surfaces of APC's after any pathogen infection. The limits to this, and the patterns of responsivity that are imposed by the particular MHC gene alleles present in an individual, are called **restriction**, and are extremely important determinants of **host susceptibility** to particular pathogens (a good example is differential susceptibility to HIV infection, and of disease progress, associated with possession of certain alleles of the HLA-B genes in the MHC class I group)[14]. Only T-cells through their **unique T-cell receptors** (TCR) can recognise the antigens presented by APCs: cytotoxic T-cells (marked as such by containing the surface co-receptor membrane protein **CD8**) recognise MHC class I-bound antigenic peptide fragments, while T-helper cells (bearing the co-receptor marker **CD4**) recognise antigens bound to MHC class II molecules on presenting cells.

Activation of T-cells (the **inductive phase** of adaptive immunity) is a complex, highly regulated set of processes, to prevent accidental assaults on the body's own tissues (normally completely abrogated by developmental mechanisms not covered here) and to lessen the likelihood that pathogens co-evolving with human populations will find ways of subverting or even eliminating the arsenal of complementary attacking possibilities made possible by the highly evolved human adaptive immune system. The activation or inductive site is lymphoid tissue, whether this be in the intestines (gut-associated lymphoid tissue, GALT), regional lymph nodes or the spleen. **Immature dendritic cells** are the "professionals" in antigen presentation as described above, continuously sampling with great sensitivity both soluble microbial products (through micropinocytosis) and the whole microbes themselves, recognising the pathogens through Toll-like receptors or because they have been opsonised (see above), and internalising them through phagocytosis for antigen processing and presentation through (MHC class II) or after becoming infected by internally replicating organisms (MHC class I). In either case, they become motile and migratory in their behaviour, so that they very rapidly end up in lymphoid tissue, mainly via lymph drainage pathways. Certain pathogens (e.g. HIV) can also be externally bound to dendritic cells in MHC class I-restricted ways that also cause migration and are stable enough to survive the journey to lymphoid tissue. **Maturing dendritic cells** secrete cytokines including chemokines, attracting to, and activating other cell types at, the site of infection (see above, innate immunity), while pre-emptively upregulating T-cell co-stimulatory molecules on their surfaces, such as $CD4^+$.

**Mature dendritic cells** once in lymphoid tissue are potent activators of T-cells, each being able to activate 100–3000 T-cells. Productive interaction is initiated when T-cells bear the "correct" TCR encounter and bind to APCs bearing the matching antigenic peptide fragment signalling the presence in the body of a particular pathogen. The





number of co-receptors and adhesion-enhancing molecules provided by both interacting cells increases to intensify the close binding of the two cell types, until, via highly complex signalling pathways, the proliferation and lymphoblastic transformation of the affected T-cells into specific **effector T-cells** of the adaptive immune system is induced. These cells may be of two types, dependent on complex differential cytokine stimulation patterns**: Th 1**, assisting mainly with cytotoxic reactions, and **Th 2**, focusing inter alia on B-cell differentiation. Effector $CD8^+$ T-cells (cytotoxic T-cells) kill cells bearing MHC class I-bound, pathogen-derived antigenic fragments, as already described. Effector $CD4^+$ T-cells (T helper cells) amongst other actions are able to activate macrophages bearing MHC class II-bound, pathogen-derived antigenic fragments, in the presence of interferon-gamma secreted by the engaged T-cells, and by co-stimulation by their $CD4^+$. These **activated macrophages** have greatly enhanced anti-microbial capacity through enhancement of the phagolysosomal pathway, induction of "respiratory bursts" (destructive oxygen radicals- see above), stimulation of nitric oxide formation, and increased formation of antimicrobial proteins such as **defensins**[15]. The fact that all this activity is directed by antigen-specific effector T-cells only at macrophages presenting pathogen-derived antigens keeps damage to host tissues potentially caused by secreted immune mediators to a minimum.(It is important, however, to realise that certain chronic infections, like those caused by pathogenic mycobacteria like those which cause tuberculosis and leprosy, can be associated with considerable tissue destruction by immune mechanisms which can become lethal if massive haemorrhages occur or vital structures are affected).

Some highly repetitive pathogen antigens (especially polysaccharide antigens) can stimulate resting B-cells into producing antibodies without the involvement of effector T-cells. Most B-cell responses, however, require T helper functions to enable the full expression of antibody-associated phenomena needed in humoral adaptive immune responses, including the ability to **switch the kind of antibody molecule** being produced (important for mucous membrane-lined cavities in the body where **immunoglobulin A, IgA,** is needed as a transmucosally acting **secretory antibody** capable of attacking pathogens at their site of entry[16]). Resting B-cells internalise such pathogen-derived antigen-immunoglobulin complexes as are formed in the initial, relatively ineffective stages of a humoral response, and peptide fragments presented on MHC class II molecules are then recognised by T-cells with specificity towards the same antigen; this, in the presence of co-receptors and cytokines, results in B-cell activation, differentiation into plasma cells, and production of high-affinity antibodies, all as already described. The T-cell stimulation also permits **affinity maturation** to take place, so that the antibodies formed at the height of the infection, and "remembered" in memory cells, are the most effective ones. Antibodies are powerful agents of anti-pathogenic defence: they bind to and neutralise

soluble products (e.g. toxins) released by pathogens, to form (often) insoluble complexes that can be removed by phagocytes; they bind to target antigens on microbial surfaces, efficiently opsonising the particulate organisms for phagocytic attack; they potentiate the action of natural killer cells by identifying and tagging cells harbouring replicating infecting agents; and they abet complement attack (see above).

Activated lymphocytes are generally shortlived and undergo apoptosis as a naturally occurring mechanism limiting the immune responses concerned. Some survive, however, and continue life as $CD62^{+}$ memory cells, able to mature into a proliferated population of specifically targeted effector cells as soon as the offending antigen/pathogen is again encountered in the body. In chronic infections such as HIV and tuberculosis, however, the effector CTLs fail to clear the body of the infecting organism and progressively dwindle in number and effectiveness – they become exhausted. A surface protein called PD-1 accumulates in these circumstances and responds to a circulating cytokine PD-1L to produce the "exhausted" phenotype. Fortunately, interrupting the PD-1D-1L interaction (with an antibody) can "revive" the lymphocytes[17], as has been shown in HIV-infected subjects[18].

## The "hormones" of immunity, acting locally and systemically[9, 19]

The crucial roles of **cytokines** in human immunity have been high-lighted throughout the above account. They are secreted by many of the cells in the immune system, in different combinations, amounts and sequences, are generally short-acting, both locally and systemically, and exert their often multiple and interactive effects by binding to specific receptors on target cells. They amplify reactions, and distribute them spatially. They inhibit some reactions and enhance others; their effects are the resultant of their own concentrations and those of their receptors. Some are **chemokines** ( causing motile cells to move along a chemical gradient of cytokine concentration; others inhibit local viral infectivity **(interferons**), while others act at a distance on large organs to affect macro-metabolic changes to support the local immune processes **(pro-inflammatory cytokines**). Cytokines accompany and modulate inter-cellular interactions during immune responses, in an indispensable way, at very low effective concentrations; their rapid turnover nevertheless means that the total energy cost to the body of full-blown "cytokine storms" can be high. The total mass of lymphoid tissue in the adult human body has been estimated to be over 1 kg; while much of this is not necessarily active in an infection with one pathogen, because of the specificity of adaptive immune responses, cells dealing with the multiple antigens present in every infection do predominate as a result of clonal proliferation, and increase the requirements of the active and enlarged





immune cells for fuel (in the form of glucose and glutamine at approximately 2:1, both provided in adequate amounts as part of the cytokine-stimulated "inflammatory state" ) and for precursors of nucleic acids (and folate coenzymes as well as Zn ions) for cell proliferation, and newly synthesised proteins for vital immune functions. In addition, the hostile chemical environment created by the elaboration of toxic microbicidal agents has to be countered by increased supplies of cell-protective reducing agents like vitamins C and E, as well as sulphur-containing amino acids (see above).

## Biomarkers of immune function for nutritional studies and interventions in human subjects

Considerable progress has been made in evaluating immune responsivity and the impact of long-term nutrient supply and/or deficiencies[20]. The necessarily concise description that has been given above of the integrated innate and adaptive immune systems will leave no doubt that exhaustive and precise tests and analyses will in the main be both complex and expensive. This fact should not, however, serve to prevent as good a bank of standardised, feasible and informative tests as can be developed to become widely available for the support of effective health care in South Africa.

A large number of ***ex vivo* measures** already exist for the assessment of blood-derived cells as well as, in some cases, cells collected from mucosal surfaces by lavage techniques[21]. These include measurements of phagocytosis/killing of bacteria, yeast cells or sheep red blood cells; assays of respiratory burst/microbial killing rates; NK and CTL activity on virally infected cells; lymphocyte proliferation rates under stimulation by mitogens; stimulated cytokine production, or gene expression in whole cells; IgG ( total and specific) production rates; cell-surface expression of HLA molecules or cytokine receptors (flow cytometry). ***In vivo* measures** include the size of lymphoid organs (e.g. ultrasound in the case of the thymus); cytology of lymphoid tissue; numbers and types of circulating immunocytes of various kinds, inclusing cell-surface expression of defined molecules; circulating thymulin; general plasma immunoglobulins as well as specifically targeted ones; secretory IgA in mucosal washings; circulating cytokines; delayed-type hypersensitivity (DTH) responses to antigens already experienced by subjects; resistance to infectious diseases, progression and outcomes. Widespread inter-individual and inter-group variations can be expected (see above for genetic and other causes of this).

A highly useful study was performed by the European branch of the International Life Sciences Institute (ILSI Europe) to identify the most reliable, cost-effective generally useful markers to assess the impact of nutrition on immune function in humans, including those associated with the intestine (see Chapter 7)[22]. A number of systematic potential confounding subject-specific factors were listed, including age-dependence

of immune function, sex differences, physical and psychological stress, drug history, smoking, and vaccination history. Technical confounding factors were identified as study designs, sample collection times (circadian rhythms), seasonal variations, meals and/or depleting wash-out periods, and the length of the intervention period. Immune function assays commonly in use were rated as to their biological relevance (e.g. known correlation with clinical end-points), sensitivity (within- and -between subject variation), and practical feasibility. Methods that were rated highly suitable were:

i. vaccine-specific serum antibody production;
ii. delayed-type hypersensitivity responses;
iii. vaccine-specific or total secretory IgA in saliva, and
iv. the response to attenuated pathogens.

Markers with medium suitability were NK cytotoxicity, oxidative bursts in phagocytes, lymphocyte proliferation, and cytokine patterns produced by stimulated cells. The authors suggested using a mix of highly and medium suitable markers for general use. **While this very careful and detailed work is doubtlessly valid in a European setting, no similar study under South African/African conditions has apparently ever been done, despite the crying need in a country where the public is constantly exhorted to consume a plethora of mostly untested or poorly tested, (expensive) proprietary "immunomodulators" and the like[23].**

## The issue of life-long programming of the immune system by fetal or perinatal experience

One of the most controversial but potentially influential theories of human development is that nutritional, toxicological or virological "insults" prior to birth, especially in key phases of neonatal organ growth, may cause life-long programming of body systems such as immune responses, metabolic patterns and cardiovascular functioning that deviates from the norms of a population[24]. Careful studies conducted in the Gambia, where annual subsistence-type food production lends itself to a recurrent pattern of periods of general hunger and satiety in local communities, have suggested that temporary malnutrition of pregnant women may produce off-spring with enhanced susceptibility to infectious diseases prevalent in the population late in their lives[25], associated with stunted thymic growth and under-functioning. Other factors, such as mycotoxins also prevalent in the diminishing stored food supply during hungry periods, may, however, be partly or wholly responsible for the findings. **It is clear that proper studies of South African communities need to be performed to help assess the importance of this possibly quite general phenomenon within the general context of the much greater prevalence here of "hidden hunger" (micronutrient deficiencies) rather than protein/energy starvation.**



CHAPTER 6

# Pathogenesis of *Mycobacterium tuberculosis* infection in humans

It has been 25 years since the HIV epidemic rekindled research interest in tuberculosis (TB) in the developed world. Remarkable progress has been made since that time in elucidating the mechanisms by which *Mycobacterium tuberculosis* causes human disease[1, 2]. Nonetheless, much remains to be learned about this ancient scourge of humankind. In this chapter, the current understanding of the critical steps in the pathogenesis of pulmonary TB will be reviewed.

## *Mycobacterium tuberculosis (M.tb.)* – the Captain of all the Men of Death

The bacterium that causes tuberculosis (TB) is a slow-growing organism that has probably co-evolved with modern humans to become so successful that about one-third of all living humans are stably and dormantly infected; it is the triggering of dormant infection or transmission of organisms in large doses from one individual to another that causes active or "clinical" TB (see below). There are many strains of the organism distributed in different geographic regions, and useful genetic markers have been developed to detect local infection patterns[3]. The genome of a laboratory strain of *M.tb.* was sequenced in 1998, revealing much information about the specialised lipid and polysaccharide metabolism concerned with the formation of the thick cell envelope, large-scale biosynthesis of biologically active substances called polyketides, and even highly prevalent proteins that have highly repetitive, variable sequences, possibly permitting a source of antigenic variation over time[4]. The genome has over 4 million base pairs and codes for around 400 genes[4]. An attenuated strain (Bacillus Calmette Guerin, BCG) is used worldwide as a vaccine, effective mainly against childhood forms of TB.

## Susceptibility to active TB varies in the human population

The genetic (as opposed to environmentally determined) factors that render some people susceptible to *M.tb.* infection are very poorly understood, but a recent discovery has

revealed that a receptor on human dendritic (antigen-presenting) cells (see Chapter 5) called DC-SIGN has common polymorphisms that are more common in Eurasian than in African populations and appear to be associated with considerable resistance to the development of active TB[5]. As more such factors are expected to be discovered, the likely genetic heterogeneity of populations used to study nutritional interventions must be taken into account in study designs.

## Pulmonary TB

The commonest infection route for tuberculosis is inhalation of a very small number of droplet nuclei containing virulent tubercle bacilli[6]. Documentation of the failure of some chronically exposed individuals (e.g. household contacts of a TB patient) to develop an infection indicates that the inhaled organisms may be inactivated before an immune response (i.e. conversion of the PPD skin test) is induced[7]. In most exposed individuals who are otherwise healthy, however, an infection does develop. The immune response controls the replication of the bacilli and the individuals remain disease-free until some immunosuppressive event later in life (e.g. HIV infection, immunosuppressive drug therapy, advanced age) induces endogenous reactivation TB[8]. This chronic infection is referred to as **"latency" or "persistence**" and is estimated to affect one-third of the world's population[9].

The conditions under which *M.tuberculosis* remains alive in the tissues for decades, and the factors which trigger its reactivation, remain unsolved mysteries[10, 11]. Much has been learned, however, about the mechanisms by which mycobacteria subvert the antimicrobial functions of macrophages and persist in the face of adverse conditions in the host[12, 13]. Recent attempts to study the gene expression profiles of mycobacteria exposed to adverse conditions, either in vitro or in vitro, and signature-tagged mutagenesis approaches to identifying mycobacterial genes necessary for persistence in experimental animal models, are beginning to shed some light on these important questions[14, 15]. Pulmonary TB may also develop following exogenous re-infection in a previously infected person[16]. The relative contributions of primary TB, endogenous reactivation TB, and exogenous re-infection TB to the spectrum of disease appear to vary widely in different geographical settings.

In primary pulmonary TB, the inhaled mycobacteria likely encounter the alveolar macrophage as their initial host target cell[2]. Direct infection of alveolar (Type II) epithelial cells may also occur, however, the contribution of epithelial cells to the initial host-pathogen interaction is not clear[17]. Mycobacterial constituents are known to serve as ligands for the binding and internalisation of tubercle bacilli, and for various pathogen-recognition receptors (e.g. Toll-like receptors) on macrophages, leading to the activation of those cells





and the production of pro-inflammatory cytokines (e.g. TNF-α) and chemokines[18-21]. The roles of various adhesion proteins, chemokines, and chemokine receptors in the trafficking of immune cells into infectious foci are topics of intense investigation[22]. Other cells of the innate host defense system, such as natural killer (NK) cells, may also play a role in promoting activation of alveolar and blood-borne monocyte-macrophages in this early phase of the infection[23]. During the ensuing inflammatory response and following initial replication in the alveolar macrophages, mycobacteria are taken in the draining lymph to the regional lymph nodes, where they replicate further. It is in the lymph nodes where mycobacterial antigens are first presented to naive $CD4^+$ and $CD8^+$ T-cells, likely by infected dendritic cells which are capable of stimulating a $CD4^+$ Th1 effector cell response[24, 25]. After a short period of replication in the pulmonary lymph nodes, viable mycobacteria leave the nodes in the efferent lymph, enter the blood stream via the thoracic duct, and appear in the spleen and other organs[26]. Concomitant with extra-pulmonary dissemination, which apparently occurs in all infected individuals, the host begins to express evidence of a $CD4^+$ T-cell-mediated immune response (e.g. PPD skin test conversion). Eventually, viable bacilli make their way back to the lung in the bloodstream where they infect all lung lobes uniformly in a process known as **"haematogenous re-seeding**"[27]. Thus, the lung of every infected individual receives two inocula of mycobacteria; the initial implantation from the airway, and a second shower of bacilli from the blood. The blood-borne organisms establish so-called "secondary" granulomas, in which the organisms persist in the face of a very active immune response[28]. It is one of these secondary granulomas which are thought to be the "reactivatable site" in post-primary TB[29].

Following the induction and expansion of $CD4^+$ Th1 effector cell populations in the pulmonary lymph nodes, these effector cells leave the nodes and home to the inflamed sites of active infection in the lungs and other tissues. There, the Th1 cells are activated to produce their signature cytokines, including IFNα[30]. The combination of IFNα from the Th1 cells and TNF-α from activated phagocytes likely act synergistically to activate macrophages to inhibit the growth of the mycobacteria[1, 32]. While TNF-α is apparently essential for resistance to TB[30], it is also responsible for local tissue destruction, central necrosis of the granuloma, and impairment of Type 1 T-cell immunity[34]. Successful modulation of the intense inflammatory response in the granulomas by anti-inflammatory cytokines such as TGF-α, and IL-10 allows the host to control mycobacterial replication without compromising lung function. These cytokines are, however, also known to suppress Type 1 immunity[35]. Apparently, $CD8^+$ T-cells may also play a role in resistance to TB, by producing macrophage-activating cytokines and differentiating into effector cytolytic T-cells (CTL), although their precise contributions are unclear[36].

The anti-mycobacterial mechanisms expressed by activated macrophages, including the identification of essential effector molecules, remain to be elucidated. The ability

of the activated macrophage to overcome the pathogen-induced block of endosome maturation and phagosome-lysosome fusion is undoubtedly important[37, 38]. Induction of the receptor (VDR) for the **vitamin D** metabolite, 25-hydroxycholcalciferol (25-HCC), as well as the hydroxylase which then forms 1,25-dihydroxycholecalciferol (1,15-DHCC), appears to be part of the mechanism which help the cells to destroy the intracellular pathogen, combined as it is with the generation of the antibiotic peptide, cathelicidin (see Chapter 4, Vitamin D). **Reactive oxygen intermediates** (e.g. hydroxyl radical, singlet oxygen, hydrogen peroxide, etc) are also involved, while the role of nitric oxide (NO) is much less clear in humans than in murine models of TB[39, 40].

Co-infection with HIV changes the above scenario dramatically. While there is little evidence that HIV infection predisposes an individual to increased risk of primary *infection,* it clearly increases the risk of progression from infection to *disease* by orders of magnitude[41]. This is likely due to the adverse effects of HIV on the CD4$^+$ T-cells which are essential for control of mycobacterial growth, as well as defects in macrophage and other cell functions which are known to accompany HIV infection.



CHAPTER 7

# Pathogenesis of Human Immunodeficiency Virus (HIV) infection: moving from older to newer thinking

## HIV-1 infection of cells of the human immune system[1]

The predominant circulating Human Immunodeficiency Virus type 1 (HIV-1) causing HIV/AIDS in South Africa belongs to clade **C** and is a small RNA virus (two molecules per virus particle, or virion) packaged with a small number of proteins (both virus-coded and host-derived) within a protein-lipid membrane coat mostly physically "borrowed" from infected host cells from which it was released during productive infection. The virions prominently present on their surfaces many "studs" made up of the virus-coded HIV coat protein, gp120, anchored in the membrane coat by gp41. Much of the surface of the "studs" is covered with a dense forest of carbohydrate side-chains. The cell-surface receptor for infection of new cells by free viruses is a protein called **CD4$^+$,** which normally serves other important functions in a specific array of cells playing various roles in the immune system, mainly by resting and activated helper T lymphocytes, macrophages and dendritic cells (see Chapter 5). A **co-receptor** is required for successful infection; this can be one of the chemokine receptors CCR5 (R5 viruses) exclusively used in early stages of HIV infections, or CXCR4 (X4 viruses) sometimes used in late infective stages; one unique aspect of clade C HIV infections in South Africa, however, is that there very few X4-using viruses have been detected during the course of the disease, and most virus infectivity occurs through the use of the CCR5 co-receptor.

Internalised virus particles are unscrambled in the cellular cytoplasm and the RNA molecules transcribed into DNA versions by an error-prone and unstable, virus-derived **Reverse Transcriptase** (RT). Transcribed virally derived pro-DNA can then be stably inserted into the host chromosomal DNA through the action of another virus-derived enzyme, **integrase**, and can remain dormant there for long periods, surviving cell division through mitotic processes. The sequences can also be transcribed, by host RNA polymerase, to form large numbers of new RNA virus molecules; this re-start of

virus production is prompted by signals of which only some are known, for example the transcriptional factor NF-kappa beta which becomes active as a result of cytokine action in inflammatory states of the body, especially when the cellular redox status is poised to oxidation (see Chapter 5) or during an attack of malaria[2]. The virus RNA molecules are themselves messenger RNAs which are each translated by host ribosomes into polyproteins that are then neatly and specifically cut by a virus-derived specific **protease** to form an array of proteins, including gp120 and its stalk protein gp41, which polymerise to form the studs on the surfaces of the new virions, once each of these have been fully assembled through release from the cell membrane, taking parts of the latter with it. Other proteins formed by cutting up of the primary polyprotein are either packaged with the virus RNA molecules into released virions (e.g. Reverse Transcriptase) or remain in the infected cell as "virus-support agents" (**accessory proteins**), mainly working to evade host immune attack in a variety of ways. One of these ways is to down-regulate the amounts of specific sub-types of HLA class I molecules at the cell surfaces, effectively attenuating the tell-tale signal of virus infection normally associated with the peptide fragments presented to CTLs targeting ("hunting down") cells presenting those epitopes. The subtypes of HLA molecules that through their presence prevent cell destruction by NK cells (see Chapter 4) are not affected, so the chances of the infected cells to avoid killing by the main killer cells of both innate and adaptive immunity are significantly enhanced.

## Early events in a typical HIV infection (vaginal route)[3]

In the first hours and days of a typical heterosexual infection, a few virus particles in the semen of an infected male escape the destructive fate of countless others by gaining access to the vaginal or cervical submucosal space in the uninfected female; here they infect activated or "recently activated" $CD4^+$ T lymphocytes as well as dendritic cells and resident macrophages, all of which begin to produce new virus particles. Together, the viruses and infected cells migrate to nearby lymph nodes in increasing numbers, and from there they are disseminated to the lymphoid tissues of the whole body. When the "signal" (dose) of virus is strong enough, adaptive immune responses begin to be launched by the host, but the rate at which susceptible cells are productively infected by virus becomes enormous, bringing about a characteristically high **viraemia** (nearly always experienced by the newly infected subject as a "flu-like illness" lasting a few days), and causing the destruction of a very large proportion of the $CD4^+$ $CCR5^+$ T-cells in the body, most of them resident in the mucosal tissues of the bowel wall (see below). Most of the soluble antibodies initially directed at the HIV particles are unable to neutralise the latter because of the extensive shielding of the target epitopes by the





carbohydrate "forest" on the gp120 coat protein; attacks by NK cells are attenuated by virus-induced surface changes (see above) and those of specific $CD8^+$ CTL by similarly evasive surface changes, the adaptive response essentially coming **"too little and too late"** decisively to control the infection, but nevertheless creating breathing space by lowering the rate of new attacks on the remaining $CD4^+$ T-cells, which are themselves activated as part of the on-going adaptive response and therefore susceptible to virus infection (see below).

## The immune response to HIV infection

The acquired immune response to HIV (specifically HIV) has been studied extensively[4] and the course of adaptive immunological events from the time of transmission can be divided into the acute, early and chronic phases of infection. The two greatest challenges that HIV presents to host immunity is the **selective infection and depletion of $CD4^+$ T lymphocytes** and the **extensive viral genetic variability** due to accumulated mutations in, and recombinations of the viral genome. After 25 years of extensive study, systematic correlates of immune protection still remain elusive, which in part is related to a still-limited full understanding of HIV pathogenesis. After acute HIV infection, the robust cellular immune responses do contribute to the greater than 100–1000-fold drop in viraemia from the typical acute levels of several million viral RNA copies/ml plasma. This is coincident with the generation of primary cellular responses of virus-specific $CD8^+$ cytotoxic T lymphocytes (CTL)[5] and it seems logical to assume that these cells are most important in the apparent "control" of the infection and the preservation of the residual pool of $CD4^+$ T-cells. In children, a different scenario occurs after in utero, peripartum or postnatal transmission, in that systemic viraemia peaks several logs higher than in adults, and there a slow decline of viraemia over the first 4 years of life. The immature nature of immunity in neonates along with a greater number of $CD4^+$ T-cells probably accounts for this larger viral burst and slower decline. It is known that high peak or set-point levels of viraemia, after the acute high viraemia has subsided, are associated with a more rapid course of infection leading to AIDS[6], where it is known that the overall status of immune activation is related to the resurgent viraemia which then occurs.

In addition to virus-specific $CD8^+$ T-cells, $CD4^+$ T-cells appear also to be critical for immune control[7]. Monkey models of chronic SIV infections have established that virus-specific $CD4^+$ T-cells play an essential role in maintenance of effective immunity[8]. It is likely that the immune response to HIV in humans has these same requirements, and enhanced proliferation of HIV-specific $CD4^+$ T-cells in individuals who maintain long-term control of HIV replication[9] and in patients treated for acute infection with potent

antiretroviral therapy[9–11], reflects the functions of these cells in helping to control virus replication. Functionally competent HIV-specific CD4+ T-cells secrete cytokines and influence/enhance both CD8+ T-cell and B cell-dependent antibody responses. A large number of CD4+ cells are infected in the bowel wall, mainly because the bulk of the total body CD4+ pool resides within lymphoid tissue around the gastrointestinal tract (see below). In subtype C HIV infection, it is rare to find anything other than a CCR5-using viral population, and preferential infection therefore occurs of CCR5-bearing CD4+ T-cells and macrophages. Direct killing of CCR5+ CD4+ T-cells within the gut[12, 13] and memory CD4+ T-cells in multiple tissues[14] by HIV has been postulated as the main mechanism for the depletion of these cells during SIV infection in monkeys. This situation also applies to HIV-induced depletion of CD4+ cells within the human gut[15].

The swiftest and probably the most effective immune response in an individual infected with HIV is a robust CD8+ T-cell response, where these cells have a specificity directed at numerous virus epitopes. Experiments that have used various species of monkeys have shown that when CD8+ T-cells are depleted during acute infection with SIV, persistent high viraemia results[16]. When CD8+ T-cells have been depleted during chronic, or established, infection, this is associated with a sudden increase in viral load and set point[16, 17]. The relationship between the initial drop in viraemia during acute infection and the appearance of CD8+ class I restricted CTL[18, 19] is a very strong indication of the antiviral effect of CD8+ T-cells and provides compelling evidence for this as the probably dominant effector mechanism of reducing viraemia at the acute stage of infection. Indeed, in vitro studies have shown that CD8+ CTL can lyse infected cells before new virions are produced, thus inhibiting virus replication[20, 21], and they also release soluble factors with anti-HIV properties[22-24]. A critical role for CD8+ T-cell responses is also inferred from population data demonstrating evidence of HIV adaptation to HLA class I restricted responses[25].

In addition to the rapid cellular immune responses to primary HIV infection, active humoral immunity becomes apparent through increased titres of anti-Gag antibodies, which are used to measure seroconversion to the HIV-infected state. Some neutralizing antibodies, or functional antibodies, are able to block HIV entry into CD4+–bearing cells; they mainly target the highly variable V3 loop of gp120, its CD4+–binding domain, or the more conserved gp41 transmembrane protein[26]. Although high titres of such neutralizing antibodies have been shown to prevent infection in, animal models and the role of these responses in viral containment in HIV-infected people are questionable[27]. During acute infection, these responses appear following the initial drop in viraemia, while abrogation of B-cells during SIV infection leads to delayed emergence of neutralizing antibodies but no change in early viral kinetics. Passive transfer of neutralizing antibodies in monkeys can, however, protect against





intravenous and mucosal challenge of SIV[28]. Virus sub-populations that can infect new hosts appear to be more susceptible to neutralising antibodies in vitro than are the predominant populations in the infecting donor, but subsequent mutational changes in the virus in its new host mostly nullify this factor[29]. Neutralising antibodies raised by vaccination could thus in principle be important for prevention of viral infection, but antibodies are relatively ineffective in countering a new HIV infection as it becomes established.

## The micro-evolution of HIV in a single human body[30]

HIV as an infecting RNA virus is subject to the compulsory step of reverse transcription catalysed by its self-encoded, highly error-prone Reverse Transcriptase, RT (about one base per thousand is mis-coded to form a **mutant** viral DNA-genome). In addition, RT uses both infecting virus RNA molecules (and possibly more, if the cell is super-infected by more than one virus particle) to complete a single DNA copy for integration into the host genome, often switching from one strand to the other **(recombination**). Host RNA polymerase is also subject to errors, so more mutations may be generated in multiple daughter transcripts which are made when the latent integrated virus is activated for replicative production of many new virions (see above). A further cause of rapid micro-evolution of HIV is the defensive role of a host-derived protein (called APOBEG 3G) which may be packaged into the virions released from productively infected cells and functions as an RNA-cytosine deaminase to alter the sequences within individual free virions [31]. All these processes ensure that new viruses are constantly generated (and proportionately to the rate of overall replication), to be instantly tested for selection in the hostile host immune environment. Antibody and CTL attacks, as well as anti-retroviral drugs, drive the micro-evolutionary processes by allowing resistant virus sub-populations rapidly to "grow out" in the vast crowd of wild-type and mutant HIV particles, probably differentially in various locations in the body. The **micro-evolution of HIV** within a single infected subject is what brings about resistance to anti-retroviral drugs, special types of pathophysiology in organs such as the skin and the brain, and general persistence of infection- everything that happens is "directed" to surviving the immune attacks of the host. There are indications that it is only a (small) sub-population of HIV particles that retains the competence to infect new hosts, thus preserving the basic model of the virus-human interaction over long periods. HIV infection has been called a "soap opera" in that "a lot happens but fundamentally everything stays the same" – the virus as a human pathogen retains its main features as a slow, chronic, ultimately fatal infection spread by direct contact with different body fluids at different rates.

## Humans are differentially susceptible to HIV infection and its rate of progression[32]

Amongst the millions of people who have been infected with HIV since 1980, a spectrum of infection prevalence and progression rates has clearly emerged. Within any population, and between different populations, there are individuals with increased susceptibility and others with increased resistance, who may in either group progress to AIDS slowly or quickly. Generally, however, increased susceptibility to becoming infected has accompanied the trait of more rapid progression to AIDS. Many gene loci have been found that variously affect virus entry and intracellular replication, host innate immunity and especially adaptive immune processes.

The widely distributed "**host resistance factors**" so far identified include specific deletion variants in the co-receptor CCR5; gene dosage variations of one of its natural chemokine ligands; restriction factors that inhibit virus-handling pathways in the cell; the cytosine deaminase system based on APOBEG 3G (see above); controlling factors for NK effectiveness. Additionally, is has been shown that specific Human Leukocyte Antigen (HLA) types, which are genetically governed by X-linked genes, are associated with rapid or delayed progression of disease. HLA are fundamental to all T-cell and NK cell activity, where the presence or absence of expression will dictate immune responses through virally-derived epitope presentation on the surface of HIV-infected or antigen presenting cells. It has been shown that HLA-B27, -B57 or a variant of –B58 (B5801) are associated with long term non-progression or delayed onset of AIDS, and better clinical outcomes[33]. One of the likely reasons for this association is that these specific HLA can present epitopes that cause a higher level of T-cell function than other epitopes, which leads to better viral control and preservation of $CD4^+$ T cell counts. These epitopes may also be found in regions of the HIV genome that are invariant and that do not undergo mutations in response to immune pressure.

In the context of this report, these genetically determined variations in host susceptibility are important because they will constitute "background noise" in any clinical trial or other investigation of clinical HIV progression. Fortunately, the laboratory means to establish the nature of possible resistance or susceptibility genes in human subjects are being created through active research and will impact positively on this problem.

## The link between HIV infection, $CD4^+$ T cell depletion and lymphoid tissues

The important components of an immune response to HIV are good functional $CD4^+$ T-cells, active HIV-specific $CD8^+$ T-cells, and (possibly) broad levels of neutralising





antibodies. This represents a classic view of anti-viral immunity and is mainly derived from investigations of peripheral blood. Despite detailed knowledge, however, we do not fully understand pathogenesis and which aspects of immunity correlate with protection or, conversely, which aspects of immunity are absent that result in progression to AIDS.

Following the above-described immune response during the acute stage of infection in adults, there is a gradual loss of $CD4^+$ cells from the peripheral blood that may take several years to reach the low levels (less than 200 cells/ml) that are currently used to signify the onset of AIDS. Initially, it was thought that HIV was directly killing $CD4^+$ T-cells which accounted for the loss. This is controversial; however, because there was always much more virus detectable than the actual number of HIV-infected $CD4^+$ cells, or the percentage of infected $CD4^+$ cells in the blood. This led to proposals for indirect models of $CD4^+$ cell depletion, including apoptosis and/or immune activation-induced cell death. In the latter model, HIV infection as a process leads to increased T-cell activation in primarily the lymphoid tissues, which is where the bulk of HIV is sequestered after infection; the activated $CD4^+$ T-cells not only become targets for HIV infection, but are mostly (physiologically) short-lived even if uninfected, leading to the eventual depletion of the population. (The molecular overlap of the binding site on $CD4^+$ for MHC II and HIV gp120 might account for this activation-induced depletion[34], as $CD4^+$ is an integral component of the array of signalling molecules required for T-cell activation.) The residual memory cells are recruited into activated state, over and over, by chronic antigen persistence. This model can account for $CD4^+$ cell depletion in the face of the large numbers of $CD8^+$ T-cells which are present in the same lymphoid tissues, because HIV gp120's affinity for $CD4^+$ would be expected to corrupt $CD4^+$- but not $CD8^+$-mediated cellular activation, leading to $CD4^+$ cell apoptosis exclusively.

Incidentally, chronic alcohol consumption by monkeys' leads to impaired control of SIV replication, mainly as an "escape effect" associated with lowered numbers of CTLs and higher relative numbers of virus-susceptible $CD4^+$ T cells in the gut wall[35].

## HIV infection and changes in gut-associated lymphoid tissue (GALT)

The lymph nodes serve as the platform for the induction of immune responses, and are structurally arranged into discrete anatomical compartments of tissue and cells (see Chapter 5). Naïve cells arrive in regional lymph nodes in lymph through lymphatics, or in central nodes through the peripheral blood circulation, entering through afferent vessels, whereafter B-cells encounter antigen within the germinal centres and T-cells do so in the paracortical region (see Chapter 5). The microenvironment of lymph nodes allows B and T-cells to be presented with epitopes derived from invading pathogen, ensuring that adaptive immune

responses can occur. While **GALT** constitutes the majority proportion of lymphoid tissue in the body, and contains the majority of immunocompetent cells, its inductive foci, the discrete lymphoid follicles also called Peyer's patches, are available to "sample" only locally encountered foreign organisms and/or antigens. The proximity of such extensive lymphoid tissue along the intestines reflects the special nature of the hugely folded internal surface as a key interface between the host and an environment that contains not only vast numbers of microorganisms but is also subject to the continual passage of a large mass of heterogeneous food-derived material. That this is a continual on/off "battlefield" is attested to by the fact the effector arm of the immune system is extensively distributed throughout the submucosal space in the entire bowel, in the lamina propria. In this way, approaching two-thirds of the body's entire immune system is arranged defensively along the entire intestinal wall[36].

One of the main functions of the intestinal immune system is thus to discriminate on the part of the host body between harmful and harmless/beneficial organisms and antigens. "Oral tolerance" is a concept that suggests that the host as here defined may extend to more than the human tissues of a person but include substantial numbers of bacterial species or other microorganisms that are symbiotic for the true human host (reviewed in[37], also see Chapter 5). Strong protective immunity around the gut is essential to prevent invasion by pathogens or dietary proteins, or to normally tolerated commensal organisms, that can lead to acute, subacute or chronic infectious, inflammatory or allergic disease[38]. The processes of exclusion of "unwanted" elements are based mainly on the continual action of secreted mucosal antibodies (IgA and IgM) in the mucus-covered surface layer, and to a lesser extent within the epithelial cells and the submucosa, together with constant sampling of intestinal contents by specialised M-cells in the lymphoid follicles, leading to priming of B-cells and dendritic cells within the follicles, migration of these through lymphatic drainage to regional lymph nodes, and circulation in the peripheral blood to central lymphoid tissues. The effector cells finally "home" to the lamina propria of the gut, where the B-cells secrete IgA and IgM antibodies, and the $CD4^+$ and $CD8^+$ T-cells engage with such organisms and antigens as may have succeeded in entering the submucosa. There is evidence that cytokine-mediated inflammation is normally kept to a minimum during this low-level "policing" of the extensive "border", possibly through the controlling influence of $T_{regs}$[39] and the involvement of cytokine-stimulated mast cells[40].

## GALT events and progression of HIV infection

Studies from SIV infection in monkeys, used as a proxy for understanding HIV infection and pathogenesis, has shown that SIV rapidly and selectively infects and destroys memory $CD4^+$ T-cells co-expressing the HIV-co-receptor CCR5 in the gut lamina propria of the gut[12, 13, 41-44]. Similarly, loss of intestinal memory $CD4^+CCR5^+$ T-cells has been shown in





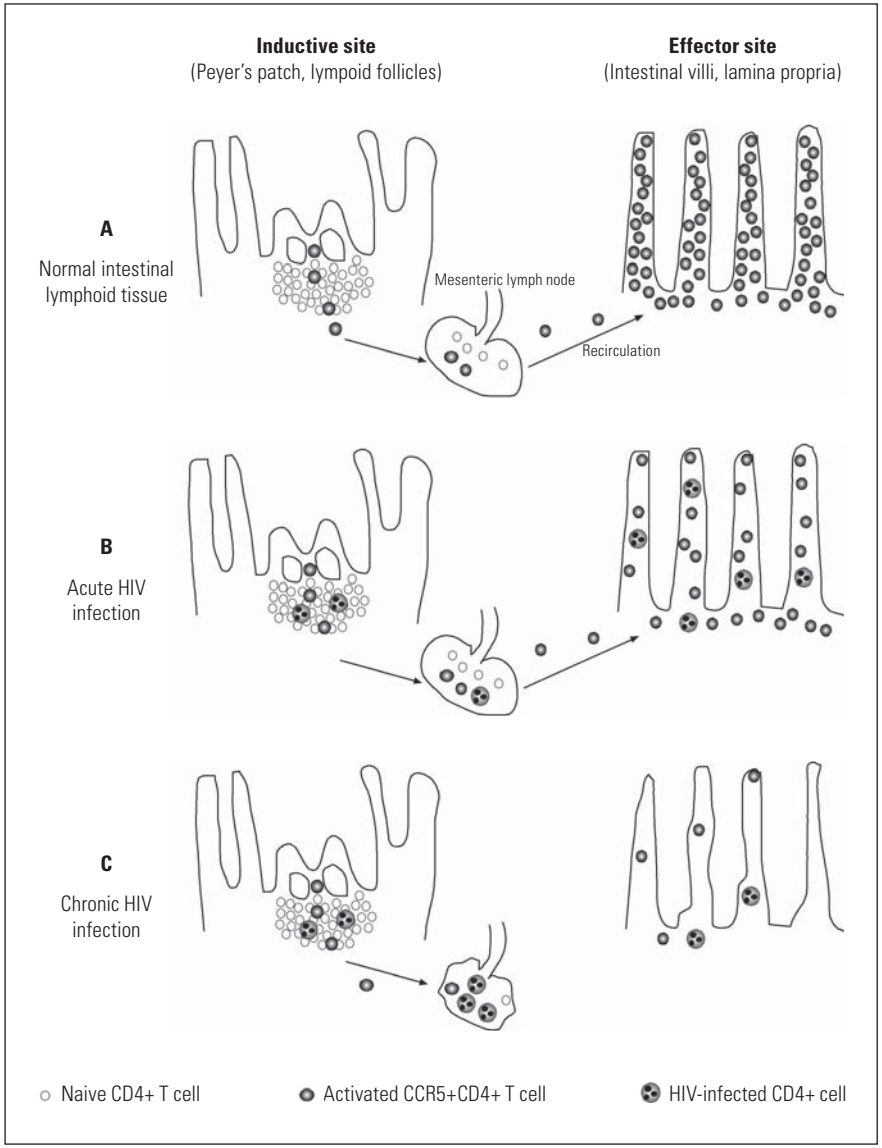
humans in HIV infection[14, 44]. Many mechanisms of $CD4^+$ depletion during SIV and HIV infection have been proposed, the majority of which, in addition to direct viral killing of infected cells, propose additional indirect mechanisms of $CD4^+$ killing, involving a variety of mechanisms including "bystander apoptosis" and/or immune activation[3]. An interesting proposal is that multiple HIV particles preferentially bind $CD4^+$ molecules on cell surfaces and interrupt late signalling steps in T-cell activation by antigen-presenting macrophages or dendritic cells, inducing apoptotic death pathways[45]. The finding that massive $CD4^+$ depletion occurs in the gut during acute SIV/HIV infection has raised the possibility that persistent $CD4^+$ depletion after HIV infection is due to the irreparable loss of the greater part of the large $CD4^+CCR5^+$ T-cell pool in GALT, and that continued destruction of replacement $CD4^+$ T-cells is possibly the likely reason for the overall $CD4^+$ T cell decline also reflected in the blood. Further studies in Macaque monkeys have shown that the rate of memory $CD4^+$ T-cell turnover in mucosal tissue, after SIV challenge, directly determines the rate of disease progression, and suggest that maintenance of a gut mucosal $CD4^+$ memory T-cell population adequate to control HIV and other infections is vital for longest-possible viral control[44]. This is a key concept for understanding the role of memory T-cells in chronic HIV infection and in the context of associated gut disturbances and diet (see below).

There are two, not mutually exclusive ways of examining the issue of gut integrity in HIV infection and in relation to diet and nutrition. The first is that HIV infection itself, for example through direct viral effects[36], may result in negative perturbations of normal gut integrity and of the immunosurveillance function of GALT, leading to gut malfunction (chronic "sterile" and/or infective diarrhoea) and associated malabsorption of food and/or loss of micronutrients. The second is that dietary factors damaging the gut epithelium may lead to increased susceptibility to HIV infection, specifically through the gut wall portal believed to be used by the virus in neonates. "Abrasions" of the gut have been postulated as one mechanism by which breast-milk mixed with formula feeding of their infants by HIV-infected mothers leads to increased transmission of HIV to the child[46]: the study concerned showed that exclusive breast-feeding carried a significantly lower risk of HIV transmission than mixed feeding, and a risk similar to that associated with no breast-feeding at all. The two approaches merge in the unresolved issue (see below) as to whether dietary "insults" in HIV-infected adults may aggravate virus-induced bowel malfunction or accelerate net losses of memory T-cells from the lamina propria.

## A synthesis of HIV infection and impaired intestinal immunity

In **Figure 1**, the stages of intestinal lymphoid tissue are shown to depict events that may follow in the wake of HIV infection. In (A), normal intestinal lymphoid tissue, naïve

*Figure 1: Anatomical arrangement of the inductive site of normal immune responses in Peyer's patches and lymphoid follicles in GALT and CD4+ T cell distribution in the lamina propria upon HIV infection (adapted from Veazey & Lackner[49] and Cohen[50].*








CD4+ T-cells reside in the inductive sites of the lymphoid follicles. Immune responses are initiated in the follicles and mesenteric lymph nodes (MLNs), where T-cells are unavoidably exposed to small but significant quantities of a variety of antigens normally found in the intestinal tract, such as dietary and microbial antigens. The CD4+ T-cells become activated, co-express CCR5, and ultimately (see above) migrate to the effector sites in the lamina propria, where they reside in large numbers[36]. It has recently been shown that the Vitamin A metabolite, retinoic acid, can facilitate this cell migration to the effector sites and aid immunity in the intestine[47, 48]. In acute HIV infection (B), the activated CCR5+ CD4+ T-cells are targets for HIV infection and replication and die either through direct infection or powerful bystander effects that coincide with peak viraemia in the plasma and the emergent rise of CD8+ virus-specific in the circulation. The eventual rapid decline in plasma viraemia may not be principally caused by the effector CD8+ T-cells, as previously thought, but rather to the greatly diminished numbers of target CCR5+ CD4+ T-cells residually resident in the gut wall. As HIV infection progresses to the chronic stage, which is for a time characterized by persistent viral infection more-or-less counter-balanced by replacement of new T-cells migrating into the bowel wall, net loss of CCR5+ CD4+ T-cells (C) ensues when the rate of T-cell activation, in the face of the immune suppressive effects of $T_{regs}$ in the lamina propria against the background of a general "oral tolerance regime" of a normal bowel wall, provides enough targets for viral replication and associated apoptotic T-cell deaths to overcome the defences, so to speak, and usher in the slow or rapid decompensating phase of grossly immune-deficient AIDS, leading to death from repeated, progressively more serious opportunistic infections or cancers in various locations.

The widely held notion that robust T-cell immunity to HIV in the peripheral circulation, as outlined earlier in this chapter, may exert strong pressure on viral replication such as to maintain a steady-state of viral replication resulting in a set-point of viraemia, may now well turn out be a misplaced judgement. Indeed, recent data from Reynolds, *et al*[51] have shown that the identified strong CD8+ virus-specific responses in monkeys after they were given SIV were minimal or not found in GALT, but rather occurred mostly in the cervico-vaginal tissues. The conclusions made were that specific strong CD8+ T-cell immunity initiated by the host cannot prevent the huge CD4+ T-cell depletion occurring in gut tissue, and the responses identified in the peripheral circulation are too late and too little to obviate viral persistence in this location, and disease progression as described above. **Steady-state T cell activation within the effector sites of the GALT, due to continued gut-derived antigen encounter, may be the single most important factor which maintains a pool of CD4+ T-cell targets for HIV infection and replication, supporting viral persistence and the eventual failure of viral control by the body.**

M**icrobial translocation** in the gut during chronic HIV infection has now been shown to be one of the major driving forces behind immune activation and maintaining the pool of activated $CD4^+$ T-cells that can be productively infected by the virus[52]. The levels of **plasma lipolysaccharide (LPS**, an endotoxin to the human body derived from Gram-negative bacteria resident as part of the gut flora), in HIV-infected individuals during the chronic stage of disease and during AIDS have been found to be much higher than in healthy HIV-negative controls or in subjects newly infected with HIV. Plasma LPS appeared to be derived from bacteria crossing into the blood circulation from the intestine and colon during HIV infection, probably as a result of mucosal malfunction induced by the virus, directly or indirectly. Similar types of bacterial translocation have also been observed in patients receiving radiation therapy and in those that suffer from inflammatory bowel disease. Concomitant with increased plasma LPS was increased plasma soluble CD14, a receptor molecule found on the surfaces of monocytes and shed upon in vivo activation by LPS. Interestingly, HIV-infected individuals who were able to control viral replication and appeared clinically healthy had higher levels of endotoxin-core antibodies that seemed to neutralize the chronic inflammation-activating effects of LPS in the systemic circulation. Along with data to show that provision of antiretroviral drug treatment resulted in lowered plasma LPS levels as well as lowered T-cell activation, the study has provided evidence that it may be the continuing presence of HIV in GALT that causes regular microbial translocation resulting in a degree of immune activation that maintains viral replication in susceptible cells and ultimately drives the slow collapse of the immune system, as described above. The plasma levels of an acute-phase (inflammatory) protein , C-reactive Protein (CRP), has recently been positively associated with progression time to AIDS in HIV-infected subjects[53]. **The crucial link that needs to be made is whether different nutrients/dietary components affect gut mucosal integrity in HIV-infected individuals in systematic or individually different ways, which could influence or determine the course of HIV pathogenesis through effects on the basic processes of viral persistence and net $CD4^+$ T-cell depletion, by influencing the rate of microbial translocation.**

The notion that chronic HIV infection may manifest as a form of inflammatory bowel disease urgently requires the bringing together of two, possibly three major fields on enquiry in the health sciences in HIV medicine/biology, clinical/scientific gastroenterology (specifically where it relates to chronic inflammatory disorders), and nutrition (specifically where this relates to the burgeoning field of probiotics and prebiotics (see Chapter 4).

Much is now known, for example, about the underlying molecular and genetic basis of Crohn's Disease[54], and therapy involving drugs such as 5-aminosalicylic acid[55] and elemental or polymeric diets (in children) is well-characterised[56]. It may be relevant that





5-aminosalicylates, which have become a standard, effective therapy for inflammatory bowel disease, have also successfully been used in the context of HIV-related enteropathy[57]. Beneficial effects of probiotics and/or prebiotics in numerous clinical situations[58-60] may well be replicable in chronic HIV infection.

It is possible that the topic of diet and nutrition in relation to the HIV pandemic may acquire a new and exciting dimension that goes beyond traditional pre-occupations with nutrient deficiencies actively to explore therapeutic approaches aimed at the optimum functioning of the intestines in infected subjects, generated in a productive, interdisciplinary environment.

# Special considerations of infancy and childhood

CHAPTER 8

# Nutrition, HIV infection and active TB in infants and children

HIV and TB are infectious diseases that contribute significantly to infant and child morbidity and mortality in developing-country settings. The pathogenesis of HIV infection may depend on both virological factors (viral strain and phenotype) and host factors (genetic and nutritional). Nutritional deficiencies may play a role in the pathogenesis of HIV infection partly because of the role of some micronutrients as anti-oxidants and/or in immune function (see Chapter 7).

## Nutrition, infection and immunity in infants and children

Any nutrient deficiency, if sufficiently severe, will impair resistance to infection[1]. The relative immaturity of the immune system in infants, and to a lesser extent in children[2-4], means that the relationship between immunity and nutrition is more complicated than in adults. The possibility that lifelong programming of the immune system can result from nutritional and/or other insults during fetal development have been discussed in Chapter 5. One of the known effects of foetal malnutrition is defective thymic growth, which may never be reversed postnatally and exerts long-range effects in later life. Diets for infants that are deficient in calories, protein, vitamin A, pyridoxine, biotin and zinc can result in a de novo loss of thymic cellularity[5]. In children with kwashiorkor and marasmus, there is a defect in T cell maturation, with a preferential loss of helper/inducer T4 cell subsets[5]. Protein-energy malnutrition results in a reduced number and function of T cells as well as phagocytic cells, and the secretory immunoglobulin A (IgA) antibody response is reduced. Zinc deficiency is associated with impairment of cell-mediated immunity, as revealed by lowered lymphocyte stimulation responses, decreased circulating $CD4^+$ and $CD8^+$ T-cells, and decreased chemotaxis of phagocytes[6] (see Chapter 7).

## Breast-feeding and mucosal/adaptive immunity[7]

Human foetuses acquire maternal antibodies (mainly IgG) passively via the placenta from the mother, and perhaps also from swallowed amniotic fluid via gut IgG receptors,

but the ability to take up antibodies from the gut stops variably after birth (up to 2 years), following the so-called "gut closure" of the receptor-based route concerned. Immediately after birth, the mucosal surfaces, only one cell layer thick, are bombarded by many microorganisms and protein antigens (especially if bottle-feeding is used). The **passive immune protection** afforded by residual maternal antibodies (lost by catabolism in the first two months) is key to the protection of the newborn infant at this stage.

Infants become very dependent on **breast-feeding** after loss of the maternal IgG stock, particularly the rich supply of **secretory IgA (SIgA) and IgM (SIgM**) ( 12g/l and 0.6 g/l respectively in colostrum, and about a quarter of this in breast-milk). The specificities of these soluble antibodies integratedly reflect antigenic stimulation of the mother, including pathogens in both airways and intestines, and are therefore particularly protective against the same pathogens likely to be encountered by the infant. Factors of **innate immunity** are also provided, including lysozyme, lactoferrin, peroxidase and mucins. Small numbers of immune cells of various types are also secreted in breast-milk, and may play a role in the immune stabilisation of the gut mucosa in the infant, possibly by secretion of cytokines, some of which are present in the milk as secreted, together with growth factors.

After passive immunity has ceased, and while breast-feeding continues (or is replaced by formula-feeding) the infant depends on its own **adaptive immune response** to fend off microorganisms. At the mucosal surface this is expressed mainly as the production and subsequent transepithelial secretion of SIgA and SIgM by activated B-cells and plasma cells which are capable of "immune exclusion" of pathogenic microbes, i.e. preventing these from gaining access to the submucosa or from establishing a productive infection there. This highly effective humoral immunity is supported by cell-mediated responses, mainly through the critical roles played by antigen-presenting dendritic cells and macrophages, as well as helper T-cells in initating humoral, antibody-based responses. The colonisation of the intestines by microbial flora is highly contributory to the development of well-functioning gut-associated lymphoid tissue.

## HIV acquisition in infants and children

HIV transmitted from mother to child accounts for the majority of paediatric HIV infections occurring in South Africa[8, 9]. Mother-to-child transmission (MTCT) of HIV can occur in utero, during labour and delivery, or through postnatal consumption of breast milk[10], with the bulk of transmission events currently occurring in the intrapartum period[11–13]. Transmission rates vary from less than 2% in the developed world (mainly due to the use of highly active antiretroviral therapy (HAART), elective caesarean section and adequate formula-feeding) to more than 30% in the developing world where there





is no or little access to antiretroviral prophylaxis and where prolonged breast-feeding is standard[14]. MTCT rates also vary in South Africa from 2% to 30%, depending on use of interventions to prevent MTCT and the duration and method of breast-feeding[15-21].

## Role of nutrition in MTCT

**Low birth weight** is the most important general determinant of infant mortality and morbidity[22, 23]. Compared with the pregnancy outcomes of uninfected women, HIV-infected women are more likely to display intra-uterine growth retardation, preterm delivery and low birth weight. Adverse pregnancy outcomes become even more pronounced in women with symptomatic HIV infection[24]. Actual weight loss during pregnancy, as compared with women who gained 167 g/week or greater, increases the risk of intra- and early post-partum transmission of HIV and adverse pregnancy outcomes[25].

Anaemia and low plasma levels of vitamin A have been associated with increased rates of MTCT of HIV[26], but it is still unclear whether nutritional deficiencies play a specific role in the aetiology of HIV transmission. Antenatal vitamin A supplementation of HIV-infected women increased birth weights as compared with a control group, decreased the incidence of low birth weight, and decreased the proportion of infants who were anaemic at 6 weeks old, but did not affect perinatal HIV transmission[27]. However, a different study demonstrated an increased risk of HIV transmission through breast-feeding in women who received vitamin A supplementation[28]. There are no data on the effects of vitamin A supplementation when antiretroviral prophylaxis for MTCT is used.

Zinc supplementation of HIV-infected pregnant women has not been found to affect perinatal HIV transmission or any other birth outcomes, but impacted negatively on haemoglobin levels, red blood cell counts and packed cell volumes[29, 30]. In HIV-infected women, low plasma selenium levels were associated with increased risks of fetal death, child death and intrapartum HIV transmission[31].

## Role of breast-feeding in HIV transmission

HIV can be transmitted from mother to child through breastmilk [19, 20, 32-34], and while some studies have addressed this question[35, 36] the exact mechanism by which transmission occurs is not fully understood. For example, it is unclear whether infection takes place through entry of cell-free virions or entry of cell-associated HIV (both forms of HIV have been detected in colostrum and breast-milk), or whether the portal is the tonsils or the upper intestine itself. Cell-free virus could penetrate the mucosal lining of the gastrointestinal tract of infants by infecting inter-epithelial dendritic cells and be sampled by M cells of the Peyer's patches, or it could enter the submucosa directly by the kinds of mechanisms that allow intact

proteins to traverse the immature mucosal barrier. Alternatively it could penetrate through damaged mucosal foci. An *in vitro* study showed that HIV-infected immunocytes stimulated enterocytes to engulf HIV particles present on their surfaces[36], and it appears there may be both cell-associated and cell-free mechanisms of transmission.

Breast-feeding as opposed to formula-feeding approximately doubles the risk of vertical transmission, and in populations where breast-feeding continues into the second year, the additional risk is a further 15–20%[37]. The risk of transmission through breast-feeding depends on the age of complete cessation of breast-feeding, the stage of maternal disease, breasthealth, and the modality of feeding. In the Breastfeeding and HIV International Transmission Study (BHITS), 42% of HIV-infected children had been infected via breast-feeding when they had reached 4 weeks of age[38].

## Factors affecting disease progression in children

Progression of HIV infection in children may depend on the timing of transmission[39], the inoculum size of the virus[40, 41], the phenotype of the virus[42], and immune functioning[43]. Among the host factors that affect the pathogenesis of HIV infection is nutritional status involving either free radicals and/or more general nutritional/immunological factors.

Activated macrophages and neutrophils play a role in the killing of micro-organisms through the generation of reactive oxygen intermediates such as superoxide radicals; hydrogen peroxide and hydroxyl radicals[44] (see Chapter 7). These reactive oxygen intermediates oxidiSe nucleic acids, create chromosomal breaks, peroxidise lipids in cell membranes, and damage structural proteins and enzymes. The generation of reactive oxygen intermediates by immune-effector cells or injured tissues is counteracted by the anti-oxidant defence system: oxidative stress occurs when there is an imbalance between pro-oxidants and anti-oxidants, and the overproduction of reactive oxygen intermediates result in pathology. Nuclear factor κB (NF-κB) is a transcriptional promoter of proteins that are involved in the inflammatory response and acute-phase reaction. NF-κB is bound to factor I κB in the cytoplasm in its inactive form, but various factors such as tumour necrosis factor-α (TNF-α) and reactive oxygen intermediates can cause the release of NF-κB from IκB, after which NF-κB translocates to the nucleus and binds to promoter sequences in DNA, amongst them those that regulate the transcriptional activation of HIV (see Chapter 7). The cellular anti-oxidant glutathione inhibits the activation of NF-κB, and may thereby dampen HIV replication: HIV-infected adults with low levels of glutathione in their $CD4^+$ T-cells have decreased survival[45], providing some rationale for the use of *N*-acetylcysteine (an oral pro-drug form of glutathione) in HIV infection.

As discussed in Chapter 10, **micronutrients** such as vitamins A and E and zinc are involved in normal immune function, and micronutrient deficiencies may compromise





host immunity to HIV infection and associated infections and thereby hasten disease progression. Vitamin A plays a central role in children in the growth and function of T- and B-cells, antibody responses, and maintenance of mucosal epithelia, including that of the respiratory, gastrointestinal and genitourinary tracts[46]. Zinc plays an important role in the growth and development and function of neutrophils, macrophages, natural killer cells, and T and B lymphocytes[47]. Vitamin E influences the function of T-cells, B-cells and phagocytic cells, and may also protect immune effector cells against oxidative stress[48]. Vitamin $B_6$[49], selenium[50] and folate[51] may also influence immune function, but further work is needed regarding the role of these micronutrients in the pathophysiology of paediatric HIV infection (see Chapter 4).

## The effects of infant feeding on survival

Because HIV can be transmitted through breast-feeding, all HIV-infected women who are pregnant need to be informed of this risk and that avoidance of breast-feeding should be avoided if replacement feeding is acceptable, feasible, affordable, safe and sustainable. If replacement feeding is deemed to attribute a greater risk of mortality than the risk of HIV acquisition via breast-feeding, exclusive breast-feeding with early weaning (4-6months) can be recommended. In certain settings, non-breast-fed infants have been found to have a greater risk of dying than infants who were predominantly or exclusively breast-fed[52]. Recently, in a non-randomised observational study[53] conducted in slums in New Delhi, India; shanty towns in Lima, Peru; and rural villages in the Kintampo district of Ghana demonstrated that infants who were not breast-fed or who were partially breast-fed were more likely to die from gastroenteritis and respiratory infection than predominantly breast-fed infants. This study also showed that infants who were not breast-fed were at a substantially higher risk of all-cause hospitalisation. The impact of HIV on mortality and morbidity was not assessed in this study.

## Infant feeding in the context of HIV

Studies conducted in Africa among infants born to HIV-infected mothers, have demonstrated no increases in mortality in infants who have been formula-fed[54]. In Nairobi, a randomised clinical trial of breast-feeding versus formula-feeding[55] to prevent MTCT of HIV found similar mortality rates among infants who were formula-fed as compared with those who were breast-fed, even after adjusting for HIV infection status; in addition, this study found that the rates of diarrhoea or pneumonia were similar in infants assigned to be formula-fed or breast-fed. In a recent randomised clinical trial in Botswana[56], the mortality distributions at 18 months were not significantly different

between the infants who were breast-fed or formula-fed. At 7 months, however, the cumulative infant mortality was higher in the formula-fed group than in breast-fed group. The PETRA study[21], conducted in Tanzania, South Africa and Uganda, demonstrated a significantly lower an infant mortality rate that was significantly lower in South African than East African sites; in the South African sites, only 53% of women breast-fed, as opposed to 97% of women in the East African sites. In a study conducted in Soweto[19] in a population of HIV-infected mothers, 2.2% of all breast-fed infants and 3.8% of all the formula-fed infants died before 12 weeks of age (no statistical difference). In the same study, when comparing overall transmission from HIV-infected mothers to their infants, the rate of being infected at 12 weeks was 14.8% in the breast-fed group as opposed to 9.4% in the formula-fed group (log-rank test: p-value = 0.007), suggesting that the excess risk of acquiring HIV postnatally through breast-feeding was about 5%.

Models have been applied to assess the impact on mortality of various infant-feeding strategies implemented for the prevention of MTCT of HIV in various settings. Formula feeding appeared to be highly effective in settings with high HIV seroprevalence and reasonable levels of child survival, but dangerous when infant mortality rates were high (>50/1000) or the protective effects of breast-feeding substantial[57]. In another modelling exercise, three categories of breast-feeding (optimal breast-feeding, complete avoidance of breast-feeding, and early cessation of breast-feeding) were compared in terms of HIV transmission and infant mortality[58]; the lowest frequency of adverse outcomes appeared to occur if none of the HIV-seropositive women breast-fed their infants but all HIV-seronegative women breast-fed optimally, given that infant mortality rates were below 100/1000, and the relative risks of dying were set at 2.5 for non-breast-fed compared with optimally breast-fed infants.

Most recently, a non-randomised cohort intervention study on a large number of births to HIV-infected mothers in KwaZulu-Natal has shown that when such mothers breast-fed exclusively, their babies had a lower risk of postnatal HIV infection than did babies who also received replacement liquids, and especially so when solids were included in the feeds[59]. The infant mortality in breast-fed babies was also half that of replacement-fed babies. Importantly for policy, a large proportion of the cohort of mothers was able to achieve exclusive breast-feeding for 6 weeks (82%) and 3 months (67%), aided by well-designed and effective counselling and others forms of support.

## Natural history of paediatric HIV infection

In the developed world, before the use of HAART, a bimodal distribution of survival of HIV-infected children was described, with approximately 10–20% of children being "rapid progressors" (usually dying of AIDS before 4 years of age), while the remaining





80–90% had survival patterns typical of adults with AIDS, with a mean survival of approximately 9–10 years[60]. Before the use of HAART, 17–25% of infants died within the first 8 months after birth, with 75% of children still being alive at 6 years of age, and 50% still alive by 9 years of age[60].

The survival of children infected with HIV in Africa appears to be much shorter than that in Europe or the USA. In Uganda, the mortality rate of children with a laboratory diagnosis of HIV was 336/1000[61]. Median survival was 21 months, with 66.2% of children dying by 36 months of age. In Malawi, the mortality rate per 1000 person-years of observation in HIV-infected children was 339.3 as opposed to 46.3 among uninfected children born to HIV-infected women[62]. In Zimbabwe, HIV-infected women had a perinatal mortality rate 2.1 times greater than uninfected women, a still-birth rate 1.6 times greater, and a neonatal mortality rate 2.7 times greater[63]. Neonates born to HIV-infected women had an increased mortality rate (133/1000) compared with neonates born to uninfected women (40/1000) in Abidjan[64], and a decreased 12-month survival rate in Brazzaville[65]. In a pooled analysis from Africa, it was found that by one year of age, 35.2% of HIV-infected infants and 4.9% of HIV-exposed but uninfected infants had died[66].

Data on survival of HIV-infected children in South Africa are available in the case of detailed birth-cohort studies. In Durban, 35% of children who were infected perinatally died in their first year of life[67], while in a study conducted in Soweto[19], 6.9% of HIV-infected infants died within 100 days after birth compared with 1.5% of HIV-exposed but uninfected infants (p<00001)[52]. At Tygerberg Hospital in Cape Town, 16.8% of HIV-infected children died over a 5-month period; in this cohort, most children were malnourished, with those under 2 years of age having significantly lower growth indicators than those over 2 years of age[68].

## The role of nutrition in childhood HIV infection

A combination of insufficient macro- and micronutrient intake resulting from anorexia, nausea, vomiting, diarrhea and malabsorption, associated with impaired storage and altered metabolism of nutrients as well as elevated energy expenditure during secondary bacterial and/or systemic opportunistic infections often leads to both wasting and micronutrient malnutrition during HIV infection in children[69]. One-third of symptomatic HIV-infected children displayed carbohydrate malabsorption[70]. Deranged metabolism associated with HIV infection has included increased hepatic lipogenesis[71], increased protein turnover[72] and increased insulin sensitivity[73]. Unlike starvation, which is associated with a predominant loss of body fat, HIV infection is predominantly associated with a loss of protein as measured by lean body mass[74], which

has been associated with increased mortality[75, 76]. Excessive loss of protein, coupled with inadequate intake and absorption, as well as an increase in the body demand for protein related to cellular proliferation in the immune system readily results in a vicious cycle of protein imbalance in rapidly growing children. This predisposes to immune deficiency resulting in intercurrent infections, which further exacerbate the shortfall of protein required for sustaining cellular integrity and function.

## Mechanisms for malnutrition in HIV-infected children

Malnutrition and nutritional problems in HIV-infected infants and children can be the consequence of a number of mechanisms, often operating simultaneously or synergistically[77]. **Loss of appetite and poor dietary intake** are regarded as among the most important causes of growth failure or weight loss associated with HIV infection, but HIV also increases the body's nutritional needs.

Upper gastrointestinal ulcers due to viruses or of idiopathic origin are common, and decrease food intake because they cause pain during eating[78]. Inflammation and ulceration of the upper gastrointestinal tract has been found in up to 70% of HIV-infected children undergoing endoscopies[79], and is often related to opportunistic infections that lead to anorexia because of odynophagia, dysphagia, or abdominal pain that is associated with eating. Primary anorexia may also contribute to inadequate intake; although the role of cytokines as mediators of anorexia is controversial[80] (there is evidence that increased cytokine production (e.g. interferon-alpha, interleukin-1, interleukin-6 and TNF-$\alpha$) may be associated with anorexia[81]. TNF-$\alpha$ also delays gastric emptying which can also increase anorexia[82]. HIV encephalopathy, present in up to 16% of children with HIV infection[83] may also reduce energy intake considerably. Many medications that HIV-1-infected children are required to take may result in gastric irritation, vomiting, nausea and diarrhoea[84].

Gastrointestinal mucosal dysfunction because of local HIV infection or from secondary enteric infections can lead to **malabsorption** of both macro- and micronutrients. Several studies have reported impaired carbohydrate, fat and protein absorption in patients with HIV/AIDS[85, 86], with the extent of malabsorption not always correlated with the degree of malnutrition[87]. Furthermore, while the impaired absorption may not always be clinically evident, it may imperceptibly contribute to worsening of the disease. Fat malabsorption and protein-losing enteropathy in HIV-infected children are usually the result of severe enteritis caused by secondary infections. Many micronutrients are also malabsorbed in children with AIDS-related gastrointestinal disease, including zinc, selenium, calcium, magnesium, vitamins A and $B_{12}$, folic acid, thiamine and vitamin D. Diarrhoea resulting from infection may be persistent (lasting more than 14 days), predisposing to severe





malnutrition and death. (Diarrhoea may also be caused by antiretroviral therapy and other medications, particularly antibiotics). Gastrointestinal bleeding due to mucosal ulcerations leads to loss of nutrients secondary to blood loss. Opportunistic infections affecting the gastrointestinal tract, including the hepatobiliary system and the pancreas, may also result in various types of malabsorption.

**Increased energy utilisation** in children with HIV is related both to viral load and to coinfections and comorbidities. Asymptomatic chronic viral infections can predispose patients to secondary infections, which can significantly alter energy utilisation patterns. There are, however, few good studies of energy expenditure in HIV-infected children. Available data show no differences in resting energy expenditure or of total energy expenditure between children with growth failure and those with normal growth rates[88-95].

**Psychosocial factors**[96–98] may also contribute towards malnutrition in HIV-infected children, who often live with ill parents or aged relatives with limited access to social services and support. An unstable home environment and inadequate emotional and social support has been shown to influence growth negatively in both HIV-infected and uninfected children by many mechanisms, including growth hormone deficiency. Caregivers can influence the functional status of HIV-infected children positively and/or negatively.

## Growth patterns in HIV-infected children[99–115]

A number of abnormal growth patterns occur in HIV-infected children, ranging from symmetrical retardation of weight and height to severe wasting (low height-for-weight) with normal height. These differences in growth patterns can probably be explained by the variable clinical disease patterns occurring in HIV-infected children, related to factors such as viral load and the frequency and severity of coinfections. Higher HIV viral load increased the risk of growth failure in children. Factors such as lower $CD4^+$ T-cell count, infectious complications such as pneumonia and diarrhoea, (prior) maternal drug use during pregnancy, and exposure to antiretroviral therapy (non-protease inhibitor) have been associated with growth problems. Cytokines may be responsible for some of the growth, metabolic and immunological effects associated with HIV infection. Factors that contribute to wasting syndrome (defined as a net weight loss of at least 10% of body mass) include hypermetabolism, opportunistic infections and associated anorexia, increased protein catabolism, gastrointestinal disease, malabsorption, and imbalance between the pro- and anti-inflammatory cytokines produced by mononuclear cells.

Regarding the growth of HIV-infected children, a study conducted in Durban examined children born to HIV-1-seropositive women, followed up in respect of growth parameters from birth to early childhood[114]. Mean Z-scores were calculated for weight-

for-length, weight-for-age and length-for-age and, if they were low, the children were regarded as wasted, malnourished or stunted, respectively. At the end of the study, there were 48 infected and 93 uninfected children under observation. While there were no significant differences between the two groups at birth, the infected group had early and sustainedly lower mean Z-scores for length-for-age and weight-for-age but not for weight-for-length, reaching statistical significance at ages 3, 6 and 12 months for length-for-age, and at 3, 6 and 9 months for weight-for-age. Infected children who died early had more severe stunting, wasting and malnutrition than did infected children who survived. Infected children born to HIV-infected women have early and sustained stunting and are malnourished but not wasted. Generally, children with rapidly progressive disease had both stunting and wasting, and were more severely affected.

A study conducted in Cape Town looked prospectively at anthropometric and micronutrient indicators of 60 stable, HIV-infected children (median age 25 months) in an economically deprived setting[115]. Investigations included $CD4^+$ counts, anthropometry and plasma levels of albumin, transthyretin, retinol-binding protein (RBP), vitamins A, $B_6$, E and $B_{12}$, as well as folate, zinc and copper. Thirty-two per cent had mild, 48% moderate and 20% severe clinical features, and 80% were moderately or severely immunosuppressed. Twenty-eight per cent had a weight Z-score <-2.0 and 58% a height Z-score <-2.0. Many children had biochemical features of micronutrient deficiencies: low albumin (70%), transthyretin (100%), RBP (85%), vitamins A (80%), $B_6$ (37%), E (37%) and $B_{12}$ (5%), zinc (20%) and copper (25%). Sixty-two per cent had two or more trace element or vitamin deficiencies. There was a weak association between micronutrient status and disease status. Micronutrient concentrations did not correlate with chronological age, height-for-age or weight-for-age. Plasma CRP was elevated in 53% but this did not correlate with any of the micronutrient concentrations. Micronutrient deficiencies were more common and micronutrient concentrations lower in children over 24 months of age.

The evidence to support the theory of inadequate energy intake being responsible for poor childhood growth in an outpatient setting is surprisingly inconclusive. A large, cohort study found that stable HIV-infected children whose growth rate was below that of a control group received well over the recommended daily allowance of energy and protein, similar to the non-infected control children (higher intake was, however, associated with improvement in weight and fat mass). Other smaller studies have shown differences in energy intake between HIV-infected and non-infected children, suggesting that inadequate intakes may relate to growth differences.

Children infected with HIV show progressive declines in lean body mass over time, while measures of fat stores remain constant, yet low. Although clinical manifestations may take time to appear, the onset of growth changes begin soon after birth (within the first 1 to 3 months). Sequential follow-up has showed that growth in HIV-infected





children remained below that of age- and gender-matched uninfected children. Because growth failure often precedes secondary infections in HIV-infected infants and children, inadequate protein deposition may be an early manifestation of infection by the virus; children with HIV infection, but without secondary infection, have negative protein balance because of an inability to down-regulate protein catabolism. Furthermore, the acute-phase protein (APP) response elicited by HIV infection is characterised by higher concentrations and synthesis rates of positive APPs without lower concentrations of some negative APPs (see chapter 4).

Poor growth is associated with an increased risk of mortality in HIV-infected children. In one study, HIV infection appeared to be a stronger predictor of mortality among children hospitalised with pneumonia, and who were wasted, than in those who were not. Nutritional status therefore needs to be assessed at regular intervals as part of the management of HIV infection in children, to prevent stunted growth and development. The simplest approach to assessment to date has been serial weight measurement, with the more comprehensive nutritional assessment including (1) anthropometric measurements of body composition; (2) biochemical measurements of serum protein, micronutrients, and metabolic parameters; (3) clinical assessment of altered nutritional requirements and social or psychological issues that may preclude adequate intake; and (4) measurement of dietary intake (see Chapter 4).

## The effects of tuberculosis on HIV infection[116–134]

The importance of tuberculosis as a cause of childhood morbidity and mortality is controversial only because of limitations in the tools available for diagnosing pulmonary tuberculosis in children. Tuberculosis is a leading cause of death in children under 5 years of age in sub-Saharan Africa[116]. HIV-infected individuals are at an increased risk of developing TB; immunocompetent adults have a 10% lifetime risks of developing TB after infection with *Mycobacterium tuberculosis* compared with an annual risk of 10% among HIV-infected adults. Among immunocompetent children infected with *M. tuberculosis* the risks of developing TB disease and of developing the most severe forms of TB disease (miliary and meningeal disease) are much higher than among adults. In general, the younger the child with TB infection is, the greater the risk of progression to disease[117]. Most cases of pulmonary TB among South African children (both HIV-infected and HIV-uninfected) occur in children under two years of age (median ages of 12 and 14.5 months, respectively)[118]. In 2004, at least 50% of children aged 0–9 years in a peri-urban township in South Africa who developed TB disease were also found to be HIV-infected[119]. In a study conducted at a primary health care level in Cape Town, HIV-infected children were more likely to have complicated Ghon focus and disseminated disease than non HIV-infected

children (OR 10.9, 95% CI 3.2–35.9)[120]. In a study conducted in clinics in Johannesburg and Cape Town, the overall incidence of TB in HIV-infected children was found to be 3.2/100 person-years (95% CI 2.0–4.9). In HIV-infected children receiving antiretroviral therapy, the incidence of TB was reduced (8.3/100 person years) as compared with those children not on antiretroviral therapy (14.4/100 person-years), despite those on therapy being initially far more immuno-compromised[121].

Although there are no data available regarding the risk of development of TB disease in children coinfected with TB and HIV, it would seem reasonable to assume an even higher risk than that observed in adults, particularly for those children living in countries with a high prevalence of TB[122]. Among TB patients in South Africa, the HIV seroprevalence rates among in those younger than 18 months old and those between 10 and 14 years old were 52% and 14.7%, respectively[123]. It is possible that these variations reflect the increasing HIV seroprevalence rates among young children, as the older children may have been born at a time when overall national (South African) seroprevalence rates were lower. Data from Zambia indicate that approximately 69% of children hospitalised with clinical TB were co-infected with HIV[124]. TB was the fifth most common diagnosis among hospitalized children at this institution, with five percent of children admitted having TB. Similarly, in a study in Cote d'Ivoire, 23.4% of children with TB were co-infected with HIV[125]. At a time when approximately 4% of the birth cohort in South Africa were estimated be to be HIV-infected, 42% of all children in whom TB was diagnosed had concurrent HIV infection[126].

*M. tuberculosis* is also increasingly being recognised as an important cause of severe acute pneumonia in children in sub-Saharan Africa; it was isolated in 8% of children hospitalised for acute pneumonia in three separate studies among HIV-infected and uninfected children[127–129]. TB can aggravate the immunological deterioration of HIV-infected individuals, and thus hasten HIV disease progression[130]. Globally, the case fatality rate from TB is estimated to be 23%, but it exceeds 50% in regions such as Africa where the prevalence of HIV infection is extremely high[130]. There are conflicting data regarding the importance of tuberculosis as a cause of death based on post-mortem studies performed in Africa: while pulmonary tuberculosis was identified in 4–12% of HIV-infected children who died in South Africa and Botswana[132, 133], more recently a study from Malawi associated tuberculosis with 20% of all deaths[134]. The proportion of deaths due to tuberculosis was particularly high after 12 months of age. Tuberculosis was the second most common pathology in children dying between 12 and 17 months of age and was identified in 31.6% and 36.8% of HIV-infected and uninfected children, respectively. Additionally, tuberculosis was the most common associated cause of death in HIV-uninfected children older than 18 months of age, and the second leading cause of death in HIV-uninfected children across all age-groups.



# Clinical evidence

CHAPTER 9

# The effects of nutritional interventions in HIV/AIDS: Macronutrients

In this chapter we consider the evidence from human studies on the effects of nutritional intervention on clinical outcomes in people infected with HIV. The first part of this chapter focuses on the potential benefits of macronutrients (i.e. carbohydrates, proteins and fat which are required by the body in large amounts). This is followed by a review of the evidence concerning micronutrients (i.e. essential elements, such as vitamins and minerals, required in small quantities). We emphasise findings from randomised controlled trials as these provide the most reliable evidence on the effects of treatment highlighting other types of studies where relevant (See Chapter 3: Evidence-based policy and recommendations).

## Background

HIV/AIDS has many faces. There are major differences between HIV-infected individuals in developed and developing countries with regard to income level, availability of adequate nutrition and access to basic healthcare or life-prolonging antiretroviral treatment. In addition, patients vary in terms of their response to the virus, stage of the disease, susceptibility to secondary infections, nutritional status and individual response to the various treatments received. These complexities must be taken into account when formulating recommendations and guidelines on nutrient and energy supplementation for HIV-infected individuals.

## HIV-associated weight loss and wasting

Weight loss and wasting, two features commonly associated with HIV infection, are independent contributing factors to poor clinical outcomes in people living with HIV/AIDS[1]. A 10% or greater loss of body weight over a year is not uncommon in HIV-infected people, but there is a high degree of variability in the extent of weight loss and wasting. In most cases, acute weight-loss episodes are associated with secondary infections[2].

Once the secondary infections are successfully treated and energy intake is increased sufficiently, patients are able to regain weight and remain weight-stable[3]. Instances of chronic weight loss are normally associated with secondary gastrointestinal infections and subsequent malabsorption[2] (see Chapter 7).

Alterations of the metabolic system commonly occur in people with HIV infection. **Whole-body protein turnover** is up to 25% higher in untreated HIV-infected individuals than in HIV-negative controls[4]. The increased protein turnover is positively correlated with resting energy expenditure (REE)[5] and increased protein turnover, with net protein loss, results in cachexia. This condition is characterised by the loss of body cell mass, the "work-generating" tissues in the body. Insulin clearance and sensitivity are also increased in untreated HIV-infected individuals[6], while those on HAART develop insulin resistance. Furthermore, lipid metabolism is altered in HIV-infected individuals, particularly those on antiretroviral treatment. Fat oxidation is increased in both treated and untreated HIV-infected people.

In uncomplicated starvation the body slows down its metabolic processes, with minimisation of resting energy expenditure (REE) and total energy expenditure (TEE), and conservation of the body's protein at the expense of the fat stores. By contrast, in HIV infection resting energy expenditure is increased in asymptomatic, and even more so in symptomatic patients[7, 8], with both fat and protein stores being oxidised to fuel the body's energy requirements. A recent meta-analysis found that **resting energy expenditure**, expressed per kg fat free mass (REE/FFM), was significantly higher (~11%) in HIV-infected individuals than in healthy HIV-negative controls[9]. Sub-group analyses showed that the REE/FFM of symptomatic HIV-infected individuals tended to be higher than that of asymptomatic weight-stable HIV-infected individuals, although the difference was not statistically significant[9]. There is also some evidence to suggest that the type of secondary infection might influence changes in the resting energy expenditure[10].

Despite the observed increase in REE/FFM in HIV-infected individuals, **total energy**) was found to be significantly reduced in HIV-infected, rapidly **expenditure (TEE** weight-losing men[7, 11]; the finding of decreased TEE in a group of obviously very ill individuals must be interpreted with caution, however, as this outcome may not hold true in asymptomatic HIV-infected individuals. Nonetheless, if TEE is decreasing, or at the very least not changing, the cause of the weight loss and wasting seen in HIV-infected people requires explanation.

Scarcity of food (particularly in the developing world), loss of appetite, and decreased absorption of energy appear to be crucial factors associated with HIV-related weight loss and wasting. In areas of the world where food security is an issue it is important to ensure that all individuals and in particular those who are HIV-infected, are provided with sufficient nutritious food. In such areas those most vulnerable are people who are





too ill to go to work to earn money for food or to tend their crops or livestock. In this context the provision of adequate food, may contribute to patients maintaining their weight and facilitate the use of antiretroviral drugs.

Decreased appetite, a common complaint of many individuals living with HIV, is most profound in the later stages of infection[12]. The precise physiological reason behind this response is not clear, although it is probably related to the anorexic effect that the proinflammatory cytokines exert on the body (see Chapter 4)[13].

**Malabsorption and diarrhoea**, secondary to disruption of the architecture and function of the intestines, are also frequently encountered in people with HIV infection (see Chapter 7). These individuals may further decrease their food intake to minimise the episodes and severity of the diarrhoea[14, 15]. Carbohydrate malabsorption, (particularly in children)[16] and fat malabsorption are commonly reported in HIV infection. Malabsorption of nutrients and the associated decrease in energy intake thus further contribute to the observed weight loss.

## Nutrition and immunity in HIV-infected individuals

The relationship between nutrition and immunity is well established and individuals with poor nutrient status are known to be more susceptible to infections. The interplay between nutrition and immunity is particularly evident in HIV-infected individuals. In this context, poor nutrient status worsens an already compromised immune system increasing vulnerability to opportunistic infections, which in turn leads to deterioration in nutrient status. Malnutrition therefore acts as both a cause and a result of HIV disease progression.

A balanced diet provides the macronutrients (and its constituent parts, for example amino acids and triglycerides and fatty acids), micronutrients and energy required for optimal growth and development (see Chapter 4). Macronutrients (i.e. carbohydrates, proteins and fat), required by the body in large amounts (i.e. tens to hundreds of grams), provide the body with energy and essential building blocks for the construction of cell membranes, organelles and proteins. In a balanced diet the total energy requirements should consist of approximately 50–55% carbohydrates (for example, fruit, vegetables, breads, cereals, rice and pasta), 10–20% protein (for example, chicken, fish, beans and peas, nuts and seeds) and 30–35% fat (oil, butter, cheese). Nutrient and energy requirements will vary depending on age, gender, level of physical activity, pregnancy and lactation needs and health status.

In order to ameliorate weight loss and wasting, HIV-infected individuals should ensure that the basic nutrient and energy requirements are met. On the basis of the findings that REE/FFM of HIV-infected individuals is increased by approximately

10%[9], one might assume that in order to prevent weight loss and wasting, food intake should be increased accordingly to meet these increased energy demands. Furthermore, REE/FFM has been shown to increase dramatically in HIV-infected individuals with secondary infections. The appropriate amount and type of food to eat during or post infection is not yet known with certainty, but it is considered prudent to monitor patients' body weight carefully during this period providing adequate nutritious food as needed.

## Evidence for the benefits of macronutrient supplementation

It is reasonable, in principle, to assume that nutritional interventions administered to people with HIV infection will maintain or enhance defence against infection (decrease the risk of infection), promote recovery from opportunistic infection and improve quality of life and survival. Various nutritional interventions including food (high energy, protein or fat diets), oral supplements with specific nutrients, enteral and total parenteral therapy, appetite stimulants and anabolic hormones (growth hormone) have accordingly been recommended for HIV-infected individuals.

In this section we summarise the available evidence on whether oral nutritional supplementation with macronutrients is effective for reducing negative clinical outcomes in HIV-infected individuals. As discussed in Chapter 3, such evidence should ideally be informed by blinded (masked) randomised controlled trials with adequate patient follow-up. Furthermore, the studies should be of sufficient power to detect differences between experimental and control groups in clinically important endpoints. While randomised studies will provide the most reliable evidence on what does or does not work, the results obtained will apply to specific patient groups and settings, and care will be needed in extrapolating their findings to different populations. In the absence of randomised trials, well-conducted, non-randomised trials and observational studies or expert opinion may provide guidance on what nutritional interventions may be of value but these methods are less reliable as they are more susceptible to bias.

## Randomised, placebo-controlled trials

A recent Cochrane systematic review* has evaluated the evidence concerning the effects of orally administered macronutrients (in the form of a balanced diet or dietary

* Mahlungulu S, Grobler LA, Visser MME, Volmink J. Nutritional Interventions for reducing morbidity and mortality in HIV infected individuals (Cochrane review in press)[53-57].





supplements high in protein, fat or carbohydrate) in reducing morbidity and mortality in HIV-infected individuals[17]. Despite an exhaustive search for randomised trials, only eight small studies, with a total of 486 participants, could be found that met the pre-specified inclusion criteria. While none of the trials directly assessed the effects of macronutrients on overall or HIV-related mortality, morbidity or disease progression, findings for surrogates of these outcomes were evaluated and are provided below.

Meta-analysis found that macronutrient supplementation regardless of type (with or without nutritional counselling) significantly improved total energy intake (5 trials; weighted mean difference (WMD) 367 kcal/day; 95% CI: 217.1 to 516) and total protein intake (3 trials; WMD 17 g/day; 95% CI: 8.37 to 26.34) compared with no nutritional supplementation or placebo (with or without nutritional counselling). No evidence of an effect on body weight, fat free mass, fat mass, HIV-viral load or $CD4^+$ count was noted in this broad comparison.

More specifically, balanced nutritional supplements (consisting of 50–60% carbohydrate, 15–30% protein and 20–30% fat) aimed at improving energy intake by 600–960 kcal/day, compared with no nutritional supplements, significantly increased energy (4 trials; WMD 407 kcal/day; 95% CI: 250.2 to 563) and protein intake (2 trials; WMD 23 g/day; 95% CI 12.97 to 33.7). In comparison with various nutritional placebos, specific nutritional supplements such as an amino acid mixture containing arginine, glutamine and β-hydroxy-β-methylbutyrate[18], monohydrated L-ornithine α-ketoglutarate[19] and L-glutamine and antioxidant nutrients (ascorbic acid, α-tocopherol, β-carotene, selenium and N-acetyl cysteine)[20] significantly increased body weight (3 trials; WMD 1.93 kg; 95% CI: 0.15 to 3.71) and decreased HIV-viral load (2 trials; WMD −0.45 $\log_{10}$ copies/ml; 95% CI: −0.86 to −0.04) of HIV-infected participants.

As stated by the Cochrane authors, these findings must be interpreted with caution for a number of reasons. First, the studies involved are all relatively small (sample size 128 to 178 participants), raising the possibility that the play of chance may account for the results obtained. There are also concerns regarding the methodological quality of some of the trials, especially in terms of randomisation and exclusions from analysis. Variations in the nutritional composition of active supplements and control interventions across studies, as well as differences in disease stage of the participants in various studies (which can significantly affect intake and absorption of food) further complicate interpretation of the existence evidence.

It is worth noting that patients with opportunistic infections – the category most prone to weight loss and who, in theory, are most likely to benefit – were not assessed in any of the trials. Nonetheless, if the finding of increased energy and protein intake is valid this would be important given that decreased energy intake and accelerated protein turnover is common in people with HIV/AIDS.

The generalisability of the findings of the Cochrane review to subjects in developing countries must also be carefully considered. All of the trials were conducted in the north hemisphere and evaluated males between 30 and 50 years old who were reasonably well nourished in terms of their body mass index and most of whom were receiving antiretroviral therapy. These observations highlight some challenges in applying the trial findings to people in developing countries where nutritional status is generally poor and access to antiretroviral therapy is limited.

Two randomised, placebo-controlled trials that did not meet the inclusion criteria of the Cochrane Review investigated the effect of specific amino acid supplementation on the immune parameters of HIV-infected individuals[21, 22]. The administration of 600 mg of the amino acid N-acetyl cysteine over a period of 7 months resulted in a significant increase in immune function, both in combination with antiretroviral therapy and without antiretroviral therapy in HIV-infected adults with a $CD4^+$ count between 200 and 500, compared with a placebo[21]. A non-significant increase was noted in the supplemented group's viral load, despite the favourable changes in immune parameters. A similar effect was noted in a small pilot study in which participants (N=11, age: ±38, $CD4^+$:376-588) received either 19.6g arginine per day or equivalent placebo tablets, for 14 days. Although none of the changes were significant, the viral load of supplemented participants increased compared with that of the placebo group, despite there being an increase in the supplemented group's natural killer cell activity[22]. Both of these trials were small and the results reported are likely to have occurred due to chance.

In summary, randomised controlled trials conducted in high-income countries provide limited evidence that targeted supplementation of the diet with macronutrients compared with placebo or no supplementation increases protein and energy intake in HIV-infected patients on antiretroviral therapy. However, the effects of nutritional supplementation on mortality, morbidity, body weight and immunological parameters remain unclear.

## Controlled trials with active controls

A number of additional randomised and quasi-randomised, controlled studies have compared two or more oral nutritional supplements with each other. The majority of these studies were conducted in the USA[23–29], with three studies conducted in Africa[30–32] and one study each conducted in Germany[33], Switzerland[34] and Spain[35], respectively. The effects of various nutritional supplements on outcomes such as body weight, body composition, dietary intake, HIV-associated diarrhoea, immune parameters, viral load, quality of life and progression of the disease have been investigated in HIV-infected adults and children. The studies are grouped by intervention investigated and discussed below.





### Balanced nutritional supplements

Three small, randomised trials[23, 24, 35] compared the effects of supplementation with standard nutritional formula (ENSURE®) and a peptide-based formula (ADVERA®) in adults (age: ±38 years, $CD4^+$: 178–561, BMI: ±21), for time periods ranging from 6 weeks[24] to 6 months[23]. The peptide-based formula used has a higher caloric density, 4% more protein and 15% more fat (as a percentage of the total calories), and is enriched with n-3 polyunsaturated fatty acids. Two out of the three studies provided nutritional counselling to all participants[23, 35].

After 6 weeks of supplementation no significant difference was noted in nutrient intake, body weight or body composition between the two groups[24]. However, both nutritional formulas significantly increased total energy (ENSURE® mean difference 15% of needs.day$^{-1}$, $p<0.05$; ADVERA® mean difference 7% of needs.day$^{-1}$, $p<0.05$) and total protein intake (ENSURE® mean difference 15% of needs.day$^{-1}$, $p<0.05$; ADVERA® mean difference 17% of needs.day$^{-1}$, $p<0.05$) from baseline levels.

After three months of supplementation an increase in body weight from baseline values was noted for both formulas (ENSURE® 3.2%, $p<0.05$; ADVERA® 3.1%, $p<0.05$); although this was mainly due to an increase in fat mass[35]. Compared with baseline values, $CD4^+$ count remained stable in the standard formula group and increased significantly in the peptide-based formula group (576±403 vs 642±394 cells.mm$^{-3}$, $p<0.05$)[35].

After 6 months of supplementation, participants receiving the peptide-based formula gained a significantly greater amount of weight compared with those receiving the standard formula (mean difference between the two groups, post intervention = 5.5kg; 95% CI: 4.97 to 6.03). Furthermore, those receiving the peptide-based formula had significantly fewer unscheduled hospitalisations compared with the standard formula group (ADVERA® 0 visits vs ENSURE® 36 visits, $p<0.05$)[23].

### Medium-chain fatty acid-containing triglycerides (MCT) and long-chain fatty acid-containing triglycerides (LCT)

Two small, randomised controlled trials compared the effect of 12 days of supplementation with MCT- and LCT-based formulas on fat absorption and body weight in participants (age: 32–38 years, $CD4^+$: 63–179, BMI: ±19) in the USA[26, 27]. Compared with ingestion of LCT formula, ingestion of MCT formula significantly increased fat absorption, in both studies (4.3±5.8 vs –1.1±5.8 g.day$^{-1}$ fat absorption, $p<0.04$,[26]; 5.4±0.6 vs 12±2.6 g.day$^{-1}$ faecal fat excretion, $p<0.01$,[27]). No significant change in body weight was noted in any of the participants in either of the studies.

A further trial compared the effect of supplements containing either peptides and MCTs or whole proteins and LCTs on body weight and body cell mass of HIV-infected individuals[25]. Throughout the 4 months, both the supplemented groups and the non-

supplemented control groups received a multivitamin and mineral supplement. No differences in percent change in body weight or body cell mass were noted within or between either of the groups.

### Specific amino acids and polyunsaturated fatty acids

In a quasi-randomised, controlled trial, a six-week supplementation with fish oil bars enriched with n-3 polyunsaturated fatty acids, compared with safflower oil supplementation, resulted in a non-significant decrease in $CD4^+$ cell count in HIV-infected individuals[29]. In a randomised, controlled trial, six months supplementation with either a standard nutritional supplement or a standard nutritional supplement enriched with arginine and n-3 polyunsaturated fatty acids resulted in an increase in total energy intake, body weight and fat mass[34]. No change was noted in immune or viral parameter over the six months in either of the groups[34].

In a controlled, crossover phase trial, HIV-infected adults (N=10, age: 44±3.5, $CD4^+$: 286±47, BMI: 22.3±0.8) receiving a standard formula (liquid formula containing 500 kcal/500ml of which 17% protein, 25% fat and 53% carbohydrates) enriched with arginine, α-linoleic acid and ribonucleic acid for 16 weeks gained a significantly greater amount of weight compared with those receiving the standard formula alone (mean difference in weight between the two groups post intervention 3.4kg 95% CI: 1.51 to 5.29)[36]. While no significant changes were noted in circulating lymphocytes, serum concentrations of tumor necrosis factor-alpha receptors, sTNFR 55 (mean difference 0.63 $ng.ml^{-1}$; 95% CI: 0.39 to 0.87) and sTNFR 75 (mean difference 1.26 $ng.ml^{-1}$ 95% CI: 0.48 to 2.04), were significantly elevated following supplementation with the enriched formula compared with the standard formula[36]. While serum concentrations of sTNFR reflect TNF release and levels increase with HIV disease progression, sTNFR also act as neutalising agents limiting the systemic catabolic effects of TNF[36].

In a randomised crossover trial, malnourished (HIV-infected participants (age:37±12 yrs, BMI: 18.6±1.3, $CD4^+$: 176±203) were supplemented with a polymeric diet (consisting of whole proteins rather than specific amino acids) and regular food for two consecutive 45-day periods. Compared with regular food, supplementation with the polymeric diet resulted in significant improvements in body weight (mean difference 2.9kg 95% CI: 0.61 to 5.19) and fat-free mass (mean difference 1.59kg 95% CI: 0.09 to 3.09). No significant changes were noted in $CD4^+$ or $CD8^+$ cell count or plasma albumin levels[37].

### Ready-to-use therapeutic food in African children

The effect of ready-to-use therapeutic food has been investigated in malnourished HIV-infected and non-infected African children. Ready-to-use therapeutic food is an energy-dense liquid paste made from peanut butter, milk powder, oil, sugar, vitamins





and minerals. In a quasi-randomised, controlled trial, ready-to-use therapeutic food (energy content: 175kcal/kg/day) was compared with a ready-to-use therapeutic food supplement (energy content: 500kcal/day) and a blended maize/soy flour mix (energy content: 175kcal/kg/day) in severely malnourished (weight-for-height Z score: ± –1.8 to –2.8) HIV-infected children in Malawi (N=93, age: ±24 to 27 months)[30]. Fifty-six percent of the children reached 100% weight-for-height (by weight-for-height Z score standards based on children's height at admission), with those receiving ready-to-use therapeutic food gaining weight more rapidly and being significantly more likely to reach 100% weight-for-height (RR 1.62 95% CI: 1.01 to 2.59) than those receiving the ready-to-use therapeutic food supplement or the maize/soy flour mix. In an earlier quasi-randomised, controlled trial imported and locally produced ready-to-use therapeutic food was similarly effective in treating severe malnutrition (weight-for-height Z score: ± –2.1 to –2.2) in HIV-infected (N=78) and non-infected children (N=182)[32].

In Zambia, 4 weeks supplementation with infant formula feed (Neocate, SHS International, Liverpool, United Kingdom) containing amino acids, maltodextrin and a combination of safflower oil, coconut oil and soya oil (energy content: 70 kcal/100ml; vitamin and mineral content similar to that of breastmilk) resulted in significantly greater weight gain (weight-for-height Z score) in malnourished HIV-infected (N=106; mean difference 1.0 95% CI: 0.87 to 1.13) and non-infected children (N=90; mean difference 0.39 95% CI: 0.28 to 0.5), compared with those in the control group[31]. The control group received a liquid feed containing a mixture of skimmed milk, sugar and vegetable oil (energy content: 100kcal/100ml; vitamin and mineral content not specified) for the first two weeks, following which they were fed a high-energy protein porridge (energy content: 400kcal/100ml).

## Intervention trials without controls

Increased cytokine activity is believed to be responsible for the metabolic disturbances and subsequent weight loss associated with HIV infection. Dietary n-3 polyunsaturated fatty acids have been proposed as potential inhibitors of the cytokine-mediated weight loss. In one study, however supplementation with dietary fish oil (18g/day of MaxEPA), typically rich in n-3 polyunsaturated fatty acids, for 10 weeks did not alter food intake, body weight or body composition in stable AIDS patients (N=20, age: 41±2, BMI: 21±2, $CD4^+$: 188±74)[38].

Stack *et al.* (1996)[39] investigated the effect of high-energy, high-protein oral, liquid, nutritional supplementation and nutritional counseling, administered for 6 weeks, on the body weight of HIV-infected adults with or without secondary infections (N=17, age: 38±9, BMI: ±21, $CD4^+$: 99±110). The intervention either improved (10 patients; mean weight gain ± SD=2.9±1.2) or maintained (2 patients) the body weight of most

participants. Four out of the five participants who lost weight had developed secondary infections after enrollment in the study.

In a group of HIV-infected outpatients (N=54) receiving intensified oral nutritional intervention, weight maintenance and weight gain was achieved in 57% and 33% of the group, respectively[40]. A 3-month supplementation with a polymeric (whole protein-based) diet, designed to provide 1000–1500 kcal/day, did not have any effect on the body weight of malnourished, clinically stable HIV-infected individuals (N=34)[41]. Sixty-six percent of the participants were lost to follow up, primarily due to intolerance of the diet.

## Cross-sectional studies

Weight loss of greater than 5% body weight[41] and a loss of body cell mass greater than 30%[42] are both associated with disease progression and mortality in HIV-infected individuals. Cross-sectional studies, however investigating the relationship between energy intake and weight loss in HIV-infected individuals have shown varying results. For instance, a significant positive correlation between energy intake ($r=0.67$, $p<0.01$) and weight ($r=0.48$, $p<0.01$) and $CD4^+$ count in HIV-infected men (N=35) was noted indicating that a decline in weight and energy intake is associated with HIV/AIDS disease progression[43]. In contrast, Woods *et al*, 2002[44] reported a significant inverse relationship between energy intake and $CD4^+$ cell count in a large cohort of HIV-infected men and women (N=516; $r= -0.16$, $p=0.001$): men and women with the lowest $CD4^+$ counts ($<200/mm^3$) had the highest energy intake. Despite their increased energy intake, this group had the lowest body weight and body mass index (BMI) compared with that of the higher $CD4^+$ count categories. The group may have increased energy intake in an attempt to compensate for this weight loss. An increase in viral load or the presence of an opportunistic infection may explain the decrease in $CD4^+$ cell count. Furthermore, the therapeutic regime of the participants was not reported. Owing to the numerous unknowns and confounders characteristic of cross-sectional study designs, these findings must be interpreted with caution. Luder *et al.* 1995[45] found no correlation between weight loss and nutrient intake, $CD4^+$ cell count or absolute lymphocyte count in HIV-infected individuals (N=56). The major discrepancies of the above studies may be due to the limitations of cross-sectional study design (see Chapter 4 on Evidence-based policy and recommendations).

In a cross-sectional study of weight-stable HIV-infected men (N=467, age: 40±8.2, $CD4^+$: 104±58), body weight, height, muscle-building activity and protein intake were all positively correlated with body cell mass ($p<0.001$ for all)[46]. Age, number of AIDS-related disease events and carbohydrate intake had a significantly negative correlation with body cell mass ($p<0.05$ for all). This finding suggests that HIV-infected individuals might benefit from a high-protein diet by minimising the loss of body cell mass.





## Cohort studies

Cohort studies investigating the relationship between energy intake and weight loss in HIV-infected individuals have also shown conflicting results. Some studies failed to show a correlation between HIV-associated weight loss and energy intake[47-49], while other studies reported a significant inverse correlation between weight loss and energy intake[7, 15].

The relative contribution of energy intake and malabsorption on body weight loss in HIV-infected individuals was assessed by comparing the oral intake and malabsorption in HIV-infected men and women (with or without chronic diarrhoea) with HIV-negative individuals with chronic malabsorption due to small-bowel resection or disease[50]. When the level of malabsorption was controlled for, HIV-infected individuals had a significantly lower energy intake compared with individuals with chronic malabsorption ($p<0.02$). Furthermore, only in the HIV-infected group was body mass index significantly correlated with energy intake ($r=0.33$, 95% CI: 0.12 to 0.51). These results reiterate the importance of energy intake in HIV-associated weight loss and wasting.

A small cohort of clinically stable AIDS patients (N=5) demonstrated that progressive wasting is not an inevitable consequence of AIDS. By maintaining an adequate energy intake (comparable with that of HIV-negative individuals, N=11) and by down-regulating basal metabolic processes (primarily by decreasing physical activity) this group maintained body cell mass and remained weight-stable for 6 weeks[51]. In a retrospective cohort study, nutritional intervention consisting of dietary assessment, intake analysis, appropriate counselling, follow-up and the provision of supplements as needed, significantly increased body weight gain in a greater proportion of participants than in those who did not receive the intervention*. These results of an increased change in body weight must be interpreted with caution, however, as the initial mean body weight of each group was not presented by the investigators.

## Conclusions and recommendations

### Relevant to policy:

I. In people infected with HIV, weight loss and loss of body cell mass are strong predictors of poor prognosis. These features are associated with increased resting

* Mahlungulu S, Grobler LA, Visser MME, Volmink J. Nutritional Interventions for reducing morbidity and mortality in HIV infected individuals (Cochrane review in press)[53-57].

energy expenditure, accelerated protein turnover, decreased energy intake, diarrhoea and malabsorption.

II. There is limited evidence from randomised, placebo-controlled trials that macronutrient supplementation is of benefit in HIV-infected individuals. Targeted interventions with balanced nutritional supplements seem to increase energy and protein intake. There is also preliminary evidence that specific dietary supplements, such as amino acid mixtures, increase body weight and reduce HIV viral load.

III. Evidence from small trials comparing two or more nutritional supplements suggests that balanced supplementation increase body weight and that supplementation with medium-chain triglycerides is more effective than long chain triglycerides in reducing HIV-associated intestinal dysfunction and fat malabsorption. Supplementation with a whole-protein diet has been found to increase body weight and fat-free mass in HIV-infected adults. Specific amino acids and polyunsaturated fatty acids may increase energy intake, body weight and fat-free mass. Finally, ready-to-use therapeutic food is effective in reversing the poor nutritional status found in severely malnourished HIV-infected and non-infected African children.

IV. Evidence-based advice on the use of macronutrient supplementation in HIV-infected individuals in developing countries is constrained by the fact that the few randomised trials that exist have mainly been conducted in high-income countries where most patients are well nourished and have access to life-prolonging antiretroviral therapy. Further, existing trials have focused on intermediate endpoints (such as energy intake) and most have been too small to assess important clinical outcomes (such as death and morbidity). There are also substantial variations in the nutritional composition of the experimental and control interventions, the use of dietary counselling, disease stage and treatment status of the participants across studies.

**Relevant for research:**

I. High-quality, adequately powered randomised controlled trials investigating the effects of macronutrient interventions on important clinical outcomes such as morbidity and mortality in HIV-infected individuals in developing countries are urgently needed.

II. Interventions should be well defined and directed at specific target populations defined by age (adults and children), $CD4^+$ cell count, viral load, treatment status (presence and absence of treatment; type of antiretroviral therapy) and baseline nutritional status (undernourished, adequately nourished or overnourished).

III. A multi-disciplinary, collaborative approach will ensure that nutritional findings from basic science research are evaluated in well-targeted clinical studies and that effective interventions are implemented with minimum delay.



CHAPTER 10

# The effects of nutritional interventions in HIV/AIDS: Micronutrients

More than 60% of the estimated 43 million persons with HIV/AIDS worldwide live in sub-Saharan Africa, where poverty, social insecurity, food shortages and malnutrition are major problems. In 2005, an estimated 3.2 million people in the region were newly infected with HIV, while 2.4 million adults and children died of AIDS[1]. In children under the age of 5 years who live in developing countries, malnutrition has been associated with half of the 10.8 million deaths caused mainly by neonatal disorders, diarrhoea, pneumonia, malaria and HIV/AIDS[2]. Micronutrient deficiencies are widespread and are associated with increased morbidity and mortality, particularly in relation to infectious diseases[3]. This review focuses on the interaction between micronutrients and HIV/AIDS and discusses recent research findings that may have important public health implications for the management of the HIV/AIDS pandemic.

## Importance of micronutrients in general

Micronutrients are vitamins and trace elements and essential to all microorganisms, plants and animals (see Chapter 4). They play an important role in gene expression, cellular differentiation and immune function. In addition, many exhibit anti-oxidant properties. Micronutrients are therefore essential for human health and development.

Of all the micronutrients, zinc and vitamin A deserve special mention. Deficiencies of both are widespread in developing countries and contribute to the morbidity and mortality from infectious diseases.

A pooled analysis of randomised placebo-controlled trials has shown that prophylactic zinc supplementation in children was associated with a 41% reduction in the incidence of pneumonia and an 18% reduction in the incidence of diarrhoeal disease[4]. Zinc also has a therapeutic benefit in sick children through promoting recovery from acute and persistent diarrhoea, and from severe pneumonia[5].

Community-based studies conducted in various developing countries have demonstrated that periodic vitamin A supplementation (in most instances 6-monthly)

or fortification reduces all cause child mortality by at least 23% in children aged 6–60 months[6]. In addition, vitamin A supplementation in children hospitalised with severe measles significantly reduced mortality rates[7].

Effective and sustained control of micronutrient deficiencies, in particular vitamin A deficiency and zinc deficiency, have the potential to be among the most cost-effective and efficacious child-survival interventions in sub-Saharan Africa[8]. These interventions could make a large contribution toward the attainment of the Millennium Development Goal for the reduction of child mortality rates by two-thirds between 1990 and 2015.

## Micronutrients and HIV/AIDS

The hallmark of AIDS is immune suppression, resulting in acute, recurrent and chronic opportunistic infections. While HIV is the main cause for the immune suppression, other factors may contribute. These include concomitant malnutrition, including micronutrient deficiencies, and opportunistic infections, in particular tuberculosis.

Micronutrient deficiencies are common in persons with HIV infection and AIDS in both developing and developed countries[9]. They occur as a consequence of a number of factors, including reduced intake as a result of the anorexia associated with AIDS and opportunistic infections, and excessive losses in the stools in patients with diarrhoea, malabsorption and parasitic infestations[10]. These deficiencies are more pronounced in individuals with advanced disease and in situations where diets are inadequate in meeting the recommended daily requirements of micronutrients.

Recent studies have highlighted significant multiple micronutrient deficiencies in South Africans. Sixty-two percent of stable, HIV-infected children in Cape Town had two or more trace element or vitamin deficiencies; most were vitamin A deficient[11]. Another study reported significant vitamin A and zinc deficiency in adults. Levels tended to be lower in individuals with stage 3 and 4 disease, and there was a positive correlation with $CD4^+$ counts[12].

Micronutrients are essential to immune function and deficiencies may therefore act as cofactors in HIV disease transmission and progression. Deficiencies of vitamins A, $B_6$, $B_{12}$, C and E, beta-carotene, zinc, copper, selenium, magnesium and iron have all been reported to occur in association with HIV infection[13–15].

Several observational studies have shown an inverse correlation between micronutrient intake (including vitamins B and C) or multivitamin supplementation and clinical progression of HIV disease in adults[16–20]. Higher intakes of niacin, and vitamins $B_1$, $B_2$ and $B_6$ were associated with a significant 40–48% slower progression to AIDS[17] and 40–60% significant reductions in the risk of death after 8 years of follow-up[18]. Among South African men and women, daily supplementation with B-complex vitamins was





associated with lower risks of disease progression (median duration to AIDS: 72.7 vs. 32.0 wks; p=0.004; median survival: 264.6 vs. 144.8 wks; p=0.001)[20].

Dietary and supplement intake of **vitamin A** between 9000 and 20 000 IU per day in HIV-infected American men was associated with lower risk of disease progression compared with intakes below 9000 IU/day (RR=0.55; 95% CI: 0.35–0.88)[17]. Vitamin A deficiency has been associated with increased morbidity and mortality in children[21], with increased mother-to-child transmission of HIV-1[22] and subsequent infant mortality[23], and increased mortality in HIV-infected injection drug users[24].

In the United States, daily multivitamin use among 296 HIV-infected men was associated with a 30% reduction in progression to clinical AIDS (RR=0.69; 95% CI: 0.46 mother-to-child 1.03), and a significantly reduced risk for low CD4 counts (RR=0.6, 95% CI=0.4, 0.9)[16].

Excessive **zinc** losses occur in diarrhoeal disease leading to loss of the gastrointestinal epithelial integrity and absorptive power[25]. Another consequence of zinc deficiency is impaired thymolymphoid integrity and reversible immune dysfunction, particularly of T-lymphocyte cell-mediated immunity, and hence deficiency may contribute to infectious morbidity. Zinc deficiency has been associated with increased mortality in a cohort of HIV-infected American men[26]. In a prospective cohort study of 281 asymptomatic HIV-infected men in the US however, increased dietary zinc intake was associated with faster HIV/AIDS disease progression (RR for highest vs. lowest quartiles = 2.06, 95% CI 1.16–3.64)[17] and higher mortality (RR for any zinc vs. none = 1.49; 95% CI 1.02–2.18)[18]. Conversely, a nested case-control study within the same cohort found that lower serum zinc predicted disease progression independently of baseline $CD4^+$ level, age, and calorie-adjusted dietary intakes of copper and zinc[26].

**Selenium** deficiency is associated with reduced immune function in animal studies[27]. Low plasma selenium levels have been described early in the disease process and are associated with faster disease progression and higher mortality in HIV-infected children[28], and higher mortality and risk of TB in drug users[29, 30]. Among HIV-1-infected pregnant women, each 0.1 µmol/l increase in plasma selenium was related to a 5% (95% CI: 0%–9%) decreased risk of mortality[31].

The contribution of copper and manganese to immune function is not clear, but children with overt copper deficiency (Menkes' syndrome) are prone to infection[32]. The equilibrium in iron homeostasis is delicate; both deficiency and excess negatively affect immune function. As in other groups of HIV-infected subjects, anaemia is a common finding in South African HIV-infected children[33] and may enhance disease progression and increase mortality[34]. Although iron supplementation is recommended for preventing and treating anaemia in young children (0–59 months), there is no experimental evidence for HIV-infected children and very little for HIV-infected adults[35].

Inference from the above studies that micronutrient supplementation is beneficial for reducing HIV transmission or disease progression is problematic, however. In many observational studies valid markers of micronutrient status are lacking, especially in individuals with infections. Furthermore, confounding by other micronutrients is likely, as most studies only assess the status of one or a few micronutrients even though micronutrient deficiencies usually coexist. Studies assessing micronutrient intake, rather than status, may therefore be better able to account for multiple micronutrient effects[36].

## Effects of micronutrients supplemented in HIV/AIDS

Recent systematic reviews of randomised controlled trials published in The Cochrane Library have evaluated the evidence for the effectiveness and safety of micronutrient supplements in HIV-infected children and adults, and of vitamin A for reducing the risk of mother-to-child transmission (MTCT) of HIV. Irlam *et al.*[37] appraised and summarised the evidence from 18 trials of single and multiple micronutrient supplements in adults, children, and pregnant or lactating women, but did not perform a meta-analysis due to substantial clinical heterogeneity across the trials. Additionally, a systematic review and meta-analysis by Shey Wiysonge *et al.*[38] included four trials in HIV-infected pregnant women. We draw extensively on the findings of these Cochrane reviews and provide supplementary evidence from studies not included in the reviews.

### Single micronutrient supplements

Five small randomised, placebo-controlled trials (sample size 21 to 120 participants) of vitamin A or beta-carotene in adults[39–43] and one moderately sized trial of vitamin A (n=400 women)[44] found no significant benefits or adverse effects on morbidity (HIV-associated and AIDS-defining infections), mortality, viral load, or immunological markers. A small Canadian trial of large doses of daily vitamin C (1000mg) and vitamin E (800 IU) for three months showed a non-significant reduction in mean plasma viral load (–0.45 log) versus placebo (0.5 log increase)[45].

A meta-analysis of four randomised placebo-controlled trials of vitamin A supplementation in HIV-infected pregnant women in Africa found no evidence of an overall effect of vitamin A supplementation on MTCT of HIV in 3 of the trials (2022 participants; OR 1.14, 95% CI 0.93 to 1.38)[38]. A trial in Tanzania, however showed an increased risk of MTCT (OR 1.53, 95% CI 1.15 to 2.04 at 24 months)[46]. The subsequently-published results from the fourth trial, in Zimbabwe (ZVITAMBO), demonstrated no effect of either maternal or neonatal supplementation on post-natal MTCT[47].

The ZVITAMBO trial used a factorial design to compare the effect of four regimens of single-large-dose supplements of postpartum maternal (400 000 IU)/ infant (50 000 IU)





vitamin A/ placebo among 14110 mother-infant pairs. There was no significant effect on overall mortality between baseline and 24 months, but there was a reduction of 28% in supplemented infants who were HIV-uninfected at baseline but HIV-infected at 6 weeks. All three vitamin A regimens were associated with an approximate doubling of mortality in infants who were HIV-uninfected at 6 weeks ($p<=0.05$)[47].

In two randomised trials in HIV-infected children high-dose vitamin A supplementation (200 000 IU on two successive days) compared with placebo improved immune function (a 16% increase in $CD4^+$ counts, $p=0.03$)[48], and reduced immune activation following influenza vaccination (viral load mean change at 14 days: vit. A: –0.13 +/- 0.09 log copies/ml vs. placebo: +0.14 +/-0.08; $p=0.02$)[49]. Intermittent supplementation with vitamin A in a placebo-controlled trial in Durban, South Africa, halved diarrhoeal disease morbidity (OR=0.51; 95% CI: 0.27, 0.99) in a subgroup of 28 children infected with HIV[50]. High-dose vitamin A supplements (50 000 IU before 6 months, 100 000 IU between 6 and 11 months, and 200 000 IU every six months from age 1 to 5 years) given 6-monthly compared with placebo reduced all-cause mortality over two years in an HIV-infected subgroup (n=58) of children hospitalised with pneumonia in a Tanzanian trial (RR=0.37; 95% CI: 0.17, 0.84)[51]. A regimen of vitamin A (60mg retinol equivalent) every 3 months from 15 to 36 months among 181 Ugandan children halved mortality (OR=0.54; 95% CI: 0.30, 0.98) compared to placebo[52].

Zinc supplementation in a trial of HIV-infected adults on ART has shown potential benefit in reducing candida and pneumocystis-related infections, increasing $CD4^+$ counts ($p<0.05$), and improving or stabilising body weight ($p<0.01$), without any adverse effects[53]. This trial did not use a random method of allocation however, and was therefore excluded from the review by Irlam *et al.*[37]

Supplementation with zinc for 6 months in a randomised trial of 120 HIV-infected children in Durban, South Africa, was safe and reduced the incidence of acute diarrhoea diagnosed at clinic visits (RR = 0.51; $p=0.001$)[54]. In another trial among HIV-infected South African children in Cape Town, 3mg of zinc daily for 6 months reduced the frequency of both acute respiratory tract infections and episodes of diarrhoea. The effect of zinc on diarrhoea and acute respiratory tract infections was greater than that of a multiple micronutrient mixture, which was itself no better than placebo (unpublished data, Heloise Buys 2006). A placebo-controlled trial in 159 Peruvian adults with HIV and persistent diarrhoea found no effect of dietary zinc supplementation on the persistence and severity of diarrhoea at two weeks[55].

A randomised trial of zinc supplementation compared with placebo given to Tanzanian women daily from 12 to 27 weeks of gestation until 6 weeks after delivery demonstrated no effect on pregnancy outcomes or immunological indicators[56]. In this study there was a rise from baseline to 6 weeks postpartum in haemoglobin, red blood

cell count and packed cell volume in women in both groups, but these increases were all significantly lower in the group receiving zinc group than in those on placebo.

In a further placebo-controlled trial, daily selenium supplements of 200 μg for 12 months among 186 drug users decreased the risk of CD4⁺ counts declining below 50 cells/μl (p=0.01), and reduced the hospitalisation rate for opportunistic infections, HIV-related conditions and psychiatric disorders combined (overall RR=0.40; 95% CI: 0.21–0.75)[57]. Hurwitz *et al.* evaluated a similar regimen for 9 months in a placebo-controlled trial of 262 HIV-infected adults, and found that greater serum levels predicted decreased type 1 viral load ( p<0.02), which predicted increased CD4⁺ count (p<0.04)[58].

### Multiple micronutrient supplements

A randomised placebo-controlled trial of high doses of vitamins A, C and E, zinc, and selenium daily for 2 weeks in Zambian adults showed no effect on the response to albendazole of persistent diarrhoea[59]. The patients had advanced HIV disease with diarrhoea-wasting syndrome, however, and absorption of the micronutrients may therefore have been inadequate.

In Thailand a randomised trial in 481 HIV-infected outpatients of a commercial supplement containing 18 micronutrients and taken twice daily for 48 weeks was associated with a non-significant reduction in mortality compared with placebo, which was most pronounced in the subgroup of 96 participants with a low CD4⁺ count (<200 cells/mm$^3$) (HR=0.37; 95% CI: 0.13, 1.06)[60]. There were no effects on CD4⁺ count or viral load however, suggesting that the reductions in mortality could be due to reductions in the risks of other infections or the maintenance of lean body mass[36].

A large randomised factorial-design trial among 1078 pregnant and lactating Tanzanian women found that high-dose multivitamins (mainly vitamins B, C and E and excluding vitamin A) reduced the risk of progression to stage 4 disease or AIDS-related mortality by 29% over the entire 4–8 year supplementation and follow-up period (RR = 0.71; 95% CI: 0.51 to 0.98) compared with no multivitamins (vitamin A only or placebo). Mean CD4⁺ counts were significantly higher by 48 cells/mm$^3$ (95% CI: 10 to 85) in the multivitamin-supplemented versus placebo group, and viral load significantly lower (–0.18 log; 95%CI: –0.32 to –0.03)[61], which probably also accounted for the reductions in episodes of HIV-related morbidity. Vitamin A alone had no effect on the above outcomes. Multivitamins improved weight gain during pregnancy (mean difference during the third trimester = 304 g; 95% CI: 17 to 590)[62], and reduced the risk of adverse pregnancy outcomes, including foetal loss (RR=0.61; 95% CI: 0.39 to 0.94), prematurity (RR=0.61; 95% CI: 0.38 to 0.95 for severe preterm births), and low birth weight (RR=0.55; 95% CI: 0.38 to 0.81)[63]. Children born to mothers receiving multivitamin supplementation during pregnancy had higher CD4 counts (mean difference = 153 cells/μl; 95% CI: 67.6 to 238.4) and less diarrhoea (RR=0.83; 95% CI: 0.71 to 0.98 at 24 months)[64], as well as improved growth during the first two years of life (weight-for-age: mean difference = 0.42; 95% CI 0.07 to 0.77)[65] compared with placebo. Mortality was reduced among the children of supplemented women with low lymphocyte counts (<1340/ mm$^3$) (RR=0.30; 95% CI: 0.1 to 0.92 at 24 months)[46].

A randomised, placebo-controlled trial among Zimbabwean women found that multimicronutrient supplementation was associated with higher birth weight overall (49 g; 95% CI: –6 to 104). The effect was greater for the third of the population that was HIV-infected than among the HIV-negative women, although the interaction was not significant[66].

## Potential adverse effects of micronutrient supplements

Vitamin A supplementation has been associated with a 50% increased risk of HIV vertical transmission in a Tanzanian trial in pregnant and lactating women, who were randomly assigned to vitamin A or multivitamins excluding vitamin A[46], and a reduction in the benefits of micronutrient supplementation[61]. The findings of a more recent Zimbabwean trial of vitamin A in mother-infant pairs, which showed higher mortality in infants who were HIV-negative at 6 weeks, has raised further concerns about the safety of universal maternal or neonatal vitamin A supplementation in HIV-endemic areas[47].

In general high-dose vitamin A supplements are safe in children. Minor adverse effects have included vomiting and bulging fontanelle in children born to HIV-infected women[50], and a risk of acute diarrhoea in normally nourished (RR=1.37; 95% CI: 1.06–1.79) and in growth-stunted children (RR=1.84; 95% CI: 1.16–2.90) hospitalised with pneumonia[51]. Modest adverse effects after 3 days have been reported in HIV-uninfected children recovering from pneumonia[67]. In this randomised placebo-controlled trial, children receiving vitamin A (n=48) had lower blood oxygen saturation, higher prevalence rates of retractions, and auscultatory evidence of consolidation, and were more likely to require supplemental oxygen than children in the placebo group (n=47). No differences were seen in duration of hospitalisation or in chest x-ray changes 14 days after admission. No deaths occurred, and toxicity of vitamin A was not seen. These findings have not been reported in clinical trials of vitamin A therapy in HIV-infected children, however.

Experimental data suggest that vitamin A increases viral replication *in vitro*[68], which provides a rationale for the adverse effects reported in these studies. It is reassuring to note, however, that a number of clinical studies comparing vitamin A with placebo that have monitored viral load and CD4⁺ counts have not demonstrated such an effect[49, 43, 69]. The presence of effect modifiers such as iron supplementation, malaria treatment

and prophylaxis, dietary intake of other nutrients, and other infections has been suggested[36] as an explanation for the increased risk of MTCT associated with vitamin A supplementation in the Tanzanian trial.

There is also a concern that zinc may potentiate HIV replication, since the HIV-Tat protein and the HIV nucleocapsid NCp7 proteins are strongly zinc-dependent[70]. Evidence from one observational study suggested that high-dose zinc supplementation was associated with increased HIV/AIDS disease progression and mortality in HIV-infected adults[18]. Two studies in children demonstrated no adverse effects in terms of increase in viral load or reduction in $CD4^+$ counts, however[54] (unpublished data, Heloise Buys, 2006).

## Conclusions and recommendations

### Relevant to policy:

I. Micronutrient deficiencies are common in people with HIV/AIDS and are more pronounced in individuals with advanced disease and in those with inadequate diets. Such deficiencies may hasten disease progression, increase mortality, and facilitate MTCT of HIV.
II. Observational studies have shown a direct correlation between micronutrient intake (especially vitamins A and B, multivitamins, zinc and selenium) and favourable clinical outcomes in patients with HIV infection. However, observational studies lack valid markers of micronutrient status, and the effects of micronutrient deficiencies are prone to confounding by other factors, including micronutrient interaction.
III. Based on limited evidence from randomised trials, supplementation with vitamin A/beta-carotene does not seem to have any significant beneficial or adverse clinical effects in HIV-infected non-pregnant adults. In HIV-infected children receiving vitamin supplements, reductions in morbidity and mortality have been reported, but these conclusions are based either on small trials or subgroup analyses. Vitamin A possibly increases the risk of vertical transmission in HIV-infected pregnant and lactating women, and the risk of mortality in infants of supplemented mother-infant pairs.
IV. The concerns about the safety of universal maternal and neonatal supplementation in HIV-endemic areas may have important policy implications for South Africa.
V. There is sound evidence that multivitamin supplementation (excluding vitamin A) in HIV-infected pregnant women reduces the risk of disease progression, AIDS-related mortality and adverse pregnancy outcomes.
VI. Results derived from a few small trials indicate that zinc supplements given to HIV-infected children are safe and effective in reducing morbidity, but zinc supplementation in HIV-infected pregnant women seems to have no benefit and may be harmful to the women.
VII. Finally, the evidence-base on the effects of micronutrient supplementation in people with HIV is remarkably limited. It seems reasonable at this stage to support the recommendations of the WHO that everything possible should be done to promote and support adequate dietary intake of micronutrients at Individual Nutrient Intake Level (INL98) levels[71], while recognising that this may not be sufficient to correct nutritional deficiencies in all HIV-infected individuals. In situations where micronutrient deficiencies are endemic, these nutrients should be provided through food fortification or micronutrient supplements where available that contain at least 1–2 INL98s.

### Relevant to research:

I. There is a critical need for adequately powered studies to answer questions related to the efficacy and safety of micronutrient supplements in people with HIV infection in both the medium and long term. Apart from vitamins, attention should be given to other promising interventions, such as zinc and selenium.
II. Research should focus on both asymptomatic HIV-infected individuals and those with more advanced disease (with and without ART therapy) and should take into account the special needs of children and adults, including pregnant women. It is important to determine how the effects of micronutrients in immunocompromised individuals differ from those in people with normal immune function
III. Future research should determine whether HAART initiation restores micronutrient concentrations, independent of inflammatory markers, and whether micronutrient supplements affect HIV-related outcomes in HIV-infected persons receiving HAART[72]
IV. The optimal composition and dosage of various supplements requires investigation, as preparations can vary considerably and may not have equivalent effects.
V. All nutritional interventions to improve the health and well-being of persons living with HIV/AIDS need to be optimised and research into identifying optimal interventions and operational strategies is therefore required. Such research should not be to the detriment of antiretroviral treatment, as this remains the one intervention to date that has consistently been shown to reduce morbidity and mortality associated with HIV/AIDS.

CHAPTER 11

# The influence of nutrition on the risk and outcomes of tuberculosis

This chapter critically reviews the scientific data supporting the contention that malnutrition is an important risk factor for TB, and that nutritional intervention in conjunction with appropriate chemotherapy may contribute to improved clinical outcomes in malnourished patients. It focuses principally on published observations in humans, with occasional reference to appropriate studies conducted in highly relevant animal models.

## Introduction

*Mycobacterium tuberculosis* is a highly evolved human pathogen. Over millennia, this pathogen and its human host have adapted to each other to an astonishing degree, resulting in an impasse in which the organism can reside for decades within the tissues of most infected individuals[1]. While the cellular and molecular determinants of this stand-off have not been elucidated, it is clear that a successful immune response is able to keep the microbe in check in most individuals, albeit without eliminating it completely[2]. Any immunosuppressive condition, such as HIV infection, malnutrition, aging, etc., may tip the balance in favor of the pathogen, resulting in reactivation tuberculosis (TB)[3]. Nutritional deficiencies, in particular, are known to affect adversely precisely those immunological mechanisms that are crucial for successful control of mycobacteria, namely the functions of T-lymphocytes and a variety of phagocytic cells[4]. This chapter therefore focuses on the relationship between malnutrition and TB.

*A priori*, one could hypothesise several ways in which nutritional deficiencies could affect the prevention and management of TB. Malnutrition has cognitive and behavioural consequences that could increase the risk of primary infection, i.e. consequences that would respond to nutritional rehabilitation – apathy, intellectual impairment, inactivity, etc. Therefore, while we tend to concentrate on the molecular and cellular mechanisms by which malnutrition can affect the risk of TB, we must also keep in mind that malnutrition may affect TB risk in other ways.

Theoretically, malnourished individuals might exhibit increased susceptibility to primary, pulmonary infection with *M. tuberculosis*. It is clear that not all individuals exposed to an infectious case of TB (e.g. household contacts) become infected as indicated by a conversion of their tuberculin (purified protein derivative, PPD) skin test to positive, raising the possibility that innate mechanisms of resistance may be impaired by nutritional insufficiency[5]. Innate resistance may involve the enhanced anti-mycobacterial functions of alveolar macrophages or lung dendritic cells which are activated via Toll-like or other pathogen receptors[6] and which could, at least theoretically, be impacted by nutrient deficiencies. However, there is little evidence that the intrinsic susceptibility to infection is altered by nutritional status. Evidence to support the contention that malnutrition may not affect the establishment of primary infection comes from studies of chronically protein-deficient guinea pigs that demonstrated no alteration in the number of tubercles resulting from low-dose aerosol exposure to virulent *M. tuberculosis*: the same number of inhaled, retained bacilli resulted in pulmonary granulomas in both well-nourished and malnourished guinea pigs[7]. This conclusion must be tempered by the fact that malnourished individuals are more likely to be anergic. PPD skin test reactivity is likely to underestimate their prevalence of infection.

In populations residing in countries with endemic bovine tuberculosis, malnutrition may affect the risk of gastrointestinal tuberculosis following the consumption of unpasteurised dairy products. Deficiencies of protein and calories have a profound impact on the gastrointestinal mucosa, e.g. flattening of microvilli, atrophy of Peyer's patches, which could facilitate mycobacterial invasion of the mucosal barrier and increased risk of peritoneal TB, scrofula, mycobacteraemia and disseminated TB. This is another condition that would respond to nutritional rehabilitation.

Another way in which malnutrition could alter the pathogenesis of TB would be to increase the risk of progression from infection to primary disease in the short term, or to increase the risk of reactivation disease in the long term. This aspect of pathogenesis appears to be most profoundly affected by nutritional deficiencies. The progression from infection to disease is prevented in most healthy individuals by a successful adaptive immune response involving cooperation between populations of T lymphocytes and phagocytes[8]. Malnutrition is known to impair precisely these immune responses[4], hence allowing sub-clinical infection to develop into full-blown clinical illness. Several epidemiological studies relating specific nutrient deficiencies to clinical tuberculosis are reviewed below.

Nutritional status also may affect progression from TB infection to disease by altering the availability of essential nutrients to meet the metabolic requirements of the pathogen as well as the person. For example, increased severity of TB (and HIV) has been observed in persons with hemochromatosis due to drinking traditional beer fermented in iron





vessels[9]. *M. tuberculosis* requires iron and obtains it within the host by means of various iron scavenging mechanisms that must compete with highly avid host iron-binding proteins (e.g. lactoferrin, transferrin) to be effective[10, 11]. Depending upon the relative availability of iron to the pathogen and host, the effect of iron deficiency or excess may be beneficial for the microbe or the patient. Iron chelation therapy has even been suggested for TB patients and HIV-infected individuals with iron overload[12]. Although not as well studied, the consequences of the availability to the pathogen of other essential nutrients in a malnourished individual could provide a non-immunological mechanism by which nutritional deficiencies alter progression from infection to disease in TB.

Still another way in which nutritional deficiencies could impact on TB is by interfering with appropriate chemotherapy in diseased individuals. Current anti-mycobacterial drug regimens are highly effective if given properly to individuals infected with drug-sensitive strains of *M.tuberculosis*[13]. However, concurrent malnutrition could blunt the effects of these antibiotics, which must be administered for several months to cure the patient. Since malnutrition-induced loss of some immune functions is reversed rapidly upon correction of the nutritional deficiency[14], nutritional intervention in conjunction with appropriate chemotherapy could improve the outcome in malnourished TB patients. Several intervention trials have been conducted and are summarised below.

Finally, malnutrition could interfere with the protective efficacy of BCG vaccine, thus increasing the disease burden in vaccinated populations suffering from nutritional deficiencies. BCG vaccine is thought to work by inducing the same array of protective T lymphocyte responses which are responsible for preventing the progression from infection to disease in non-vaccinated individuals[15, 16]. Nutrient deficiencies could therefore also impact on vaccine-induced immunity. Unfortunately, no clinical studies of the effect of nutrient deprivation on BCG vaccine efficacy have been conducted. Most of the information related to the loss of BCG-induced protection in malnutrition comes from experimental animal studies[17], reviewed below.

## Tuberculosis causes malnutrition

A clear understanding of the relationship between malnutrition and TB, particularly in establishing a cause-and-effect link between nutrient deficiencies and clinical disease, is complicated by the so-called "chicken *vs* egg" conundrum. Malnutrition may predispose to TB; however, TB also causes malnutrition. Some of the cardinal signs and symptoms of TB (e.g. wasting, anemia, loss of lean and fat mass, etc) are also signs of malnutrition. Patients with concurrent HIV infection tend to be even more malnourished[18]. Many cross-sectional and case-control studies of nutritional status in TB patients are therefore confounded by the fact that some of the nutritional aberrations observed are the result of,

rather than the cause of, the mycobacterial disease[19, 20]. Tuberculosis results in anorexia, cachexia, and asthenia produced, in part, by the fever and other consequences of exorbitant production of tumour necrosis factor-α (TNF–α) and other pro-inflammatory cytokines produced by a well-intentioned immune system in the face of persistent infection with *M. tuberculosis*[21]. In addition, TB is often associated with other co-morbid conditions that also affect nutritional status and disease risk, such as HIV, diabetes mellitus[22] and alcoholism[23]. The challenge of distinguishing between predisposing nutritional deficiencies and disease-induced malnutrition is therefore formidable.

Nutritional deficiencies are thus generally associated with increased risk and severity of tuberculosis.

## Ecological studies

Research on the interaction of nutrition and TB before 1950 was reviewed by Rich[24] and in the exhaustive treatise by Scrimshaw, Taylor, and Gordon published in 1968[25]. Many of the early studies in humans had flaws in sampling, sample size, measurement and definition of nutritional deficiency, assessment of infection, unmeasured confounding variables, and analytical methods. Most of them were ecological studies which, while suggesting a connection between malnutrition and TB, could not confirm causality. Nutritional deficiency in populations is linked to many other adverse circumstances that may be strong, independent risk factors for TB. With that caveat, several published studies have demonstrated a link between the risk of TB and malnutrition in populations affected by famine, war, natural disasters, poverty, mass migration, and confinement in prisons or ghettos. These studies reveal the difficulties of conducting controlled studies of TB and malnutrition in human populations, and take advantage of historical circumstances. The results suggest putative causal links that can be investigated further. These ecological studies do not attempt to distinguish deficiencies of specific nutrients; rather, they address the multiple simultaneous nutritional deficiencies that commonly afflict malnourished humans. However, the effects of malnutrition cannot be distinguished from the effects of confounding variables such as poor housing, overcrowding, lack of medical care, poor hygiene, massive social disruption, etc.

For example, sharp increases in TB morbidity and mortality in Paris[26] and Germany during the two World Wars[27–29] cannot be ascribed solely to pervasive malnutrition, but may have been due to loss of public health infrastructure, crowding, and psychosocial stresses that were part of these wars. Similarly, the heroic studies carried out by Jewish physicians in the Warsaw ghetto during the Second World War do not control for the extreme crowding, psychological stress, and catastrophic social circumstances[30–32]. While the role of malnutrition independent of other circumstances cannot be isolated





in these studies, they constitute a large body of observation that supports the contention that inadequate nutrition adversely influences either the incidence or the severity of TB, or both.

Three of these ecological studies strongly suggest that nutrition, isolated from other circumstances, played a direct role in TB morbidity and mortality. During most of the First World War, neutral Denmark exported the bulk of its meat, fish, poultry and dairy products to the extent that the local diet suffered. Tuberculosis rates climbed during that time in Denmark. However, subsequent to the German blockade of Denmark in 1918, these foods became available to the Danes and TB rates plummeted in comparison with the neighbouring countries still in conflict[33]. The second study reported that the unusually high rate of TB among the recruits at the Trondheim, Norway, Naval Training School in the early 20th century decreased dramatically when their diet was fortified with dairy products, cod liver oil, whole-wheat bread, and fresh fruits and vegetables. Tuberculosis morbidity dropped to the prevailing level for free-living young adults in the same population[34]. The third study documented a striking difference in TB morbidity between British and Russian prisoners of war (POW) held in German camps during the Second World War. The British POW received Red Cross food supplements amounting to 30 g protein and 1000 kcal per day and exhibited a TB rate of only 1.2% compared to the Russian POW, who had a TB rate of 15% to 19%. Both groups shared the same living and working conditions and, presumably, risk of infection. In the malnourished prisoners, the onset of disease was more rapid, the clinical course was more severe, and mortality was common[35].

Finally, McKeown suggested that the decline in TB mortality in England and Wales from 1770 to 1900 was probably due to the improvement in the nutritional status of the population[36]. The death rate from TB at the beginning of the 19th century was approximately 40/1000 person-years, and it declined to 14/1000 by the end of the century. After excluding alternative explanations (i.e. advances in medicine, natural selection), McKeown concluded that a change in the environment over more than a century was responsible for the decrease in TB, and that diet was the most likely environmental factor[37]. This contention is supported by a report showing that while TB mortality decreased in England and Wales during the period of 1850–1910, mortality due to cholera and dysentery did not. During this interval, income increased slightly but crowding did not change[38].

## Case series

Documenting the correct temporal sequence between the onset of malnutrition and the development of TB would provide evidence supporting a cause-and-effect relationship.

Patients undergoing gastrointestinal bypass surgery for morbid obesity experienced rapid weight loss and malabsorption due to their shortened bowel. During the post-surgical period, the incidence of TB was much higher (1–4%) in patients than the expected incidence among historical or concurrent population control groups[39–44]. Partial gastrectomy for ulcer disease led to malnutrition and predisposed men to TB (in that temporal order)[45]. Subsequently, a number of studies have documented gastrectomy-induced malnutrition as a risk factor for TB[46–48].

A similar increase in TB incidence has been observed in patients with inflammatory diseases such as rheumatoid arthritis who received treatment with various TNF-α blocking agents[49]. As pointed out above, exorbitant production of TNF-α in clinical TB results in wasting in patients, but it is also known to be an essential cytokine for the containment of mycobacteria in the host. Neutralising the pro-inflammatory effects of TNFα reduces symptoms and signs of autoimmune diseases and probably improves the nutritional status of treated patients. However, blocking TNF-α activity also reactivated latent infection and may have increased the progression to TB disease in exogenously infected individuals, depending upon the nature of the anti-TNFα agent utilised[50].

## Cross-sectional and case-control studies

In cross-sectional and case-control studies, patients with and without active TB are compared in terms of their concurrent nutritional status[51–61]. However, as stated above, TB itself *causes* physiological and metabolic changes resembling malnutrition. Such studies *do not* prove that pre-existing malnutrition contributes to the development of TB because there have been no accurate measurements of antecedent nutritional status in comparable cases and controls, but they do demonstrate the nutritional aberrations that accompany TB.

Several studies examined micronutrient status in TB patients and compared them with healthy individuals in a cross-sectional design, or before and after chemotherapy in a prospective design. In some studies, the effect of HIV status on micronutrient levels within the TB patients was examined. Karyadi *et al*.[62] reported that TB patients were more anemic and had lower plasma concentrations of retinol and zinc than controls, and these abnormalities were exacerbated in patients with other indices of general malnutrition (e.g. low body mass index [BMI]). In a study in Ecuador, Koyanagi *et al*.[63] observed that TB patients had significantly lower serum concentrations of zinc, retinol and selenium associated with an acute phase reponse. Similar observations were made in Malawi[64], where more than 800 TB patients demonstrated deficiencies in circulating selenium, carotenoids and vitamin A, and these deficiencies were exacerbated in the most severely wasted group (BMI<16). Interestingly, there were no significant differences in plasma micronutrient concentrations between the HIV-infected and HIV-uninfected





TB patients. A second paper from the same research group[65] confirmed that low plasma selenium levels were associated with anemia in TB patients, as were high HIV loads and elevated IL-6 concentrations.

A similar cross-sectional study was carried in out in Ethiopia[66] in 155 TB patients, 74 of whom were co-infected with HIV. HIV co-infection was associated with lower serum zinc and selenium concentrations and an elevated copper/zinc ratio compared with TB patients without HIV. After the intensive phase of antibiotic therapy, serum levels of both selenium and zinc had improved in both patient groups. A beneficial effect of anti-mycobacterial therapy was also reported in a study of paediatric TB patients in India[67]. Prior to therapy, these children had markedly reduced levels of plasma zinc, irrespective of their general nutritional status, and there was significant improvement after 6 months of anti-TB therapy. Turkish investigators[68] also observed a significant improvement in serum zinc (which increased) and copper/zinc ratios (which decreased) after 2 months of anti-TB therapy in 22 adult patients.

Anti-oxidant vitamins have been associated with clinical TB in several recent cross-sectional studies. A study of Ethiopian TB patients, with and without HIV, reported that serum concentrations of vitamins C, E and A were significantly lower in patients than in healthy controls. High malonaldehyde concentrations, an indicator of overall oxidant stress, were associated with increased clinical severity of TB, and these parameters were exacerbated in co-infected individuals[69]. Similar results were obtained in 159 Russian TB patients, who were found to have reduced dihydroascorbic acid levels compared with control patients with pneumonia[70]. Wiid *et al*.[71] observed significantly lower total antioxidant status (TAS) in TB patients compared with community controls, and TAS values increased during anti-mycobacterial chemotherapy. Similar results were seen with vitamin A and zinc levels, but not with vitamin E. The vitamin A status of 100 HIV-infected and HIV–uninfected TB patients was studied in Tanzania before and after the intensive phase of anti-TB therapy[72]. The authors reported that vitamin A levels were low in TB patients and improved with therapy in HIV-negative, but not in HIV-infected patients. HIV infection was also associated with low vitamin A status in healthy controls. In India, Ramachandran *et al*.[73] observed low serum vitamin A levels in 47 newly-diagnosed TB patients compared with household contacts and healthy controls. Their vitamin A status improved significantly following anti-TB therapy without the need for vitamin A supplementation. Reduced serum concentrations of vitamin A were observed in HIV-infected, TB patients in Rwanda, and were lowest in patients who had experienced recent wasting. Unfortunately, no HIV-uninfected TB patients or healthy control groups were included in that study[74].

Another vitamin that has been linked to TB is **vitamin D** because of its importance as a macrophage-activating hormone. Two cross-sectional studies examined the dynamics

vitamin D in lymphocytes and macrophages from patients with TB compared with controls. T lymphocytes, predominantly $CD4^+$ cells obtained by bronchoalveolar lavage from TB patients, expressed specific receptors for the directly bioactive hormonal form (i.e. $1,25(OH)_2$ vitamin $D_3$), but not its precursor $25(OH)D_3$[75]. Purified T lymphocytes from all patients with TB produced $1,25(OH)_2D_3$ which correlated closely with that produced by lavage cells. Since $1,25(OH)_2D_3$ can improve the capacity of macrophages to kill mycobacteria, the authors concluded that macrophage activation by vitamin D may contribute to anti-tuberculosis resistance[76]. In addition, a few studies have detected vitamin D receptor genetic polymorphisms that are associated with vitamin D deficiency and increased incidence of TB. Two studies demonstrated that TB patients of Asian and African origin in the United Kingdom were significantly vitamin D deficient[77], and that vitamin D receptor polymorphisms were found among vegetarians from Gujarati, India[78]. Two additional studies, one in India[79] and the other in Africa[80] also found vitamin D deficiency associated with receptor polymorphisms in TB patients. Recently, *in vitro* studies of human macrophages have begun to reveal the mechanisms that may underly the link between vitamin D deficiency and TB. Cellular immune functions that depend on vitamin D include the induction of important innate macrophage functions via Toll-like receptor ligation of mycobacterial cell surface molecules[81], the mycobacteria-specific activation of T lymphocytes by infected macrophages[82], and the critical fusion of phagosomes containing mycobacteria with lysosomes within infected macrophages[83] (see also Chapter 4: Human Nutrition) .

## Cohort studies

The unique strength of cohort studies is that nutritional status is measured prior to the onset of TB. Only two cohort studies, both assessing vitamin C, examined the relationship between micronutrients and TB incidence. Getz *et al*.[84] examined 1100 men for the onset of TB by clinical, radiographic and laboratory criteria for up to 5 years. Plasma levels of both vitamins A and C were low in most men who developed active TB compared with those who did not. Investigators in Finland[85] randomised 26.975 healthy males to supplementation with tocopherol, beta-carotene, both or neither, to determine the impact of these anti-oxidants on cancer, and followed up the subjects for a mean 6.7 years. The data were analysed for a relationship between the intake of vitamin C and vitamin C-rich foods and a diagnosis of TB. Increased intake of vitamin C and fruits and vegetables was associated with an adjusted relative risk of TB of 0.4 (95% confidence interval 0.2–0.7).

Indicators of general nutritional status were analysed as part of the long-term follow-up of participants in the large-scale BCG vaccine trials in Georgia and Alabama.





Comstock and Palmer reported that the incidence of TB was 2.2 times higher in children with 0–4 mm subcutaneous fat than in those with >10 mm subcutaneous fat[86]. Cegielski *et al*. examined the relationship between under-nutrition and the incidence of TB based on data from a nationally representative population of adults in the US from 1971 to 1987. Baseline data on nutritional status were derived from the first National Health and Nutrition Examination (NHANES-1), a cross-sectional survey of the US population from 1971 to 1975, after excluding persons with previous TB. The NHANES-1 Epidemiological Follow-up Study (NHEFS) followed up more than 95% of the adult subjects of NHANES-1 for a median of 9 years to relate health outcomes to baseline characteristics. Individuals with a BMI, average skin-fold thickness, or upper arm muscle area in the lowest decile of the population suffered an increased risk of TB from six- to ten-fold, controlling for other known risk factors[87]. Of all the cohort studies, this is the only one that was (1) based on a representative sample of any national population, (2) excluded patients who had TB before enrollment (or that developed shortly after enrollment), and (3) used multivariable analysis to isolate the effects of nutritional status independent of other known risk factors for TB. In later analyses of these data, the BMI cut-off point for under-nutrition in the US at the time these data were collected (<20.0 vs. >20.0) was compared with the cut-off point that is currently used in the US (<18.5 vs. >18.5) and the same results were obtained. Defining under-nutrition in absolute terms rather than in relative terms may make these results more broadly applicable to populations outside of the US.

Palmer *et al*.[88] studied the relationship of TB incidence to PPD hypersensitivity in 68,754 US Navy recruits from 1949 to 1951. During 4 years of follow-up, 109 developed TB. These investigators related the risk of TB to a weight-height index calculated from the entrance medical examination on a stratified random sample of 1138 subjects. TB incidence was 75/100 000 for those 15% or more below the median weight for their height and decreased to 19/100 000 for those at least 5% overweight for their height ($p< 0.01$). The trends were the same regardless of the degree of tuberculin sensitivity. Edwards *et al*.[89–90] extended Palmer's study to over 823 000 Navy recruits, and found that TB developed three times more often in young men 10% or more below their ideal body weight than those 10% or more above it. Despite some methodological flaws[91], these studies are important because of the large sample size and consistency of the findings. Curiously, these authors did not interpret low weight-for-height as an indication of malnutrition. Rather, they concluded that there was an association between "body build" and risk of TB disease[88–90].

Although ignoring the obvious implications of low weight-for-height as an indicator of malnutrition seems counter-intuitive, the concept of body build as an independent risk factor for tuberculosis has been reported by others[92]. A mass radiography screening programme for TB in Norway[93] covered 42% to 85% of the population depending upon the

age group. Height and weight were measured accurately for nearly 80% of those screened, and over 1.7 million individuals were followed up for a mean of 12.1 years. The incidence of pulmonary, but not extrapulmonary, TB declined logarithmically with increasing BMI. The age-adjusted incidence of new pulmonary TB was five times higher in the lowest BMI category than in the highest. The author argued that this association was a function of body build and did not discuss nutrition. Comstock[94] suggested that body build may affect pulmonary mechanics and, thereby influence susceptibility to pulmonary TB, but no data are available to support this hypothesis. It seems unlikely that body build by itself predisposes to or protects against TB with no link to general nutritional status.

## Intervention trials

Intervention trials, especially randomised, placebo-controlled trials (RCT) of the effect of specific nutritional supplements on the response to conventional TB chemotherapy, provide the strongest evidence of a nutritional effect on TB and suggest a practical manner in which to apply this knowledge. One caveat in the interpretation of such trials of nutrients as adjuncts to anti-TB therapy is that the drugs exert a rapid and dramatic effect on bacillary loads and, therefore, on clinical status. Modest improvements resulting from the correction of a nutritional deficiency during therapy may be difficult to detect statistically. Under these circumstances, the fact that significant effects of nutritional intervention have been documented is remarkable.

Downes[95] reported the results of a controlled trial among the families of African-American TB patients in New York City between 1941 and 1946. One hundred and ninety four families were allocated alternately (i.e. non-randomly) to receive vitamin and mineral supplements (treated group) versus no supplements (control group) along with the health department's intensive nutrition education programme. The two groups were similar in prior attack rates and mortality from TB, prevalence of primary and re-infection TB at the start of the study, sputum smear positivity among the index cases, and relation of the index case to the rest of the family. In addition, the groups did not differ in terms of their income, the degree of crowding, and food habits. After 5 years of follow-up, the risk of TB in the control group was 2.8 times the risk in the vitamin and mineral-supplemented group. However, when only those who actually took the supplements for the entire follow-up period were included in the analysis, the risk of TB in the control group was 5.9 times higher than the treated group. Therefore, vitamin and mineral supplementation appear to have substantially reduced the risk of TB among family contacts of active tuberculosis cases. A discussion that factors which support even a stronger conclusion in favor of a beneficial effect of dietary supplementation from this study has been published previously[91].





More recently, a number of trials of nutritional intervention during conventional chemotherapy of TB patients have been published. In one RCT[96], 80 Indonesian patients with TB were found to have significant indices of malnutrition, including low BMI, and low plasma retinol and zinc. They were assigned to receive both retinol and zinc supplements (treated group) and placebo in addition to their standard anti-TB drugs. Significant improvements in sputum conversion and resolution of radiographic abnormalities were observed in the treated group, in association with a significant increase in plasma retinol after 6 months of therapy. Beginning at 2 weeks post-therapy, the percentage of patients with negative sputum smears was significantly higher ($p<0.01$) in the micronutrient-treated group (23%) compared with the placebo group (13%). Mean reduction in lesion area as determined radiographically was significantly greater in the micronutrient-treated group after two months of therapy ($p<0.01$). Furthermore, plasma retinol concentrations were correlated inversely with a reduction in mean lesion area at 6 months ($r=-0.367$; $p=0.02$). Another RCT in a large (n=499) population of TB patients in Tanzania[97, 98] examined the effect of supplementation with either zinc alone, multiple micronutrients (MMN, vitamins A, B, C, D, E and minerals Se, Cu), MMN + zinc, or a placebo. Approximately 43% of each group was HIV-infected, and all received standard anti-mycobacterial chemotherapy. After 8 weeks of therapy, neither supplement had a significant effect on sputum culture positivity: however, the patients receiving the MMN experienced a significant improvement in body weight. HIV status had no influence on the outcome[97]. In a later publication on the same study, the authors reported that after 8 months of therapy, the group receiving the combined nutritional supplements (zinc + MMN) had a significantly reduced mortality (RR=0.29; CI: 0.10–0.80), but only in TB patients who were co-infected with HIV[98].

Hanekom *et al*.[99] conducted a RCT of vitamin A supplementation in 85 South African children with TB who were not co-infected with HIV. Children were given either 200 000 IU of retinyl palmitate or placebo on day 0 and day 1 after initial clinical evaluation and then followed up during 3 months of conventional anti-TB therapy. Nearly two-thirds of the patients were vitamin A-deficient at the beginning of the study, and the deficiency was more pronounced in children with extra-pulmonary disease. Vitamin A status improved in both groups during chemotherapy, but supplementation had no significant effect on the outcome of therapy as measured by weight gain, radiological improvement, serum biochemical parameters, etc. In a subsequent publication from the same RCT[100], these authors reported that vitamin A supplementation did decrease significantly the circulating levels of CD30, a protein biomarker of a type 2 cytokine response. Plasma CD30 concentrations were decreased by a factor of 0.99+/-0.02 in vitamin A-supplemented children, compared with 1.05+/-0.02 in the placebo group ($p=0.02$). These results indicate that vitamin A supplementation may promote the establishment of a beneficial type 1 cytokine profile in children with TB.

Three other RCTs examined the impact of supplementing zinc, iron or vitamin D on the outcome of chemotherapy in TB patients. In the first study[101], 66 HIV-infected TB patients in Singapore who were receiving antiretroviral and anti-TB therapies were assigned to 28 days of oral zinc sulfate supplements or placebo. The authors examined several parameters of TB-specific immunity, including PPD-stimulated IFNγ production, and found no significant effect of zinc supplementation. Perhaps this is not surprising since nearly all (94%) of the subjects exhibited normal plasma zinc levels at baseline. In another study[102], 117 adult, male TB patients in India with anaemia were enrolled in an RCT of iron supplementation during the first 2 months of conventional anti-TB therapy. Follow-up assessment was conducted at 1, 2 and 6 months after initiation of the trial. The authors reported improvement in haematological status as the TB disease improved, but supplemental iron appeared to have no additional beneficial effect on treatment outcome as determined principally by degree of radiographic abnormality that was still present after 6 months of standard chemotherapy. Finally, 24 newly diagnosed paediatric TB patients in Egypt were enrolled in an RCT to examine the effect of vitamin D supplementation (1000 IU/day for 8 weeks) on the outcome of anti-TB therapy[103]. Although the authors reported that most of the children were vitamin D-deficient at baseline and serum levels of 1,25 $(OH)_2D_3$ improved in both groups during chemotherapy, supplementation did not affect this parameter. However, the supplemented group showed significant radiological and clinical improvement at follow-up compared with the placebo group. Body weights of vitamin D-supplemented children increased significantly at 8 weeks after treatment (3.3 kg) compared with the placebo group (2.2 kg) ($p<0.05$).

Inducible nitric oxide (NO) is a critical proximal mediator of anti-mycobacterial resistance in rodent models of TB[104]. The central role of NO in human TB remains controversial; however, some supportive evidence has been published recently[105]. Since the amino acid arginine is a precursor in the biosynthetic pathway of NO production, a case can be made for the value of arginine supplementation to improve infectious disease outcomes[106]. An RCT of oral arginine supplementation was conducted in 120 HIV-infected and HIV–uninfected Ethiopian TB patients[107]. The patients received either 1 g/day of arginine or placebo daily for 4 weeks along with standard anti-TB therapy and clinical outcomes were assessed at 8 weeks. Arginine supplementation resulted in significant improvement in serum arginine levels, weight gain, sputum conversion rate, and reduction of symptoms compared with the placebo group, but *only* in HIV-uninfected patients. The percentage of patients with cough was reduced significantly ($p<0.05$) in the arginine-supplemented group (25%) compared with the placebo group (65%). The percentage of patients with negative sputum smears was significantly higher in the supplemented patients (100%) compared with the placebo group (85%) ($p<0.05$). No treatment effect was observed in HIV co-infected patients.





Two additional recent RCTs of macronutrient supplementation during TB therapy may be worth noting, although both were quite small. Paton *et al.*[108] gave a high-energy oral nutritional supplement (600–900 kcal/day) for 6 weeks to 19 HIV-uninfected TB patients in Singapore in conjunction with standard anti-TB therapy. Compared to 17 control TB patients who received nutritional advice but no supplements, the treated group exhibited significant increases in weight gain, total lean body mass, and grip strength. Unfortunately, no disease-specific results were reported in this publication, so the effect of supplementation on the outcome of TB treatment cannot be assessed. In a second small RCT[109], investigators in Mexico City compared the clinical responses of 10 HIV-uninfected TB patients who received a cholesterol-rich diet (800 mg/day) with those of a group of 11 TB patients who consumed a control diet containing 250 mg/day of cholesterol during the first 8 weeks of standard anti-TB chemotherapy. Respiratory symptoms improved in both groups: however, the rate of sterilisation of sputum cultures increased significantly and sputum production decreased significantly in the patients consuming a high-cholesterol diet. At two weeks, the proportion of patients with negative sputum samples on the high-cholesterol diet (91%) was much higher than in the placebo group (20%) ($p<0.002$). The bacillary load in the sputum (measured in log10 colony-forming units per ml) was much lower in the cholesterol-supplemented patients (0.05) than in the placebo patients (3.4) ($p<0.0002$). The cholesterol content of the membranes of macrophage vesicles (i.e. phagosomes, lysosomes) has been shown to affect the ability of the phagocytes to suppress the intracellular growth of mycobacteria[110], although the authors did not measure that parameter in cells from their patients.

## Nutritional deficiencies and TB chemotherapy – a bidirectional interaction

Many of the studies reviewed above document the fact that a substantial proportion of TB patients are malnourished at diagnosis, especially in high-burden countries, either because of pre-existing nutritional deficiencies or because of the adverse metabolic effects of the disease itself. Furthermore, malnutrition has clearly been shown to increase the risk of death early in the course of treatment in spite of the institution of appropriate chemotherapy. For example, Leimane *et al.*[111] demonstrated, among patients with multi-drug resistant (MDR) TB, worse outcomes of chemotherapy in patients with a BMI < 18.5, independent of other factors. Although low BMI may be a sign of worse disease, these authors controlled for disease severity by multivariable logistic regression.

Several of the aforementioned intervention trials support the conclusion that dietary supplementation with specific macro- and micronutrients can have a significant beneficial

impact on both nutritional status and treatment outcomes in TB. The goal of nutritional interventions should be to provide adequate energy and protein to compensate for the elevated resting energy expenditure and catabolic state associated with TB with or without HIV, to support the extensive cellular proliferation and protein production associated with the anti-mycobacterial immune responses, to allow repair of tissue damage in the lungs and elsewhere, and to replenish somatic reserves. In addition, as the studies above clearly show, supplementation with specific nutrients (e.g. vitamin A, vitamin D, zinc, arginine) may be required to correct specific deficiencies and to promote proper immune cell functions.

There are other ways in which nutritional status can interact with TB chemotherapy, however. For example, an essential first-line anti-TB drug, isoniazid (INH) interferes with vitamin $B_6$ metabolism by blocking the formation of the coenzyme form of the vitamin[112]. In the absence of vitamin $B_6$ supplements, a significant proportion of TB patients treated with INH-containing regimens will experience peripheral neuritis, particularly persons otherwise predisposed to peripheral neuropathy such as those with diabetes and those who consume excessive alcohol[113]. Patients treated with cycloserine, an important second-line drug used in the treatment of MDR TB, should also receive supplemental vitamins because of central nervous system side-effects (psychosis, depression) also related to pyridoxine metabolism[114]. Other anti-TB drugs, such as ethionamide and para-aminosalicylic acid, frequently cause gastrointestinal disturbances, including nausea, vomiting and anorexia, which can impact negatively on the patient's nutritional status. In addition, several TB drugs, including INH, rifampicin, and pyrazinamide, can cause hepatitis characterised by symptoms of anorexia, nausea and vomiting and decreased nutrient intake. In its more severe forms, hepatitis results in disturbances in carbohydrate, protein and lipid homeostasis that clearly impact on the patient's metabolic status and, indirectly, their nutritional status[115].

## Nutritional deficiencies interfere with the protective efficacy of BCG vaccine

The protective efficacy of BCG vaccine varies widely in countries around the world, with the lowest degree of protection often seen in high-burden tropical counties[116]. It has been suggested that malnutrition might impair the ability of BCG to protect against pulmonary TB[117]. Although no clinical trials to test this hypothesis directly have been conducted to date, several prospective studies have evaluated the effects of malnutrition on the delayed-type hypersensitivity (DTH) responses following BCG vaccination. While there is considerable controversy over the validity of DTH as a quantitative biomarker of vaccine-induced resistance, there is general agreement that failure of the vaccine to induce T-lymphocyte-mediated immunity suggests the host response is suboptimal.





Satyanarayana *et al*.[118] showed that milder grades of malnutrition did not affect the DTH response to PPD 6 months after immunisation with BCG, but that children with kwashiorkor were skin test negative. Chandra and Newberne demonstrated that the DTH response to PPD and other antigens was impaired by protein energy malnutrition in children and adults[119]. Among TB patients in that study, the degree of PPD skin test reactivity was directly proportional to serum transferrin levels, a sensitive indicator of protein status. Similarly, malnourished individuals did not develop skin test responses to PPD as frequently after BCG vaccination, nor were the reactions as large as those observed in well-nourished, BCG-vaccinated children. Importantly, this negative effect on BCG vaccine-induced cell-mediated immune responses was observed even in modest protein energy malnutrition[120–121].

Perhaps the strongest evidence for malnutrition-induced loss of BCG vaccine protective efficacy has come from studies conducted in a highly relevant guinea pig model of low-dose pulmonary TB[122]. The pathogenesis of TB in this model following the deposition of a few virulents, *M. tuberculosis* into the alveolar spaces by means of a specially designed aerosol chamber mimics essentially all of the important aspects of tuberculosis in humans[123]. Moderate, chronic protein deficiency in guinea pigs results in a dramatic loss of T-cell functions following BCG vaccination, including much smaller PPD skin tests and reduced lymphocyte responses *in vitro*[124]. More importantly, following pulmonary challenge with virulent *M. tuberculosis*, protein-deficient guinea pigs were unable to form mature, well-circumscribed granulomas in the lungs[125] and expressed significantly less BCG-induced resistance in the lung and spleen[126]. It should be noted that the profound loss of T-cell-mediated resistance that accompanies chronic dietary protein deprivation in this model is substantially and rapidly reversible. BCG-vaccinated guinea pigs maintained on a low-protein diet during the entire 6-week period post-vaccination, but given a normal diet beginning on the day of virulent pulmonary challenge, displayed PPD skin test reactivity and vaccine-induced control of bacillary loads in the lungs and spleens 2–4weeks later that were indistinguishable from BCG-vaccinated animals that had never been protein-deficient[127].

## Conclusions and Recommendations

### Relevant to policy:

I. Findings from observational studies suggest that nutritional support of undernourished populations at high risk of TB (e.g. young children, household contacts of TB patients, health care workers, institutionalised populations, the elderly) may reduce the incidence of TB in such groups in both low-burden and high-burden countries.

II. Although the risk of TB in severe malnutrition may be higher than in mild or moderate malnutrition, severe malnutrition occurs in a very small fraction of the population

even in poorer countries, except in famine, war, or natural disaster-type situations. Mild to moderate proteinenergy malnutrition or micronutrient deficiencies may affect large fractions of the population at risk for TB so that prevention efforts will not be highly successful if they target only severely undernourished groups.

III. There is limited evidence from randomised controlled trials (RCTs) that supplementation with particular macronutrients (to meet energy and protein needs) and micronutrients (such as vitamin D, arginine and protein) during conventional anti-TB therapy are of value, especially in patients who are demonstrably deficient in those nutrients. Some RCTs have shown clear improvements in nutritional and general health status in nutrient-supplemented patients receiving appropriate TB chemotherapy, while others have also demonstrated more rapid recovery from TB in supplemented patients. Recent evidence about the role of subclinical vitamin D deficiency in weakening resistance to progressive *M.tb* infection at the level of macrophages provides a biologically plausible basis for a potentially effective preventive and therapy-supportive measure in certain susceptible populations.

IV. It is clear that malnutrition *per se* is a condition that impacts health on negatively on so many levels that a justification to feed malnourished people on the basis of their increased risk of any single disease (e.g. TB) is only a small part of the rationale. Unfortunately, however, it is not realistic to imagine that country-wide (much less worldwide) nutritional deficiencies will be addressed effectively in the foreseeable future. Therefore, scarce resources in TB-endemic countries must be focused where they are needed most, or under circumstances where they will have the greatest impact. One could debate whether nutritional intervention on a population basis (among malnourished people at risk for TB, as noted above) or on a clinical basis to improve outcomes of TB treatment (i.e. among TB patients) would be the most effective use of the available resources in high-burden countries. The data supporting the former are compelling, while the argument for the latter remains somewhat equivocal. A recommendation for providing nutritional support to undernourished contacts of TB patients and for patients with MDR and XDR TB therefore seems warranted on the basis of available scientific evidence.

**Relevant to research:**

I. Given a disease as old as tuberculosis, the lack of research on the specific nutrients that are most beneficial and the conditions under which nutritional interventions are warranted, in terms of cost and effectiveness, is truly astonishing.

II. Prospective studies should also facilitate the validation of biomarkers of malnutrition-induced immunosuppression in TB during both disease onset and recovery. The development of valid nutritional biomarkers will allow the identification of the





subset of at-risk individuals most in need of dietary intervention either to prevent progression from TB infection to disease or to maximise the response to therapy in those already diseased. Scarce resources could thus be expended where they are most likely to produce a beneficial health outcome.

# Recommendations for policy and research priorities

CHAPTER 12

# Recommendations for policy and practice

Many international guidelines and policies, including those of the World Health Organization, have acknowledged the important interfaces between nutrition, poverty, HIV and TB. In 2001, the South African National Department of Health produced the first "National guidelines on nutrition for people living with TB, HIV and AIDS, and other chronic debilitating conditions", and in 2006, in the face of new developments and scientific information; the Department developed new guidelines for comment and input. The comprehensive HIV Plan that was approved by the South African Cabinet in 2003 recognised the importance of nutrition in the care of people living with HIV. Building on this, the newly developed National Strategic Plan for HIV has in 2007 also prioritised food security and nutrition in its recommendations.

Unfortunately, recent public debates about the value of certain foodstuffs and supplements in the management of HIV and AIDS, as well as claims of benefit and cure arising from unproven diets and therapies, have caused confusion within communities and among health care workers about what really is best nutritional practice for people living with HIV or TB. The objective of this ASSAf report is to examine nutritional influences on human immunity and HIV and TB, and in so doing to inform policy and practice in South Africa in an evidence-based way. In this chapter, the evidence scrutinised through this scientific review has been translated into policy recommendations for consideration by policy makers, managers, clinicians, and people living with HIV and TB. South Africans represent a population widely varying in nutritional and immunological status, both in terms of current/recent situations and long-term pre-histories, some of which may leave their mark for decades, even lifelong. In addition, there is increasing evidence of genetic heterogeneity amongst individuals and groups, which may alter response to nutritional interventions. All of these factors will influence the outcomes of nutritional interventions at both the individual and the population levels and make it imperative that major recommendations for interventions are properly monitored and evaluated.

The optimum diet or food combinations, as well as specific nutrient needs of HIV- or TB-infected persons, are not yet sufficiently well understood and known. This

review has alarmingly revealed a striking dearth of strong evidence to support detailed nutritional recommendations with any degree of conviction. For this reason, the policy recommendations have of necessity to be general and limited in scope and should be read together with the research recommendations in Chapter 13. The recommendations for policy can nevertheless be regarded as the evidence-based consensus views of this expert committee, which generally concur with the expert views from a variety of WHO consultations where these are available.

## Summary of findings in this Report that are relevant to policy and guidelines

### With respect to macronutrients:

1. *In people infected with HIV, weight loss and loss of body cell mass are strong predictors of poor prognosis. These features are associated with increased resting energy expenditure, accelerated protein turnover, decreased energy intake, diarrhoea and malabsorption.*
2. *A systematic review of randomised, placebo-controlled trials found that macronutrient supplementation is of benefit in HIV-infected individuals. Targeted interventions, with balanced nutritional supplements, seem to increase energy and protein intake. There is also* ***limited*** *evidence that specific dietary supplements, such as amino acid mixtures, increase body weight and reduce HIV viral load.*
3. *Evidence from small trials comparing two or more nutritional supplements suggests that balanced supplementation increases body weight and that supplementation with medium-chain triglycerides is more effective than long-chain triglycerides in reducing HIV-associated intestinal dysfunction and fat malabsorption. Supplementation with a whole-protein diet has been found to increase body weight and fat-free mass in HIV-infected adults. Specific amino acids and polyunsaturated fatty acid may increase energy intake, body weight and fat-free mass. Finally, there is* ***some*** *evidence that ready-to-use-therapeutic food is effective in reversing the poor nutritional status found in severely malnourished HIV-infected and non-infected African children.*
4. *Overall, evidence-based advice on the use of macronutrient supplementation in HIV-infected individuals in developing countries is constrained by the fact that the few randomised trials that exist have mainly been conducted in high-income countries where most patients are well nourished and have access to life-prolonging antiretroviral therapy. Further, existing trials have focused on intermediate endpoints (such as energy intake) and most have been too small to assess important clinical outcomes (such as death and morbidity). There are also substantial variations in the nutritional composition of the experimental and control interventions, the use of dietary counseling, disease stage and treatment status of the participants across studies.*





### With respect to micronutrients:

1. *Micronutrient deficiencies are common in people with HIV/AIDS and are more pronounced in individuals with advanced disease and in those with inadequate diets. Such deficiencies may hasten disease progression, increase mortality, and facilitate mother-to-child transmission (MTCT) of HIV.*
2. *There is evidence from observational studies of a direct correlation between higher micronutrient intake (especially vitamins A and B, multivitamins, zinc and selenium) and slower progression to AIDS. Observational studies lack valid markers of micronutrient status: however, and the effects of micronutrient deficiencies are prone to confounding by other factors, including micronutrient interactions.*
3. *Systematic reviews of randomised, placebo-controlled trials have evaluated the effectiveness and safety of micronutrient supplements in HIV-infected children and adults, and of vitamin A for reducing mother-to-child transmission of HIV. Supplementation with vitamin A/ beta-carotene does not seem to exert any significant beneficial or adverse clinical effects in HIV-infected non-pregnant adults. In HIV-infected children receiving vitamin supplements, reductions in morbidity and mortality have been reported, but this evidence is based on small trials or subgroup analyses. Vitamin A possibly increases the risk of vertical transmission in HIV-infected pregnant and lactating women, and the risk of mortality in infants of supplemented mother-infant pairs.*
4. *There is evidence from a large randomised trial that multivitamin supplementation (excluding vitamin A) in HIV-infected pregnant women reduces the risk of disease progression, AIDS-related mortality and adverse pregnancy outcomes.*
5. *There is evidence, derived from a few small randomised trials that zinc supplements given to HIV-infected children are safe and effective in reducing morbidity, but zinc supplementation in HIV-infected pregnant women seems to have no benefit and may be harmful to the women.*
6. *Finally, the evidence-base on the effects of micronutrient supplementation in people with HIV is remarkably limited. It seems reasonable at this stage to support the recommendations of the WHO that everything possible should be done to promote and support adequate dietary intake of micronutrients at Individual Nutrient Intake Level (INL98) levels, while recognising that this may not be sufficient to correct nutritional deficiencies in all HIV-infected individuals. In situations where micronutrient deficiencies are endemic, these nutrients should be provided through food fortification or micronutrient supplements where available that contain at least 1–2 INL98s.*

### With respect to nutrition and TB:

1. *There is evidence from observational studies in both low-burden and high-burden countries that nutritional support of undernourished populations at high risk of TB (e.g. young children, household contacts of TB patients, health care workers, institutionalised populations,*

*the elderly) who receive nutritional support have a lower incidence of TB than those who do not.*

2. *Although the risk of TB in severe malnutrition may be higher than in mild or moderate malnutrition, severe malnutrition occurs in a very small fraction of the population even in poorer countries, except in famine, war, or natural disaster-type situations. Mild to moderate protein energy malnutrition or micronutrient deficiencies may affect such large fractions of the population at risk for TB that prevention efforts will not be highly successful if they target only severely undernourished groups.*
3. *There is evidence from randomised controlled trial that supplementation with particular macronutrients (to meet energy and protein needs) and micronutrients (such as vitamin D, arginine and protein) during conventional anti-TB therapy is of value, especially in patients who are demonstrably deficient in those nutrients. Some trials have shown clear improvements in nutritional and general health status in nutrient-supplemented patients receiving appropriate TB chemotherapy, while others have also demonstrated more rapid recovery from TB in supplemented patients.*
4. *It is clear that malnutrition per se is a condition that impacts health on negatively on so many levels that a justification to feed malnourished people on the basis of their increased risk of any single disease (e.g. TB) is only a small part of the rationale. Unfortunately, however, it is not realistic to imagine that countrywide (much less world-wide) nutritional deficiencies will be addressed effectively in the foreseeable future. Therefore, scarce resources in TB-endemic countries must be focused where they are needed most, or under circumstances where they will have the greatest impact. One could debate whether nutritional intervention on a population basis (among malnourished people at risk for TB, as noted above) or on a clinical basis to improve outcomes of TB treatment (i.e. among TB patients) would be the most effective use of the available resources in high-burden countries. The data supporting the former are compelling, while the argument for the latter remains somewhat equivocal. A recommendation for providing nutritional support to undernourished contacts of TB patients and to patients with MDR and XDR TB therefore seems warranted on the basis of available scientific evidence.*

## The policy context

### The Policy Environment

The HIV/AIDS and TB epidemics in South Africa are associated with widespread poverty, unemployment, hopelessness and despair, resulting in early morbidity and mortality of potentially economically productive adults, AIDS orphans, HIV-infected children, households headed by children and elderly grandparents forced into caregiver roles. Nutritional interventions should target all stages of the life-cycle, should include





efforts to improve food and nutrition security on both national and community levels, should include education on healthy food choices leading to balanced diets, and, at the individual level, should include therapeutic or medical nutritional care of primary and secondary infections. It is clear that malnutrition *per se* is a condition that impacts health on negatively on so many levels that a justification to feed malnourished people on the basis of their increased risk of any single disease is only a small part of the rationale.

The issue of food security and poverty alleviation is recognised as a cornerstone of the government's 2007 National Strategic Plan for HIV and AIDS. The Department of Health recognised the need for a multisectoral approach to food security in their 2005 operational plan for "Comprehensive HIV/AIDS care, management and treatment." Resources and knowledge to implement such care are still limited, however.

There is **no high-level scientific** evidence that good nutrition will influence transmission of HIV infection, but there is **some evidence** (mainly indirect and circumstantial) that optimal nutritional status in infected individuals may slow the rate of progression of HIV to AIDS. The same level of evidence suggests that responses to drug treatment (including ARVs) for HIV infection and for both primary and secondary infections and side effects of drugs can be improved by therapeutic nutritional interventions. There is evidence from observational studies suggesting that nutritional support of undernourished populations at high risk of TB (e.g. young children, household contacts of TB patients, health care workers, institutionalised populations, the elderly) may reduce the incidence of TB in such groups in both low-burden and high-burden countries. **There is, however, no evidence and no biological reason to suspect that any diet, food, nutrient, non-nutrient (phytochemicals) or combinations of these can replace the proper use of orthodox drug treatment for either TB or HIV.**

### Public confusion about the role of nutrition in the management of HIV and TB

General access to antiretrovirals in South Africa commenced in 2004, but despite enormous efforts to roll out treatment it is estimated that at best one-quarter of those requiring ARVs are currently receiving them. There are several reasons for this delay, including fear of HIV testing, fear of taking ARVs fostered by adverse publicity, and infrastructure and human resource constraints within health services. While there are fewer stigmas associated with TB infection, nonetheless only two-thirds of individuals on TB treatment complete their course of therapy. As the numbers of HIV- and TB-infected patients continue to rise, the potential to exploit frightened people with claims of disease protection or cure has increased. The marketplace has been flooded with claims that various foodstuffs, food supplements and micronutrients can prevent, alleviate or cure HIV or TB, or strengthen the immune system. Senior community and political leaders have expressed concern about potential toxicities of ARVs, while

simultaneously promoting alternative nutritional therapies for benefit or cure of HIV. Within communities and for individuals infected with HIV and TB, this has created enormous confusion and lack of trust in orthodox health practitioners and in orthodox medicines. While there is widespread support for further research into indigenous medicines or plants that might have a therapeutic role, little has been done to support such recommendations. **An effectively dual system of approval of medicinal claims has developed, in which orthodox medicine is evaluated with all the rigour of the regulatory environment, while traditional medicines and alternative therapies continue to claim efficacy without being properly evaluated by the responsible legal and regulatory institutions.**

### Uncertainity among healthcare workers

Against the background of confused nutritional messages, health care workers at all levels have found themselves unclear about what basic nutritional advice to offer patients and communities, as well as what nutritional interventions they should be prescribing as part of an integrated approach to treatment. Health care workers are not being trained about nutrition, and lack confidence when it comes to giving nutritional advice or to prescribing food supplements or micronutrients. There is a national shortage of nutritionists and dieticians, which further impedes the delivery of comprehensive clinical services, as well as limiting support to the development and implementation of integrated programmes.

The panel cannot emphasise strongly enough that in the especially problematic context of HIV infection and active TB it is unethical and unacceptable to recommend any treatment for which there is no proof that it would be beneficial and no convincing proof that it would do no harm.

## Policy recommendations

**Noting** that impaired growth in children is common and related *inter alia* to food insecurity of pregnant mothers, infants and children, **it is recommended that the implementation of the existing integrated nutrition programme of the Department of Health is evaluated and that it is adequately resourced for implementation to address undernutrition in all vulnerable groups, but especially in women and very young children**[1]. It also is recommended that the possibility of augmenting Primary School Nutrition Programme (PSNP) of the Department of Education and other childhood feeding schemes with ready-to-use fortified, therapeutic foods, as an integral part of their feeding regimens, be explored in terms of effectiveness, cost, feasibility and general logistic requirements.





The issue of pregnant and breast-feeding mothers is currently in flux. The current WHO recommendation (2005) states that "HIV-infected mothers are advised to avoid all breast-feeding and use replacement feeding when it is acceptable, feasible, affordable, sustainable and safe to do so. Otherwise, exclusive breast-feeding is recommended during the first months of life and should then be discontinued as soon as it is feasible and replacement feeding can be provided safely." The WHO mentions, however, that "the mother's choice should always be respected and supported". A recent study from rural and semi-rural KwaZulu-Natal has shown that HIV transmission is significantly lower in mothers who exclusively breastfeed compared with those who use mixed feeding, it would seem that while the safe exclusive bottle-feeding may be a safe food option for more sophisticated areas and/or populations, initial exclusive breast-feeding may be a safer option for rural or impoverished areas. **It is recommended that an urgent national expert consultation is convened to develop national guidelines for the feeding of infected infants that take into account all relevant studies especially but not only the Paediatric Food-Based Dietary Guidelines for South Africa for all children by the SA Nutrition Society.**

**Noting** that there is evidence that nutritional support of undernourished populations at high risk of TB (e.g. young children, household contacts of TB patients, health care workers, institutionalised populations, the elderly) may reduce the incidence of TB in such groups in a high-burden country, **it is recommended that resources are directed to ensure food security based on locally available, affordable and traditional foods to vulnerable populations.**

**Noting** that there is evidence that macronutrient supplementation is of benefit in HIV-infected individuals, **the nutritional care of people infected with HIV should focus on diversified diets including locally available, affordable and traditional foods, complemented by appropriate, locally acceptable macronutrient supplements.**

While the evidence-base on the effects of micronutrient supplementation in people with HIV is remarkably and alarmingly limited, **it is recommended that everything possible should be done to promote and support adequate dietary intake of micronutrients at INL98 levels**, while recognising that this may not be sufficient to correct nutritional deficiencies in all HIV-infected individuals. In situations where micronutrient deficiencies are endemic, these nutrients should be provided through food fortification or micronutrient supplements, where available, that contains at least 1–2 INL98s.

**Noting** that there is evidence that multivitamin supplementation (excluding vitamin A) in HIV-infected pregnant women reduces the risk of disease progression, AIDS-related

mortality and adverse pregnancy outcomes; **it is recommended that HIV-infected pregnant women are offered multivitamin supplementation (without Vitamin A) at INL98 levels**.

**Noting** that there is evidence that supplementation with particular macronutrients and micronutrients during conventional anti-TB therapy are of value, and that there is evidence that nutrient-supplemented patients receiving appropriate TB chemotherapy show clear improvements in nutritional and general health status, and that supplementation may contribute to more rapid recovery from TB, **it is recommended that the nutritional care of individuals with active TB should focus on adequate diversified diets including locally available, affordable and traditional foods**. In addition, the use of appropriate, locally acceptable macronutrient supplements is recommended, especially for those patients who are demonstrably deficient in these nutrients. Nutritional interventions for patients with TB should extend to their close contacts and families as well (see recommendation 3).

**Noting** that there are false and/or unproven medicinal claims being made about the potential of certain products to prevent or treat HIV or TB**, it is recommended that the existing legislation and regulations are enforced for all products claiming medicinal benefit with respect to HIV or TB**.

**Noting** that there may be potential for certain traditional or herbal products to be developed for therapeutic benefit in the prevention, treatment or symptomatic relief of HIV or TB, **it is recommended that government identifies accessible, ethical and scientifically valid ways to accelerate the investigation of such products**.

**Noting** that there is a general lack of knowledge or confidence among health care workers with regard to giving advice to those infected with or at risk of contracting HIV or TB, **it is recommended that many more nutritionists and dietitians should be trained and employed and utilised in all programmes addressing the two epidemics, than is the case currently, and that the nutritional knowledge of all health care workers in community, clinic and hospital settings should be improved and extended**.



CHAPTER 13

# Recommendations for research

The ever-increasing global store of evidence-based knowledge is the key to rational action and effective policy, but it must be used with caution. Simply transferring the conclusions of studies and insights gained in settings that differ from one's own may turn out to be misleading, yet points of departure that incorporate such information are likely to be very helpful in achieving understandings and recommendations that are in fact locally valid and appropriate. South Africans represent a population widely varying in nutritional and immunological status, both in terms of current/recent situations and long-term pre-histories, some of which may leave their mark for decades, even lifelong. In addition, there is increasing evidence of genetic heterogeneity among individuals and groups, some of which are becoming amenable to laboratory characterisation.

An important conclusion (and point of debate) arising from the heterogeneity of the population is that epidemiological and/or interventional studies ideally should aim to take into account possible "hidden" stratifications of the population concerned, even when care has been taken in terms of selected inclusion criteria, thereby permitting what is called in epidemiology "clean evaluations". Such an approach is designed to ensure that the recommended studies are "conventionally" sound but also include design parameters based on the best possible characterisation of underlying heterogeneities (i.e. "**explanatory trials**").

While explanatory trials are doubtlessly valuable from a scientific point of view, they may often not be feasible (due to the high cost of screening and selecting study participants). They may also not be useful clinically due to the rarified setting in which studies are often conducted and their tendency to small sample sizes, which may preclude the evaluation of "hard outcomes". Health care decision-makers need to know whether an intervention makes a difference to outcomes that are important to patients (typically death and quality of life measures), not only to scientists (who often study surrogate markers only). There is therefore a need to go beyond explanatory studies to "**pragmatic trials**" (i.e. trials with more liberal selection criteria, conducted under real world conditions and evaluating important clinical outcomes).

The best solution to the dilemma of choosing between explanatory and pragmatic trials is to embed the essence of explanatory trials in the design of pragmatic trials, by ensuring, as far as is possible, that the internal stratifications are detected within the "liberally included" sample, so that the benefits of both approaches can be derived.

These considerations highlight the need for a progression from bench research, to observational and "proof of concept" clinical studies, to large-scale effectiveness trials, in coordinated programmes. This approach serves as a framework for organizing the Study Panel's **recommendations for research:**

1. **Well-designed and informative clinical and epidemiological studies are urgently needed to generate and test hypotheses in relation to nutritional support for HIV-infected subjects ( both those not being treated with antiretrovirals, and those that are), and patients exposed to, or suffering from active tuberculosis. Large pragmatic, randomised trials, should be conducted in which explanatory designs are embedded as far as is possible. The studies should separately target adults, children and pregnant women.**

The Study Panel is frankly appalled by the dearth of reliable and truly informative studies of nutritional influences/interventions on the course and outcomes of the pandemic chronic diseases addressed in this report. Even in the rare cases when the high-level study designs have been theoretically satisfactory, and therefore have been included in the analyses, flaws can almost always be detected in the underlying detail, either as hidden (but in principle detectable) stratifications within cohorts, or as omissions of key measurements needed to understand mechanisms and facilitate interpretations.

Remediation of this major problem is a high priority, and will require a broad and multi-faceted intervention across the entire system. We cannot accept with equanimity the finding that a handful of studies/trials in an area as deservedly high-profile and pervasive important as nutrition in relation to HIV infection and clinical TB co-exists with a veritable plethora of clinical trials conducted worldwide and nationally in the case of drugs used to produce incremental improvements in the treatment, for example, of common chronic cardiovascular or allergic conditions.

We have discussed approaches to evidence that are likely to be most useful in this country for strengthening the evidence on which to base public policy and practice in the focus area of this report (Chapter 3). This has obvious implications for the optimal study designs and scope of further investigations that are so urgently needed, as briefly mentioned above and described in the rest of this chapter. We believe that the approval and funding of proposals should in future take these considerations into account (see Policy Recommendations, Chapter 12) and that a deliberate and concerted attempt





should be made to energise and educate researchers who will participate in this national endeavour, and to coordinate their work as far as this is compatible with effective practice and performance at the coalface.

A critical requirement is for skilled and informed scientific input into clinical and epidemiological studies and into the enormously important area of translating research findings into practicable, affordable reliable and robust concepts and tools at the coalface of patient support and treatment. This report shows how an understanding of advances in nutritional and immunological knowledge is essential both to study design and to interpretation, and subsequently to implementation and monitoring in field situations of all kinds.

Apart from the fundamental relationships between theory and study design, all other aspects of clinical and epidemiological investigation will also urgently need to be strengthened – from people capable of designing and running adequately powered trials to improved regulatory mechanisms, to translational skills to turn valid findings into implementable policies and practices in the field. Since a number of large-scale vaccine trials will be done in South Africa during the next decade some nutritional input into their design is highly desirable, if only to establish a basis "nested" controlled studies (see above).

With the above in mind, we proceed systematically to list the studies or programmes of research that we think are necessary to permit effective and affordable nutritional interventions to be applied in South Africa to stemming the tide of the "three epidemic" described in Chapter 2.

2. **The indicators of both vitamin and inorganic micronutrient depletion and repletion in individuals and populations need to be much better defined.**

Examination of the much of the evidence-base included in this report reveals that many, perhaps most of the studies have proceeded without a reasonable understanding of the actual nutritional status of the subjects included in the studies concerned, nor has the complexity of what is 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 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 (see above), and blood concentrations of vitamin 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 coenzymes 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 coenzymes 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 coenzyme 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' (now called the 'Average Nutrient Requirement'), 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." (quoted from Chapter 4).

The big problem for investigators, which needs to be addressed in the design and execution of their studies, is therefore 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 is likely to see improved functioning (including fighting infection and minimising its deleterious effects), while in the second, beneficial effects in respect of infection are unlikely to ensue as the supplementation mainly brings the body's reserves up to the steady state. This generalised view has to be modified when there is no steady state, for example when extensive losses of micronutrients are occurring on a regular basis as a result of chronic diarrhoea. It must also be remembered that each of the micronutrients will have an individualised effect on the spectrum of depletion-repletion. In pioneer studies, where all the stops have to be pulled out in order to establish the mechanisms involved in an intervention, for later simplification in the implementation phases, such information is essential.

It is evident that antiviral treatment of HIV infection, which almost always has markedly positive effects on the state of well-bring of the subject, with increased appetite, decreased bowel disorder and associated malabsorption, etc., will itself bring about a "repletion process" if there was deficiency at the time when treatment was started





and if an adequate diet is being taken. Careful characterisation of this kind of whole-body process would be useful in developing comprehensive dietary guidelines.

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

In cases where micronutrients are not precursors of cellular coenzymes, such as the vitamins which act as antioxidants in various locations, the above-described model of a depletion/repletion spectrum has to be modified, as the dose-response curves are not likely to be saturational, but rather continuously linear or (more usually) peaking to a maximum benefit before becoming toxic (see Chapter 1). There may be cases, such as vitamin A (see below) where there is a mix of both kinds of systems due to a multiplicity of pathways and active metabolites.

These considerations apply similarly to the case of inorganic micronutrients:

"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 baseline or accelerated net losses from body stores that are unavoidable as a result of excretory and secretory processes such as urination, defaecation and sweating. Subsets of these inorganic micronutrients have been shown to be especially relevant to human immunity and resistance to infections. In general, (they) resemble vitamins in being present in particular food sources in notable amounts; in varying in 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 reflecting 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; next, symptomatic deficiency associated with lowered tissue content; and finally, serious disorder and death"(quoted from Chapter 4).

The assessment situation is also not very different from that of vitamins, when inorganic micronutrients are in question:

"As in the case of vitamins, assessment of inorganic micronutrient status requires a combination of clinical acumen and laboratory measurement. 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." (also quoted from Chapter 4).

The above-described considerations reinforce the overall conclusion that the degree to which health care personnel are able to determine where exactly their patients/consultees are situated with respect to individual micronutrients, must be substantially improved, through a major research effort and concomitant public policy development.

As stated in Chapter 5:

"Because dietary history combined with food tables, 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 features to be looked for include various anthropometric measures, such as body weight, body mass indices and standardised skin-fold thickness, especially valuable if observed over time; 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 and mineral deficiency syndromes. Laboratory tests that have become preferred for reasons of affordability, feasibility, reliability, relevance and conclusiveness 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 or mineral 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 micronutrient 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."

This leads naturally to the following conclusion:

"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 micronutrients) is very common (see Chapter 10) 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." (quoted from Chapter 5).

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

A good example of an obvious need in the system is the full exploration of the possibility that improvements in nutritional status because of better food security have, 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 is urgently necessary."

The Panel accordingly attaches 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 interventional studies where study designs can be improved and interpretational difficulties overcome. This cannot 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.

**3. Reliable and accessible biomarkers are urgently needed to assess immune function in nutritional studies and in interventions in human subjects.**

Malnutrition has been generically associated with "poor immunity" since pioneer studies were conducted in the 1950s–60s; reasonably specific immunodeficiency syndromes arising for example from severe protein-energy deficiency have later been detected by a variety of available immunological investigations (see Chapters 5 and 11). The field of

immunology has advanced by leaps and bounds since then, however, and a huge variety of test and measurements have become possible. To some extent, their very multiplicity has become a problem, as any of the isolated *in vivo* or *ex vivo* measurements available can be linked to effects observed in clinical studies or trials, as "evidence of immunomodulation". The integrated functioning of the immune system in terms of both innate and adaptive immunity has become difficult to assess as its observed complexity increases. The Panel recommends that strenuous efforts should be made to end the situation where any and every effect is thought/said to be significant and "proof of benefit":

"Considerable progress has been made in evaluating immune responsivity and the impact of long-term nutrient supply and/or deficiencies… Precise tests and analyses will in the main be both complex and expensive. This fact should not, however, serve to prevent as good a bank of standardised, feasible and informative tests as can be developed to become widely available for the support of effective health care in South Africa.

A large number of *ex vivo* measures already exist for the assessment of blood-derived cells as well as, in some cases, cells collected from mucosal surfaces by lavage techniques[19]. These include measurements of phagocytosis/killing of bacteria, yeast cells or sheep red blood cells; assays of respiratory burst/microbial killing rates; NK and CTL activity on virally infected cells; lymphocyte proliferation rates under stimulation by mitogens; stimulated cytokine production, or gene expression in whole cells; IgG (total and specific) production rates; cell-surface expression of HLA molecules or cytokine receptors (flow cytometry). *In vivo* measures include the size of lymphoid organs (e.g. ultrasound in the case of the thymus); cytology of lymphoid tissue; numbers and types of circulating immunocytes of various kinds, including cell-surface expression of defined molecules; circulating thymulin; general plasma immunoglobulins as well as specifically targeted ones; secretory IgA in mucosal washings; circulating cytokines; delayed-type hypersensitivity (DTH) responses to antigens already experienced by subjects; resistance to infectious diseases, progression and outcomes. Widespread inter-individual and inter-group variations can be expected..." (quoted from Chapter 5).

The Panel has noted with interest the efforts made in Europe to address the problem of providing the broad health community with an idea of what tests are actually most appropriate, reliable, affordable and informative:

"A highly useful study was performed by the European branch of the International Life Sciences Institute (ILSI Europe) to identify the most reliable, cost-effective generally useful markers to assess the impact of nutrition on immune function in humans, including those associated with the intestine. A number of systematic potential confounding subject-specific factors were listed, including age-dependence of immune function, sex differences, physical and psychological stress, drug history, smoking, and vaccination history. Technical confounding factors were identified as study designs,





sample collection times (circadian rhythms), seasonal variations, meals and/or depleting wash-out periods, and the length of the intervention period. Immune function assays commonly in use were rated as to theirbiological relevance (e.g. known correlation with clinical end-points), sensitivity (within and between subject variation), and practical feasibility. Methods that were rated highly suitable were:

i. vaccine-specific serum antibody production;
ii. delayed-type hypersensitivity responses;
iii. vaccine-specific or total secretory IgA in saliva; and
iv. the response to attenuated pathogens.

Markers with medium suitability were NK cytotoxicity, oxidative bursts in phagocytes, lymphocyte proliferation, and cytokine patterns produced by stimulated cells. The authors suggested using a mix of highly and medium suitable markers for general use. While this very careful land detailed work is doubtlessly valid in a European setting, no similar study under South African/African conditions has apparently ever been done, despite the crying need in a country where the public is constantly exhorted to consume a plethora of mostly untested or poorly tested, (expensive) proprietary 'immunomodulators' and the like". (quoted from Chapter 5)

The Panel accordingly recommends that a nationally coordinated effort is made in South Africa to determine the optimum *in vivo* and *ex vivo* methods reliably and informatively to assess the general status of the immune system in nutritional interventions, in addition to the specialised tests such as $CD4^+$ counts which are in standard use in relation to HIV infection. The results of the investigation should be published as national guidelines, including translation into practicable field tools, supported by enabling measures in the National Laboratory Health Service (NHLS) and the system generally. Regular updating will obviously be necessary.

**4. A major effort must be made to integrate and deepen our understanding of the functional interactions between HIV infection, gut immune mechanisms and the intestinal microbiota.**

Spectacular progress has recently been made in locating the most extensive and significant aspects of HIV infection in the intestinal wall. The low-grade but relentless immune battlefield in the mucosa seems to be a key site both of continuing viral replication and the source of varying but chronic systemic inflammation. Bringing the skills of research gastroenterologists and dieticians/nutritionistsо bear on these issues will be extremely valuable, with possible new ameliorating or even therapeutic agents in the offing. To quoted from Chapter 8:

"Microbial translocation in the gut during chronic HIV infection has now beenshown to be one of the major driving forces behind immune activation and maintaining the pool of activated $CD4^+$ T-cells that can be productively infected by the virus. The levels of plasma lipolysaccharide in HIV-infected individuals during the chronic stage of disease and during AIDS have been found to be much higher than in healthy HIV-uninfected controls or in subjects newly infected with HIV... Concomitant with increased plasma LPS was increased plasma-soluble CD14, a receptor molecule found on the surfaces of monocytes and shed upon in vivo activation by LPS. Interestingly, HIV-infected individuals who were able to control viral replication and appeared clinically healthy had higher levels of endotoxin-core antibodies that seemed to neutralise the chronic inflammation-activating effects of LPS in the systemic circulation… The plasma levels of an acute-phase (inflammatory) protein, C-reactive protein (CRP), have recently been positively associated with progression time to AIDS in HIV-infected subjects. The crucial link that needs to be made is whether different nutrients/dietary components affect gut mucosal integrity in HIV-infected individuals in systematic or individually different ways, which could influence or determine the course of HIV pathogenesis through effects on the basic processes of viral persistence and net $CD4^+$ T-cell depletion, by influencing the rate of microbial translocation."

Much can already be said for the burgeoning field concentrating on the gut microbiota and the possibility of extensive influences on human health, as well as new approaches to countering gut-sourced inflammation. To quote from Chapter 7:

"There are indications that both local (gut) and systemic immunity can be affected by interactions between 'normal' bacteria (non-pathogenic commensals) and the gut epithelium, but experimental evidence is so far mostly restricted to certain probiotics (exogenously introduced beneficial bacteria) and prebiotics (food components that selectively support the outgrowth of beneficial bacteria), both of which appear to be able to modulate immune signalling in the mucosal wall, as well as immune interactions between the gut and the distributed general immune system."

We advocate an extensive national programme of research capacitation in this multi-disciplinary area of focus, now considered crucial to HIV infection and its complications, and to a lesser extent also to (rare) forms of tuberculosis and obviously also to malnutrition in all its manifestations. This would include:

i. bringing together gastroenterologists, immunologists and nutritionists/dieticians in joint studies, and sharpening up and expanding diagnostic tests of intestinal function;
ii. developing the capacity to characterise the microbiota present in both the small and the large intestines of individuals and populations, quantitatively and qualitatively, and to determine longitudinal responses during follow-up and treatment scheduling;
iii. understanding the systematic relationships between diets of various kinds and the intestinal microbiota, as well as gut function and inflammation;
iv. establishing why there may be malabsorption of specific nutrients in asymptomatic HIV-infected individuals, whether this is selective, and whether compensatory change in the bowel micro-architecture/biochemical physiology take place, and with what significance for morbidity and mortality;
v. characterising the short- and long-term effects of selected probiotics and prebiotics, of various kinds and at various dose levels, on gut microbiota in various kinds of subjects, including those with and without HIV infection;
vi. performing controlled clinical trials of the possible therapeutic value of selected probiotics and/or prebiotics on HIV-infected subjects at varying stages of the disease and with varying intestinal symptoms and complications;
vii. examining the possible therapeutic value in HIV-infected subjects of specific anti-inflammatory agents ( such as 5-aminosalicylic acid ) and/or specialised diets that have proved to be effective in chronic inflammatory bowel disease;
viii. improving the reliability and informativeness of direct or proxy tests of immune functioning in the intestinal mucosa;
ix. documenting the relationships between intestinal symptoms and signs, on the one hand, and the progression of HIV infection to its final end-stages, on the other; and
x. ensuring that the role of already well-accepted opportunistic intestinal pathogens is better understood in the context of newer conceptual frameworks of local immune defences and accompanying systemic changes.





The discovery that the gastrointestinal tract is the probably major site of the critical reactions that maintain HIV productively in its host has opened up an entirely new front in the nutrition and HIV debate, with the emphasis broadening from nutritional themes based on "classic" macro- and micronutrients to promising new areas of food composition and supplementation. The Panel believes that South African scientists should be able to contribute much that is beneficial to its enormous HIV-infected population by exploring a new frontier that holds enormous promise.

**5. 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 polypharmacy 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 usually expensive supplements justifies investigations aimed at detailed scientific understanding of the consequences of their regular ingestion:

"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" (quoted from Chapter 4).

The Study Panel therefore 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) multivitamin preparations. Are the consequences of self-administration of (commonly available) 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 INL98" 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 INL98s 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, and to turn the knowledge thus gained into optimised public policy and more effective individual care.

**6. A better understanding is needed of the significance of lifelong programming of the human immune system arising from fetal "insults"**

One of the most controversial but potentially influential theories of human development is that nutritional, toxicological and/or virological "insults" prior to birth, especially in key phases of neonatal organ growth, may cause lifelong programming of body systems such as immune responses, metabolic patterns and cardiovascular functioning that deviates from the norms of a population. The Panel believes that the evidence for a systematic, programmed "stunting" of the immune system of malnourished people through this mechanism is not yet firm enough to become an accepted part of thinking in the field. The apparent association of chronic degenerative and metabolic diseases in middle-aged adults with fetal malnutrition (the "Barker Hypothesis") is one thing; maintaining that the immune system of an adult will "under-perform" in the face of concurrent adequate nutrition and general health care is another. Nevertheless, it is clear that proper studies of South African communities need to be performed to help assess the importance of this possibly quite general phenomenon within the general context of the high prevalence here of "hidden hunger" (micronutrient deficiencies) as opposed to protein/energy starvation.

**7. Recent developments in the understanding of some micronutrients are so important that they merit thorough study and further interventional trials.**

### Vitamin A

The physiological transformations, translocations and and diverse roles of this vitamin (or, better, vitamer complex) increasingly resemble "metabolic maps"; their actions being multiple, complex and highly variable, and dependent on a plethora of cellular proteins. A relevant example is the very recent discovery of a specific and obligatory action of dendritic cells in the gut wall in forming retinoic acid as a mediator (together with cytokines) of B and T lymphocyte differentiation and homing to the gut-associated lymphoid tissue (GALT); vitamin A-deficient mice failed adequately to perform these essential immune defence functions in respect of both humoral (mucosal secretion of IgA) and cell-mediated immunity (see Chapter 7).

The fact that active vitamin A repletion has proved helpful in many childhood diseases, including HIV infection, and (potentially) unhelpful in preventing mother-to-

child transmission of the same virus, points to the need more fully to understand the exact situation with respect to this vitamin-"protohormone" in the bodies of subjects included in clinical trials, on the one hand, or in clinic-going populations, on the other.

### Vitamin D

This is another vitamin-"protohormone" which has an immensely complex network of pathways and actions throughout the body, including 1,25-cholecalciferol synthesis and vitamin D receptor (VDR) induction followed activation of the Toll-like receptors (TLR) of macrophages infected with *Mycobacterium tuberculosis* (Mtb), associated causally with the generation of an antibiotic peptide called cathelicidin and enhanced microbial killing. We need to know whether many South Africans are unable to synthesise enough of their own vitamin D, with possible lowering of serum 1,25-cholecalciferol levels and increased susceptibility to tuberculosis. Do dietary intakes have a bearing on this? The relationship between this nutritional factor and other genetic susceptibility factors such as alternate DC-Sign alleles needs further study.

Randomised controlled clinical trials of vitamin D supplementation are obviously necessary in active tuberculosis, making sure the design includes the detection of subjects with sub-clinical hypovitaminosis, and, if there are such, determining if possible the reasons for this status. Intersections with calcium intake and status will need to be established

### Folate

The consequences of folate deficiency are not restricted to cells of the haemopoietic system, but are encountered by epithelial cells of the gastrointestinal and genitourinary tracts as well, these tissues also containing cells with high natural turnover rates. HIV infection involves adaptive or compensatory T-cell proliferation which may reach a point at which folate deficiency becomes factor, perhaps acting synergistically with other causes of lymphocyte destruction.

The Study Panel therefore recommends careful studies of folate metabolism in HIV-infected persons, to detect abnormalities, and to establish increased demand and/or decreased absorption (specifically of conjugated folate forms) due to intestinal aberrations.

### Zinc

A central clinical feature of zinc deficiency in humans, already detectable on marginal regular daily uptakes, and well documented especially in children, is increased susceptibility to infectious diseases, through impairment of multiple mediators of both cell-mediated and humoral immunity. The Panel recommends a special focus on zinc





in prospective well-controlled and randomised clinical trials, as it constitutes a good example of the main precept that such trials should be firmly based in an understanding of the prior and ongoing depletion-repletion status of each participant.

### Selenium

The role of this trace element is to become part of a number of important enzymes in the form of constituent seleno-methionine or seleno-cysteine residues (these are also the forms of selenium that are ingested as effectively micronutrient components in protein foods) acting mainly in anti-inflammatory and/or anti-oxidant mechanisms. Because seleno-enzymes are important for maintaining an anti-oxidant environment in HIV-infected cells sufficient to limit transcriptive generation of new virus genomes and particles, carefully designed trials are needed to assess their possible significance in the nutritional support of those HIV-infected persons who are deficient in the element. The cause of the toxicity of excess dietary selenium must also be better understood in this specific context.

**8. We need to factor into population-directed studies the genetically difference to susceptibility to HIV and TB infection of different members of the population**

A pitfall of many clinical trials is the acceptance of a "blank slate" model of the population under study. We must take into account the fact that a spectrum of infection prevalences and progression rates has emerged in the case of both HIV and *M.tB* infections. Within any population, and between different populations, there are individuals with increased susceptibility and others with increased resistance, who may in either group progress to AIDS or develop TB slowly or quickly. Generally, however, increased susceptibility to becoming infected with HIV has accompanied the trait of more rapid progression to AIDS. Many gene loci have been found that variously affect virus entry and intracellular replication, host innate immunity and especially adaptive immune processes, and affect the clinical course of the infection accordingly.

The widely distributed "host resistance factors" include HLA markers that are associated with long-term non-progression or delayed onset of AIDS, and better clinical outcomes, while other markers have the reverse effects. Determination of the HLA marker status of subjects in a trial is feasible in a good research design. In the case of susceptibility to tuberculosis, DC-SIGN alleles can be determined that can be associated with a seven-fold variation in disease incidence, other things being equal. In the context of this Report, these genetically determined variations in host susceptibility are important because they will constitute "background noise" in any clinical trial or other investigation of clinical HIV progression. Fortunately, the laboratory means to establish the nature of other possible resistance or susceptibility genes in human subjects are being created through active research and will impact positively on this problem.

9. **The elevation of the research agenda to the national level in the field of nutrition and immunity means that this focus must be given special attention in the current (strategic) enhanced resourcing of science and technology in South Africa.**

There can be no doubt but that the national interest requires a deliberate and coordinated focus on pandemic infectious diseases and their nutritional components. This means that research chairs, centres of excellence, special training programmes (as in astrophysics and information technology), international collaborations, etc. should include in their calls for proposals the priority that this critically important field enjoys (see Chapter 12).



CHAPTER 14

# Collation of existing guidelines from the World Health Organization, the Southern African HIV Clinicians Society and the National Department of Health

The consideration of the available evidence in this report about the role of nutrition in HIV/AIDS and TB, leading to the above recommendations for policy and research, has highlighted the gaps in our knowledge of the effects of exposure to specific diets, foods, nutrients, combinations of nutrients, nutrient supplements and other substances (especially in plant foods) on the health outcomes in infected people. The report further showed that these knowledge gaps are partially related to the difficulties in designing appropriate studies because of the complexities of dietary interventions and heterogeneities in infected subjects and controls, as well as a lack of comparable measurements of health outcomes.

These gaps in our knowledge (and therefore in available evidence), further exacerbated by the stigmatisation of HIV/AIDS, should however, not prevent the formulation of recommendations to health professionals, people living with the infections, and those in households, families and communities responsible for the care of infected people, on how to apply nutritional principles as part of this care. Three "authoritive" bodies have made such recommendations: the World Health Organization[1], the Southern African HIV Clinicians Society[2], and the South African National Department of Health, Nutrition Directorate[3]. All three organisations have based their recommendations on the limited literature about the role of nutritional status in HIV/AIDS and active TB, as well as general knowledge about the role of nutrition in health and disease, especially the relationships of nutrients with immune function. The latter two sets of recommendations (2, 3) also include practical advice on food security, food safety (hygiene) and therapeutic care of patients with secondary infections and those on medication for primary and secondary infections. Both these sets of recommendations use the South African Food Based Dietary Guidelines[4] with appropriate additions and amendments, to promote healthy, balanced

nutrition. The Department of Health has gone one step further and included advice on the use of traditional herbal treatments and remedies, with an acknowledgement that their effects are largely unknown and a warning that they should be used in moderation. The Department of Health recommendations also include specific advice on how to cope with nutrient-drug interactions and how to minimise side-effects of drug treatment.

The purpose of this Chapter is not to give detailed, practical nutrition recommendations for people infected with HIV and/or with active TB (since that has been done in the three reports mentioned), but rather to focus on the principles that should underlie nutritional recommendations for practice, originating from the careful considerations of all available evidence in this report as well as the realities of the South African situation.

## Principle 1

**Optimum nutrition at the population (public health) level is necessary for prevention of the spread of HIV/AIDS and TB, and at the level of individuals to improve health, quality of life and response to drug treatment, but it cannot directly prevent transmission of these infections or cure them or supervening infections**

There is widespread acceptance that malnutrition is part of a vicious cycle of poverty, underdevelopment, lack of education, and an intergenerational lack of development of cognitive skills and "human capital", all factors collectively contributing to the HIV/AIDS and TB pandemics. To break this cycle, these factors should be addressed simultaneously and in concert, and nutritional interventions should focus on all stages of the life cycle, but especially aim to improve the nutritional status of expecting mothers and their unborn babies. There is no direct, hard scientific evidence that good nutrition will influence transmission of the infections, but (so far mainly indirect and circumstantial) evidence does indicate that the arte of progression of HIV to AIDS will be slower in individuals with optimum nutritional status. The same level of evidence suggests that responses to drug treatment of both primary and secondary infections and side-effects of drugs can be improved by therapeutic nutritional interventions. **There is, however, no evidence and no biological reason to suspect that any diet, food, nutrient, non-nutrient (phytochemicals) or combinations of these can replace the judicious use of drug treatment of these infections.**

## Principle 2

**Recommended nutritional interventions should do more good than harm.**

The already mentioned lack of evidence and gaps in our knowledge, combined with unreported, unpublished results from clinical experience and traditional practices, as well





as the desperation of critically ill people suffering from stigmatised diseases, have spawned a plethora of recommendations regarding the beneficial effects of specific foods, traditional plants and their extracts, herbs and spices, as well as supplements, in the prevention and treatment of HIV/AIDS and active TB. These "alternative or dissident" recommendations, often expressed by political leaders and by practitioners claiming positive results from their treatments, have received wide press coverage at home and abroad. It may well be that there are unknown and unrecognised substances in edible and medicinal plants with these unproven putative beneficial effects. **However, until these suggested remedies have been proven to do more good than harm the panel cannot support their use**. For example, the putative beneficial effect of garlic in "strengthening" the immune system may well be true, but the SA HIV Clinicians Society[2] warns that "various deleterious side effects are associated with the use of garlic supplements" in HIV/AIDS. Other unconventional treatment strategies identified by the Society[2] that "might be beneficial" but with a concern that "they could be harmful" are virgin olive oil, African potato, onion, spirulina, Sutherlandia frutescens, and several phytosterols. The Department of Health's recommendations[3] list 12 commonly used herbs and spices with putative benefits, advice on how to use them, and cautions about amounts and when not to use them (e.g. cinnamon in pregnancy). The list also advises against the use of garlic by people who are taking antiretroviral drugs, as garlic may inhibit absorption of these drugs[3].

The same principle is also valid for micronutrient supplements in excess; therefore, except for vitamin A in children, all three sets of nutrition recommendations (1–3) advise that upper limits of recommended nutrient intake levels should not be exceeded. Therefore, even in the light of limited evidence, recommendations to the public should be responsible**. In the special problematic context of HIV infection and active TB, it is unethical to recommend any treatment of which there is no proof that it would be beneficial and no convincing proof that it would do no harm.**

## Principle 3

**Nutritional interventions to address the HIV/AIDS and TB pandemics should be part of a holistic, comprehensive, integrated approach, including both public health and therapeutic nutrition strategies and actions.**

The HIV/AIDS and TB epidemics in South Africa are associated with widespread poverty, unemployment, hopelessness and despair, resulting in early morbidity and mortality of potentially economically productive adults, AIDS orphans, HIV-infected children, households headed by children and elderly grandparents forced into caregiver roles. Nutritional interventions should therefore target all stages of the lifecycle, should include efforts to improve food and nutrition security at national and community level, should

include education on healthy food choices leading to balanced diets, and, at the individual level, include therapeutic or medical nutritional care of primary and secondary infections. The Department of Health[3] recognised this multisectoral approach in their operational plan for "comprehensive HIV/AIDS care, management and treatment". Resources and knowledge to implement such care are limited, however. The practical implications are that many more nutritionists and dietitians should be employed and utilised in all programmes addressing the epidemics, and that the nutritional knowledge of all health care workers in community, clinic and hospital settings should be improved and extended.

## Principle 4

**Nutritional care of people infected with HIV and/or with active TB should focus on diversified diets including locally available, affordable and traditional foods. The widespread micronutrient deficiencies endemic to South Africa, the characteristic wasting of infected persons, and the known effects of the infections on food intake and nutrient turnover (absorption, metabolism and losses) dictate the use of fortified foods, however, as well as macro- and micronutrient supplements at safe levels.**

There is agreement in the three sets of the above-mentioned nutrient recommendations that a food-based, diversified diet approach is the first choice. The "optimal" specific nutrient needs of infected persons are still not totally clear; there are for example indications from an epidemiological study which included asymptomatic HIV-infected subjects[5, 6] that the mainly plant-based, prudent diet recommended as the optimal diet to prevent both under- and overnutrition (in which saturated fat is largely replaced by unsaturated fats) may not be the optimal diet for infected persons. Nevertheless, the aims of dietary recommendations are to improve and maintain the best nutritional status possible, of as many persons as possible. Wasting of infected persons (involuntary weight loss ≥ 10% of initial body weight) and impaired growth in children indicate that the actual needs for energy-providing macronutrients (protein, fat and carbohydrate) are not being met. A food-based approach should be accompanied by the use of appropriate, locally acceptable macronutrient supplements. All three sets of guidelines[1-3] also promote the use of multimicronutrient supplements with practical advice in two[2, 3] on how much and when to take them.

## Principle 5

**Established, well-described steps and protocols should be followed in public health nutrition interventions and in the therapeutic nutritional care of patients.**

There is an extensive literature on the reasons why many well-meaning food and nutrition interventions at the public health level fail. The ways to ensure success have





also been described, including following established protocols of assessment of existing situations, analyses of all contributing factors, appropriate actions, evaluation of effects and outcomes of these actions, and adjustments of actions when necessary. Similarly, there are established protocols and algorithms for the therapeutic nutritional care of patients, starting with nutritional screening that will guide follow-up actions. It is unnecessary to emphasise that these protocols and frameworks should be followed in the case of HIV-infected and TB-infected persons, and appropriate support from families and communities is a given. However, because these diseases are still stigmatised in South Africa, health care workers should be sensitive on how to involve households, families and communities and how to mobilise social support for affected individuals.

## Principle 6

**HIV-infected pregnant women, lactating mothers and their babies need special advice and nutritional care to ensure best possible outcomes.**

The Department of Health[3] gives detailed advice for the nutritional care of pregnant and lactating women based on the acknowledgement that "nutritional care and support for the pregnant and lactating mother infected with HIV may minimise the impact of the disease, delay disease progression and allow mothers to remain productive and able to take care of themselves and their families". As in non-infected women, weight gain during pregnancy of infected women, especially during the second and third trimesters, should be carefully monitored (to ensure an approximately 1 kg gain per month during this period). Increased needs for calcium, iron, vitamin C and folic acid should be addressed with a diversified food-based approach where possible and appropriate supplements if not feasible. The Department advises that all HIV-infected pregnant women should be provided with a daily multi-micronutrient supplement at one nutrient intake value (they express it as one INL98), and warns that high-dose vitamin A supplements should not be given to pregnant women as they "can cause birth defects."

The evidence of mother-to-child transmission of HIV during breast-feeding has been systematically reviewed in this report (see Chapter 8). The dilemma is that breast-feeding as opposed to formula-feeding (replacement feeding) creates a risk of post-natal transmission but replacement feeding in resource-poor countries contributes to high infant mortality. Therefore, the WHO[1] has to date recommended that "HIV-infected mothers are advised to avoid all breast-feeding and use replacement feeding when it is acceptable, feasible, affordable, sustainable and safe to do so. Otherwise, exclusive breast-feeding has been recommended during the first months of life and should then be discontinued as soon as it is feasible and replacement feeding can be provided safely." The WHO[1] mentions, however, that "the mother's choice should always be respected

and supported". The Department of Health[3] recommends either exclusive breast-feeding (for 6 months) or exclusive replacement feeding, and leaves the choice with an informed mother: "If the mother is HIV-infected she should be provided with the correct information for her to make the best feeding choice for the health of her child in order to reduce the risk of mother-to-child transmission of HIV"[3].

HIV-infected women who are pregnant should receive infant feeding counselling that aims to empower them to decide on the best infant feeding practice for her, her infant and her family and should take into consideration amongst other things, access to clean water and an uninterrupted supply of formula and primary health care support. Women should be supported in their infant feeding choices. The modeling exercises to assess the impact on mortality of various infant feeding strategies discussed in Chapter 8, showed the lowest frequency of adverse outcomes if **no** HIV-infected mother breast-fed and **all** non-infected mothers breast-fed optimally, given that infant mortality rates were below 100 per 1000 live births and relative risks of dying set at 2.5 for non-breast-fed compared with optimally breast-fed infants. The feeding solution in a well-resourced setting would be to provide safe, ready-to-use replacement feeding to infants of HIV-infected mothers. In most resourced-contrainst South African setting, however, exclusive initial breast-feeding of infants for as long as possible, preferably for at least 3–4 months (corresponding to the maternity leave period usually available for working mothers) appears to be the general aim. What is undesirable according to the most recent definitive finding is the initial mixed feeding post-natally especially when combined with solids (see Chapter 8). An urgent national expert consultation is obviously needed (see Chapter 12) as well as revision of the WHO guidelines (the WHO has already issued an amended advice). The national consultation should be part the AIDS National Strategic Plan NSP) Process that is already ongoing.

Nutrition and pathogenesis of HIV and active TB in infants and children have been extensively discussed in Chapter 8 of this report; the Department of Health guidelines[3] give detailed advice on care and feeding of infants and older infected children. The principles are basically the same as those for adults, with an understanding that small children cannot take care of themselves. This emphasises the need to educate caregivers, which often would be older children or grandparents in HIV-affected households.

## Conclusions

Optimising the nutritional status of individuals and populations will probably help to stem the spread of the HIV/AIDS and TB pandemics. It will improve the health and quality of life of infected persons by minimising the effects of the infections on nutritional status, improving responses to drug treatment and improving adherence to these drugs through minimising side-effects.





The optimum diet or food combinations, as well as specific nutrient needs of infected persons, are not yet sufficiently well understood and known. It is acknowledged that the gaps in our knowledge, leading to perhaps "over-careful," general, and limited practical recommendations, but this may improve with more appropriate research (see Recommendations in Chapter 12). The above approach should be updated and adjusted as this knowledge become available.

# Concluding section

CHAPTER 15

# The way forward – concluding remarks

The Panel at one stage considered giving its report the title **"Knowing and Helping"** to help emphasise the enormous importance of applying relevant and reliable knowledge in order to help the large number of people in South Africa afflicted by the triple epidemic of malnutrition, HIV infection and active TB. The Study Panel has discovered to its intense disappointment and alarm that **the base of reliable evidence relating to nutritional influences on the course of the two rampant infectious diseases is woefully small**. This contrasts dramatically with the huge cloud of often acrimonious controversy that hangs over the subject and has become a source of widespread concern in, and about, the government, both within and outside the country. To adapt Henry Kissinger's remark about academic conflicts being so bitter because the stakes are so small, it seems that the controversy about nutrition and the two pandemic chronic infections has been so bitter because the (actual) knowledge base was so small. Without **knowledge,** and specifically knowledge that is based on reliable evidence obtained within the local, relevant context, the suffering people, and the extended nation of which they are so large a part, cannot be effectively **helped.**

Since an Academy has to be careful not to be seen to prescribe from an apparently pure ivory tower, let us admit that the failure to establish a knowledge base that can properly guide policy must lie to a large extent with the knowledge-producing sector itself. Instead of setting up perfectly feasible and obviously necessary high-powered interventional trials in the (regrettably) large population of affected subjects, most clinical investigators are spending their time repeating clinical trials already done many times elsewhere, on new drugs with (possibly) incremental benefits within a competitive market replete with other effective drugs. Thus, to our surprise, **the loudness of the controversy about nutritional approaches to HIV infection has not elicited a wave of well-designed local studies that would have given this Panel the right grist for its mill.**

Some of our recommendations for research involve cooperative action to establish and use benchmarks for detecting and assessing functionally significant micronutrient

deficiencies, and for assessing functionally significant immune deficits in people who are chronically malnourished, with or without infections. This kind of cooperation is really quite common in groups of specialised health-care professionals who regularly generate and publish consensus guidelines for the treatment of asthma, hypertension or duodenal ulcers. **Here we seek a more advanced degree of cooperation in which the tools to establish an evidence base have first to be cooperatively generated, so that soundly conducted trials, of both the pragmatic and explanatory kind, can be used to build a consensus on guidelines informed by local context and feasibility considerations, and equitably implemented in the whole affected population.**

**The Study Panel has been unable to give its full attention to the issue of nutritional support and therapy for HIV-infected people who are on long-term, probably lifelong antiretroviral therapy**. The WHO Technical Reports of 2005 provide excellent evidence-based guidelines based on the aggregate world literature and experience; South African caregivers are in a good position to develop collective insights into local conditions and considerations in this area, which will assume ever-greater importance as the roll-out of antiretroviral therapy is extended to the great majority of those who need it. As in the case of anti-tuberculosis therapy, more and more food-drug interactions are likely to be observed, but **we must emphasise that this factor is vastly outweighed by the sustained and beneficial effects of the drugs on the overall functioning of nearly all treated subjects, including better food intake, less malabsorption, less whole-body inflammation, and normalised caloric balance.**

The Panel has also not been able to give attention to **the vast and complicated field of biologically active phytochemicals** that may or may not exert positive effects on one or the other aspect of the pathophysiology of HIV or *M.tb.* infection. We are committed to an evidence-based approach, while accepting the good advice of those who have pointed out that no controlled, randomised clinical trial has yet been performed to prove conclusively that people stepping out of an aeroplane flying at altitude will perish if they don't have a parachute strapped onto them. The reliable evidence base on the possible efficacy of assorted phytochemicals in infected subjects appears to date to be vanishingly small; this obviously needs to be corrected in the light of the history of (some) traditional (unpurified) medicines that have turned out to contain powerful drugs of proven efficacy and safety, as confirmed through unbiased, controlled and repeated testing of the pure active principles. Advances in plant biochemistry are revealing the enormous complexity of micro- and macro-constituents of individual plant species, and the evolutionary basis for the presence of so many compounds with biological activities of such varied kinds, primarily for the purposes of the continued existence of the plants themselves and not to assist a very distantly related, unspecialised primate species. **We would be wise**





**to keep an open but critical mind about the many claims made in this field, and encourage their thorough testing, using the agreed parameters of nutritional-related dysfunction and immune functionality that we have recommended should urgently be set up by means of cooperative programmes and studies, and sound and reliable trial methodologies** (see Research Recommendations, Chapter 13). In five years time a separate Consensus Study may be feasible to review the hard evidence yielded by such studies, to take us all further.

Nutrition is a difficult science because everybody can be an "expert" (and is likely to be an assertive expert), while even the most learned and experienced authority cannot easily cope with the enormous extent of human (group and individual) variation in the composition of the regular diet, in the detailed genetic make-up, and in the particulars of the life situation. **Yet the formal science of nutrition, built up over the last century or more through the skilled gathering and interpretation of publicly reported evidence, has brought us enormous benefits, has eradicated or greatly reduced much human suffering, and has made it possible to make sensible policy for many nations, including our own**. Well-planned and executed food fortification schemes and policies, well-run and -resourced Primary School Nutrition Programmes, and high-priority food security policies in general, show that the bewildering complexity of the "system" can be reduced to manageable proportions with considerable societal benefit.

Finally, the question must be answered as to why and how a science academy (which, in the words of Mr Krushchev in referring to the extent of the Pope's authority, while certainly merit-based has no army of divisions, let alone battalions) should engage with nationally controversial policy questions such as have been covered in this Report. **The Study Panel believes that the adoption in South Africa of a model of science-based advice to the nation that is provided through rigorous review of all available evidence by a multi-disciplinary team of scholars free as far as possible of vested interest or previously declared positions, in a professionally managed independent process, is a sound and wise step, and has been humbly pleased to have undertaken one of the first exercises in this direction in this country**.

Giving advice and making recommendations is one thing; implementing them and making them work to best effect, is another. Science academies in developed countries have shied away from involvement in the implementation of their recommendations, believing this to be best left to government and/or the "relevant authorities". We are not sure that this model is optimal for developing countries like our own, and that careful, continued and appropriate engagement with certain implementation processes may well be justified. The form that this involvement should take is likely to be in the realm of further science-based policy advice where this turns out to be necessary in an unpredictable and ever-changing environment.

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## Special considerations of infancy and childhood

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## Clinical Evidence

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## Recommendations for policy; research priorities

### Reference – Chapter 12: Recommendations for policy and practice

1. South African Department of Health Integrated Nutrition Programme: Policy and Implementing Guidelines, 2nd edition, January 2005, Pretoria.

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# Appendices

APPENDIX A

# About The Study Panel

## Chairperson

**Professor Barry Mendelow –** [MB BCh, PhD, FCPath (Haem), FRSSAf, MASSAf] is employed by the National Health Laboratory Service (NHLS). He qualified in Medicine at the University of the Witwatersrand in 1972, and after internship at Johannesburg Hospital, studied as a registrar in Haematology at the South African Institute for Medical Research in Johannesburg. His research was focussed on the cell biology of haematopoiesis and B-cells and he obtained a PhD on the topic in 1980. He was awarded a Fellowship from the South African Medical Research Council and the Israeli National Council for Research and Development to conduct postdoctoral studies at the Weizmann Institute, under the supervision of Professor Leo Sachs, Head of the Department of Genetics. His research activities since then have focused mainly on haematological malignancies, with special reference to the genetics of leukaemias and the pharmacological inhibition of tyrosine kinases, switching later to cell signaling abnormalities in HIV/AIDS.

His postgraduate awards include the Watkins Pitchford Research prize of the South African Institute for Medical Research (1981), the Daubenton Prize for Teaching (1985), the Phillip Tobias Prize for Teaching (1990), the Distinguished Researchers' Award of the University of the Witwatersrand (1995).

Mendelow's previous University and SAIMR/NHLS appointments include Professor and Head of Pathology at Baragwanath Hospital (1982–1983), Assistant Dean (Research) for the Faculty of Health Sciences (1996–1998), Executive Director (Research) for Wits University (1999-2000), and research manager of the South African Institute for Medical Research (2001). He was Professor and Head of the Dept of Haematology (1983-1998) and founding Professor and Head of Molecular Medicine and Haematology at the University of the Witwatersrand (1998–2003).

He has served as President of the South African Society of Haematology and as Councillor on the International Society of Haematology and President of the Federation of South African Societies of Pathology.

## Panel Members (in surname alphabetical order)

- **Dr Peter Cegielski** [MD, MPH] received his MD degree in 1984. He proceeded to Duke University Medical Center in Durham, North Carolina for residency training in internal medicine, finishing in 1987, and then fellowship training in infectious diseases and international health, finishing in 1990. From 1988 to 1990, during his fellowship, he worked as a lecturer in the Department of Medicine, Muhimbili Medical Center, University of Dar es Salaam, Tanzania. After returning to the U.S., in 1990, he joined Duke University's faculty as an Assistant Professor in the Division of Infectious Diseases and International Health, continuing there until 1994. During that time, he completed a Masters of Public Health degree from the Department of Epidemiology in the School of Public Health at the University of North Carolina at Chapel Hill. Dr. Cegielski's thesis focused on the risks of tuberculosis due to undernutrition. Subsequently he became the head of Tuberculosis Services and Research at the University of Texas Medical Center in Tyler from 1994 to 1996. Dr. Cegielski left Texas to become Field Director of Johns Hopkins University's HIV/AIDS research unit at Chiang Mai University in Chiang Mai, Thailand until 1998. After 2 years in Thailand, Dr. Cegielski joined the international activities unit of the Division of TB Elimination at the US Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. Since then, the international TB unit at CDC has tripled in size, and in 2001 he was appointed Team Leader for Drug-Resistant TB, a position he holds to this day. While at CDC, Dr Cegielski was a founding member of the Stop TB Green Light Committee in 2000 and its chairman beginning in 2004.

- **Dr Muhammad Ali Dhansay** [MB ChB, DCH, MMed (Paed), FCPaed] qualified in Medicine at the University of Cape Town in 1977. After his internship, and a senior houseman appointment in paediatrics, he served as Medical Officer at an ambulatory care hospital in an impoverished area of Cape Town, seeing mainly children. He obtained specialist degrees in paediatrics from Stellenbosch University in Cape Town and from the College of Medicine of South Africa in 1985, having also obtained a Diploma in Child Health from the latter institution. He was awarded a 2-year MRC postgraduate scholarship with the National Research Institute for Nutritional Diseases (NRIND), investigating lipid metabolism in kwashiorkor. The position allowed him to gain experience in the research process, as well as in laboratory procedures and benchwork. The MRC offered him a permanent position as specialist scientist (medical), which brought a medical perspective to the nutrition work of the NRIND. He reached the level of Chief Specialist Scientist and was appointed Director of the MRC's Nutritional Intervention Research Unit in 2004.





He has served or is currently serving on advisory committees for the SA provincial and national departments of health (e.g. 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 and the SA HIV Clinicians Society. Previously on the editorial board of the *SA Journal of Clinical Nutrition,* he is current chair of the International Union of Nutritional Sciences, member of the Advisory Board of South African Cochrane Centre, and a trustee for the International Life Sciences Institute in South Africa.

In August 2005, in addition to his current position, he was appointed Acting Executive Director for Research at the MRC, which position he still holds. As such, he is a member of the MRC Executive Management Committee and has been involved in the current strategic restructuring of the MRC. He is on the MRC Ethics Committee, chairs the Grants Committee, and has oversight of 47 MRC research units.

- **Professor Wieland Gevers** [MB ChB, MA, DPhil, DSc hons causa, FCP SA ed eundem, FRSSAf, MASSAf, FTWAS] qualified in Medicine with First Class Honours in 1960, and proceeded as a Rhodes Scholar to Oxford University to obtain the DPhil degree in 1966 under Sir Hans Krebs (regulation of liver metabolism). He subsequently spent 4 postdoctoral years in the laboratory of another Nobelist, Dr Fritz Lipmann, at the Rockefeller University in New York (biosynthesis of peptide antibiotics) before returning to South Africa in 1970.

  He was Senior Deputy Vice-Chancellor responsible for planning and academic process at the University of Cape Town from 1992 until the end of 2002, and Professor of Medical Biochemistry since 1978. He was (founder) President of the South African Biochemical Society from 1975 to 1976, and again President from 1981 to 1982. He was President of the Academy of Science of South Africa from 1998 to 2004. He is a Fellow of the Third World Academy of Sciences (elected 2002). He holds a Distinguished Teacher's Award from the University of Cape Town.

  Gevers directed MRC Research Units at both Stellenbosch University (1970–1977) and University of Cape Town (1979–1994), using biochemical, cell-biological and molecular genetic approaches to heart contractility, intracellular protein turnover and cholesterol metabolism. He was awarded the Wellcome Gold Medal for Medical Research and the Gold Medals of both the South African Society for Biochemistry and Molecular Biology, and the South African MRC. After his formal retirement from UCT at the end of 2002, Prof Gevers took up an appointment until 31 March 2005 as the Interim Director of UCT's new Institute of Infectious Disease

and Molecular Medicine. He is now the Executive Officer of the Academy of Science of South Africa.

- **Professor Clive Gray [MSc, PhD]** is a Chief Specialist Scientist and Head of the Department of HIV Immunology at the National Institute for Communicable Diseases (NICD), Johannesburg, South Africa. He received training as an Applied Biologist and specialised in immunology in 1984. His initial research was in transplantation immunology and understanding allo-recognition and innate immunity in solid organ transplantation; he was awarded a PhD in this field in 1994. Subsequently, he moved into HIV/AIDS research, seeing this as a priority in the South African context. He was awarded the prestigious James Gear Fellowship in 1995, which allowed him to work at the Center for AIDS Research at Stanford University as a Post-doctoral Fellow.

  From 1996 to 1998, Gray was involved in investigating specific cellular immunity to HIV in individuals receiving antiretroviral drug therapy. He was one of the first to publish on the restorative effects of HAART on the immune system and to describe the plasticity of the immune response. He subsequently showed that lymph node architecture also returns after effective drug therapy and suppression of HIV replication. He was also involved in the application of MHC tetramers while at Stanford, and was lead author on one of the first papers to show diminished antigen-specific $CD8^+$ T-cells in response to lowering antigen load by suppressing viral replication with HAART. He was responsible for co-coordinating one of the first workshops on HIV vaccines in Cape Town in early 1997 with the aim of initiating HIV vaccine research in South Africa. Since this time, he has been actively involved in the scientific agenda of vaccine development in South Africa and directs much of his energies to accomplishing this task.

  Currently he directs for the South African AIDS Vaccine Initiative (SAAVI) and HIV Vaccine Trials Network (HVTN) the southern African Central Immunology Laboratory at the NICD, for monitoring the immunogenicity of HIV-1 vaccines and understanding T-cell immunity in HIV-1-infected individuals. His expertise has been recognised internationally and he co-chairs the Laboratory Sciences Sub-Committee of the HVTN and review committees for the MRC and AIDS Fonds of The Netherlands. He regularly reviews articles for the *Journal of Infectious Diseases* and the *Journal of Virology*. He is also the 2004 recipient of the Elizabeth Glaser AIDS Pediatric Fund International Leadership Award, a 3-year grant to fund the Program of Enrichment in Paediatric HIV Immunology in South Africa. He holds faculty positions as Visiting Professor at the South African National Bioinformatics Institute, University of the Western Cape, and Visiting Professor in the Department of Immunology, Duke University, North Carolina, USA.





- **Professor Glenda Gray** [MB BCH, FCPaed (SA)] is an associate Professor in the Department of Paediatrics, University of the Witwatersrand, and the executive director of the Perinatal HIV Research Unit. In 1999, she was awarded a post-doctoral Fogarty Fellowship to study clinical epidemiology at Cornell University and epidemiology at Columbia University. She has been involved in pivotal PMTCT trials since 1993. She was awarded the 2002 Nelson Mandela Health and Human Rights Award for work done in PMTC in South Africa and in 2003 received the IAPAC Hero in Medicine Award. Gray is also involved in HIV vaccine research, and led the first HIV vaccine trials in South Africa. In the NIH-funded HVTN, she is the co-chair for the phase I/II committee, and the international vice-chair for vaccines for the NIH-funded IMPAACT network. She is protocol chair for the first phase IIb HIV vaccine trial involving 3000 participants in South Africa and will lead the clinical development of South Africa's first HIV vaccines that have been developed by SAAVI. She has published more than 80 peer-reviewed scientific articles and reviews in the field of HIV and is a member of the Professional and Ethical Standards Committee of the Health Faculty Sciences, a member of the Dean's Advisory Committee on AIDS, University of Witwatersrand, and a member of Academy of Science of South Africa.

- **Dr Liesl Grobler** obtained her PhD in Exercise Physiology in December 2003. She subsequently spent 2 years doing postdoctoral research (molecular mechanisms involved in the regulation of GLUT4 expression) at the UCT/MRC Exercise Science and Sports Medicine Research unit under the supervision of Dr Edward Ojuka. Liesl then accepted a part-time position at the South African Cochrane Centre where she was responsible for conducting Cochrane Systematic Reviews and teaching evidence-based health care to medical students, doctors, policy makers and the general public. During this time Liesl was employed by the Academy of Science of South Africa as the interim Study Director of this Consensus Study: Nutrition, HIV/AIDS and TB. Stellenbosch University currently employs her as the Project Manager of the HIV/AIDS, TB and malaria clinical trials registry (ATM Registry).

- **Professor Gregory Hussey** [MBChB, MMed, MSc, DTM&H, FFCH] qualified as a Medical Practitioner. He has had postgraduate training in paediatrics, public health and infectious diseases, and is registered as a sub-specialist in infectious diseases. He was appointed as the director of the newly established Institute of Infectious Diseases and Molecular Medicine at the University of Cape Town (UCT) in 2005. Prior to that, he was Professor and Head of Paediatric infectious diseases in the School of Child and Adolescent Health at UCT and consultant to the Red Cross Children's Hospital in Cape Town.

His main research interest has been in the field of vaccine-preventable diseases and he has published extensively on this subject. He is currently the Director of the South African Tuberculosis Vaccine Initiative whose mandate is to contribute to the development of novel TB vaccines. This initiative is funded by a number of groups including the Gates Foundation via the Aeras Global TB Vaccine Foundation, the European Union and the US National Institutes of Health. With the support of Oxford University and the Wellcome Trust they have commenced with the first trials of a new TB vaccine in southern Africa.

He has been a part-time World Health Organization consultant for the past 10 years and serves on a number of international and national committees including the WHO Tuberculosis Vaccine International Advisory Group, the WHO Global Advisory Committee on Vaccine Safety, the GSK International Data Safety Monitoring Committee for a new rotavirus vaccine and the South African National Advisory Group on Immunisation.

Hussey is actively involved in postgraduate education and training and capacity development of health workers. He is the coordinator of the annual week-long residential course "Developing expertise for vaccinology in Africa". In addition he is the holder of an NIH Fogarty International Global Infectious Disease Research Training Program for the period 2006 to 2010.

- **Professor David N McMurray** [BS, MS, PhD] served in the US Peace Corps in rural Kenya from 1965 to 1967, where he decided to dedicate his career to the study of the interaction between malnutrition and infectious diseases. He received his PhD degree in Medical Microbiology from the University of Wisconsin in Madison in 1972; his dissertation research involved the development of aerosol exposure technology and its application to the early evolution of the guinea pig model of pulmonary tuberculosis. He completed his post-doctoral training at the Tulane University International Center for Medical Research in Cali, Colombia in 1976. His research in Colombia focused on the impact of infant and childhood malnutrition on immune responses among the urban poor in Cali. McMurray accepted a faculty position in the Department of Medical Microbiology and Immunology at the College of Medicine at Texas A&M University in 1976. He rose through the faculty ranks to his current position as Regents Professor, which was awarded in 2000.

  At Texas A & M, McMurray established a highly successful research program that focuses on the application of the guinea pig model of pulmonary tuberculosis to the elucidation of fundamental mechanisms of vaccine-induced resistance and the impact of nutrient deficiencies on tuberculosis vaccine efficacy. His research has been funded continuously by the US National Institutes of Health (NIH) since 1978. He has published nearly 180 papers in peer-reviewed journals. He serves on the editorial boards of three infectious disease or nutrition journals, and regularly reviews grants for the NIH and other federal and international funding agencies.

  McMurray is currently the Chair of the Tuberculosis and Leprosy Panel of the US-Japan Cooperative Medical Sciences Program, on which he has served since 1990. He was a member of the World Health Organization IMMYC Steering Committee and Animal Models Task Force from 1992 to 2000, and chaired the Steering Committee from 1997 to 2000. He has been a member of the Board of Directors of the Aeras Global TB Vaccine Foundation since 2001. Aeras is a non-profit foundation, funded principally by the Bill and Melinda Gates Foundation, which is developing novel TB vaccines and field trial sites in high-burden countries such as South Africa.

- **Associate Professor Gernard Msamanga** [MD, ScD] is a public health physician with super-specialty in population sciences. Currently he is employed as Associate Professor of Community Health at Muhimbili University College of Health Sciences in Dar es Salaam, Tanzania, a position that he has occupied for the last 8 years. In his department he was the Chair also for 6 years.

  Msamanga has experience as a consultant for the Ministry of Health, USAID, WHO, DANIDA, UNICEF and AMREF. He has worked in the areas of HIV/AIDS, population and nutrition, primary health care, immunisation, health sector reforms, quality management, environment and field research management. He has published about 60 articles in peer review journals. His recent work during the last 10 years has been to examine the effect of multivitamin supplements on pregnancy outcomes, HIV transmission and disease progression.

  Currently he is the team leader for over 300 research staff including a multidisciplinary team of investigators in Dar es Salaam. He is also in charge of the PEPFAR program for ART care and Treatment of HIV/AIDS, runs as a partnership between the Muhimbili University with the Dares Salaam City and Harvard (MDH).

  He has been a member of his College's research and publications committee for over 10 years and also the Chairman for a similar committee for the school of public health. With support from German Technical Cooperation (GTZ) district health support program, he has established and coordinated since 2002, the Muhimbili Health Exchange Forum, a website that can be accessed at www.muhef.or.tz, whose mission is to facilitate exchange of health information with health staff within and outside the country with the goal to improve quality of care.

- **Professor Dan Ncayiyana** was born and educated in KwaZulu-Natal and began his medical studies at the University of Natal before being forced into exile in his third

year. He subsequently received a medical degree in 1970. Following postgraduate training at Albert Einstein College of Medicine and New York University medical school, he was Board-certified by the American Board of Obstetrics and Gynaecology, was elected Fellow of the American College of Obstetrics and Gynaecology, and practiced in the US from 1970 to 1983. Back in South Africa, he served as Dean of Health Sciences, and later Vice-Chancellor of the University of Transkei. Subsequently, he was Deputy Vice-Chancellor at the UCT from 1996 to 2001 and Vice-Chancellor of the merged Durban University of Technology from 2001 to 2005.

Ncayiyana has been editor of the *South African Medical Journal* since 1993, serves on the Editorial Board of the *British Medical Journal* and of the web-based *Medscape*, and is chair of the Editorial Board of the HSRC Press. He was founder member and first secretary of the World Association of Medical Editors (WAME) and, more recently, was founder member of the Forum for African Medical Editors (FAME).

He is an Honorary Fellow of the Colleges of Medicine of South Africa (FCM SA) and a Member of the Academy of Science of SA (MASSAf), and has been honorary Professor of Obstetrics and Gynaecology at the universities of Cape Town and Natal. Professor Ncayiyana has previously served as consultant in higher education governance and strategic planning in a variety of Sub-Saharan African countries on behalf of the World Bank, USAID and the American Council on Education, and in health-related projects on behalf of the WHO. He is advisor to the President/ CEO of the Human Sciences Research Council (HSRC).

- **Professor Helen Rees** qualified in medicine in 1977 at Cambridge University, from where she also obtained a Masters in Social and Political Sciences for which she wrote a thesis on women and obstetric care. She specialised in General Practice and Paediatrics in London until moving to Zimbabwe in 1981, where she was in charge of the neonatal unit of Harare Central Hospital. She moved to South Africa in 1984 where she ran the Paediatric Department of Alexandra Health Centre and coordinated the rural training component of Wits University's rural outreach programme. During this time Professor Rees was an active anti-apartheid doctor, with a special interest in women's health and care of detainees, and in 1994 was responsible for coordinating women's health policy for the newly elected democratic government. In 2002 she became an alumnus of Harvard Business School.

  Rees is now the Executive Director of the Reproductive Health and HIV Research Unit of the University of the Witwatersrand, where she is also an Associate Professor in the Department of Obstetrics and Gynaecology. She is internationally recognised in the field of HIV and reproductive health. She has research interest in STIs/ HIV, microbicides, barrier methods, HIV and HPV vaccines and adolescent health,





and is an active Clinician heading a large PEPFAR grant which provides technical support to the government's HIV programmes. Rees has served on many national statutory councils, including being Chairperson of the Medicines Control Council (MCC) between 1997 and 2002, and serving on the National Health Research Ethics Committee and the Ministerial Committee for Women in Science and Technology. She was recently elected as a member to the Academy of Sciences of South African and she is also a member of the Wits University Council.

Rees serves on or chairs a number of international scientific committees including the International AIDS Vaccine Initiative, WHO's Strategic Advisory Group of Experts on Immunisation and the WHO Expert Committee on HIV vaccines and on HPV vaccines. She has served as an adviser to WHO and to UNAIDS on a range of issues including STIs and HIV, microbicides, HIV and HPV vaccines, contraception and on drug regulation, and in 2002 and 2006 she co-chaired the International Microbicides Conference.

She was made an Officer of the British Empire in 2001 for her work in the South African and international health sectors. In 2004 she became the first South African to receive the national award for 'Distinguished Scientist recognised for their outstanding contribution to improving the quality of life of women.' In 2003 she was awarded a Life Time Achievement award by Amanitare, a pan-African partnership of organisations working in the field of Women's health and rights.

- **Dr W D Francois Venter** qualified in medicine in 1993, subsequently obtaining his specialist qualification as a physician in 2000. He obtained the DTM&H and Diploma in HIV Medicine shortly after, while working for the Clinical HIV Research Unit and then subsequently the Reproductive Health and HIV Research Unit (RHRU). He is currently Clinical Director of the HIV Management Cluster, RHRU, and a lecturer in the Department of Medicine, University of the Witwatersrand.

  Venter is one of a small number of public sector HIV physicians in South Africa. He lectures extensively on HIV, has expertise in the use of antiretroviral (ARV) therapy in developing countries, and is one of the few clinicians with experience of using ARVs in public sector services. He supports a major academic hospital's HIV clinic, which treats several thousand patients on ARVs and was one of the first SA ARV rollout sites. He participated in the National and Provincial Department of Health's 2003 ARV rollout working groups developing guidelines on opportunistic illness and antiretroviral treatment, and is actively supporting the Gauteng and North-West provinces in both training and treatment. Venter is involved in a number of research grants that partner the National Institutes of Health (NIH) and the University of North Carolina. He supports several palliative cares and supports

non-governmental organisations, and participates on the Dean's AIDS Advisory Committee at the University of the Witwatersrand.

Venter is currently President of the Southern African HIV Clinicians Society (SAHCS), which has over 10 000 members throughout Africa.

- **Professor Jimmy Volmink** [BSc, MB ChB, DCH, MPH, D Phil] is Professor and Deputy Dean (Research) in the Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa and Director of the South African Cochrane Centre, Medical Research Council. He has previously held appointments as Professor and Chair of Primary Health Care at the University of Cape Town and Director of Research and Analysis at the Global Health Council, Washington DC.

  Volmink has a special interest in rigorous evaluation of the effects of health care interventions. He has extensive experience in the application of randomised controlled trials, systematic reviews and meta-analysis in evaluating strategies and therapies for the control of tuberculosis, HIV/AIDS and cardiovascular disease. He has worked with policy-makers and clinicians both globally and locally (in South Africa) to promote the use of research evidence in decisions about health care and has taught courses in evidence-based health care to students, health professionals and policy-makers in a large number of countries.

  Volmink has authored more than 100 peer-reviewed journal articles and book chapters. He serves on committees and advisory boards of a number of international organisations, including the Wellcome Trust, World Health Organization, Cochrane Collaboration, Doris Duke Charitable Foundation and the International Clinical Epidemiology Network (INCLEN). He is also a member of the advisory boards of a number of international journals and serves as a peer referee for several others. He is member of the Council of the Academy of Science of South Africa and has recently been appointed as Honorary President of the Pharmaceutical Care Management Association of South Africa.

- **Professor Esté (HH) Vorster** obtained a DSc (Physiology) with a thesis on nutrition and haemostasis. She started the Nutrition Research Group at the institution which is now the North-West University, and is at present the Director of the Africa Unit for Transdisciplinary Health Research in the Faculty of Health Sciences.

  Vorster has served as President of the Nutrition Society of South Africa (NSSA) from 1995 to 1998 and again from 2003 to 2006. She was also President of International Life Science Institute South Africa from 1997 to 2001, served on the National Board of the International Union of Nutritional Sciences (IUNS) for 15 years and ICSU for 2 years. She is a member of the IUNS Task force for the





Nutrition Transition, has served as invited expert and chair of several WHO/FAO expert consultation groups, and is part of an international group which is redefining nutritional science (The New Nutrition Science Project) and another UNU/WHO/FAO/UNICEF group that has harmonised global nutrient recommendations.

Vorster has conceptualised nutrition research as a holistic, 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 200 papers in peer-reviewed scientific journals and is author, co-author and editor of 5 books and chapters in textbooks on Human Nutrition. Vorster is the first woman who received the NSSA award for "Outstanding Contributions to Nutrition Research" (in 1996) and her group was awarded the 5-yearly NNIA award for their sustainable contribution to Nutrition Research in Africa in 2005.

APPENDIX B

# Glossary of key terms

**Amino acid:** One of 21 nitrogen-containing building blocks of proteins.

**Antiretroviral:** Pharmacological agent that inhibits replication of a retrovirus, usually referring specifically to the human immunodeficiency virus (HIV).

**Arginine:** One of 21 amino acids.

**Carbohydrate:** A form of food rich in carbon-hydrogen-oxygen bonds, which on being completely combusted (oxidised) in the body provides a moderate amount of energy per gram.

**Endemic:** Occurs commonly within a geographical region.

**Fat:** A form of food rich in hydrocarbon bonds which on being completely combusted (oxidized) in the body yields a large amount of energy per gram.

**Fatty acid:** (Usually straight) chain of hydrocarbons.

**Fortification:** The deliberate and systematic addition of a nutrient or mixture of nutrients to commonly ingested foods.

**Immunity:** The complicated system of specific cells, tissues and chemicals that constantly and highly effectively defends the body from harmful microorganisms; it has an **innate** component which is always immediately ready to act, and an **adaptive** component which takes time to become active but when it does is both very powerful and highly selective, as well as being able to "remember" the invading organisms so that a second attack can be prevented or ended very quickly.

**INL98:** Typically the quantity of a nutrient required daily for normal health.

**Interventional trial:** One in which a particular (possibly beneficial) intervention, such as administration of a foodstuff or supplement, is given to some members of cohort of subjects, but not to others who receive placebos (see below); predefined outcomes are then measured after an interval. (Optimally, such trials should be "double-blind", i.e. neither the subjects nor the investigators should know the distribution of test subjects and controls during the trial period.) (See "Randomised controlled trial" below).

**Lipid:** Fat.

**Microbiota:** A large and sustained culture of mixed microorganisms occupying a habitat in or on the body.

**Macronutrients:** Nutrients that are required in large amounts, usually tens or hundreds of grams; they comprise proteins, fats and carbohydrates.

**Malabsorption:** The inability to absorb adequate quantities of ingested nutrients or other food components, despite adequate dietary intake thereof.

**Micronutrients:** Nutrients that are required in small amounts, typically tens to hundreds of milligrams; they comprise 13 vitamins and 17 trace elements.

**MDR TB**: Multi-Drug Resistant Tuberculosis is defined as resistance to at least two of the most effective anti-TB drugs, rifampicin and isoniazid.

**Nutrient:** Component of food that is necessary for normal health, specifically to provide for normal tissue and cell growth/repair, and/or a form of energy usable by the body.

**Observational study:** Gathering (often retrospectively) of clinical information relating to particular observations, such as favourable or unfavourable outcomes or surrogates thereof to other factors such as dietary intakes.

**Pathogenesis:** The natural course and mechanisms of disease.

**Pharmacokinetics:** Information describing the absorption, distribution and fate of administered pharmacological compounds (drugs).

**Phytochemical:** Non-nutrient component of plant-derived food, many but not all of which have some form of (evolved to be useful) biological activity in their host plants, but may also have actions in animals, including humans.

**Placebo:** Sham intervention that is assumed to exert no objective effect.

**Prebiotic:** Food component that stimulates growth of probiotic micro-organisms (see "Probiotics", below).

**Protein:** Complex biological compound consisting of a string/chain of amino (ie nitrogen–rich) acids.

**Probiotic**: Ingested microorganisms that confer benefits on the host when they colonise the intestines.

**Protein energy malnutrition:** Nutritional deficiency state caused by inadequate intake of both dietary calories and amino acids.

**Randomised controlled trial:** An interventional trial (see above) where bias is eliminated by the process of prospectively assigning specified treatments and/ or controls.

**Retrovirus:** One of a group of RNA (ribose nucleic acid)-based viruses, whose life cycle commences with their ability to make DNA (deoxyribose nucleic acid) copies, which then insert permanently into the nuclear DNA of the host cell.

**Saturated fatty acid:** Fatty acid with only single bonds linking its component hydrocarbons.





**Supplementation:** The administration of a nutrient or mixture of nutrients in addition to normally ingested foods.

**Trace element:** A simple chemical element that (in this context) is also a necessary component of food, e.g. iron, selenium, zinc.

**Triglyceride:** Most common form of dietary fat, comprising a (carbohydrate) glycerol backbone to which three fatty acids are attached.

**Unsaturated fatty acid:** Fatty acid with one or more double bonds linking its component hydrocarbons.

**Vitamin:** Complex biochemical compound that as a micronutrient is a necessary dietary component of food, usually over medium to long periods.

**XDR TB**: Extensively Drug Resistant tuberculosis (resistant to all or nearly all available anti-TB agents).

APPENDIX C

# Acronyms and Abbreviations

**A**

| | |
|---|---|
| ACT | Alpha-1 chymotrypsin |
| ACTH | Adrenocorticotropic hormone |
| AGP | Alpha-1 acid glycoprotein |
| AHRQ | Agency for Healthcare Research and Quality |
| AICR | American Institute for Cancer Research |
| AIDS | Acquired immune deficiency syndrome |
| AMREF | African Medical Research and Education Foundation |
| ANC | African National Congress |
| ANR | Average Nutrient Requirement |
| APC | Antigen-presenting cells |
| APP | Acute phase proteins |
| ARI | Average Requirement Intake |
| ART | Antiretroviral Therapy |
| ASADI | African Science Academy Development Initiative |
| ASSA | Actuarial Society of South Africa |
| ASSAf | Academy of Science of South Africa |

**B**

| | |
|---|---|
| BCG | Bacille Calmette-Guérin |
| BHITS | Breastfeeding and HIV International Transmission study |
| BIA | Bioelectrical impedance analysis |
| BMI | Body Mass Index |
| BMR | Basal metabolic rate |

**C**

| | |
|---|---|
| $CD4^+$ | Cluster of differentiation 4 |
| CD8 | Cluster of differentiation 8 |

| | |
|---|---|
| CDC | Communicable Disease Control |
| CI | Confidence interval |
| CRH | Corticotropic-releasing hormone |
| CRP | C-Reactive Protein |
| CSIR | Council for Scientific and Industrial Research |
| CTL | cytotoxic T-cell |

**D**

| | |
|---|---|
| DBP | D-binding protein |
| DHA | Dehydroascorbic acid |
| DNA | Deoxyribonucleic Acid |
| DoH | Department of Health |
| DOTS | Directly Observed Therapy Short |
| DRI | Dietary Reference Intakes |
| DTH | Delayed-type hypersensitivity |

**E**

| | |
|---|---|
| EAR | Estimated Average Requirement |
| EDCTP | European and Developing Countries Clinical Trials Partnership |

**F**

| | |
|---|---|
| FAME | Forum for African Medical Editors |
| FAO | Food and Agriculture Organisation |
| FFM | Fat-free mass |

**G**

| | |
|---|---|
| GALT | Gut Associated Lymphoid Tissue |
| gp41 | Glycoprotein 41 |
| gp120 | Glycoprotein 120 |
| GRADE | Grading of Recommendations Assessment, Development and Evaluation |
| GTZ | German Technical Cooperation |

**H**

| | |
|---|---|
| HAART | Highly active antiretroviral therapy |
| HIV | Human Immunodeficiency Virus |
| HIV-1 | Human Immunodeficiency Virus type 1 |
| HLA | Histocompatibility antigens |
| HPV | Human Papillomavirus Virus |





| | |
|---|---|
| HR | Hazard Ratio |
| HSRC | Human Sciences Research Council |
| HVTN | HIV Vaccine Trials Network |

**I**

| | |
|---|---|
| IAC | InterAcademy Council |
| IAP | InterAcademy Panel |
| ICSU | International Council on Science (ICSU |
| IDD | Iodine Deficiency Disorders |
| IFNα | Interferons gamma |
| IgM | Immunoglobulin M |
| IgA | Immunoglobulin A |
| ILSI | International Life Sciences Institute |
| IL-1β | Interleukin-1 beta |
| IL-6 | Interleukin-6 |
| IKS | Indigenous Knowledge Systems |
| INCLEN | International Clinical Epidemiology Network |
| INH | Isoniazid (also called isonicotinyl hydrazine or isonicotinic acid hydrazide) |
| INL | Individual Nutrient Intake Level |
| IU | International Unit |
| IUNS | International Union of Nutritional Sciences |

**L**

| | |
|---|---|
| LPS | Lipopolysaccharides |
| LBW | Low Birth Weight |

**M**

| | |
|---|---|
| MALT | Mucosa Associated Lymphoid Tissues |
| MASSAf | Member Academy of Science of South Africa |
| MCC | Medicines Control Council |
| MDG | Millennium Development Goal |
| MDR | Multi Drug Resistant |
| MHC | Major histocompatibility complex |
| MMN | Multiple micronutrients |
| MRC | Medical Research Council |
| Mtb | M.tuberculosis |
| MTCT | Mother-to-child transmission |

**N**

| | |
|---|---|
| NCD | Non-communicable diseases |
| NCp7 | Nucleocapsid protein 7 |
| NDoH | National Department of Health |
| NEPAD | New Partnership for Africa's Development |
| NF-κB | Nuclear factor κB |
| NICD | National Institute of Communicable Diseases |
| NIH | National Institutes of Health |
| NIV | Nutrient Intake Values |
| NHANES-1 | National Health and Nutrition Examination |
| NHEFS | NHANES-1 Epidemiological Follow-up Study |
| NHLS | National Health Laboratory Service |
| NK | Natural killer |
| NO | Nitric oxide |
| NRIND | National Research Institute for Nutritional Diseases |
| NSSA | National Science Supervisors Association |

**O**

| | |
|---|---|
| OR | Odds Ratio |

**P**

| | |
|---|---|
| PD-1 | Programmed Death-1 |
| PEM | Protein Energy Malnutrition |
| PEPFAR | Presidential Emergency Plan for AIDS Relief |
| PETRA | Positron-Electron Tandem Ring Accelerator |
| PMTCT | Prevention of Mother to Child Transmission |
| PMN | Polymorphonuclear neutrophil |
| POW | Prisoners of War |
| PPD | Purified protein derivative |
| PRI | Population Reference Intake |
| PSNP | Primary School Nutrition Programme |
| PUFA | Polyunsaturated fatty acids |

**R**

| | |
|---|---|
| RBP | Plasma retinol binding protein |
| RCT | Placebo-controlled trials |
| RCT | Pandomized controlled trials |
| RDA | Recommended daily allowances |
| RDR | Relative dose response |
| REE | Resting energy expenditure |
| RFP | Requests for-proposals |
| RHRU | Reproductive Health and HIV Research Unit |
| RNA | Ribonucleic acid |
| RNI | Reference Nutrient Intake |
| RR | Relative Risk |
| RT | Reverse Transcriptase |

**S**

| | |
|---|---|
| SAA | Serum amyloid A |
| SAAVI | South African AIDS Vaccine Initiative |
| SAHCS | Southern African HIV Clinicians Society |
| SAIMR | South African Institute of Medical Research |
| SAVACG | South African Vitamin A Consultative Group |
| SD | Standard Deviation |
| SIgA | Secretory IgA |
| SIgM | Secretory IgM |
| SIV | Simian immunodeficiency virus |
| STI | Sexually transmitted infections |

**T**

| | |
|---|---|
| T | Thymus |
| TAS | Total antioxidant status |
| TB | Tuberculosis |
| TCR | unique T-cell receptors |
| TE | Total Energy |
| TEE | Total energy expenditure |
| TGFα | Transforming growth factor α |
| THUSA | Transition and Health during Urbanization of South Africans |
| TLR | Toll-like receptors |
| TNF-α | Tumour necrosis factor |
| TWAS | Academy of Sciences of the Developing World |

**U**

| | |
|---|---|
| UN | United Nations |
| USAID | United State Agency for International Development |
| UNAIDS | Joint United Nations Programme on HIV and AIDS |

| | |
|---|---|
| UNICEF | United Nations International Children Educational Fund |

**V**

| | |
|---|---|
| VAD | Vitamin A deficiency |
| VDR | Vitamin D receptor |

**W**

| | |
|---|---|
| WAME | World Association of Medical Editors |
| WCRF | World Cancer Research Fund |
| WHO | World Health Organization |
| WHZ | Weight for height Z-score |

**X**

| | |
|---|---|
| XDR | Extensively Drug Resistant |



APPENDIX D

# The Council of the Academy Of Science of South Africa (ASSAf)

## Steering Academy activities and taking responsibility

The affairs of the Academy are governed by a Council comprising 12 members, each of whom holds office for four years. ThE Council is elected by the Members every two years. For the sake of continuity, six members continue to serve a further term, while six new members are elected once they have been nominated according to the constitutional mechanism. To provide a better balance of race, gender or disciplinary area, the Council can coopt additional members from persons who were nominated for election to the Council for the current term.

The members of the 2005–7 ASSAf Council are; **Dr Rob Adam, Prof Patricia Berjak** (Vice President), **Prof Robin Crewe** (President), **Prof Vivian de Klerk, Prof Manfred Hellberg, Prof Jonathan Jansen** (Vice President), **Prof Benito Khotseng** (General Secretary), **Prof Sunil Maharaj, Prof Chabani Manganyi, Prof Daniel Ncayiyana, Dr Francis Petersen, Prof Priscilla Reddy, Prof Peter Vale** (treasurer)**, Prof Jimmy Volmink**.

The Secretary of the ASSAf Council is the Memberships Officer, **Mr Ntsheu Mangena**

APPENDIX E

# About the academy

## Objectives

### Scientific thinking for the good of society

According to the Act the **objectives of the Academy** are:

- to promote common ground in scientific thinking across all disciplines, for example the physical, mathematical, life, human, social and economic sciences;
- to encourage and promote innovative and independent scientific thinking;
- to promote the optimum development of the intellectual capacity of all people;
- to provide effective advice and facilitate appropriate action in relation to the collective needs, opportunities and challenges of all South Africans; and
- to link South Africa with scientific communities at the highest levels, in particular within Africa, and further afield.

## Vision

### An engine of excellence in scholarship and intellectual cooperation

ASSAf aspires to be the apex organisation for science and scholarship in South Africa, internationally respected and connected, its membership simultaneously the aspiration of the country's most active scholars in all fields of scientific enquiry, and the collective resource making possible the professionally managed generation of evidence-based solutions to national problems.

## Mission statement

### Clarifying the niche of the Academy

Like democratic South Africa in general, ASSAf aspires to play both a national and an international role, particularly with respect to the African continent. We see the Academy as usefully at arm's length from government and other organised sections of

the state, comprising an assembly of excellent scholars from many disciplines who are well-networked both nationally and internationally, and have shown their interest in and capacity for promoting the development of a prosperous and a fully enabled society. Membership of the Academy (by election) is both an honour and an obligation to work individually and collectively (as the Academy) to ensure that decision making requiring scholarly scrutiny and analysis is based on the best and most integrated understandings and insights available to the country. The Academicians thus represent an organised, independent but responsive scholarly voice to help guide the development of the country and its people.

### The mission of ASSAf is thus to;

- become increasingly associated in the mind of the nation with the highest levels of scholarly achievement and excellence in the application of scientific thinking for the benefit of society;
- consolidate its infrastructure and capacity, and to expand and mobilise the membership to ensure that scholars from a full disciplinary spectrum are available for its work, and that these are indeed both thinkers and doers, willing to put significant effort into the Academy's activities;
- embark on a programme of systematic studies of evidence-based issues of national importance, some proposed by government or other sectors, and some identified by the Academy itself;
- develop a sound and robust methodology for constituting study panels, organising their work, including conferences and workshops, and producing authoritative reports that are well-disseminated and have significant impact;
- publish science-focused periodicals, especially a multidisciplinary journal of high quality (the *South African Journal of Science*) and a science magazine that will showcase the best of South African research to a wide national (and international) audience (*Quest – Science for South Africa*); and to promote the development in South Africa of an indigenous system of research journals of internationally recognised quality and usefulness;
- develop productive partnerships with other organisations, especially (but not only) the departments of Science and Technology, Education, Health and Agriculture; the National Advisory Council on Innovation; science councils; higher education institutions, etc., with a view to the building of capacity in science and its applications within the National System of Innovation (NSI);
- create new and diversified sources of funding for the sustainable functioning of an independent Academy;





- communicate effectively with the general and specific publics, as well as with partners and sponsors;
- develop a plan for the expansion of the activities of ASSAf in partnership with the national science academies of other countries, including contracted partnership with the US National Academies; and
- play a significant role in the international science system, particularly in Africa, through organisations such as the InterAcademy Panel (IAP) and the InterAcademy Council (IAC), the Academy of Sciences of the Developing World (TWAS), the International Council on Science (ICSU), as well as the Network of African Science Academies (NASAC), all in the context of the New Partnership for Africa's Development (NEPAD).

## Members

### Core asset of the Academy (each styled "MASSAf")

After nomination by four existing Members (at least two of whom do so from personal knowledge of the candidate), new Members of the Academy are elected in a secret ballot. The normal criterion for election is significant achievement in the advancement or application of science, and, in addition, Members should be persons who can be expected significantly to assist the Academy in achieving its objectives. By October 2006, ASSAf had over 250 Members drawn by self-categorisation from the earth, economic, life, mathematical, physical, social, technological, education, and agricultural sciences as well as the humanities.

## International connections

### Crucial catalyst for Academy-type activities

ASSAf is an active member of the IAP, a growing organisation that embraces the national science academies of over 90 countries. The Academy of Sciences for the Developing World now has an office in Africa based in Nairobi, and the Network of African Science Academies, of which the President of ASSAf is a Vice-President, is also located in that city.

ASSAf became an "intense partner" of the US National Academies (together with the Nigerian and Ugandan Academies of Science) as part of the African Science Academy Development Initiative (ASADI), receiving a substantial 5-year grant to build its capacity for generating evidence-based advice for the government and the nation in general.

## Strategic plan and policy development

### The way to go

ASSAf has developed a comprehensive strategic plan following a thorough process for identification of its strengths, weaknesses, opportunities and threats. Through its governing Council, the Academy has developed policies and guidelines for its activities. The initiation of the ASADI partnership with the US National Academies prompted the generation, proposal and adoption of the following items:

- Guidelines for proposals of science-based topics in terms of the ASSAf Act
- Guidelines for proposals of science-based topics (project proposals)
- Guidelines for the appointment of study panels and forum steering committees
- Policy on conferences
- Formation of a Committee on Science for the Alleviation of Poverty (first example of an ASSAf "Board") Panel for the Consensus Study on Nutritional Influences on Human Immunity, with special reference to clinical tuberculosis and HIV infection (first ASSAf Consensus Study).
- ASSAf's strategic plan and the Academy's policies and guidelines are publicly featured on the ASSAf website at http://www.assaf.org.za

## Research publishing

### The core of the quality assurance system for the dissemination of research findings

The Academy of Science of South Africa signed a contract in 2001 with the Department of Science and Technology (DST) for various activities in connection with the "strategic management" of research journals published in South Africa. The first component was a comprehensive study of the present and best-possible future role of research journals published in South Africa, now completed through the release of a full report in March 2006, with evidence-based recommendations, and a range of follow-up project integration and implementation strategies.

## SAJS

### Publishing the *South African Journal of Science*: a Nature for South Africa

The *South African Journal of Science* is the leading multidisciplinary research journal in Africa, and features a great diversity of original work by researchers throughout the country and abroad, concentrating on articles that have an appeal that is wider than that of single disciplines. Among the highlights of the volume published in 2005 were articles





featuring the research at historically black universities supported by the Royal Society-NRF bilateral programme. The journal appears six times a year, and is accessible online as one of the e-publications managed by SABINET.

## Quest

### Publishing Quest: A quarterly magazine of high quality, presenting science for South Africa

The Academy publishes the national science magazine *Quest: Science for South Africa* that was launched in 2004. Quest serves as a platform for communication about scientific research done in South Africa. It strives to showcase South African science in action, and is aimed at the broad scientific community, decision-makers, the public, students, and especially the senior grades at secondary schools.