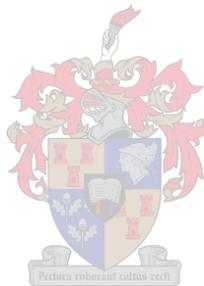


**EFFECTS OF A MICRONUTRIENT, GLUTAMINE, PRE- AND
PROBIOTIC ENRICHED LIQUID SUPPLEMENT ON NUTRITIONAL
STATUS AND IMMUNITY OF ADULTS WITH HIV/AIDS:
A PILOT STUDY**

Roy D Kennedy

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Masters in Nutrition



Research Study Leader:

Prof Demetre Labadarios

Confidentiality:

Grade A

December 2003

DECLARATION OF AUTHENTICITY

I, Roy Donovan Kennedy, hereby declare that the work contained in this thesis is my own original work and that I have not previously, in its entirety or in part, submitted it at any university for a degree.

Signature:

Date: 14/11/2003

ABSTRACT

INTRODUCTION: The objective of this pilot study was to evaluate the effects of a new micronutrient, glutamine, pre- and probiotic enriched liquid nutritional supplement on the nutritional status and immunity of adults living with HIV/AIDS. The study was designed as a prospective randomised double-blind placebo-controlled trial. Subjects were HIV-infected male and female adult volunteers (n = 47) from a community-based hospice centre in a peri-urban area in a resource-poor setting and were included irrespective of duration or clinical stage of HIV/AIDS. None of the subjects received antiretroviral therapy.

METHOD: The intervention involved the daily ingestion of 40g (200 ml reconstituted) of either the enriched test product or an isocaloric carbohydrate placebo for a period of 12 weeks. Anthropometric assessment (weight, height and triceps skinfold thickness; mid-upper arm, waist and hip circumferences) was performed at baseline and thereafter every 4 weeks (4 times). Biochemical (serum total protein, serum albumin and C-reactive protein) and haematological (full blood count and immunophenotyping) assessment was performed at baseline and again after week 12.

RESULTS: Statistical analysis of baseline values was performed with Wilcoxon two-sample tests for comparison between the supplemented and placebo groups. Outcomes were evaluated using analysis of variance with Shapiro-Wilk tests and thereafter either pair-wise t-tests or sign tests (for nonparametric data) were used. Thirty-two subjects completed the trial, 14 in the supplemented group and 18 in the placebo group. Weight increased significantly in the supplemented group (2.73 ± 3.53 kg, $p = 0.013$). Triceps skinfold thickness increased significantly in both the supplemented ($p = 0.047$) and placebo group ($p = 0.001$). No other significant

anthropometric change was observed. Serum albumin increased significantly in the supplemented group ($p = 0.003$) and was associated with a significant decline in C-reactive protein ($p = 0.028$). Haemoglobin decreased significantly in both groups. A significant decline in CD4+ count was observed in the placebo group while the decline in the supplemented group did not reach significance.

CONCLUSION: Oral nutritional supplementation in limited quantities was well tolerated for a period of 3 months. This study demonstrated that an enriched nutritional supplement was able to promote weight gain and ameliorate hypoalbuminaemia and possibly inflammation in adults living with HIV/AIDS in the short to medium term. The enriched nutritional supplement does not appear to have an effect on the immunity of people with HIV/AIDS. The small sample is a limitation of the study and the conclusions pertain to the test product as a whole and not to any of its respective ingredients. Although further studies are required to evaluate long-term feasibility, these findings suggest that the use of an enriched nutritional supplement has a role in the management of weight loss in persons with HIV/AIDS.

OPSOMMING

INLEIDING: Die doel van hierdie loodsstudie was om die uitwerking van 'n nuwe mikronutriënt, glutamien, pre- en probiotika verrykte voedingsaanvulling in vloeistof vorm te ondersoek. Die studie is ontwerp as 'n prospektiewe ewekansige dubbelblinde plasebogekontroleerde toets. Proefpersone was MIV-geïnfekteerde manlike and vroulike vrywilligers ($n = 47$) van 'n gemeenskapsgebaseerde hospitium in a semi-stedelike gebied in 'n hulpbron-arme omgewing. Proefpersone is ingesluit ongeag die duur of kliniese graad van MIV/VIGS. Geen proefpersoon het antiretrovirale behandeling ontvang nie.

METODE: Die intervensie het die daaglikse inname van 40g (200 ml gerekonstitueer) van óf die toetsproduk óf 'n isokaloriese koolhidraatplasebo gedurende 'n 12 week periode behels. Antropometriese evaluering (gewig, lengte en trisepsvelvoudikte; midbo-arm-, middel- en heupomtrekke) is uitgevoer met aanvang en daarna weer elke 4 weke (4 keer). Biochemiese (serum totale protein, serumalbumien en C-reaktiewe protein) en hematologiese (volbloedtelting en immunofenotipering) evaluering is uitgevoer met aanvang en weer na 12 weke.

RESULTATE: Statistiese verwerking van basislyndata is gedoen deur middel van Wilcoxon twee-steekproef toetse waarmee vergelyking tussen die aangevulde en plasebogroep uitgevoer is. Studiegevolge is geëvalueer deur verspeidingsanalise met behulp van Shapiro-Wilk toetse waarna óf paargewyse t-toetse óf tekentoetse (vir nie-parametriese data) gebruik is. Twee-en-dertig proefpersone het die studietydperk voltooi, 14 in die aangevulde groep en 18 in die plasebogroep. Gewig het betekenisvol toegeneem in die aangevulde groep (2.73 ± 3.53 kg, $p = 0.013$). Triseps velvoudikte het betekenisvol toegeneem in beide die aangevulde ($p = 0.047$) en die plasebogroep ($p = 0.001$). Geen ander betekenisvolle antropometriese

veranderinge is waargeneem nie. Serumalbumien het betekenisvol gestyg in die aangevulde groep ($p = 0.003$) en het gepaard gegaan met 'n betekenisvolle daling in C-reaktiewe proteïen ($p = 0.028$). Hemoglobienwaardes het in beide groepe betekenisvol gedaal. 'n Betekenisvolle daling in CD4+ telling is waargeneem in die plasebogroep terwyl die daling in die aangevulde groep nie betekenisvol was nie.

GEVOLGTREKKING: Mondelinge voedingsaanvulling van 'n beperkte hoeveelheid was goed aanvaar en verdra oor 'n 3-maande tydperk. Hierdie studie toon dat 'n verrykte voedingsaanvulling in staat is om gewigstoename te bevorder en om hypoalbuminemie en moontlik ook inflammasie te verlig in volwassenes met MIV/VIGS oor 'n kort tot medium tydperk. Die verrykte voedingsaanvulling blyk nie 'n effek op die immuniteit van mense met MIV/VIGS te hê nie. Die klein steekproef is 'n beperking van die studie en die gevolgtrekkings is slegs van toepassing op die toetsproduk as 'n geheel en nie op enige van die onderskeie bestanddele daarvan nie. Hoewel verdere studies nodig geag word om langtermyn uitvoerbaarheid te ondersoek, dui hierdie bevindinge daarop dat die gebruik van 'n verrykte voedingsaanvulling 'n rol speel in die beheer van gewigverlies in persone met MIV/VIGS.

DEDICATION

For T. and everyone who is living bravely with the virus,
especially the children.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ARC	Agricultural Research Council (South Africa)
ARV	antiretroviral
BMAMA	bone-free mid-upper arm muscle circumference
BMI	body mass index (Quetelet's index)
CD3+, 4+ and 8+	the presence of the respective receptors on the surface of T-lymphocytes usually required for efficient HIV infection, CD4+ lymphocyte depletion is the main immunological disturbance with HIV/AIDS
CDC	Centre for Disease Control (USA)
CRP	C-reactive protein, an acute phase protein, plasma levels of which rise rapidly in response to acute inflammation and an indicator of acute infection
d	day
FBC	full blood count
GALT	gut-associated lymphoid tissue
Hb	haemoglobin
HIV	human immunodeficiency virus, the cause of AIDS
HIV/AIDS	refers to HIV infection at any stage of the disease, including AIDS, emphasising the link between HIV and AIDS
MAMA	mid-upper arm muscle area
MAMC	mid-upper arm muscle circumference
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
Medunsa	Medical University of South Africa
<i>MIV</i>	<i>menslike immuniteitsgebrekvirus</i>
MUAC	mid-upper arm circumference
MRC	Medical Research Council (South Africa)
n	number, referring to sample size
NGO	non-government organisations
NHLS	National Health Laboratory Services (South Africa)
RBC	red blood cells

RBW	relative body weight
RDA	recommended daily allowance
SABS	South African Bureau of Standards
s-Alb	serum albumin
SD	standard deviation
s-TP	serum total protein
TSF	triceps skinfold thickness
VIGS	<i>verworwe immuniteitsgebreksindroom</i>
WBC	white blood cells
WHO	World Health Organisation
WHR	waist:hip ratio, an indicator of health risk
\bar{x}	mean

LIST OF DEFINITIONS

enterotropic formula	a chemically adapted supplemental nutritional formula resulting in improved digestion and absorption, also known as semi-elemental formula
Frankfort plane	anatomical placement of the head in line with the spine, the lowest margin of the socket of the eye forming a horizontal line with the level of the <i>tragion</i> of the ear
immune-enhancing	the effect of improving immune function
immunonutrition	the use of specific nutrients to improve immune function
peptide-enhanced formula	a supplemental nutrition formula adapted to include di- and tripeptides, which facilitate absorption
prebiotics	non-digestible food ingredients that stimulate the growth of probiotic microorganisms in the colon
probiotics	live microorganisms occurring naturally in the human gut, which when ingested, improve the balance of intestinal flora and positively affect the functioning of the intestinal tract and general health
T-cells/lymphocytes	the group of lymphocytes, which are the main target cells of HIV infection
ω -3 fatty acids	a group of long-chain polyunsaturated fatty acids with a double bond situated between the third and fourth C-atoms from the ω -end, also referred to as n-3 fatty acids

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CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1 MALNUTRITION AND HIV/AIDS

Sub-Saharan Africa is worst hit by the human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) pandemic. It is estimated that there are more than 25 million infected people in this region, accounting for 70% of global cases.¹ The majority of these HIV-infected people live in South Africa, where the prevalence of HIV infection may be as high as 19,2%², with 40% of adult deaths being ascribed to acquired immunodeficiency syndrome (AIDS)-related disease.³ The impact of HIV/AIDS is felt at all levels of society, in health systems, the workforce, the economy, and human development.^{4,5}

Malnutrition is a common consequence of HIV infection and recent weight loss is a diagnostic and classification criterion of HIV/AIDS. The relationship between HIV/AIDS, malnutrition and wasting is cyclic and has been well described in publications over the last decade or more.^{6,7,8} Nutritional status is compromised by reduced and/or inadequate food and nutrient intake^{9,10}, malabsorption and increased nutrient losses caused by gastrointestinal dysfunction¹¹ and increased nutritional requirements due to infection and fever^{12,13} (Figure 1.1). Malnutrition by itself is accepted to contribute to the frequency and severity of opportunistic infections associated with HIV/AIDS.¹⁴ Nutritional status has been identified as a major factor in survival^{15,16,17} and failure to maintain body cell mass leads to death at 54% of body mass.^{18,19}

Malnutrition and wasting is a universal consequence of HIV/AIDS. It is documented that the maintenance of body weight, and in particular lean body tissue, impact positively on survival in HIV/AIDS.¹⁹ In the face of increasing symptoms and opportunistic infections as the disease progresses, nutritional management of HIV/AIDS should focus on the support of food and nutrient intake in an effort to maintain body weight. Documented evidence of the benefit of food-based nutrition

intervention in HIV/AIDS is lacking.⁸ The implementation of adjunctive or alternative nutrition intervention strategies may be required and a great need exists for the examination of the effects of oral nutritional supplements on nutritional status and immunity.

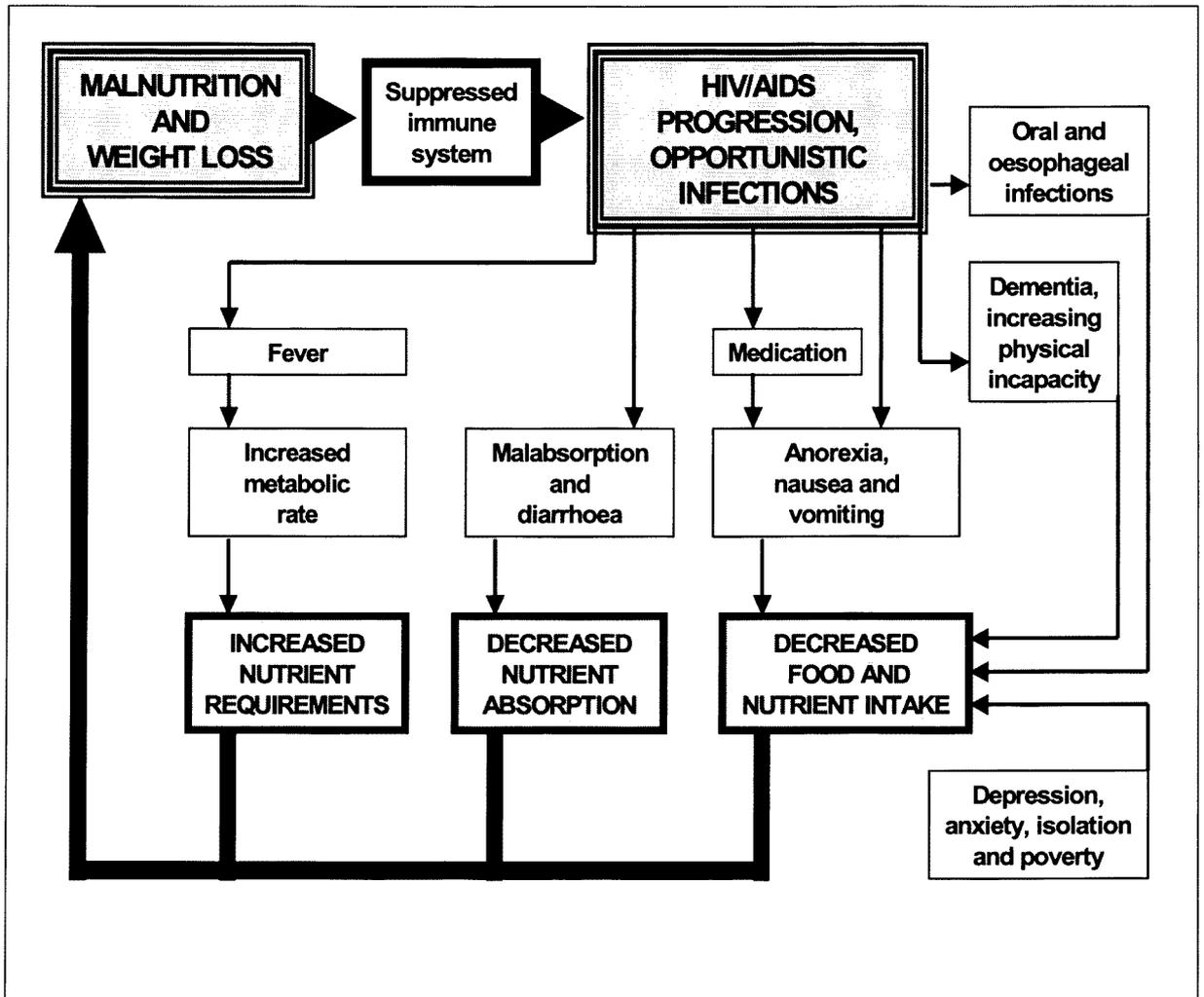


Figure 1.1: The vicious cycle of malnutrition and HIV/AIDS

From: Visser M, Kennedy RD. Patient-centred care: Diet and nutrition. In: Wilson D, Naidoo S, Bekker L-G, et al. Eds. *Handbook of HIV medicine*. Cape Town: Oxford University Press, 2002: 413

1.2 REVIEW OF NUTRITION INTERVENTION STRATEGIES FOR HIV/AIDS

1.2.1 Nutrition counselling

The effectiveness of this nutrition intervention has been documented^{20,21} and is considered paramount to the treatment of HIV/AIDS.²² However, nutrition counselling and oral nutritional supplementation are generally considered equally effective in increasing energy intake in malnourished persons with HIV/AIDS.^{23,24} Nutritional supplements accompanied and augmented by nutrition counselling have positive effects that manifest as reduction in protein catabolism and repletion of lean body tissue, but no effect on CD4+ count as a measure of immunity.²⁵

Nutrition intervention for HIV/AIDS that includes intensive counselling supports the maintenance of not only body weight, but also lean body tissue and the latter effects may last long after the intervention.^{25,26} Nutrition supplementation is best approached as an adjunct to dietary counselling in the nutrition management of people living with HIV/AIDS.

1.2.2 Nutritional supplements

A small number of studies have evaluated the effects of nutritional supplements on the nutritional and immune status in people living with HIV/AIDS. The subjects in these studies were mostly male and all the studies were conducted in the presence of antiretroviral (ARV) therapy. Due to their small sample sizes and unrepresentative composition the findings from these studies cannot be generalised.²⁷

In one such study it was possible to achieve weight gain with an energy-providing supplement enriched with multivitamins and minerals in subjects with severely depleted immune systems (CD4+ count < 200 cells/mm³) at baseline.²⁸ It is however not possible to distinguish whether the increased energy intake had any effect on weight gain over and above that of the multivitamins and minerals alone. Nutritional supplementation with a complete polymeric feed can achieve significant weight gain

in people with HIV/AIDS, but it does not have an effect on nutritional status as measured by serum albumin or immunity as measured by CD4+ and CD8+ counts.²⁹ Although it has been shown that an energy- and protein-rich oral liquid supplement is of benefit to weight maintenance³⁰, adherence to polymeric feeds for HIV/AIDS wasting is known to suffer with long-term use.³¹ Ongoing nutritional supplementation with polymeric feeds is thus best prescribed in limited quantities to ensure maximum adherence.

A small number of studies have indicated improved clinical outcomes, but there exists a paucity of data on the effectiveness of supplemental nutrition on nutritional status and immunity in HIV/AIDS.^{8,30} With oral liquid nutritional supplements it is possible to provide extra energy at times when food intake is compromised. Energy-providing nutritional supplements, with or without additional nutrients, can make a meaningful contribution to the care of people living with HIV/AIDS by supporting body weight. It has not yet been shown that nutrition supplementation improves immunity or outcome in HIV/AIDS.

1.2.3 Immune-enhancing and specially adapted supplements

Although safe for consumption by persons with HIV/AIDS, evidence for the inclusion of immunomodulating components in nutritional supplements in this context is not conclusive.^{32,33} When immunomodulating components are provided as part of an energy-supplying nutritional supplement, there is no additional benefit in terms of weight gain or immunity in HIV/AIDS.³⁴

Supplementation with individual immune-enhancing components, such as L-glutamine (with which the test product is enhanced) and arginine, has promoted weight gain³⁵ and improved immunity with increased CD3+ and CD8+ counts and reduced viral load in HIV-infected people.³⁶ Glutamine, supplemented together with antioxidants, is able to increase body weight and body cell mass and is considered a cost-effective approach to the management of weight loss in HIV/AIDS.³⁷

A study investigating an ω -3 fatty acid-enriched enterotropic peptide-based formula showed no change in immunity as measured by peripheral and jejunal mucosal CD3+, CD4+ and CD8+ counts, although weight remained stable.³⁸ A later study involving the same product showed an increase in CD4+ count.³⁹ Both of these studies refute an earlier suggestion that enterotropic peptide-based feeds may provide superior nutritional management in HIV-infection.⁴⁰ Specially adapted, semi-elemental oral supplements may however be of benefit in patients with chronic malabsorption due to HIV/AIDS.⁴¹ Supplementation with fish oil containing ω -3 fatty acids produces a weak anticytokine action, but it is not able to overcome the metabolic and nutritional consequences of HIV/AIDS.⁴²

The results are conflicting and the most recent study concluded that neither standard nor immune-enhancing formulas had any significant effect on nutritional status or immunity with HIV/AIDS.³² The latter study was conducted in the presence of ARV therapy, which does not apply to the current South African HIV/AIDS scenario and may have diminished the effectiveness of immunonutrition. The study was not blinded and with a small sample size (3 groups comprising 19, 26 and 21 subjects each completed the study) it may not have been possible to detect minor therapeutic effects. A serious limitation of this study is the lack of a true control group, since all the subjects received dietary counselling, which could have lead to change in background dietary behaviour. Substance abuse, associated with immune suppression, was not excluded from the sample and may have rendered the sample unresponsive to immunonutrition. There exists a definite need for more research into immunonutrition in people living with HIV/AIDS, particularly in the absence of ARV therapy, which was not freely available in South Africa at the time of the study.

1.3 RATIONALE FOR THE TEST PRODUCT

1.3.1 Description of the product

The product under investigation (Appendix 1) is a soya-based liquid supplement. Based on scientific evidence, not always pertaining to people living with HIV/AIDS, the manufacturer enriched the test product to include multiple micronutrients (Appendix 2) to address known nutritional deficiencies associated with HIV/AIDS and to support the immune system. The minerals are amino acid chelated for improved absorption. It is also enriched with L-glutamine, and prebiotics (Appendix 3) and probiotics.

The manufacturer recommends an intake of one feed per day that consists of one sachet (40g of dry powder) mixed with 200 ml of cold water. Due to its food-based macronutrient content the product supplements the diet with energy (663 kJ/d) and protein (6.9 g/d). The efficacy of the test product has not been tested but, on the basis of available evidence, it may be helpful in addressing HIV/AIDS wasting, particularly in populations with marginal or reduced energy intake, and it may enhance immune function.

1.3.2 Cost benefits of the test product

The recommended price of this product competes very well with nutritional supplements marketed by the pharmaceutical industry. Medical aid schemes in South Africa do not cover the costs of nutritional supplements that are often required in the management of chronic debilitating conditions, which include HIV/AIDS. The availability of low-cost nutritional supplements offers the possibility of affordable nutrition support to people living with HIV/AIDS in resource-limited surroundings. By enriching such supplements with nutrients and other dietary components, which may support the immune system, the potential exists for added health benefits. This product is well positioned in the marketplace to make it affordable not only for use in home-based care, but also accessible to HIV/AIDS supporting NGOs wishing to include nutritional supplements in food parcels for people living with HIV/AIDS.

1.3.3 Possible health benefits of the test product

1.3.3.1 Benefits of micronutrient enrichment

Micronutrient deficiencies have often been reported with HIV/AIDS^{7,43} and accelerated disease progression and increased morbidity and mortality are associated with such deficiencies.^{44,45} A number of studies have reported a deceleration of HIV/AIDS disease progression with micronutrient supplementation.^{43,46,47} Current information suggests that multi-micronutrient supplementation for decelerating disease progression may be more beneficial than single nutrients.⁴⁷ The test product is enriched with multivitamins to 100% and minerals to 15% of the 1989 recommended dietary allowance (RDA) for adults provided in one portion per day. Higher levels of vitamin A and zinc supplementation in particular are associated with accelerated progression of HIV/AIDS and should be avoided.^{48,49}

Due to its micronutrient content the test product has the potential to address micronutrient deficiencies associated with HIV/AIDS. The benefits may be seen in terms of improvement of parameters of nutritional anaemias commonly found with HIV/AIDS and may also be evident in terms of improved immunity, which is associated with a number of micronutrients, in particular vitamin A, selenium and zinc.

1.3.3.2 Benefits of glutamine enrichment

Glutamine, usually a non-essential amino acid, has been reclassified as “conditionally essential” in catabolic stress situations such as trauma and sepsis. It serves as fuel for the growth and proliferation of the cells of the intestinal mucosa and is important to enhance absorption, which is impaired with HIV/AIDS. Glutamine supplementation in animals has been shown to be involved in the maintenance of intestinal mucosal integrity resulting in improved barrier function to prevent the translocation of pathogens.^{50,51,52} In humans parenteral glutamine supplementation is safe, maintains

intestinal permeability and leads to improved nitrogen balance and clinical outcome after surgery.⁵³

HIV/AIDS is associated with glutamine deficiency⁵⁴, impaired intestinal integrity and increased gut permeability.⁵⁵ The benefits of glutamine as a single nutrient supplement in people living with HIV/AIDS have been described in terms of enhanced absorption⁵⁵, weight gain and improved immunity.³⁶

In the area of critical care, from which most of the supporting evidence originates, glutamine supplementation is still debatable after many years of research. Minimal information is available for HIV/AIDS where glutamine supplementation does not seem to outweigh the benefits of energy supplementation.³⁴ It is thus suggested that glutamine is best provided as part of an enriched nutritional supplement for HIV/AIDS rather than an isolated nutrient, as is the case with the test product.

1.3.3.3 Benefits of probiotic enrichment

Based on evidence of their viability in the human gut, the test product is enriched with the probiotic microorganisms *Lactobacillus acidophilus* and *Bifidobacterium bifidus*.⁵⁶ Probiotics play a role in aiding absorption and synthesising micronutrients to the benefit of the host and also stabilise the luminal environment, whereby it contributes to preventing bacterial diarrhoeal disease.^{58,67} In this regard probiotics have also been found beneficial in the treatment of HIV/AIDS-associated diarrhoea.⁵⁹ *Bifidobacterium* is considered safe for human consumption^{60,61} and *Lactobacillus* provides a favourable environment for its proliferation.

The ability of probiotics to influence the intestinally based immune system by stimulating the gut-associated lymphoid tissue (GALT) has recently been the focus of increasing scientific attention.⁶² *Lactobacillus* has been reported to enhance the natural immunity of the gut.⁶² Probiotics have also been reported to lead to increased numbers of circulating white blood cells and may influence immunity by stimulating the production of certain cytokines, which are involved in T-cell production.⁶³ One large study (n > 4000) reported a possible direct inhibitory effect of probiotics on HIV

infection in women, but prudently warns the reader against cause-and-effect conclusions.⁶⁴

Although little information is available on the role of probiotics in HIV/AIDS in particular, there is no reason to believe that their reported benefits will not apply to people living with HIV/AIDS where malabsorption and diarrhoea are common intestinal disorders. There is also particular interest in the possible benefits that probiotics may offer in terms of immunity.

1.3.3.4 Benefits of prebiotic enrichment

Prebiotics are non-digestible oligosaccharides including inulin and oligofructose, which, by resisting digestion in the small bowel, are available for bacterial fermentation in the colon. This fermentation process produces short-chain fatty acids, which are hypothesised to be involved in the mechanism underlying the immunomodulating effect of prebiotics. Other possible mechanisms are the direct contact of probiotic bacteria, their proliferation stimulated by prebiotics, with the immune cells in the intestine, and modulation of mucin production.⁶⁵

Preliminary data from animal studies suggest that prebiotics are able to modulate immune parameters in GALT, secondary lymphoid tissue and peripheral blood. This is a relatively new area of investigation and the immune-modulating effect of prebiotics is generally attributed to its ability to selectively modify the gut flora.^{65,66,67} As such it is considered an essential adjunct to the probiotics contained in the test product.

To move beyond this limited perception of prebiotics in symbiosis with the gut flora, further research is required to explore the immune-enhancing effect of prebiotics per se. Nearly all the studies investigating the effects of prebiotics on immunity were based on animal models and it is not possible to draw conclusions on the immune effects of various dietary fibres in humans. Human studies are of course limited by the fact that peripheral blood parameters are mostly used for immune assessment, while it is recognised that immunity may be affected at various sites in the body,

which are not practical for assessment.

1.4 SIGNIFICANCE OF THE STUDY

The evaluation of effective, affordable and acceptable nutrition interventions is particularly important in South Africa where ARV therapy, available in the industrialised world, still remains unaffordable and inaccessible to most people living with HIV/AIDS. Although effective in decreasing HIV/AIDS-related mortality and morbidity, ARV therapy does not eliminate the wasting seen with HIV/AIDS⁶⁸ and therefore does not diminish the role of nutrition intervention. All studies published to date have investigated nutritional supplementation as an adjunct to ARV therapy, including protease inhibitors. It is not possible to distinguish between the effect of ARV therapy and nutrition supplementation in these studies. In contrast, this pilot study is unique in that it investigated the effects of nutritional supplementation in HIV/AIDS without the benefit of ARV therapy.

Most previous studies were performed in the United States of America and a smaller number in Europe. No study on oral nutritional supplementation in Africa or other developing parts of the world has thus far been published. Only one prospective, longitudinal study was previously attempted in South Africa on the effectiveness of supplemental feeding in persons with HIV/AIDS not receiving ARV therapy.⁶⁹ The study was curtailed by a poor follow-up rate, possibly related to the stigma attached to HIV/AIDS, which rendered the results inconclusive. The results of the pilot study presented here therefore contributes not only to the care of people living with HIV/AIDS in South Africa, but also to the relatively small body of information on the subject internationally.

This independent research was commissioned by the manufacturer as part of its research and development programme of a new product. Appendices 1 and 2 provide the composition of the product. The results of this study will be of direct benefit to the manufacturer in the marketing and development of their product. Considering the possible effects of HIV/AIDS on expendable income and household food security, it is important to scientifically ascertain the effectiveness of any new

product before health claims are made.

1.5 STUDY AIMS

The primary aim of this pilot study was to investigate the effects of a multiple micronutrient, glutamine and pre- and pro-biotic enriched liquid supplement on the:

- 1 nutritional status of people living with HIV/AIDS by means of anthropometric and biochemical assessment over a period of 12 weeks.
- 2 immunity of people living with HIV/AIDS by means of CD3+, CD4+ and CD8+ counts and their ratios over a period of 12 weeks.

1.6 HYPOTHESIS

It was hypothesised that the test feed would have no effect on either the nutritional status or the immunity of people living with HIV/AIDS.

CHAPTER 2

METHODOLOGY

2.1 STUDY DESIGN AND ETHICS

2.1.1 Type of study

The study was designed as a prospective, randomised, double-blind, placebo-controlled clinical trial. The research project was registered as a pilot study.

2.1.2 Ethical considerations

A research protocol (Appendix 4) for this pilot study was submitted to and approved (Ref No 2002/C/102) by the ethics committee of the Health Science Faculty of the University of Stellenbosch, Tygerberg, South Africa. Confidentiality was ensured throughout the study process.

All costs incurred in the execution of the study were covered by a grant from the manufacturers of the supplement, African Dynamics Pty (Ltd), Pretoria, South Africa, and did not include incentives for subjects or remuneration for the researcher or staff involved in the study.

2.1.3 Intervention

2.1.3.1 Test product and placebo

The subjects were required to consume a single portion per day of either the test product, *B-immune*[®] (African Dynamics), or a placebo for a period of 12 weeks. One sachet (40g) of dry product mixed with 200 ml of cold water constituted one portion.

Each subject was provided with a calibrated mixer container to standardise preparation of the product. The mixing procedure was physically demonstrated during the baseline visit.

One portion of the test product (Appendices 1 and 2) provided 663 kJ, 6.9 g protein and was enriched with vitamins, minerals, L-glutamine, and pre- and probiotics. The placebo (Appendix 5) was developed to match the test product in volume, taste, colour and consistency. It contained 655 kJ of energy, but was devoid of the enrichment provided in the test product. Maltodextrin was used to expand the bulk of the dry placebo to match the test product. An energy-empty placebo constituted very low dry volume and did allow for identical packaging required for blinding.

2.1.3.2 Assessment and follow-up

Baseline data was collected and the subjects were followed up every 4 weeks until completion of the study after 12-weeks of intervention. Anthropometric assessment was performed at baseline and each of the three follow-ups, while biochemical and haematological assessment occurred only at baseline and at week 12 (Figure 2.1). The supplement or placebo was distributed to the subjects in 4-week supply parcels distributed at baseline and again at week 4 and week 8 of the study period. In the event where subjects were unable to attend follow-ups, volunteer workers from the hospice delivered the parcels to their homes.

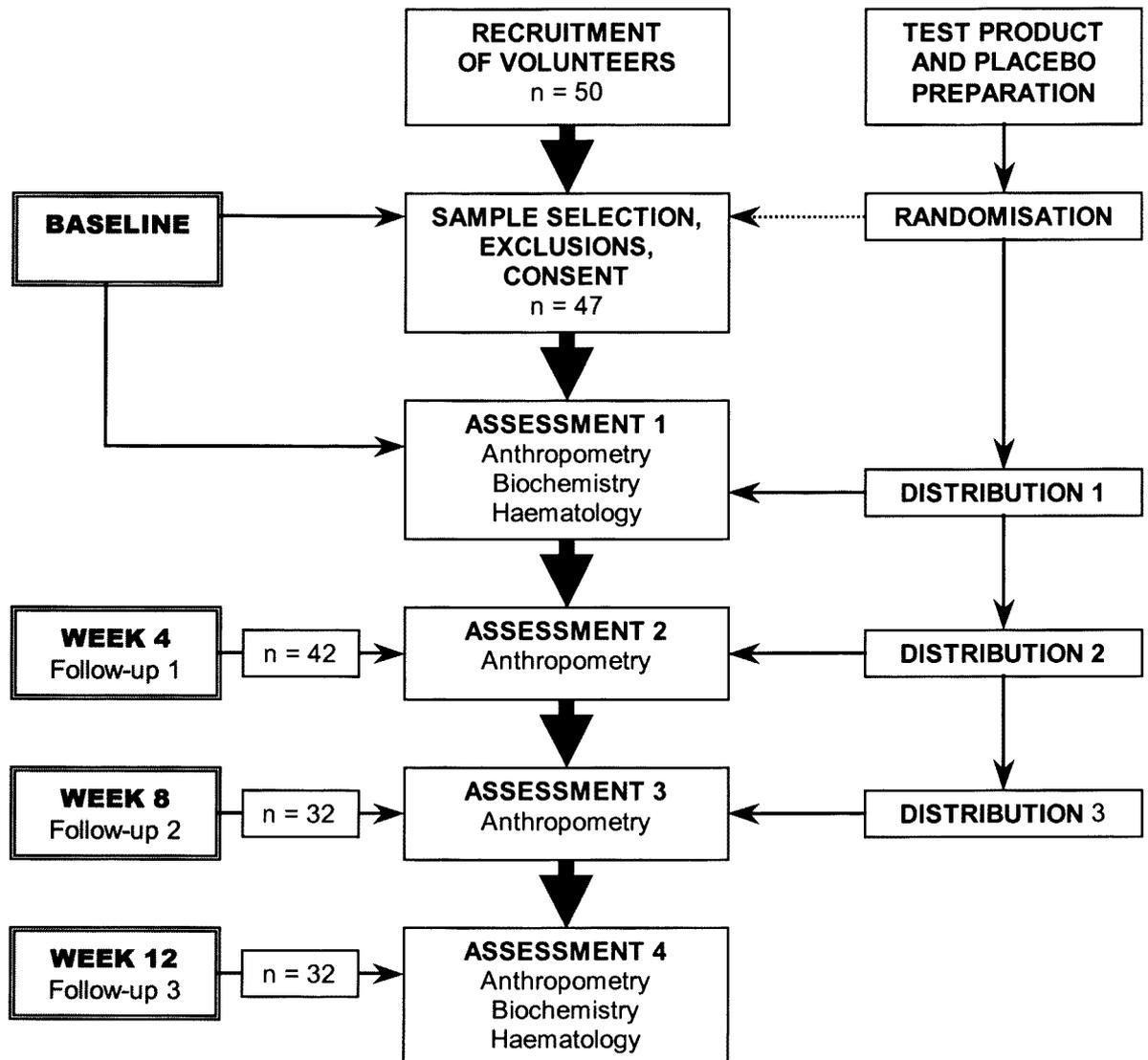


Figure 2.1: Flow diagram of the research study

2.1.4 Randomisation and blinding

A 12-week supply of 25 sets each of the test product and the placebo was prepared by the manufacturer in indistinguishable, unlabeled packaging. The manufacturer randomised the sets by lottery and retained exclusive access to the feed identification code. The identification code was broken only after completion of the study. The 50 randomised sets were delivered to the researcher in a single batch with consecutive numbering. Each set was divided into three 4-week supply parcels for convenience of distribution at baseline and weeks 4 and 8 follow-up visits. Study numbers were

consecutively allotted to subjects upon entry to the study and matched with the identically numbered pre-randomised supply parcels. Both the subjects and the researcher were blinded to the identity of the feeds. Each subject received an identification card (Appendix 5) containing only his/her study number and appointments for follow-up visits.

2.2 SAMPLING AND INDUCTION

2.2.1 Sampling method

A sample of 50 volunteers was recruited among ambulatory, disclosed HIV-infected adult clients (> 18y and < 60y) attending the community-based Moretele Sunrise Hospice at Hammanskraal in the North-West Province of South Africa, 50 km north of Pretoria. Provided they met the inclusion criteria, all the clients of the hospice were invited to participate in the study. No limitation was placed on the number of volunteers at entry, and after active recruitment by hospice staff and volunteer community workers, this initial number represented exhaustion of the available ambulatory client base of the hospice at the time the study was initiated. The subjects routinely visit the hospice once per week, which enabled induction of the sample over two sessions, one week apart, to accommodate time and laboratory constraints.

2.2.2 Selection criteria

HIV infection was the primary inclusion criterion for this study. Clients are referred to the hospice with written notification of their HIV status after testing positive at surrounding community clinics operated by the provincial health service. Re-testing for confirmation of HIV status was not considered necessary for this study. Subjects were included regardless of duration or stage of HIV infection and none of the subjects received ARV treatment or reported taking any other nutritional supplements. The age range for inclusion was from 18 to 60 years. Pregnant

women, and volunteers who were too weak to participate fully in the trial, were not included.

2.2.3 Written consent

A research assistant proficient in the local languages assisted the subjects with completion of a written consent form (Appendix 6) and each subject received a personal identification and appointment card (Appendix 7). All procedures and requirements were explained in either English or the subject's home language and none of the subjects eligible for entry into the study refused consent. To enable the researcher to trace the subjects by study number, minimal personal information was collected. Only the researcher had access to the subject information records to ensure confidentiality (Appendix 8) and alone was able to identify and trace the subjects when necessary.

2.2.4 Instructions to subjects

Besides instructions for the mixing of the product and consumption of a single portion once daily of either the test product or placebo as prescribed, subjects were advised to continue with their usual routine and were requested to report initiation of other nutritional or immune-enhancing supplements during the study period. A signed distribution record (Appendix 9) was kept for baseline and the first two follow-up visits during which distribution took place. Subjects were provided with 28-day supplement consumption record diaries (Appendix 10) between follow-up visits and were required to return the completed records. The research dietitian used the returned intake records to monitor consumption during follow-up visits. Subjects were provided with contact details of both the researcher and the research assistant and requested to report any problem encountered with the research protocol. Because many subjects do not have access to telephones they were also encouraged to communicate via the support staff of the hospice, with whom they were familiar.

2.3 DATA COLLECTION

2.3.1 Anthropometry

An experienced registered dietitian performed all anthropometric assessments using standard procedures⁷⁰ at baseline and week 4, 8 and 12 follow-up visits. All measurements were performed twice, the average recorded and the process repeated in the case of discrepancies. Height, weight, triceps skinfold thickness (TSF) and mid-upper arm (MUAC), waist and hip circumferences were measured. A Seca[®] balance-beam scale with stadiometer was used to measure height to the nearest 0.5 cm and weight to the nearest 0.1 kg. Subjects were measured and weighed in one light layer of outer clothing, with shoes and headdress removed, and all measurements were performed between 10h00 and 12h00 to control for circadian variation. The study was completed during the warm summer months and similar clothing was evident throughout. Height measurement was done with the subject standing with feet together, upright and the head placed in the Frankfort plane.⁷¹ The scale was regularly controlled for zero reading between measurements.

Plastic skinfold calipers (Slim Guide[®], Creative Health Products, Plymouth, Michigan, USA) were used to measure TSF to the nearest 0.5 mm on the right or dominant arm with the arm relaxed and extended to the side. The mid-acromial-radial level was measured and marked and TSF and MUAC were measured at this level. MUAC was measured on the exposed, relaxed arm along a horizontal plane at the marked level, the tape being pulled taught without exerting pressure.

Waist and hip circumferences were measured in the horizontal plane with the tape pulled tight over light clothing, but not causing indentation. Waist circumference was measured at the midpoint between the lower costal border and iliac crest, arms at side. Hip circumference was measured at the widest point of the posterior (gluteal) protuberance, feet together and gluteal muscle relaxed. An automatically retractable fibreglass tape was used to measure all circumferences.

2.3.2 Biochemistry

A registered nursing professional collected venous blood samples according to the specimen collection protocol (Appendix 11) using 5 ml capacity vacuum tubes without anticoagulant treatment (BD Vacutainer[®], Preanalytical Solutions, Plymouth, UK), at baseline and at the end of week 12. The specimens were immediately refrigerated and coagulated blood samples were delivered to the laboratories of the Department of Chemical Pathology, Medical University of South Africa (Medunsa), in insulated, covered containers within 4 hours of collection. Same-day centrifugation and harvesting and freezing of the serum were performed. Frozen baseline samples were stored at -12° C for a maximum of three weeks for batch analysis, the time it took to complete each of the baseline and 12-week follow-up specimen batch collections.

All instruments were calibrated according to manufacturer's procedures and all relevant quality control measures with Decision[®] Liquid Comprehensive Chemistry Control Serum (Beckman Coulter, Fullerton, CA, USA) were within limits ($\bar{x} \pm 2SD$). Routine 2-hourly Decision[®] Level 2 and Level 3 controls are run at this National Health Laboratories Services facility. Additional dedicated Decision[®] Level 2 controls were implemented for each batch analysis of this pilot study. Serum total protein (s-TP) concentration was determined by means of a rate biuret method. Serum albumin (s-Alb) concentration was measured with a timed endpoint method using albumin reagent. In the reaction, albumin combines with bromine cresol purple (BCP) dye to form a coloured product. Both s-TP and s-Alb were measured with the Synchron CX[®] 7 Delta (Beckman Coulter). C-reactive protein (CRP) was measured with the Immage[®] Immunochemistry System (Beckman Coulter) that utilises rate nephelometry to measure the rate of increase in light scattered from particles, the result of complexes formed during an antigen-antibody reaction, suspended in solution.

2.3.3 Haematology

A registered nursing professional collected venous blood samples according to the specimen collection protocol (Appendix 11) using 4 ml capacity anticoagulant-treated (7.2 mg K₂EDTA) vacuum tubes (BD Vacutainer[®]) at baseline and at the end of week 12. Taking account of circadian variability, specimen collection occurred according to a specimen collection protocol (Appendix 10) within the same 2-hour window period on both occasions. Blood samples were immediately refrigerated and delivered to the laboratories of the Department of Haematology, Medunsa, in an insulated, covered container within 4 hours of collection where same-day analyses were performed.

Full blood counts (FBC) were performed on an Advia[®] 120 (Bayer Corporation, Diagnostics Division, Tarrytown, NY, USA) automatic haematology analyser which analyses 100 µl of whole blood and provides results within one minute. Calibration was performed according to manufacturer's procedures and regular external quality control procedures of NHLS.

Immunophenotyping was performed with a Beckman Coulter XL[®] desktop flow cytometer (Beckman Coulter), flow cytometry being the method of choice for CD4⁺ monitoring in HIV/AIDS.⁷² Specimens were treated with *Q-Prep*[®] (Beckman Coulter) solution, which lyses red blood cells and fixes the membranes of white blood cells. Thereafter 10 000 lymphocytes were automatically analysed for CD3⁺, CD4⁺ and CD8⁺ subsets. The results were printed in numeric and graphic form, providing absolute values for CD4⁺ and CD8⁺; CD3⁺ percentage; and CD3⁺: CD4⁺, CD3⁺: CD8⁺ and CD4⁺: CD8⁺ ratios.

Internal quality control measures are performed according to manufacturer's (Beckman Coulter) procedures with their Flowcheck[®] laser lamp alignment procedure twice daily and Immunotrol[®] test kit which controls results on a test specimen as part of an international quality assurance programme. External quality control procedures are regularly performed with Immunotrol[®] in conjunction with a 19-centre NHLS pilot project. All relevant quality control measures were within limits ($\bar{x} \pm 2SD$).

2.4 STATISTICS

2.4.1 Calculation of derived parameters

Derived parameters were calculated using the following standard formulas:^{71,73}

Body mass index (BMI) (kg/m²):

$$\frac{w(\text{kg})}{\text{height}(\text{m})^2}$$

Relative body weight (RBW) (%):

$$\frac{\text{actual body weight}}{\text{reference weight}} \times 100$$

Waist:hip ratio (WHR):

$$\frac{\text{waist circumference}}{\text{hip circumference}}$$

Mid-upper arm muscle circumference (MAMC) (cm):

$$\text{MUAC} - (\pi \times \text{TSF})$$

Mid-upper arm muscle area (MAMA) (cm²):

$$\frac{[\text{MUAC} - (\pi \times \text{TSF})]^2}{\pi \times 4}$$

Bone-free mid-upper arm muscle area (BMAMA) (cm²):

Females:	MAMA – 6.5 cm ² (females)
Males:	MAMA – 10 cm ² (males)

2.4.2 Statistical analysis

Data was captured electronically with Microsoft Excel[®] and controlled for precision of data transfer with regular cross-referencing. The University of Stellenbosch appointed a consultant statistician to assist with data analysis using SAS System for Windows[®], Release 8.02 (SAS Institute Inc., Cary, Ill., USA). Means (\bar{x}) and standard deviations (SD) were calculated for all parameters. Baseline data for the supplemented and placebo groups were compared with the two-sided Wilcoxon two-sample test. A pairwise t-test was used to compare baseline and final follow-up data. Normality of distribution was tested with Shapiro-Wilk tests and nonparametric tests were performed with the sign test. McNemar tests were used to compare prevalence of malnutrition before and after intervention. The level of significance was set at $p < 0.05$ and applied to all tests.

CHAPTER 3

RESULTS

3.1 SAMPLE CHARACTERISTICS

3.1.1 Sample description

Twenty-six and 24 volunteers were recruited during the first and second induction sessions respectively, one week apart. Of the 50 volunteers recruited, 47 subjects were included at baseline (Table 3.1), after exclusions for pregnancy (2) and advanced HIV/AIDS progression (1) where neurological complications rendered the volunteer incapable of full participation and it was considered unethical to subject the volunteer to the rigours of the study. The pre-randomised feed allocation placed 22 subjects in the supplemented group and 25 in the placebo group. The research site is located in a historically black settlement area and all the subjects were black, with an age range of 19 to 56 years ($31.62 \text{ y} \pm 7.94$). The subjects had been aware of their HIV status for anything from 4 months to 7 years ($2.29 \text{ y} \pm 1.82$). No reliable information was available for duration of HIV infection and the lack of clinical data in the community-based setting of this pilot study renders specified CDC disease stage classification⁷⁴ impractical.

3.1.2 Sample retention and drop-out

During the first follow-up visit (week 4) 42 subjects (89%) returned while 5 subjects were absent due to employment commitments (2), relocation (1) and lost contact (2). At the second follow-up (week 8) 32 subjects (68%) returned, one subject had deceased, one was too weak to attend, 2 had work obligations and 6 were unaccounted for. Subjects who missed follow-ups had their feed parcels delivered to their homes and the intake records of the previous 4 weeks were collected where possible. During the home visits it became apparent that these subjects had experienced disease progression or secondary disease, which contributed to their

inability to attend previous follow-ups. During subsequent follow-up visits the research dietitian interviewed returning subjects regarding compliance. No subject voluntarily requested discontinuation.

The third and final follow-up (week 12) also included 32 subjects (68%). Baseline and final 12-week follow-up data was available for 32 of the original 47 subjects. This total final sample, which completed the 12-week trial, comprised 14 subjects in the supplemented group and 18 subjects in the placebo group. It is for these 32 subjects that baseline and 12-week comparison is reported. The 27 (84%) female subjects constituted a majority, with only 5 (16%) males included in the trial.

3.1.3 Intervention tolerance and compliance

Blinding remained intact throughout the follow-up period and in only one case of a cohabiting couple, belonging to both groups respectively, was suspicion about the “supplement” reported. The majority of subjects initially returned their intake records (37 of 42 at week 4; 17 from the supplemented group and 20 from the placebo group), but this tapered off with the second follow-up visit (20 of 32 at week 8; 11 from the supplemented group and 9 from the placebo group) and even more so with the final follow-up visit (12 of 32 at week 12; 8 from the supplemented group and 4 from the placebo group). Excellent compliance with the dietary intervention was reported in the returned records. Both the test product and placebo were well accepted by the subjects and no-one reported discontinuation due to the nature of the intervention. The few ailments reported over the 12-week period included nausea (1 in supplemented group), vomiting (2 in supplemented group), diarrhoea (2 in each group), constipation (1 in placebo group), abdominal pain (2 in each group), tonsillitis (1 in placebo group) and unexplained fever (1 in placebo group). None of these were reported to be directly associated with the intervention and on the converse two subjects reported “feeling better” and two having improved appetite, all from the supplemented group. Too few data were obtained from the intake records to warrant analysis, and no conclusion can be drawn in terms of clinical outcomes. A low educational level and illiteracy in this community contributed to the lack of adequate information provided on the appearance of symptoms. No subject

at any time reported taking nutritional supplements other than the intervention required for this trial.

3.2 BASELINE DATA

3.2.1 Baseline group comparison

Baseline values (Table 3.1) were compared with the two-sided Wilcoxon two-sample test and indicated that the two groups in this convenience sample were well matched. MCHC ($p = 0.027$) was the only variable found to be significantly different between the groups. The CD8+ count for one subject in the placebo group diverted more than +2SD from the mean and was excluded. This exclusion did not affect the mean significantly and the subject was referred the local hospital for further clinical assessment.

Table 3.1: Baseline characteristics of the subjects in the study (n = 47)

Variables	Reference values and units	Supplemented group \bar{x} (%) or $\bar{x} \pm SD$	Placebo group \bar{x} (%) or $\bar{x} \pm SD$	Total sample \bar{x} (%) or $\bar{x} \pm SD$
n	-	22 (47)	25 ^a (53)	47 ^b
Female (F)	-	20 (43)	19 (40)	39 (83)
Male (M)	-	2 (4)	6 (13)	8 (17)
Age	y	32.05 \pm 7.32	31.25 \pm 8.59	31.62 \pm 7.94
Known duration of HIV+ status	y	2.56 \pm 2.14	2.06 \pm 1.50	2.29 \pm 1.82
Height	m	159.41 \pm 7.98	160.34 \pm 6.73	159.90 \pm 7.28
Weight	kg	51.06 \pm 12.88	53.30 \pm 10.95	52.25 \pm 4.93
BMI	> 18 kg/m ²	19.98 \pm 4.29	21.37 \pm 5.43	20.72 \pm 4.50
TSF ^d	16.5(F), 12.5(M) mm	10.34 \pm 5.69	10.04 \pm 6.12	10.18 \pm 5.86
MUAC ^d	23.2(F), 25.3(M) cm	24.11 \pm 4.5	25.54 \pm 4.55	25.26 \pm 4.36
MAMC	cm	21.69 \pm 2.73	22.39 \pm 3.41	22.06 \pm 3.10
MAMA	cm ²	37.98 \pm 9.55	40.75 \pm 12.13	39.45 \pm 10.97
BMAMA	cm ²	31.16 \pm 9.50	33.41 \pm 12.05	32.35 \pm 10.88
Waist	cm	72.93 \pm 9.97	73.30 \pm 8.21	73.14 \pm 8.97
Hip	cm	89.48 \pm 11.02	92.04 \pm 13.05	90.84 \pm 12.08
WHR	0.8 (F); 1.0 (M)	0.82 \pm 0.05	0.80 \pm 0.06	0.81 \pm 0.06
s-TP	64 - 84 g/l	90.32 \pm 9.16	94.46 \pm 16.80	92.48 \pm 13.70
s-Alb	35 - 52 g/l	31.96 \pm 7.77	31.00 \pm 7.69	31.46 \pm 7.66
CRP	< 10 g/l	13.18 \pm 15.25	18.61 \pm 31.40	16.01 \pm 24.90
WBC	4.8 - 10.8 x10 ⁹ / μ l	6.76 \pm 5.68	4.32 \pm 1.58	5.50 \pm 4.08
RBC	4.2 - 6.1 x10 ¹² / μ l	3.75 \pm 0.68	3.64 \pm 0.74	3.77 \pm 0.63
Hb	12 - 18 g/dl	11.27 \pm 2.39	10.98 \pm 2.60	11.37 \pm 2.33
MCV	80 - 99 fl	87.71 \pm 6.41	87.40 \pm 7.27	87.54 \pm 6.80
MCH	27 - 31 pg	29.74 \pm 3.19	30.01 \pm 2.87	29.88 \pm 3.00
MCHC ^e	33 - 37 g/dl	33.26 \pm 1.05	34.00 \pm 2.01	33.65 \pm 1.65
Lymphocytes	0.9 - 5.2 x10 ⁹ / μ l	2.66 \pm 4.19	1.43 \pm 0.94	1.93 \pm 2.43
CD3+	65 - 91%	76.27 \pm 10.21	71.88 \pm 13.86	73.98 \pm 11.58
CD4+	500 - 1500/mm ³	268.36 \pm 216.70	212.88 \pm 12.55	228.94 \pm 213.25
CD8+	230 - 800/mm ³	806.78 \pm 373.90	696.50 \pm 600.33 ^c	747.96 \pm 513.27 ^c
CD3+: CD4+	30 - 65%	14.86 \pm 9.38	13.70 \pm 11.18	14.26 \pm 20.26
CD3+: CD8+	17 - 35%	55.24 \pm 10.21	53.29 \pm 14.86	54.20 \pm 12.80
CD4+: CD8+	1.0 - 3.5:1	0.31 \pm 0.23	0.32 \pm 0.41	0.31 \pm 0.34

^a Biochemical and haematological assessment for n = 24 only, one set of baseline blood specimens lost.

^b Biochemical and haematological assessment for n = 46 only, one set of baseline blood specimens lost.

^c One divergent value (> +2SD) excluded.

^d WHO reference values.

^e Significant difference between groups ($p = 0.027$) with Wilcoxon two-sample test.

Abbreviations: BMI = body mass index, TSF = triceps skinfold thickness, MUAC = mid-upper arm circumference, MAMC = mid-upper arm muscle circumference, MAMA = mid-upper arm muscle area, BMAMA = bone-free mid-upper arm muscle area, WHR = waist/hip ratio, s-TP = serum total protein, s-Alb = serum albumin, CRP = C-reactive protein, Hb = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration.

3.2.2 Evaluation of baseline nutritional status and immunity

Although unexplained weight loss of 10% is a diagnostic criterion for HIV/AIDS, the subjects were not routinely monitored and no data was available for weight loss prior to the study. To assess body weight at baseline, Relative Body Weight (RBW)⁷¹ was calculated using the lower limit of the mid-range (25th percentile) of sex-, age- and race-specific reference values⁷⁵ as reference weight, which roughly corresponds with BMI of 20 to 22. Low body weight (RBW < 90%) was present in 67% (14) of the subjects/volunteers in the supplemented group (\bar{x} = 87%) and 58% (14) of the placebo group (\bar{x} = 88%). Overweight (RBW > 120%) was present in only one subject in each of the two groups. Using BMI < 18 kg/m² as diagnostic indicator for undernutrition, 41% (9) of the supplemented group and 16% (4) of the placebo group were underweight. Two subjects, one from each group, for whom date of birth were not available, were excluded from age-specific evaluations. Although all subjects had WHR within the reference range, this was not used to assess nutritional status at baseline but to detect redistribution of body fat at the end of the study.

TSF was evaluated with sex, age and race specific reference values.⁷⁵ Values below the 5th percentile were indicative of malnutrition in 38% (8) of the supplemented group and 29% (7) of the placebo group. MUAC below the sex and age specific 5th percentile⁷⁶ was found in 38% (8) of the supplemented group and 42% (10) of the placebo group. For comparison with other studies, WHO reference values⁷⁷ were also employed, and percentage of reference values was calculated for TSF and MUAC at baseline. These evaluations produced higher malnutrition prevalence figures with 85% (18) of the supplemented group and 80% (19) of the placebo group below the respective TSF reference values. For MUAC 43% (9) of the supplemented group and 38% (9) of the placebo group, were below the WHO reference value. Expressed as a percentage of the WHO standard, mean TSF was well below standard for both the supplemented and placebo group at 59% and 64% respectively. MUAC was slightly above the WHO reference with mean percentage of standard reaching 108 in the supplemented group and 109 in the placebo group. The difference in results of TSF and MUAC evaluation indicate that the arm circumference remained intact relative to the apparent subcutaneous fat depletion in the sample.

Baseline mean s-Alb below the reference range (< 35 g/l), traditionally considered an indicator of malnutrition, for both groups was indicative of chronic inflammation, which was present equally in 52% (11) of the supplemented group and 52% (13) of the placebo group. Elevated mean CRP > 10 g/l indicated the presence of inflammation and the possibility of acute infection in both groups, in 33% (7) of the supplemented group and 32% (8) of the placebo group. Mean total lymphocyte count, an indicator of both nutritional status and immunity, was within the reference range for both groups. In 28% (13) of the sample, representing 19% (4) of the supplemented group and 36% (9) of the placebo group, total lymphocyte counts were below the reference range when assessed individually.

Anaemia (females, Hb < 12 g/dl; males, Hb < 13 g/dl)⁷⁸ was common at 76% (16) in the supplemented group and 52% (13) in the placebo group. White blood cell counts were within or near the reference range, but red blood cell counts were decreased in both groups. Hypochromic, microcytic anaemia was present in 8 (38%) subjects in the supplemented group and 6 (24%) in the placebo group. Macrocytic anaemia occurred in 5 (24%) subjects in the supplemented group and 5 (20%) in the placebo group.

Nearly all the subjects were immuno-compromised with only 5 having CD4+ counts within the reference range. Severely depleted (CDC 1993)⁷⁴ CD4+ counts < 200 cells/mm³ occurred in almost half (47%; 21 subjects) of the total sample at baseline, specifically in 43% (9) of the supplemented group and 52% (13) of the placebo group. Further indicative of poor prognosis, mean CD4+: CD8+ ratio was also substantially below the reference range in both groups.

The sample was at an advanced stage of HIV/AIDS disease progression with nearly half of the subjects having AIDS-defined disease.⁷⁹ No significant difference existed for immunity parameters between the two groups at baseline (Table 3.1), yet a greater proportion of subjects in the placebo group had clinically advanced HIV/AIDS.

3.3 STUDY OUTCOMES IN TERMS OF OBJECTIVES

3.3.1 Nutritional status outcomes

3.3.1.1 Outcomes in the supplemented and placebo groups

Only the data for the 32 subjects who completed the study period were used for comparative outcomes analysis. Shapiro-Wilk tests with normality established at $p < 0.05$, indicated normally distributed data in most of the variables. This implies that the t-test, valid for small samples from normal populations in the presence of equality of variance, could be used to compare change within and between the groups. Pairwise t-test comparison between baseline and final 12-week follow-up data within the supplemented and placebo groups respectively indicated that change occurred in all variables though few changes were statistically significant ($p < 0.05$) (Table 3.2).

Among the anthropometric parameters weight increased significantly by 2.73 ± 3.53 kg ($p = 0.013$) in the supplemented group while a non-significant weight loss of 0.27 ± 5.05 kg occurred in the placebo group (Figure 3.1). Change in body weight did not result in significant change in BMI in either group. TSF increased significantly by 0.89 ± 1.52 mm ($p = 0.047$) in the supplemented group and similarly by 0.88 ± 1.56 mm ($p = 0.001$) in the placebo group (Figure 3.2). No significant change was observed in either group for any other anthropometric parameter.

In the supplemented group s-Alb increased significantly by 2.50 ± 3.92 g/l ($p = 0.033$) compared to an insignificant decline in s-Alb in the placebo group (Figure 3.3). In contrast to the insignificant increase in CRP in the placebo group, CRP declined significantly by 8.04 ± 12.56 g/l ($p = 0.028$) in the supplemented group (Figure 3.4).

The baseline anaemia worsened with a decline in Hb by 0.56 ± 0.95 g/l ($p = 0.047$) in the supplemented group and 0.95 ± 1.26 g/dl ($p = 0.007$) in the placebo group. MCH and MCHC decreased significantly by 2.19 ± 3.04 pg ($p = 0.024$) and 1.16 ± 1.15 g/dl ($p = 0.002$) respectively in the supplemented group and similarly by 1.87 ± 1.40 pg

($p < 0.0001$) and 1.93 ± 1.44 g/dl ($p < 0.0001$) respectively in the placebo group. Neutrophils decreased significantly by $0.94 \pm 1.50 \times 10^9/\mu\text{l}$ ($p = 0.036$) in the supplemented group, whereas in the placebo group there was no significant change, but monocytes decreased significantly by $0.07 \pm 0.68 \times 10^9/\mu\text{l}$ ($p = 0.001$) (not shown in table).

Table 3.2: Comparison of nutritional status at baseline and 12-week follow-up visit (n = 32)

Parameters	Reference values and units	Supplemented group n = 14 $\bar{x} \pm \text{SD}$			Placebo group n = 18 ^a $\bar{x} \pm \text{SD}$		
		Baseline	Week 12	Change	Baseline	Week 12	Change
Weight	kg	49.19 ± 13.77	51.92 ± 13.97	2.73 ^b ± 3.53	54.39 ± 6.28	54.13 ± 8.63	-0.27 ± 5.05
BMI	> 18 kg/m ²	19.26 ± 4.74	20.27 ± 4.67	1.02 ± 1.40	22.04 ± 4.02	21.17 ± 3.40	-0.87 ± 3.09
TSF	mm	8.71 ± 5.71	9.61 ± 5.86	0.89 ^b ± 1.52	10.97 ± 5.68	11.86 ± 6.01	0.88 ^b ± 1.56
MUAC	cm	24.11 ± 4.50	24.89 ± 4.00	0.78 ± 1.84	26.24 ± 3.05	26.28 ± 3.00	0.04 ± 1.08
BMAMA	cm ²	30.03 10.70	31.54 8.98	1.51 5.09	34.72 ± 9.34	33.77 ± 8.11	-0.943 ± 3.24
S-Alb	35 - 52 g/l	30.93 ± 8.73	33.43 ± 8.83	2.50 ^b ± 3.92	32.24 ± 5.52	31.35 ± 5.81	-0.88 ± 4.39
CRP	< 10 g/l	16.66 ± 17.25	8.62 ± 11.12	-8.04 ^b ± 12.56	12.72 ± 26.95	15.58 ± 43.21	2.85 ± 51.95
Hb	12 - 18 g/dl	11.27 ± 2.39	10.71 ± 2.07	-0.56 ^b ± 0.95	11.44 ± 2.35	10.49 ± 1.82	-0.95 ^{b,c} ± 1.26
MCV	80 - 99 fl	89.51 6.51	88.67 5.53	-0.84 4.21	86.98 7.92	87.52 6.68	0.63 5.98
MCH	27 - 31 pg	30.62 ± 3.45	28.15 ± 2.20	-2.19 ^b ± 3.04	30.04 ± 2.90	28.17 ± 2.57	-1.87 ^{b,c} ± 1.40
MCHC	33 - 37 g/dl	33.22 ± 0.94	32.06 ± 1.16	-1.16 ^{b,c} ± 1.15	34.09 ± 1.72	32.16 ± 2.57	-1.93 ^{b,c} ± 1.44

^a Biochemical and haematological assessment for n = 17 only, one set of baseline blood specimens lost.

^b Significant change ($p < 0.05$) with pairwise t-test.

^c Significant change ($p < 0.05$) with sign test.

Abbreviations: BMI = body mass index, TSF = triceps skinfold thickness, MUAC = mid-upper arm circumference, BMAMA = Bone-free mid-upper arm muscle area, s-Alb = serum albumin, CRP = C-reactive protein, Hb = haemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration.

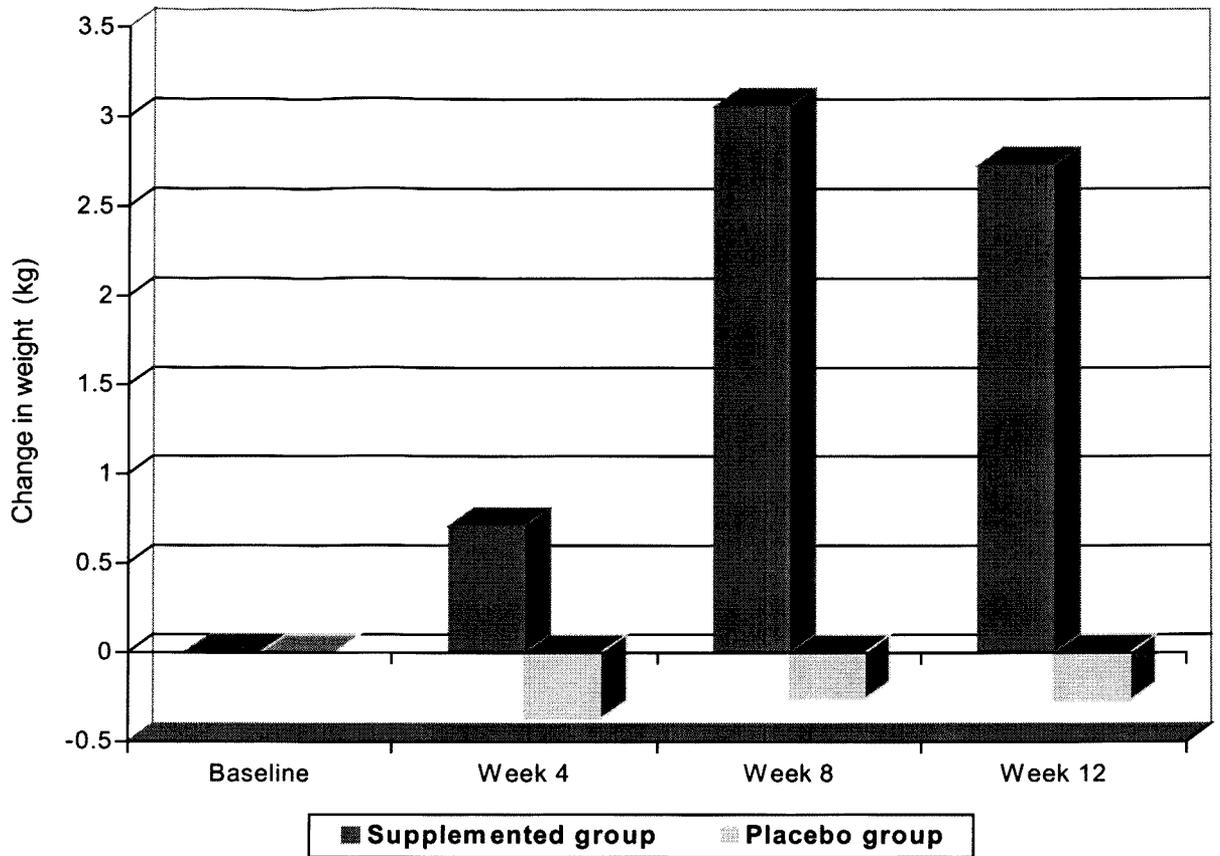


Figure 3.1: Change in weight at follow-up visits over the study period (n = 32)

Significant increase at 12 weeks in supplemented group ($p = 0.013$) with pairwise t-test.

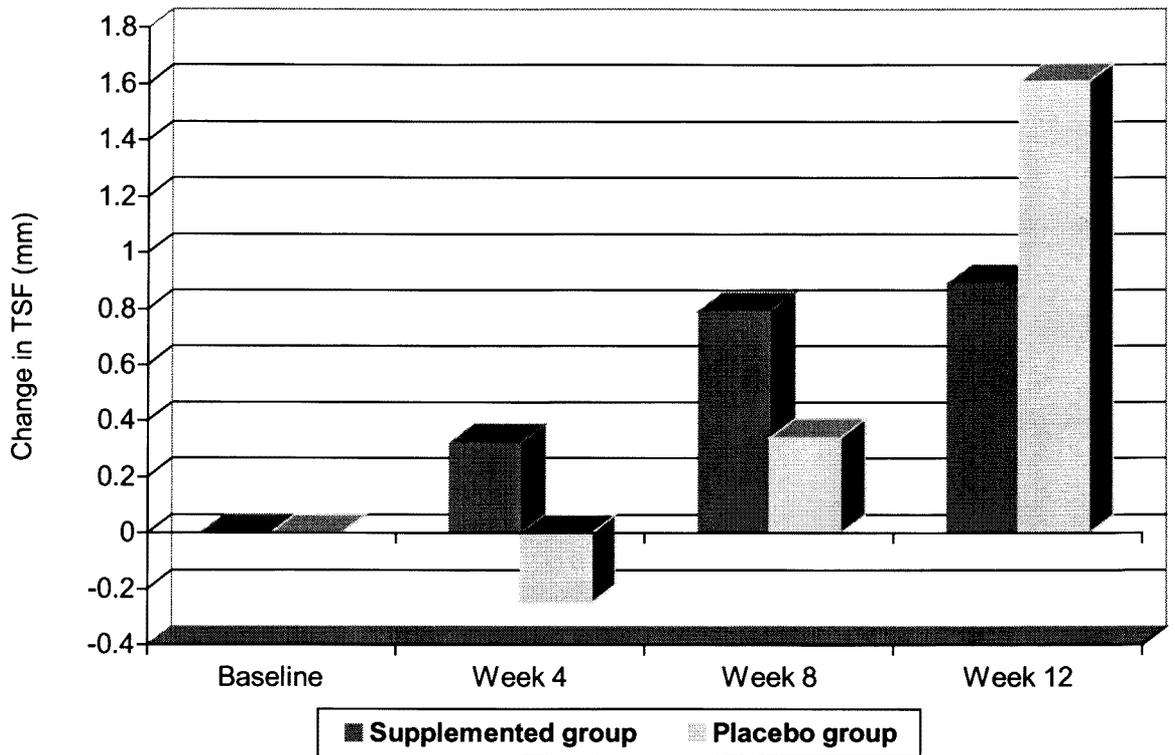


Figure 3.2: Change in triceps skinfold thickness at follow-up visits over the study period (n = 32)

Significant increase at 12 weeks in supplemented group ($p = 0.047$) and placebo group ($p = 0.001$) with pairwise t-test.

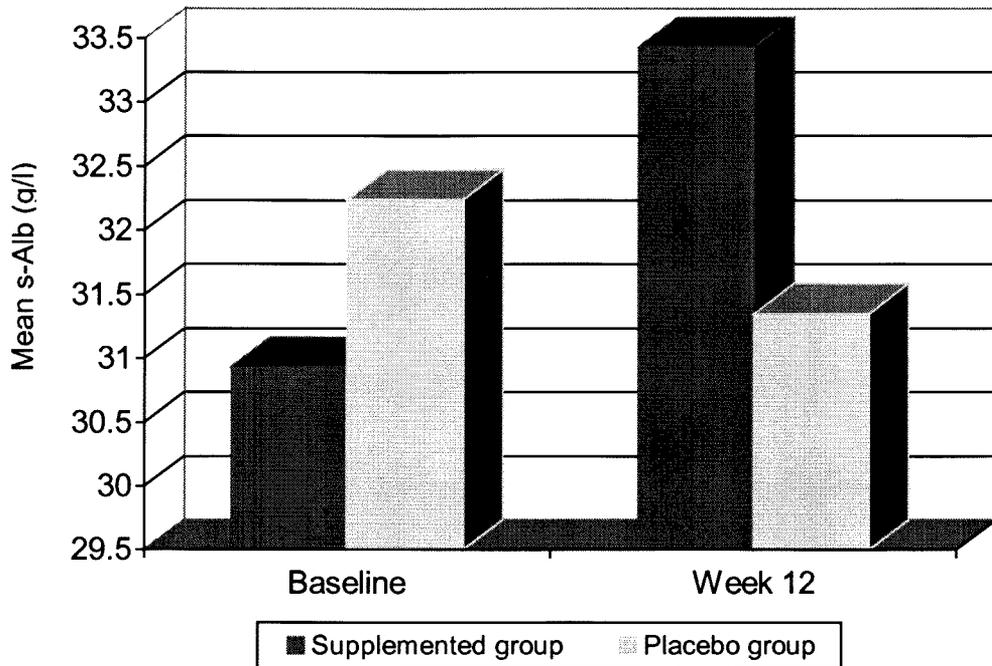


Figure 3.3: Mean serum albumin at baseline and at 12-week follow-up visit (n = 31)

Significant increase in supplemented group ($p = 0.033$) with pairwise t-test.

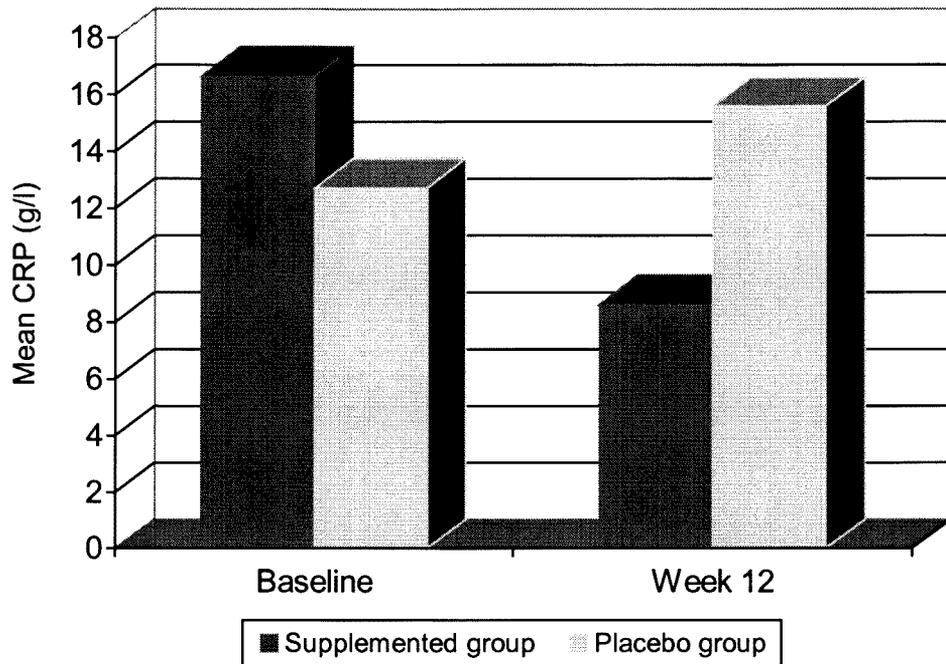


Figure 3.4: Mean C-reactive protein at baseline and at 12-week follow-up visit (n = 31)

Significant decrease in supplemented group ($p = 0.028$) with pairwise t -test.

BMI values, for example, were not distributed normally in the sample at baseline ($p = 0.008$). Although distribution reached normality at follow-up visits, it may have contributed to unequal variance between the groups. Non-parametric tests are advisable in such cases of unevenly distributed data in small samples. The two groups were subsequently also compared for each variable with the non-parametric sign test. In comparing the sign test results with those of the t-test for change between baseline and week 12, the weight increase in the supplemented group marginally misses significance ($p = 0.057$) while none of the other anthropometric results is affected. The reduced Hb in the placebo group was marginally significant ($p = 0.049$) and the significance of the decrease in MCH in the placebo group and MCHC in both groups was confirmed. The decrease in monocytes in the placebo group was also significant ($p = 0.004$) with the sign test.

Although data collected at week 4 and week 8 follow-up visits were included for analysis, no significant interim changes occurred and these data are therefore not reported in this thesis.

3.3.1.2 Within-subject and within-group outcomes

Using the General Linear Model procedure of SAS, a number of anthropometric parameters were shown to have responded significantly within subjects during the study period and also in some cases significantly differently in the two groups. Weight responded marginally significantly differently ($p = 0.049$) in the two groups (Figure 3.5), while MUAC ($p = 0.022$) (Figure 3.6) and BMAMA ($p = 0.004$) (not shown in table) showed very strong time-by-group effects within subjects. The weak change in TSF within subjects over time ($p = 0.049$) was not group-dependent, and though change in the placebo group was larger than that in the supplemented group, this was not significant.

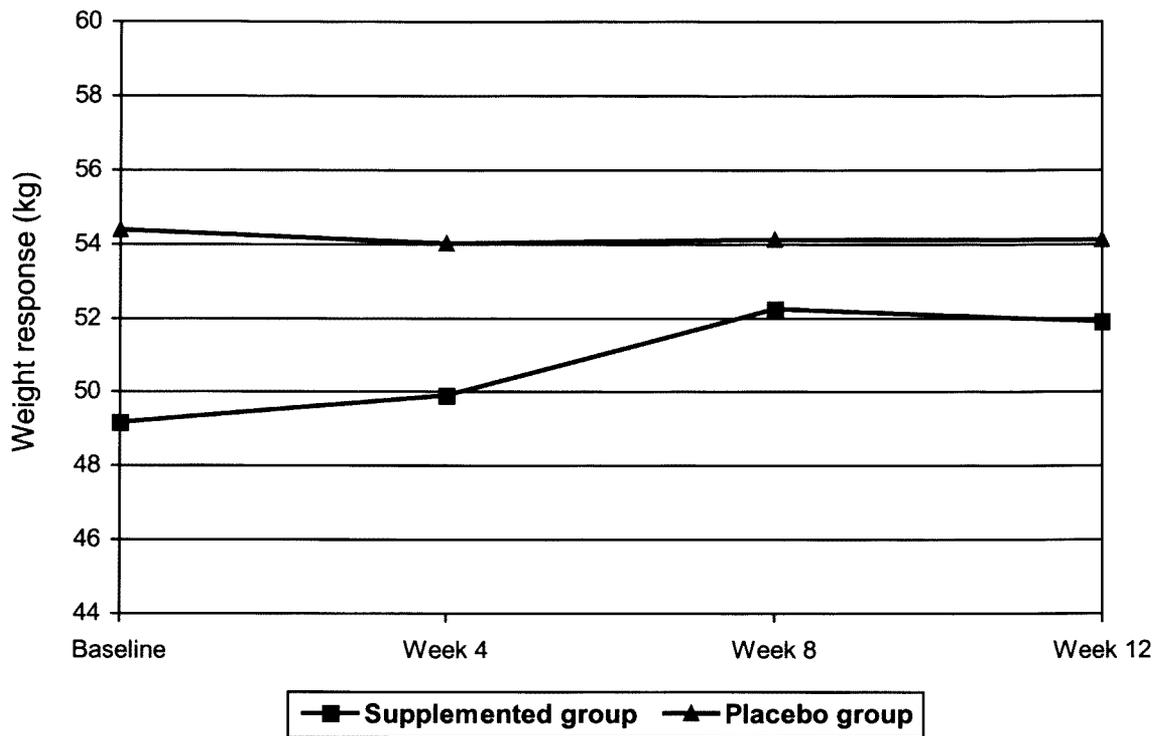


Figure 3.5: Weight response over the study period (n = 32)

Significant in supplemented group ($p = 0.049$) with two-sided Wilcoxon two-sample test.

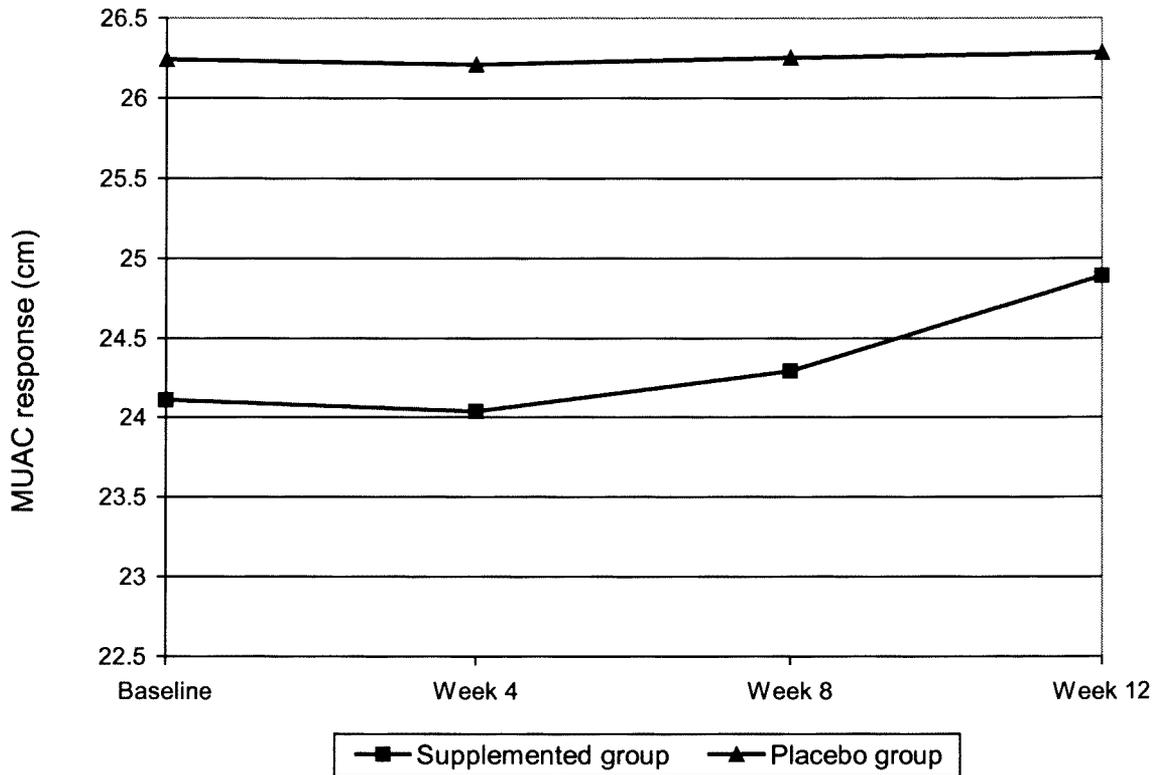


Figure 3.6: Mid-upper arm circumference response over the study period (p =32)

Significant in supplemented group ($p = 0.022$) with two-sided Wilcoxon two-sample test.

MUAC and BMAMA increased in the supplemented group and decreased in the placebo group and although these changes were not significant in themselves, the differences between the two groups were significant ($p = 0.022$ and $p = 0.004$ respectively). This may be indicative of a relative maintenance of lean body tissue in the supplemented group. WHR did not change significantly in either group and does not indicate redistribution of body fat.

S-Alb was the only biochemical marker to respond significantly within subjects and where the difference in change between the groups was also significant ($p = 0.033$) (Figure 3.3). It would appear that the test product was able to ameliorate the extent of hypoalbuminaemia associated with HIV/AIDS.

Among the haematological measures, Hb change was time ($p = 0.001$), but not group, dependent while both MCH ($p < 0.0001$) and MCHC ($p < 0.0001$) responded markedly differently within subjects independent of the group. The test product did not affect the extent of the anaemia present from baseline and relatively rapid deterioration was observed in both groups over the study period. The difference in response of neutrophil count in the two groups was significant ($p = 0.036$), the increase in the supplemented group **could be** indicative of the ability of the test product to improve the neutropaenia commonly associated with HIV/AIDS, which may reduce risk of infection. Monocytes also responded significantly ($p = 0.001$), although this effect was not group dependent.

3.3.2 Immunity outcomes

With pairwise t-tests none of the immunological parameters (Table 3.3) in either group changed significantly over the study period and none of the changes were significantly different between the groups. Using the sign test, the decrease in CD4+ count proved to be marginally significant in the placebo group ($p = 0.049$). Although of greater magnitude, the decrease in the supplemented group remained insignificant. CD3: CD8 ratio also decreased significantly ($p = 0.007$) in the placebo group only. Both of the above indicate significant longitudinal immune depletion with HIV/AIDS progression over the study period in the placebo group, but not the

supplemented group. The test product may have contributed to the maintenance of a relatively stable CD4+ count in the supplemented group during the study.

Table 3.3: Comparison of immunity at baseline and 12-week follow-up visit (n = 31)

Parameters	Reference values and units	Supplemented group n = 14 $\bar{x} \pm SD$			Placebo group n = 17 $\bar{x} \pm SD$		
		Baseline	Week 12	Change	Baseline	Week 12	Change
Total lymphocytes	0.9 - 5.2 $\times 10^9/\mu\text{l}$	2.66 ± 4.19	2.11 ± 2.15	-0.55 ± 2.13	1.56 ± 0.96	1.45 ± 0.90	-0.11 ± 0.72
CD3+	65 - 91%	75.43 ± 10.47	75.57 ± 10.28	0.14 ± 6.93	72.42 ± 13.86	75.35 ± 10.51	2.94 ± 8.07
CD4+	500 - 1500 cells/mm ³	255.57 ± 230.44	237.00 ± 170.68	-28.79 ± 86.48	212.88 ± 184.35	198.59 ± 187.25	-14.30 ^a ± 53.91
CD8+	230 - 800 cells/mm ³	647.46 ± 301.19	698.62 ± 329.26	51.15 ± 175.96	801.29 ± 666.52	727.18 ± 674.77	-74.12 ± 561.70
CD3+: CD4+	30 - 65 %	14.43 ± 8.62	14.50 ± 8.44	0.07 ± 2.40	15.00 ± 11.17	15.12 ± 11.29	0.12 ± 5.63
CD3+: CD8+	17 - 35 %	53.77 ± 7.25	54.23 ± 7.42	0.46 ± 6.17	52.53 ± 15.90	54.82 ± 14.83	2.29 ^a ± 9.45
CD4+: CD8+	1.0 - 3.5:1	0.29 ± 0.16	0.29 ± 0.16	-0.01 ± 0.08	0.37 ± 0.45	0.33 ± 0.39	-0.04 ± 0.18

^a Statistically significant ($p < 0.05$) with sign test.

Immunological outcomes were also observed with individual subject assessment at the end of the study. In only one (7%) of the supplemented subjects did the total lymphocytes count remain below reference values, while there was an increase in prevalence to 28% (5) in the placebo group. After the 12-week intervention CD4+ counts < 200 cells/mm³ were present in 36% (5) of the supplemented group compared to 59% (10) of the placebo group. This may represent a relatively more stable immunity in the supplemented group than in the placebo group. Deterioration of CD4+: CD8+ ratio (indicative of disease progression) occurred in both groups, although not significantly in either group. All subjects, but one from the placebo group, had values below the reference range by the end of the study for. The findings on immunity in this study indicate that nutrition supplementation **alone**, in the absence of ARV therapy, is not sufficient to curb the steady progression of HIV/AIDS.

CHAPTER 4

DISCUSSION

4.1 THE STUDY AND ITS LIMITATIONS

4.1.1 Comparison with other studies

This pilot study is the first prospective randomised double-blind placebo-controlled clinical trial to evaluate the effects of nutritional supplementation in people living with HIV/AIDS in Africa and one of only a few in the world.^{27,80} As such, even with a relatively small sample size, the baseline data contributes to the limited body of data on the nutrition status of people living with HIV/AIDS in Africa.^{9,77} It also provides evidence on the outcomes of nutritional supplementation with a new micronutrient, glutamine, pre- and probiotic enriched liquid nutritional supplement on the nutritional status and immunity in people living with HIV/AIDS.

The average study duration of nutrition intervention studies in HIV/AIDS reviewed to date was 15 weeks.^{27, 80} The majority of the subjects (68%) in this study completed the 12-week intervention period, which compares well with other studies of this nature³⁴, even though it was neither clinic- nor outpatient-based. Similar to other studies⁸¹, subject compliance and adherence to the study programme was satisfactory as reported in intake diaries. No adverse effects of the intervention were reported throughout the study period. This may be partly explained by the fact that the subjects voluntarily subscribed to the prolonged oral nutritional supplementation in the belief that benefit was to be derived. In addition, they were also appreciative of the nutritional assessment and the assessment of immunity, which they would experience for the first time and at no cost to themselves. This study confirms the feasibility of prolonged oral nutritional supplementation in people living with HIV/AIDS.^{31,35}

ARV therapy was not yet freely available in South Africa at the time of the study and none of the subjects received ARV therapy. The results of previous studies are often

confounded by the presence of ARV therapy, which was sometimes initiated during the research project. Subjects receiving ARV therapy at baseline and who continued to do so throughout the study gained significantly more weight.²⁸ The introduction of free ARV therapy is imminent in South Africa and studies in ARV-free populations may not be possible in the future. This implies that the independent role of nutritional supplementation on body weight in people living with HIV/AIDS may in future not be possible to distinguish.

4.1.2 Limitations of the study

Although the study was a prospective double-blind randomised controlled trial, considered to be the gold standard for strength of evidence, certain limitations nevertheless call for caution in the interpretation of the results. The small sample size is a major limitation, which limits analysis of the data and may be responsible for obscuring outcomes within subgroups and differences between the groups.

Except for the fact that all the subjects were HIV-infected, no additional clinical data was available. The research was not performed in a clinical facility and no medical records were available for the subjects. It is not possible to correlate the findings of this study with the presence of fever, diarrhoea or opportunistic infection, all of which would be expected to influence outcome. Although weight loss is often associated with opportunistic infections and recovery from opportunistic infection is associated with increased lean body mass²⁴, it has been reported that opportunistic infection does not necessarily influence weight loss or the response of body weight to nutritional counselling in HIV/AIDS.⁸² Without adequate clinical evaluation and diagnosis, no conclusions can be drawn from this study with regard to the occurrence of opportunistic infection and its association with weight loss. In the absence of ARV therapy it is likely that opportunistic infections may have played a role in subjects who lost weight.

Voluntary HIV-status assessment is rare in this community where patients are usually tested only once they are ill. Precise duration of HIV infection in the sample was therefore not known and disease stage classification was not possible at baseline.

The data was however compared for subjects with CD4+ counts either below or above 200 cells/mm, but no difference was apparent. Up until this study, immunity (CD4+ counts) had never been assessed in any of the subjects.

Nutritional status data prior to the baseline evaluation was not available. No prior weight record existed and the extent of HIV/AIDS wasting preceding this study is unknown. It is possible that weight gain may have been more easily achieved in subject with prior weight loss than in subjects with previously stable weight. Earlier studies selected subjects with stable weight in order to exclude underlying weight loss as a confounding element.²⁸ In this study baseline body weight was not an exclusion criterion. Given the small sample size it was not feasible to stratify the sample in terms of baseline weight. No difference was apparent when the data was compared for subjects with BMI either below or above 18 kg/m².

The study was conducted in the absence of background vitamin and/or mineral supplementation and subjects reported excellent compliance with the study intervention. In contrast to other studies of this nature²⁸, baseline and background energy and nutrient intake was not collected. Percentage weight gain is reported not to vary by background level of energy intake²⁸ and this is not necessarily a limitation of this study where subgroup comparison in the small sample would not have been feasible. On the other hand, it has been reported that nutritional supplementation may improve background nutritional intake expressed in terms of nitrogen:energy ratio.³⁵

Although no socio-economic data was collected for the sample, it can be safely assumed that the subjects, who were mostly unemployed, may have had marginal background dietary intakes. This sets this study apart from previous studies in more developed countries where background intake was in one instance even found to be high in comparison with the normal population.²⁸ In the latter study, it was thought that a normal to high background energy intake is likely to render the addition of 2 100 kJ (500 kcal) with a dietary supplement insufficient to result in significant weight gain.²⁸ The test product in this study added a mere 660 kJ to the diet, which is not considered adequate to bring about observable weight change per se over the short duration of the present study.

The test product was enriched with various components, and it is not possible to distinguish the individual effects, if any, of any of these components from this study. All findings pertain to the liquid nutritional supplement as a whole.

4.2 THE FINDINGS

4.2.1 Baseline findings

At baseline the majority of the sample was underweight when evaluated in terms of relative body weight and BMI. As indicated by the evaluation of other anthropometric parameters, undernutrition was not adequately identified in the subjects by weight assessment alone. Depending on the standard used, evaluation of anthropometric parameters produced varying prevalence values in the sample, which all point toward a general picture of undernutrition in both groups. This relatively depleted baseline nutritional status distinguishes this study from others in which the sample was known to be weight-stable in the period prior to the study^{28,34} and in which undernutrition was not prevalent in the study population employed.³⁴

No statistically significant difference was found between the two groups for any of the anthropometric parameters, but with individual nutritional assessment the supplemented group seemed worse off when compared to individuals in the placebo group in terms of prevalence of underweight and undernutrition based on anthropometric parameters at baseline. When biochemical indicators were employed, undernutrition was confirmed in half of the subjects, equally present in both groups. Acute infection may have been present in one out of three subjects as indicated by elevated CRP equally in both groups. Anaemia, both hypochromic, microcytic and macrocytic, was also common at baseline.

4.2.2 Study outcomes

The extent of body weight gain over a 12-week period in the supplemented group was on a scale comparable to the findings of two studies of longer duration^{40,35} and exceeded the weight gain observed in other studies.^{23,83} This study therefore supports the routine use of an enriched liquid nutritional supplement in order to prevent weight loss in people living with HIV/AIDS, at least in the short to medium term. The feasibility of nutritional supplementation as a cost-effective adjunctive treatment for weight loss in HIV/AIDS was previously reported.²⁴ The convenience of commercial liquid nutritional supplements makes them preferable over the cumbersome preparation of energy-dense meals.²⁴

Body weight increase in the supplemented group was associated with increases in both lean body mass and fat mass as indicated by the significant increases in both MUAC and TSF. This corresponds with earlier findings where fat gain predominated.^{24,34} Such fat gain is associated with food intake in excess of requirements in people living with HIV/AIDS.⁸⁴ Dietary intake data was not available in the present study and the intervention introduced too small an amount of energy to the diet to explain the weight gain on the grounds of energy excess alone. The latter statement implies that perhaps one or more of the supplemented ingredients of the nutritional supplement may have played a role.

In an effort to minimise error, a single experienced observer was responsible for all anthropometric assessments throughout the study. The significant increase in TSF in the placebo group has no rational explanation. It is possible that the subjects, realising they were being assessed, may have consciously or subconsciously increased their dietary intake in an effort to obtain an outcome to satisfy themselves and/or the researcher.

Another meaningful finding of this study is the significant increase in s-Alb in the supplemented group associated with a significant decrease in CRP. These findings are indicative of the amelioration of the hypoalbuminaemia often associated with HIV/AIDS and simultaneous amelioration of inflammation in the group supplement with the test product. These two findings together indicate that acute infections may

have been attenuated in the supplemented group, and that the enriched supplement may be involved in the reduction of inflammation resulting from HIV and other infections. A similar increase in s-Alb was previously reported in malnourished people living with HIV/AIDS who received enteral nutritional supplementation.⁸⁴ S-Alb is associated with survival¹⁵ especially in women and improvement in albumin status is particularly pertinent to this almost exclusively female sample where increased s-Alb in the supplemented group may be a powerful independent predictor of increased survival.⁸⁵

The test product had little effect on haematological parameters including anaemia, which was present at baseline, and deteriorated significantly during the study period. This would imply that the level of micronutrient supplementation in the recommended dose of the test product is insufficient to address clinical anaemia associated with HIV/AIDS. Although available data do not contraindicate iron supplementation in developing countries where HIV/AIDS and iron deficiency anaemia coexist⁸⁶, iron supplementation in HIV/AIDS may activate HIV expression and possibly worsen immunosuppression.⁸⁷ Until more is known about safe levels of iron supplementation in HIV/AIDS, it is not advisable to increase the level of iron supplementation beyond that which is already contained in the test product.

None of the immunity parameters was significantly affected by the intervention. In contrast to earlier studies^{35, 39,40}, the findings of the present study do not support the prescription of nutritional supplementation with potentially immune-enhancing enrichment to boost the immune system of people living with HIV/AIDS. One very recent study³⁹ reported a significant increase in CD4+ count in subjects with HIV/AIDS taking an immune-enhancing nutritional supplement for a period equivalent to this study. Similar findings were however not found in either earlier or later studies on the same product.^{32,40} The findings of this study thus reflect the trend found in earlier research. It should be noted that other studies usually provided higher levels of energy supplementation at around 2 100 to 2 520 kJ (500 - 600 kcal) and it is not known whether the extra energy alone may be responsible for at least some of the reported findings.

By the end of the study the nutritional status of the subjects in the present study had changed significantly as indicated by a number of anthropometric, biochemical and haematological evaluations (Table 4.1). Despite the mean increase in weight in the supplemented group, the prevalence of RBW below standard increased slightly in this group. Reassessment using BMI shows a decrease in the prevalence of underweight in the supplemented group by the end of the study. These conflicting results warrant scrutiny in terms of comparability of studies when different reference standards are used.

Table 4.1: Prevalence of undernutrition in the subjects in the study at baseline and 12-week follow-up visit (n = 32)

Indicator and standard	Supplemented group n = 14			Placebo group n = 18 ^a		
	Baseline	12 weeks	Effect	Baseline	12 weeks	Effect
	% below standard	% below standard		% below standard	% below standard	
RBW < 90%	67	71	↑	58	61	↑
BMI < 18 kg/m ²	50	36	↓	6	11	↑
TSF < 5 th percentile	50	50	-	39	22	↓
TSF < WHO standard	64	50	↓	44	17	↓
MUAC < 5 th percentile	57	43	↓	33	22	↓
MUAC < WHO standard	51	36	↓	22	17	↓
s-Alb < 35 g/l	50	43	↓	59	71	↑
CRP > 10 g/l	43	33	↓	24	19	↓
Lymphocytes < 0.9x10 ⁹ /μl	29	14	↓	29	24	↓
Hb < 12 g/dl	79	86	↑	47	82	↑ ^b
Microcytic, hypochromic anaemia	38	36	↓	24	24	-
Macrocytic anaemia	24	36	↑	20	24	↑

^a Biochemical and haematological parameters for n = 17 only, one set of baseline blood specimens lost

^b Significant increase (p = 0.03) with McNemar test.

Symbols: ↑ = increased prevalence, or undesired effect
↓ = decreased prevalence, or desired effect

Abbreviations: RBW = relative body weight, BMI = body mass index, TSF = triceps skinfold thickness, MUAC = mid-upper arm circumference, s-Alb = serum albumin, CRP = C-reactive protein, Hb = haemoglobin.

The overall picture that emerged is without doubt a general state of malnutrition at baseline, with reduction in prevalence of malnutrition over the study period as assessed with a variety of standards. The use of different parameters and standards

for assessment of nutritional status produced divergent results. Notwithstanding the benefits reported in this thesis, malnutrition persisted in the sample despite nutritional supplementation. In the absence of ARV treatment, which is the current situation in South Africa, it seems doubtful whether nutritional supplementation will result in marked positive changes in nutritional status.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSIONS

This pilot study demonstrates that a micronutrient, glutamine, pre- and probiotic enriched liquid nutritional supplement may promote weight gain and ameliorate hypoalbuminaemia, inflammation and acute infection in adults living with HIV/AIDS. The weight gain may be associated with increases in both lean body mass and fat mass. The nutritional supplement does not appear to have an effect on the immunity of people living with HIV/AIDS. The null hypothesis stating that the test product will have no effect is rejected in terms of nutritional status, but upheld for immunity.

This study was limited by its small sample size. The conclusions cannot be generalised, but can only be considered preliminary warranting further investigation. As a pilot study the sample was not intended to be representative of the South African HIV-infected population. The conclusions are applicable only to the sample population, namely black adult females living with HIV/AIDS in a peri-urban environment in a resource-poor setting. The conclusions are furthermore relevant only to situations with limited access to medical care and to the absence of ARV therapy.

In the absence of body weight data prior to the study, the findings cannot be extrapolated to weight-stable subjects. Without adequate clinical data the findings of the study cannot be associated with disease progression or extrapolated to situations of acute opportunistic infection or clinical malabsorption. Neither of the above limitations should be interpreted to imply that the use of nutritional supplementation is prejudiced or precluded in these situations.

It is concluded that the use of an enriched liquid nutritional supplement is advisable in adults living with HIV/AIDS to help prevent weight loss in the presence of malnutrition. Although dietary counselling did not form part of this study, evidence

from other studies indicates that nutritional supplementation is no better at fighting weight loss associated with HIV/AIDS and is best provided in conjunction with dietary counseling.^{23,25,88,89} On the basis of this study, no conclusions can be drawn that any specific component, with which such nutritional supplements are enriched, will have any independent effect on either nutritional status or immunity in people living with HIV/AIDS. This study does not provide evidence for the quantity of nutritional supplement required to achieve a beneficial effect and did not explore the mechanisms involved.

5.2 RECOMMENDATIONS

Additional research is required on a larger sample, possibly of a multi-centre nature, to study the effects of the enriched liquid nutritional supplement in a sample adequately representing the diverse South African population. Further studies are also required to evaluate the individual effects of the various components of the enriched nutritional supplement.

Any further studies should preferably include a strong clinical component, without which confounders such as acute infection cannot easily be eliminated. Studies done in clinical settings will allow for more improved sampling and sample stratification, particularly in terms of disease progression. While this study focused on anthropometric and laboratory parameters, it will be most useful for future research to include assessments of clinical outcomes and evaluation of quality of life.

The findings of this study contribute to the development of evidence-based nutritional messages, which are required to counter the misinformation that has appeared in the South African press.⁹⁰ Nutrition policy ought to be based on scientific evidence and should aim for the integration of messages contained in existing national guidelines on nutrition for people living with HIV/AIDS.⁹¹ Social responsibility places the onus on the food and pharmaceutical industries to ensure that health claims concerning nutritional supplements are supported by evidence.

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APPENDICES

APPENDIX 1

COMPOSITION OF THE SUPPLEMENT

Product name: *B-immune*[®]

Manufacturer: African Dynamics Pty (Ltd), Pretoria, South Africa

Ingredients: Soy protein, vegetable fat, sugar, sodium chloride, vitamins, amino acid chelated minerals, L-glutamine, carnitine, taurine, pre- and probiotics, flavourants and colourant.

Nutrition information^a:

	Per 100 g	Per 40g portion
Energy	1656 kJ	663 kJ
Protein (fat free basis)	17.1 g	6.9 g
Fat	14.2 g	5.7 g
Carbohydrates	51.2g	20.5 g
L-glutamine	5.0 g	2.0 g
Carnitine	210 mg	84 mg
Taurine	350 mg	140 mg
Prebiotics: Raftilose [®] P95 (Addendum 2)	7.5 g	3.0 g
Probiotics ^{b,c} : <i>B bifidus</i> , <i>L acidofilis</i>	2.0 x 10 ⁹ cfu	8.16 x 10 ⁸ cfu
	Per 40g portion (For persons 10 years and older)	
Vitamins	100% of RDA	
Minerals	15% of RDA	

Directions for use: 1 x 40 g sachet per day dissolved in 200ml cold water.

Flavours: Strawberry, banana and vanilla.

Storage: Store in a dry place below 25⁰C

^a Nutrient analysis performed by the South African Bureau of Standards (SABS), Pretoria.

^b Probiotic assessment performed by Agricultural Research Council (ARC), Pretoria.

^c Available at end of 6 months period unrefrigerated.

APPENDIX 2**MICRONUTRIENT CONTENT OF THE SUPPLEMENT**

MICRONUTRIENT ^a	Per 100g	Per 40g portion	% RDA ^b per portion
VITAMINS			
Biotin	250 µg	100 µg	100
Folic acid	500 µg	250 µg	100
Pantothenic acid	15 mg	6 mg	100
Nicotinamide	45 mg	18 mg	100
Vitamin A	2500 µg RE	1000 µg RE	100
Vitamin B ₁	3.5 mg	1.4 mg	100
Vitamin B ₂	4.0 mg	1.6 mg	100
Vitamin B ₆	5.0 mg	2.0 mg	100
Vitamin B ₁₂	2.5 µg	1.0 µg	100
Vitamin C	150 mg	60 mg	100
Vitamin D ₃	12.5 µg	5.0 µg	100
Vitamin E	37.5 mg α-TE	15.0 mg α-TE	100
MINERALS			
Calcium	300 mg	120 mg	15
Iron	5.3 mg	2.1 mg	15
Zinc	5.6 mg	2.3 mg	15
Selenium	20.6 µg	8.3 µg	15
Chromium	37.5 µg	15 µg	- ^c
Manganese	0.8 mg	0.3 mg	- ^c

^a Nutrient analysis performed by the South African Bureau of Standards (SABS), Pretoria.

^b RDA for persons 10y and older.

^c No RDA available.

APPENDIX 3

COMPOSITION OF THE PREBIOTIC COMPONENT OF THE SUPPLEMENT

- Product name: *Raftilose*[®] P95
- Supplier: Orafiti, Tienen, Belgium
- Description: A powder containing mainly oligofructose produced by partial enzymatic hydrolysis of chicory inulin and a food ingredient composed of oligofructose (> 93.2%) and fructose, glucose and sucrose (< 6,8% together).
- Safety: Safe. Non-toxic. Not dangerous. Excessive consumption may cause diarrhoea.
- Nutritional information^a:

	Average per 100g
Carbohydrates	5
Sugars	5
Dietary fibre	92
Protein	0
Fat	0
Vitamins and minerals	Negligible
Energy	158kcal/662 kJ

^a Information provided by supplier.

APPENDIX 4

RESEARCH PROTOCOL

TITLE

THE EFFECTS OF A MICRONUTRIENT, GLUTAMINE, PRE- AND PROBIOTIC ENRICHED FOOD SUPPLEMENT ON THE NUTRITIONAL STATUS AND IMMUNITY OF ADULTS LIVING WITH HIV/AIDS: A PILOT STUDY

RESEARCHERS

Chief researcher

Roy D Kennedy B Nutr (Stell), RD(SA)

Department of Human Nutrition, Medunsa

Co-researchers

Willa Haasbroek M Sc

Consultant Food Scientist, Gauteng

INTRODUCTION

It is estimated that 70% of the HIV positive population of the world reside in Sub-Saharan Africa (ACC/SCN 2001). In South Africa alone 40% of deaths have been ascribed to AIDS-related disease (MRC 2002). The impact of HIV/AIDS is felt at all levels of society, in the health system, the workforce, the economy and human development (ING Barings 2000, UNAIDS 1998).

The metabolic effects of HIV on the infected individual are numerous and well described (Kotler 1999). Influenced by reduced food intake, nutritional alterations and complications of HIV/AIDS, the nutritional status is greatly compromised (Piwoz and Preble 2000). Furthermore, social isolation, physical disability and unemployment impact on the progression of HIV/AIDS. HIV/AIDS and the accompanying malnutrition both suppress the immune system of the person living with the virus. Although some evidence exists for the benefits of immunonutrient

supplementation (eg glutamine, arginine and antioxidant nutrients) in physically stressed patients receiving enteral and/or parenteral nutrition support, no clear recommendations exist (Barton 1997) and very few studies have been published with regard to HIV infection (Kotler 2000).

Although it is well documented that the maintenance of body weight, and in particular lean body tissue, impacts positively on survival in HIV/AIDS (Kotler *et al.* 1989, Kotler 1999), documented evidence of the benefit of food-based nutrition intervention in HIV/AIDS is sadly lacking (Kotler 2000). Even though a small number of studies have indicated improved clinical outcomes, there is similarly a paucity of data on the effectiveness of supplemental nutrition support in HIV/AIDS (Stack *et al.* 1996, Kotler 2000). There exists a great need for the examination of the effects of supplemental oral feeds in terms of outcomes such as weight and immunity.

The availability of a new enriched food supplement on the market, offers the possibility of affordable nutrition intervention to people living with HIV/AIDS with the added benefit of added micronutrients and immunonutrients, which may support the immune system. In the light of the economic effects of HIV/AIDS and household food security, it is vitally important to ascertain the effectiveness of such a product on the nutritional status and immunity before any unsubstantiated health claims are made. A prospective, longitudinal study on the effectiveness of supplemental feeding in HIV/AIDS will contribute not only to the care of people living with HIV/AIDS in South Africa, but also internationally

AIM

The primary aim of the study is to determine the effects of a micronutrient, glutamine and pre- and pro-biotic enriched food supplement on the nutritional status and immunity of adults living with HIV/AIDS.

OBJECTIVES

The study intends to determine the effects of the enriched food supplement on:

- 1 the nutritional status of people living with HIV/AIDS by means of anthropometric and biochemical assessment over a period of 90 days.
- 2 the immune system of people living with HIV/AIDS by means of CD3+, CD4+ and CD8+ counts and their respective ratios over a period of 90 days.

HYPOTHESIS

It is hypothesised that the test feed will have no effect on either the nutritional status or the immunity of the subjects.

TYPE OF STUDY

A randomised double-blind placebo controlled study is planned where the test group will receive the enriched food supplement (the test feed) and the control group a specially formulated placebo drink without enrichment (the placebo feed).

THE SAMPLE

The convenience sample will comprise free-living adults attending the Moretele Sunrise Hospice, a community-based centre in Temba, Hammanskraal, North-West Province, located 50 km north of Pretoria. This sample will be randomly divided into a test group (those who receive the test feed) and a control group (those who receive the placebo) in a double-blind manner.

It is aimed to include at least 40 male and female subjects with HIV/AIDS independent of duration of infection. All stages of HIV infection will be included whether asymptomatic or symptomatic. Confidentiality will be ensured at all times and the subjects will be identifiable by a number consecutively allocated upon entry into study alone.

EXCLUSION CRITERIA

- The study will focus on adults only and anyone younger than 21 years will be excluded.
- The study will focus on HIV positive individuals and uninfected persons will be excluded.
- The study will focus on the effects of the enriched food supplement and persons on antiretroviral drug treatment will be excluded.
- The study aims to determine the effects of the enriched food supplement and any person who initiates the taking of other nutritional or immune-enhancing supplements during the study will be excluded.
- The study will require assurance of the regular intake of the supplemental feed. For this purpose persons in the terminal stage of the disease, who may find this problematic, will be excluded from the study. Because terminal patients are not likely to visit the clinic, it precludes the possible ethical dilemma of their exclusion from the study.
- Pregnant females will be excluded from the study.

METHOD

The manufacturers of the food supplement, *B-immune*[®] [for composition see *Appendices 1 and 2*], will supply sufficient quantities of the test and placebo feed to augment the existing diet of the subjects with one feeds per day for 84 days each. The convenience sample will be randomly divided, with one half of the subjects to receive the test feed (the complete product with micronutrient, glutamine and pre- and pro-biotic enrichment) and the other half the placebo feed. The placebo feed is formulated to be comparable in flavour and colour to the test feed. A low-energy bulking agent is used to arrive at a similar dry weight to that of the test feed, but with minimal energy content [*Appendix 4*].

The manufacturer will supply the feeds in numbered packaging (84 feeds for each number). The feeds will be pre-coded, identifying test and placebo feeds in a code not known to either the researchers or subjects. Decoding will only be done on

completion of the study and once all data had been collected. Each subject will receive a plastic mixer with marked volume measurements to ensure standard preparation of the supplement.

Before intervention, baseline data will include anthropometric, biochemical and haematological assessment of nutritional status and baseline assessment of immunological status. Upon each visit a basic health history will be taken to record health and nutritional problems.

The intervention will comprise once-daily recorded ingestion of either the test or placebo feed. The identity of the type of feed will be unknown to both the researchers and the subjects. The subjects will additionally continue with their home diets as usual. Any current or newly initiated use of nutritional or immune-enhancing supplements is to be reported.

The subjects will be provided with contact details to report any unforeseen circumstances. Subjects will be required to keep a daily record of consumption of the food supplement, problems and symptom. Any illness or infection must be recorded, as well as any factors influencing compliance with the ingestion of the supplement. A registered dietitian will assess the following anthropometric parameters at baseline and thereafter every 28 days to the completion of the study period:

- Height
- Weight
- Body mass index (calculated from the above)
- Triceps skinfold
- Arm muscle circumference
- Waist circumference
- Hip circumference
- Waist/hip ratio

The following biochemical and haematological parameters will be assessed at baseline and on completion of the study after 84 days and all blood samples will be collected by a registered nursing practitioner:

Serum total protein
Serum albumin
C-Reactive protein
Full blood count
CD3+, CD4+ and CD8+ counts (with ratios)

STATISTICS

The University of Stellenbosch will appoint a statistician, in conjunction with whom the researcher will analyse the data. Anthropometric and biochemical data will be compared with standards and normal values. Immunological assessment will be compared with diagnostic criteria used in HIV/AIDS classification. The results of the two groups will be compared using information technology based statistical programmes to determine significance of difference between the test and control groups.

LIMITATIONS

- The small study sample may limit generalisation of the results to a larger population.
- The stage of HIV infection may influence outcome in individual subjects.
- Regular visits may influence results independently from the food supplement that is being tested.

BUDGET

The manufacturers of the test feed will be responsible for the costs pertaining to the research project in full. The major costs involved relate to the biochemical and haematological assessments, which will be performed by the laboratories at Medunsa and for which the quotes run to around R 45 000 in total. The only additional costs will include traveling cost to and from the research site. No staff remuneration is planned for and equipment from the Department of Human Nutrition at Medunsa will be used free of charge.

ETHICS

- Approval for the study will be sought from the Research and Ethics Committee of the Faculty of Health Sciences of the University of Stellenbosch.
- Written informed consent [*Information and informed consent document: Appendix 5*] will be obtained from the subjects prior to commencement of the study.
- Confidentiality of information will be observed at all times and a unique coding system will be used to refer to subjects.

TIME FRAME

It is planned to initiate the intake of subjects into the study during January 2003 for the study to be completed by the end of April 2003. The final research report deadline is set for 30 June 2003.

REPORT

The study forms part of the requirements for the Masters in Nutrition degree at the University of Stellenbosch and will be reported in a dissertation. It is also planned to submit a paper on the study to a professional journal in the fields of nutrition and dietetics, hopefully internationally.

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APPENDIX 5**COMPOSITION OF THE PLACEBO**

Strawberry flavour	g/200ml	%
Total	40.000	100.000
Unidry 20 (Maltodextrin)	38.386	95.965
Cloud MB92538	0.200	0.500
TiO ₂ (Titanium dioxide)	0.200	0.500
Salt	0.200	0.500
Sweetener blend 80x (dol06)	0.150	0.375
CMC 3000 (Carboxymethyl Cellulose)	0.500	1.250
Flavourant: Vanilla 564340SPM	0.200	0.500
Flavourant: Strawberry 564.477 SPM	0.1600	0.400
Colourant: Carmoisine Red H7110	0.0040	0.010
Banana flavour	g/200ml	%
Total	40.000	100.000
Unidry 20 (Maltodextrin)	38.546	96.365
Cloud MB92538	0.200	0.500
TiO ₂ (Titanium dioxide)	0.200	0.500
Salt	0.200	0.500
Sweetener blend 80x (dol06)	0.150	0.375
CMC 3000 (Carboxymethyl Cellulose)	0.500	1.250
Colourant: Tartrazine H7061	0.004	0.010
Flavourant: Vanilla 564340SPM	0.200	0.500
Flavourant: Banana 564.006 SPM	0.130	0.325
Vanilla flavour	g/200ml	%
Total	40.0000	100.000
Unidry 20 (Maltodextrin)	38.550	96.375
Cloud MB92538	0.200	0.500
TiO ₂ (Titanium dioxide)	0.200	0.500
Salt	0.200	0.500
Sweetener blend 80x (dol06)	0.150	0.375
CMC 3000 (Carboxymethyl Cellulose)	0.500	1.250
Flavourant: Vanilla 564340SPM	0.200	0.500

APPENDIX 6

STUDY INFORMATION AND INFORMED CONSENT DOCUMENT

TITLE OF THE RESEARCH PROJECT:

THE EFFECTS OF A MICRONUTRIENT, GLUTAMINE, PRE- AND PROBIOTIC ENRICHED FOOD SUPPLEMENT ON THE NUTRITIONAL STATUS AND IMMUNITY OF ADULTS LIVING WITH HIV/AIDS: A PILOT STUDY

REFERENCE NUMBER: 2002/C/102

PRINCIPAL INVESTIGATOR: Mr R D Kennedy

ADDRESS: Department of Human nutrition
P O BOX 177
MEDUNSA 0204

DECLARATION BY PARTICIPANT

I, THE UNDERSIGNED,(name),

ID No:, hereafter called **the participant**, of

.....

..... (address).

A. HEREBY CONFIRM AS FOLLOWS:

1. I was invited to participate in the abovementioned research project, which is being undertaken by the Department of Human Nutrition, Faculty of Health Sciences, University of Stellenbosch.

2 The following aspects have been explained to me:

2.1 **Aim:** The aim of the project is to determine the effects of a food supplement on the nutrition and immunity of adults living with HIV/AIDS.

Continued overleaf/...

2.2 **Procedures:** The nature of the project requires me to take either the food supplement or a product that looks and tastes similar. Neither I, nor the investigator, will know which product I will be taking.

- The project will last for 84 days.
- It is important that I should report the taking of any nutritional supplements (vitamins or minerals, eg calcium or iron) at the beginning of the study and that I should inform the investigator when starting to take any supplements.
- I will need to visit the clinic on the first day of the project and thereafter every 28 days until the completion of the project after 84 days (a total of 4 visits).
- During the first and last visits, a 10 ml sample of blood (about 2 teaspoons) will be required to measure levels of immunity and nutrition.

2.3 **Risks:** There are no known risks or side effects of the food supplement when taken at the amount prescribed (40g or 200 ml per day) for the study. Excessive intake may lead to diarrhoea.

2.4 **Possible benefits:** The food supplement is designed to boost the immune system and improve the level of nutrition. The results of this project will benefit other people living with HIV/AIDS.

2.5 **Confidentiality:** The information collected, as well as my medical and personal information will be treated as confidential. I agree that the information may be used for publication in professional journals and that it may be included in a thesis provided my identity is kept confidential.

2.6 **Access to findings:** Upon request, the investigator will inform me of my results obtained in the project, but it will not be revealed to other persons.

2.7 **Voluntary participation/refusal/discontinuation:** Participation in the project is voluntary and I may refuse to participate in the project. The investigator may withdraw me from the project should he/she feel that it would be in my best interest or if I have not follow the agreements hereby agreed to. I understand that I will be excluded from the project if I should start to take anti-retroviral medication during this period.

3 The information above was explained to me by:

.....(name of relevant person) in English and I am in command of this language/was satisfactorily translated to me by:

.....(name of translator).

Continued overleaf/...

4 I was given the opportunity to ask questions and all these questions were answered satisfactorily.

B I HEREBY CONSENT VOLUNTARILY TO PARTICIPATE IN THE ABOVE-MENTIONED PROJECT.

Signed/confirmed at on2003
(place) (date)

.....
Signature of participant

.....
Signature of witness

STATEMENT BY OR ON BEHALF OF INVESTIGATOR

I, (*name of investigator/representative*)
declare that I explained the information given in this document to:

..... (*name of participant*);
he/she was encouraged and given ample time to ask me any questions;
this conversation was conducted in English and no translator was used/

this conversation was translated into (*language*)

by (*name of translator*).

Signed/confirmed at on2003
(place) (date)

.....
Signature of investigator/representative

.....
Signature of witness

Continued overleaf/...

DECLARATION BY TRANSLATOR

I,(name), confirm that I:

- translated the contents of this document from English into (language) to the participant;
- explained the contents of this document to the participant;
- also translated the questions posed by the participant, as well as the answers given by the investigator;
- and conveyed a factually correct version of what was related to me.

Signed/confirmed at on2003
(place) (date)

.....
Signature of translator

.....
Signature of witness

IMPORTANT MESSAGE TO PARTICIPANT

Dear participant,

Thank you for your participation in this study.

Should, at any time during the study:

- an emergency arise as a result of the research, or
- you require any further information with regard to the project, or
- you start to take other nutritional supplements,

kindly contact **Mr R Kennedy** on **082-575-6507** or **012-521-4078**

APPENDIX 7

EXAMPLE OF SUBJECT IDENTIFICATION CARD

FRONT

<p>STUDY NUMBER</p> <p>25</p>

REVERSE

<p>YOUR NEXT APPOINTMENT:</p>	
1	____/____/2003
2	____/____/2003
3	____/____/2003

APPENDIX 8

SUBJECT INFORMATION RECORD

SUBJECT INFORMATION SHEET

CONFIDENTIAL

SUBJECT NO: AD _____

SEX: M / F

Initials: _____ **Surname:** _____

Date of birth: ____/____/19____

Physical address: _____

_____ Code _____

Home telephone: (0____) ____ - _____

Work telephone: (0____) ____ - _____

Fax: (0____) ____ - _____

Cell phone: (0____) ____ - _____

Race: Black / White / Coloured / Indian

Home language: _____

Duration if infection: State number of years _____

Disclosed: No / Yes

APPENDIX 9**SUPPLEMENT DISTRIBUTION RECORD**

CLINICAL TRIAL PRODUCT DISTRIBUTION SHEET MONTH 3		
DATE	SUBJECT NO	SIGNATURE
	1	
	2	
	3	
	4	
	5	
	46	
	47	
	48	
	49	
	50	

APPENDIX 10**DAILY SUPPLEMENTAL INTAKE RECORD**

CLINICAL TRIAL INTAKE RECORD 3 (Final)				
				SUBJECT NO: AD _____
Feed No	Month	Date	Tick when taken	List any health and feeding problems in this column especially if it affects your ability to take the product
1	March	25		
2	March	26		
3	March	27		
4	March	28		
5	March	29		
6	March	30		
7	March	31		
8	April	1		
9	April	2		
10	April	3		
11	April	4		
12	April	5		
13	April	6		
14	April	7		
15	April	8		
16	April	9		
17	April	10		
18	April	11		
19	April	12		
20	April	13		
21	April	14		
22	April	15		
23	April	16		
24	April	17		
25	April	18		
26	April	19		
27	April	20		
28	April	21		

YOUR NEXT AND LAST VISIT IS ON 22 APRIL 2003

I, the participant identifiable by the number above, hereby state that the record above is a true reflection of my participation in the clinical trial.

Signed: _____

APPENDIX 11

SPECIMEN COLLECTION PROTOCOL

CLINICAL TRIAL

BLOOD COLLECTION PROTOCOL

IMPORTANT NOTICE:

- 1 Clearly mark each tube with the **subject number**
- 2 All samples must be ready for **transport to MEDUNSA laboratories at 13H00**

INSTRUCTIONS:

A Blood sample required for **Haematology**:

Collect **at least 4ml** of venous blood in an EDTA tube (**purple top**)

Mix gently by tilting the tube 6-8 times

Place sample in **cooler box**

B Blood sample required for **Chemical Pathology**:

Fill a **red-topped** tube (**5ml**) with venous blood

Place sample in **cooler box**