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Arthrospira platensis as Nutritional Supplementation for Adult Women Infected with Human Immunodeficiency Virus in Yaoundé, Cameroon

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Abbreviations

AIDS, Acquired Immunodeficiency Syndrome

ARV, Anti-Retroviral (Drugs)

Asp, *Arthrospira platensis*

BMI, Body Mass Index

CNLS, Comité National de Lutte contre le SIDA

FAO, Food & Agriculture Organisation

HAART, Highly Active Antiretroviral Therapy

HDJ, Hôpital De Jours

HIV, Human Immunodeficiency Virus

HPMC, Hydroxypropylmethylcellulose

IDA, Iron Deficiency Anaemia

IDDS, Individual Dietary Diversity Score

IQR, Inter Quartile Rang

MTCT, Mother-To-Child Transmission

OLI, Open Label Intervention

PLWHA, People Living with HIV or AIDS

RCT, Randomized Control Trial

RDA, Recommended Dietary Allowances

SIDA, Sydrome d'Immunodéficience Acquise

TAOS, Total Antioxidant Status

TC, Total Cholesterol

TG, Triglyceride

VL, Viral Load

WHO, World Health Organization

1 INTRODUCTION

The pandemic of the Human Immunodeficiency Virus (HIV) still represents a global challenge, although the United Nations Millennium Development Goal aiming to stop and begin to reverse the spreading of this disease by 2015, can be achieved. The last United Nations report presented a reduction in new infections and deaths resulting from HIV (UN 2012). According to the last World Health Organization (WHO) report, the fight against HIV/AIDS is turning from an emergency to a long-term, sustainable response of delivering HIV services (WHO 2011). The major step was taken in 1996 with the combination of three antiretroviral molecules to a Highly Active Antiretroviral Therapy (HAART), which significantly extended life expectancy (Autran, Carcelain et al. 1997). Furthermore, therapeutic access is recommended and free of charge for people living with HIV (PLWHA) who reach a CD4-lymphocytecount under 350 cells/mm^3 (WHO 2006).

Currently, UNAIDS report a world count of PLWHA of34 (31.4 – 35.9) million of whom 23.5 (22.1 – 24.8) million are living in Sub-Saharan Africa. The coverage of people in need of HAART has risen to 54% in countries of low and middle income (UNAIDS 2012).

1.1 Problem Statement and Aims

Natural products can provide a low-priced and sustainable strategy to stabilize the condition of PLWHA until they are entitled to HAART and additional support when the patients undergo lifelong therapy (Kuete 2010; Van Tienen, Hullegie et al. 2011). One natural nutrition supplement is an alga called *Arthrospira platensis* (Spirulina). Present on all continents, research, production, and commercialisation of this alga have risen in the last 20 years. The use of spirulina in the cure of various diseases is a focus of interest.

Research carried out in Asia and North America focused on its functional activity. Studies on animal models showed activation and mobilization of lymphocyte cells and regulation of transmitter proteins (Hayashi, Katoh et al. 1994; Qureshi, Garlich et al. 1996; Mao, J et al. 2000). *In vitro,* aqueous extracts of spirulina containing polysaccharides and calcium (Ca-SP) have been found to capture numerous enveloped viruses, including *herpes* virus, *cytomegalovirus*, *influenza*, and HIV, and prevent humans from infection (Hayashi, Hayashi et al. 1996; Ayehunie, Belay et al. 1998; Rechter, Konig et al. 2006). Those results and the

increasing production world widesss position Spirulina as a potentially valuable nutritional supplement (Belay 2002). Spirulina is currently sold – even to PLWHA – as a supportive treatment.

The suggestion that Spirulina could act against viruses such as HIV is used for commercialisation of the supplement as a therapeutic agent. Furthermore, publications presenting algae as a natural equivalent to the chemical HAART indicates the need to collect comprehensive information about the effect of algae in patients infected with HIV (Teas, Hebertb et al. 2004; Thanh-Sang and Se-Kwon 2010).

Point four of a WHO statement explains "there is a proliferation in the marketplace of unproven diets and dietary therapies with exploitation of fears, raising of false hopes, and further impoverishment of those infected and affected by HIV and AIDS" (WHO 2005). This observation shows the imperative need to elucidate the real effect of natural preparations such as spirulina that are already in use by PLWHA.

1.2 Human Immunodeficiency Virus (HIV)

The agent of the disease called Acquired Immunodeficiency Syndrome is HIV. The virus is a member of the lentivirus family, part of the retroviruses. The diversity of the virus in Cameroon is one of the highest in the world (Sharp and Hahn 2011). The viral history seems to be much older than commonly presumed, although it is known that indigenous populations of the Congo forest have gained mutations showing resistance against the most virulent type HIV-1 (Zhao, Ishida et al. 2012). In 2006, a relationship between the chimpanzee simian immune virus (SIV) and the pandemic sub-group major HIV-1 was found (Keele, Van Heuverswyn et al. 2006). The HIV viruses show a large diversity of types and subtypes. They can be divided in two types: HIV-1 and HIV-2. HIV-1 is more virulent and more common worldwide than HIV-2. The most prevalent type in Cameroon is HIV-1 at 80%. The major subtype M of HIV-1 is the most virulent subtype. The HIV-1 subtypes O and N represent only 1-5% of the infections in the country (Fonjungo, Mpoudi et al. 2000). HIV-2 is mostly present in western Sub-Saharan Africa. The subtype 2 stands for a direct infection from SIV sooty mangabeys (Lemey, Pybus et al. 2003).

An HIV infection is a long, progressive decrease of the immune system. The clinical course of the infection is determined by a decrease in CD4 cells. A study of an urban African cohort found a mean time lapse of 7.6 years between infection and manifestation of the disease

(Zwahlen 2008). In Africa, the majority of PLWHA have no access to or still have no need for a lifelong antiretroviral therapy. In order to achieve a sustainable answer to HIV/AIDS and to reverse HIV spread by 2015, further patient services need to be implemented, structural barriers removed, and prevention, treatment, and care optimized (WHO 2011).

- **Diagnosis**

The infection with HIV is diagnosed by the presence of antibodies against the viral antigen in the blood. In case of a positive ELISA test, a western blot is done for direct detection of the virus. This method is highly specific, detecting the gp120 protein on the viral envelope. To confirm or discover discordance, a second blood sample is taken. Again, an ELISA is conducted, and the positive result is again re-confirmed via western blot (WHO 2006).

The major markers for documenting the progress of the infection is the number of CD4 cells and the viral load (CDC 1987). The lymphocytes expressing the epitope CD4 are host cells for the virus. Their number declines, inducing a loss of cell-mediated immunity due to the intracellular multiplication of the virus. After the primary infection, the course of the infection is characterized by a long, stable phase that can last up to 10 years (Zwahlen 2008). The decline of CD4 lymphocytes can be progressive over the duration of the infection or come to an abrupt decrease after stagnation before the AIDS phase.

The impairment of immunity in the asymptotic pre-AIDS phase has still not been completely elucidated. However, immunity loss is related to the number of CD4 cells, which is also the indicator for monitoring and evaluating the therapy (Mellors, Munoz et al. 1997). The threshold of a CD4 cell population below 350 cells per mm^3 has been designated as the start for HAART therapy by the WHO. CD4 fluctuation in a patient over a day can be important and needs to be assessed by an additional marker (Hughes, Stein et al. 1994). CRP and CD38 have been postulated as monitoring markers for the stage of the infection or the effect of the therapy (Graham, Baeten et al. 2007; Nixon and Landay 2010).

Several studies have implemented the CD38 expression on CD8 cells as an intervention efficiency marker (Wilson, Ellenberg et al. 2004; Gil, Martınez et al. 2005; Ondoa, Dieye et al. 2006). An expression of CD38 is discussed in the literature as additional data for documenting the course of the infection. However, CD38 is considered to have only a minor relationship to the recovery of CD4 cells (Steel, John et al. 2008; Rosso, Fenoglio et al. 2010). Research on apes and their immune adaptation to SIV have shown that an immune system

can cope with a viral infection with no clinical outbreak (Klatt, Silvestri et al. 2012). Furthermore, ongoing research of asymptomatic carriers, called elite controllers, confirm the relationship of a non-detectable viral load and a low active immune system (Kamya, Tsoukas et al. 2011).

1.3 Relation between Infection and Nutrition

The link between nutrition and progression of the infection has been drawn as a vicious circle (Tomkins A and F. 1989). Infections have been shown to cause higher energy demand due to the activation of the immune system (Scrimshaw, Taylor et al. 1968). As the infection affects a large spectrum of physiological functions, especially the immune system, it is not only associated with an increasing energy demand but also with a decrease and imbalance of micronutrients in the blood. Micronutrient deficiencies have been documented at different stages of the HIV infection (Tang, Graham et al. 1997; Fawzi 2003; Tang, Lanzillotti et al. 2005). Deficiencies of several micronutrients have been shown to be associated with accelerated disease progression, increased mother-to-child transmission, increased genital shedding of HIV and increased mortality (De Pee and Semba 2010). One proposed link between nutrition and infection is the antioxidant status (Schwarz 1996). Increased consumption of fruits and vegetables over three months exerted beneficial effects on the systemic redox balance and immune activation parameters such as $CD38^{+}/CD8^{+}$-cells in HIV-infected persons (Greenspan 1994; Gil, Martınez et al. 2005).

Moreover, studies in the United States have shown that woman and minority groups tend to a lower dietary intake than advisable according to the Recommended Dietary Allowance (RDA). This especially affects some vital nutrients such as vitamin A, vitamin C, vitamin E, vitamin B6, iron, and zinc (Woods, Spiegelman et al. 2002). The RDA is the level of intake considered adequate to meet all nutrient needs for nearly all (95%) healthy persons (Board 2000). Furthermore, there is some evidence that micronutrient intakes at the RDA level may be insufficient for HIV-infected individuals since low micronutrient statuses have been reported in HIV-infected adults (Baum, Shor-Posner et al. 1992). The international recommendation is an increase of dietary energy intake of 10% for asymptomatic and 20 to 30% for symptomatic HIV-infected persons; micronutrients should be consumed according to the RDA (WHO 2003).

The direct relation between nutrition, metabolic complications, and HIV infection has promoted nutrition to become a central part of care for PLWHA. The place of nutrition in the HIV challenge has been recently redefined by the *American Dietetic Association*, which advocates individualized care to optimize the nutritional status (Fields-Gardner 2010). Therapeutic nutrition is generally accomplished through nutrient supplementation in order to prevent or compensate for micronutrient deficiencies. Studies about supplementation with vitamin A, B complex, C, or E have shown effects on people living with HIV, which are presented here in table 1.1.

The importance of well-balanced nutrition in the case of infections is well documented and part of international recommendations. An major problem in HIV infection is the appearance of malnutrition (UNAIDS 2001). The infection implements a burden on the nutritional status of PLWHA. In the case of a pre-existing nutritional deficiency, as often seen in sub-Saharan Africa, people suffer from the double burden of HIV and malnutrition (WHO 2005). Moreover, the spreading of HIV in sub-Saharan Africa remains related to socio-economic and wealth access inequality, which's are also key factors in nutritional status.

Efforts have been undertaken to fight emergency malnutrition with a focus on HIV as shown by the international agencies UNAIDS and WFP cooperating in the Food and Nutrition Technical Assistance project "FANTA-2"(WFP 2007; WFP 2011).

Vegetable products present a diverse and equilibrated composition of nutrients. Besides their nutritional value, herbal products also contain bioactive compounds such as phenol and diverse antioxidants. These natural antioxidants are expected to have a better bioavailability and a greater protective efficiency than synthetic nutrient products (Greenspan 1994; Preziosi, Galan et al. 1998). The consumption of fruits and vegetables has also been related to the microbiota of the gut (Greenspan 1994; Gil, Martınez et al. 2005; Serrano, Goñi et al. 2007; Schmidt, Ribnicky et al. 2008).

TABLE 1.1 ESSENTIAL NUTRIENT EFFECTS ON PEOPLE LIVING WITH HIV

Nutrients	Effect	Reference
Retinol and carotenoids (vitamin A)	-Maintenance of epithelial integrity -Enhanced monocyte differentiation and function -Improved neutrophil function -Increase in C-reactive protein during infection -Increase in T cell counts **Transmission and pregnancy outcomes** -Low serum vitamin A increases risk of mother-to-child transmission of HIV. -Low maternal vitamin A increases risk of low birth weight. -High intake of vitamin A increases vaginal and breast milk viral shedding.	(Coutsoudis, Kiepiela et al. 1992) (Mehta and Fawzi 2007) (Semba, Caiaffa et al. 1995) (Ross 2012) (Burns, FitzGerald et al. 1999) (Fawzi, Msamanga et al. 2004) (Villamor, Koulinska et al. 2010)
Bvitamins	-Use of B complex vitamins was associated with reduced progression to AIDS in South African adults. -High intake of vitamin B6 was associated with improved survival. -Low serum vitamin B12 associated with more rapid progression of HIV disease in homosexual men.	(Tang, Graham et al. 1996) (Kanter, Spencer et al. 1999) (Tang, Graham et al. 1997)
Tocopherol (vitamin E)	-Improved neutrophil phagocytosis -Lymphocyte proliferation -Increased IL-2 production -Increased natural killer cell cytotoxicity -Reduced production of inflammatory cytokines such as TNF, IL-6 -Higher serum vitamin E is associated with a one-third lower risk of progression to AIDS in HIV-infected homosexual men. -Supplementation in association with HAART is more effective in reducing viral load.	(Beharka, Redican et al. 1997) (Wang and Watson 1994) (Pekmezci 2011) (Spada, Treitinger et al. 2002) (de Souza Junior, Treitinger et al. 2005) (Tang, Graham et al. 1997)
Ascorbic acid (Vitamin C)	-Improved T and B lymphocyte proliferative responses -Reduced concentration of pro-inflammatory cytokines, including IL-6 -High dose can reduce steady-state indinavir plasma concentrations	(Hemila 1997) (Slain, Amsden et al. 2005)

The use of herbal medicine is traditionally deep-rooted in Africa. The coverage of African traditional medicine is widespread, and patients often mix traditional and Western medicine (Peltzer and Mngqundaniso 2008). The application of local herbal products can be a part of medical treatment, especially in the context of low-income countries (Atawodi 2005; Nikiéma, Djierro et al. 2009; Nkengfack, Torimiro et al. 2012). Herbal products have some advantage over synthetic products because of their diverse composition, their local production, and their common acceptance (Schmidt et al. 2008). UNAIDS has released

guidelines to formalise a collaboration between modern medicine and traditional healers in the Sub-Saharan African context (King 2006). For example, African plants have been investigated in the context of a new cancer treatment (Sawadogo, Schumacher et al. 2012). Various plants used in the care of PLWHA have been described. The implementation of traditional plants, for example the "African potato" (*Hypoxishemero callidea*) and an l-canavanine extract from *Sutherlandia frutescens* during the infection resulted in better absorption of nevirapine (Brown, Heyneke et al. 2008). The consumption of plant food also results in the intake of phytosterols, which have beneficial effects in some diseases and especially influence the immune response (Bouic and Lamprecht 1999). One major class of biologically active compounds are the polyphenols, which exert anti-inflammatory and immune-modulatory function (Cuevas, Saavedra et al. 2013). Plants used in the healthcare of PLWHA are presented in table 1.2.

TABLE 1.2: PLANTS AND THEIR EFFECTS ON HIV PATIENTS

PLANT	Effect	Reference
Garlic	-Reduces levels of saquinavir (protease inhibitor medication)	(Sussman 2002)
Aloe Vera	-Lowers blood glucose in diabetics -Reduces blood lipid in hyperlipidaemia -Might be effective against herpes and psoriasis -Might promote wound healing	(Vogler and Ernst 1999)
Curcumin	-Anti-tumour, anti-inflammatory, and anti-infectious activities -Antiviral activity, HIV-1 integrase inhibition	(Mazumder, Raghavan et al. 1995); (Sharma, Gescher et al. 2005); (Hatcher, Planalp et al. 2008)
Ginseng	-Slows CD4 T cell depletion -Antifungal, antiviral effect of ginseng protein	(Sung, Kang et al. 2005) (Ng and Wang 2001)
Tea Tree	-Effective against fuconasolzole-resistant oropharyngeal candidiasis -Antimicrobial	(Vazquez and Zawawi 2002) (Carson, Hammer et al. 2006)
Hysope	-Antiviral HIV activity of polysaccharide	(Gollapudi, Sharma et al. 1995)
St. John's Wort	-Treatment of depression -Lowers nevirapine in the blood	(Linde, Berner et al. 2005) (Piscitelli, Burstein et al. 2000)
Neen	-Anti-retroviral effect of leaf extract	(Udeinya, Mbah et al. 2004)
Propolis	-Anti-candidose	(Martins, Pereira et al. 2002)
Sutherlandia frutescens	-Antibacterial and antioxidant	(Katerere and Eloff 2005)

The WHO long ago recognized the need for research and regulation of herbal therapies (WHO 1989). Scientific efficiency and safety studies are vital for the broader implementation of herbal products. The registration of adverse events related to the consumption of herbal products improves the way these products are used and thereby improves patient safety. In

order to achieve both, the WHO started an international drug monitoring program on 'pharmacovigilance', in which observations under herbal medicines can be reported (UMC 2011).

- **Spirulina**

The current study focused on one old natural supplementation candidate: *Arthrospira,* two major species of which are consumed by humans; *A.maxima* and *A. platensis* (*Asp*). A common designation is 'spirulina'. *Asp* is a photosynthesizing cyanobacterium (originally part of blue-green algae) that grows under bright sunshine, high temperatures, and alkaline soil conditions. Originating from the regions around Lake Chad (Chad-Cameroon-Niger) and Lake Texcoco (Mexico), *Asp* is consumed by the local *Kanembou* population in Chad and was considered a high-quality food by the Aztecs in former times (Farrar 1966; FAO 2008). Owing to the absence of a cellulose membrane, spirulina presents its nutrients with a high bioavailability and is rich in high-quality proteins, lipids, vitamins, minerals, and biologically active substances(Kulshreshtha, Zacharia et al. 2008). An early interest in spirulina was based on the iconic 'single cell proteins' (Clement, Giddey et al. 1967). This interest increased, and research on its production was conducted in the 1970s. Since the expected protein gap in human nutrition lost the attention of researchers, *Asp* was also relegated to the background. At the same time, the *National Aeronautics and Space Administration* selected A*rthrospira* as a diet for its long-term space travel program, CELSS (Mahasin 1988).

Arthrospira has diverse biological activities and effects on nutritional outcomes. Clinical observations among undernourished children in Burkina Faso (Simpore, Zongo et al. 2005) and among elderly people in Korea (Park, Lee et al. 2008; Selmi, Leung et al. 2011) presented spirulina as a possible food supplement to increase the dietary quality of populations in need. Recent reviews have drawn attention to *Asp*'s therapeutic effects, such as reducing blood cholesterol, enhancing antioxidative capacity, and strengthening the immune system (Deng and Chow 2010; Ravi, De; et al. 2010).

Several investigations with *Asp* have been conducted on PLWHA in Africa. The first was studying malnourished HIV-infected children in Burkina Faso. Its showed a higher rate of nutritional recovery as well as an improvement in immunological variables in children receiving *Arthrospira* (Simpore, Pignatelli et al. 2007). In Bangi, data of a controlled study on out patients suggested that *Arthrospira* could be a strong candidate for a nutritional

supplement. However, this study could not prove any effect of *Arthrospira* on the course of the infection (Yamani, Kaba-Mebri et al. 2009). Critics wrote about this study: “they did not test body composition nor viral load and failed to demonstrate any benefits of direct improvement on immune response due to their limited experimental protocol” (Azabji, Dikosso et al. 2011). A more recent randomized single blind nutritional supplementation study conducted in Cameroon on undernourished patients starting HAART reported a significantly higher improvement in CD4 cells under *Arthrospira* supplementation in contrast to soy beans (Azabji, Ekali et al. 2011).

1.4 **Context**

The present intervention focuses on Cameroon with an HIV seroprevalence of 5.3%. It is the Central and West African country with the highest rate of HIV (UNAIDS 2010). This country is called “little Africa” and is known for its great diversity in climate, geography, religion, and populations consisting of 230 ethnic groups. This heterogeneity also impacts the regional HIV seroprevalence with 1% in the northand 10.6% in the south. The same report shows a difference in the HIV prevalence between the two megacities, Douala and Yaoundé, which are the home of 20% of the total population of the country: Douala has a HIV prevalence of 4.6% and Yaoundé one of 6.4%. The capital city also registers a gender difference of 8.8% for women and 3.9% for men (National institute of Statistic 2011). This gender difference can be found throughout the continent: HIV prevalence in women in Africa stands at 58% of all cases (UNAIDS 2012).

Since 2007 and free access to HAART medication in Cameroon, the number of patients under HAART has increased exponentially, reaching one of the highest rates in the sub-region. It reached 58% of the HIV-infected population eligible to receive HAART in June 2008 (Loubiere, Boyer et al. 2009). The national number of patients treated was 17,156 at the end of 2005 and peaked at 89,455 at the end of 2010 representing a therapeutic coverage of 38% of infected people (CNLS 2011).

1.5 Study Objectives

The current study focused on spirulina as a functional food that strengthens the immune system of patients infected with HIV. It also sought to answer the question of whether spirulina could improve the nutritional status of patients not yet under antiretroviral therapy.

- The main objective of the RCT is to evaluate *Arthrospira p.* by measuring the change in CD4 lymphocyte count compared to a placebo from baseline for up to three months.
- The second objective is to document the effect of *Arthrospira p*. on the following nutritional status markers: albumin, iron anaemia, and body weight.
- HIV infection is known for its deleterious effects on infected people. Therefore, the study will explore the course of three disease outcomes of an HIV infection at three different time points, i.e. at baseline, three months and six months. The three outcomes include the immune status, the CD8/CD38 cell ratio, and the frequency of opportunistic infections.
- The total antioxidant status of the *Asp* group will be compared to that of the placebo group.

2 Methods

2.1 Study Design

The study was an experimental, prospective, and longitudinal intervention performed as a three-month randomized double-blind and placebo-controlled intervention (Intervention I), followed by a three-month open-label extension period (Intervention II), see Figure2.1.

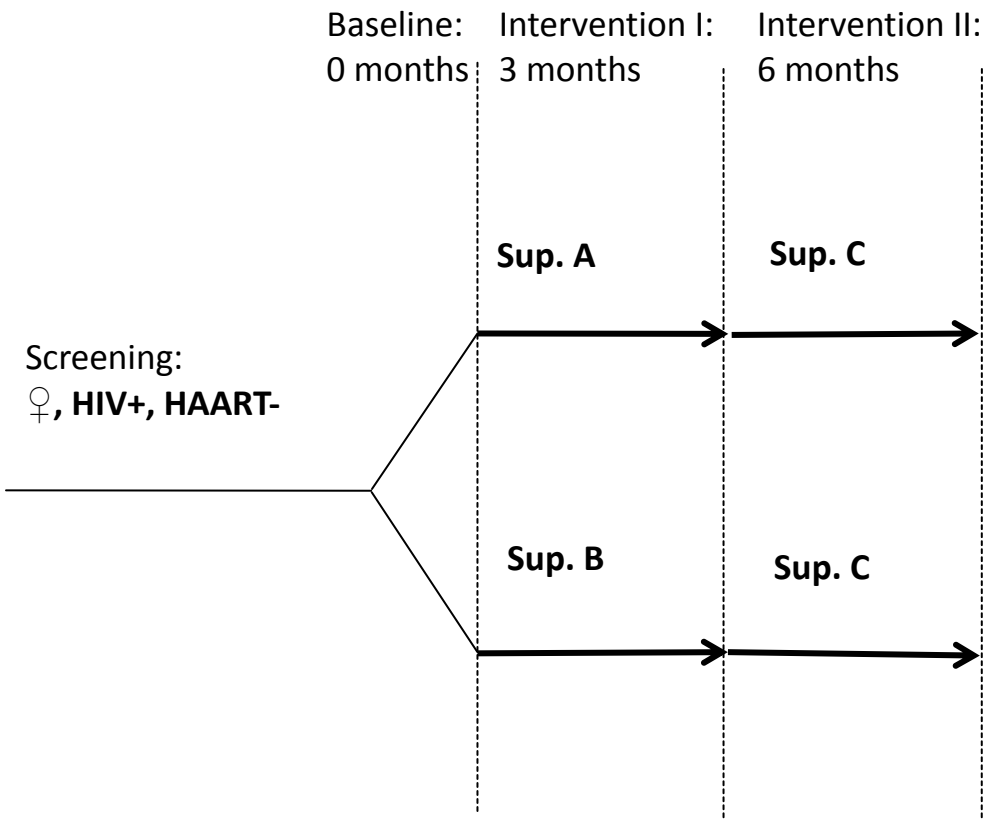

FIG. 2.1 FLOW CHART OF THE STUDY

Supplements A and B were used during the randomized control on HAART-naïve patients. Supplementation C was exclusively composed of *Asp* and was used for all patients after the RCT was completed. The figure represents the four interventions with RCT composed of Sup A and B for three months, the long-term intervention, and crossover composed of the two intervention arms from baseline to six months.

In order to answer the research question, the RCT was carried out on pre-HAART patients. After screening, the three-month RCT with pre-HAART patients started. Each participant was randomly allocated either to group A or group B (see §2.4).

The placebo for the control group contained an equal amount of protein and energy as the intervention product *Asp* (see §2.6). After three months, the randomized study ended. During the prolongation in the open label period, only *Asp* was distributed to the patients involved in the RCT in two different six-month intervention profiles; the 'crossover' originated from the placebo group, and the 'long-term' arose from the *Asp* exposure group. In order to continue the study in the open label period, the pre-HAART patients had to stay HAART naïve; this meant resuming with a CD4 greater than or equal to 350 cells/mm^3. In case of a lower count, the participant was immediately excluded from the study and referred to a physician in order to start with HAART. During the prolongation period the former randomized distribution between the RCT groups was not continued.

In addition to biomedical monitoring and the supplement bags, all participants were reimbursed for their travel expenses to the study site. The patients were asked to come and pickup their supplement bags every four weeks. The bags contained 30 daily blisters with ten pills each, 30 condoms, a pen, and one information leaflet (see appendix). The latter contained intervention information and a table on which the patient was asked to write down the number of pills taken per day and any concomitant events emerging on that day. The table helped the patient write down remarks and helped control compliance. Condoms were distributed in agreement with the patients and in accordance to the inclusion criteria to avoid pregnant participants.

- **Sample Size Calculation**

CD4 cell count is the primary outcome of this study. The sample size calculation was based on the assumption of patients (P) with a CD4 count increase of more than 100 cells/mm^3after three months compared to the baseline. The benchmark of 100 CD4 cells/mm^3 was based on previous field studies in Cameroon (Bourgeois, Laurent et al. 2005).

Null hypothesis: P_1 (intervention group) = P_2 (control group).

Sample size tables were used to estimate the value of the sample size.

These were our parameters:

Ratio intervention group/control group=1; precision = 0.05; Z_α= 1.645

At a power of 93%, **35** subjects per group (*Asp* and Placebo) were needed.

2.2 Ethical Considerations

The intervention was conducted according to the World Medical Association Declaration of Helsinki (WMA 2008). The research proposal was reviewed and approved by two independent ethical committees: the ethical clearance committee of the Faculty of Medicine of Justus Liebig University, Giessen, Germany, and the National Ethics Committee of Cameroon (n°131/CNE/SE/2010 on 16 July 2010 in Yaoundé).

- **Informed Consent**

After a positive screening based on age, proof of HIV infection, BMI, and existing contact data, the study information was read to the patient in one of the two official languages of Cameroon: French or English. In addition to a discussion during the reading, the intervention was set into a general context (Corneli, Sorenson et al. 2011). A hardcopy of the information was distributed to the patient, and a sufficient amount of time for possible feedback was offered. Patients who could not write or had problems understanding were always accompanied by a literate family member (brother, husband, mother, or sister). Once the patient had signed the informed consent statement, a screening with data collection on the medical history and the actual CD4 cell count was performed.

All original data and informed consent statements are kept under strict closure at the Institute of Nutrition at the University of Giessen, Germany. A digital copy is in the possession of the principal investigator in Cameroon.

- **Trial Funding**

The trial was mainly supported by an unrestricted grant from greenValley GmbH, Berlin Germany.

Furthermore, OCEAC/PPSAC contributed as a founding and partner agency for Central Africa. The study was presented at the OCEAC/PPSAC meeting in N´Djamena in June 2010 to share data on the effect of spirulina on HIV-infected patients.

- **Institutional Review Board**

The project was carried out under the combined supervision of **Prof Dr med. Michael B. Krawinkel** (Institute of Nutritional Sciences – International Nutrition, University of Giessen,

Germany) and **Dr med. Marcel Azabji** (Department of Physiological Sciences, Faculty of Medicine and Biomedical Sciences, University of Yaoundé, Cameroon).

In Cameroon, Dr Azabji was the principal investigator who supervised all steps of the project. Clinical data were managed by **Ndazem Djenabou**, a medical student from the team of Dr Azabjiat the Faculty of Medicine of Yaoundé. **Francois Emakam** and **Frank Winter** carried out the study's field work and collected the patient and laboratory data. Both belonged to the working group on International Nutrition in Giessen. The following institutions were involved in the project:

The 'Hôpital du Jour', the health unit for HIV/AIDS patients from the Central Hospital of Yaoundé. The current director of the unit is **Dr Charles Kouanfack**. The analysis of the clinical and biochemical markers was carried out at the Centre Pasteur Cameroun Laboratory of Virology by **Frank Winter** under supervision of **Dr Jude Kfutwah**.

Statistical analysis was guided and supervised by **Dr Rolf-Hasso Bödeker** at the Institute of Medical Statistics, University of Giessen, and **Dr Johannes Herrmann** at the Institute of Nutritional Sciences, University of Giessen.

2.3 Eligibility & Enrolment Criteria

The study population was limited to voluntarily participating individuals. Only patients registered in the study health centres could be contacted and invited to participate. Contact was established via telephone for the pre-HAART patients. Recruitment for the randomized pilot study was achieved by searching through patient files, focusing on the years 2008 to 2010. The inclusion criteria were applied during register research and at the screening.

All patient files were screened for the following criteria: female, not under HAART, age between 18 to 49, and availability of a phone number. Potential patients were contacted by phone and invited to the office for a pre-screening consultation at the HDJ. All participants meeting the inclusion criteria during the enrolment period participated voluntarily. Participants were then recruited via convenience sampling. At the screening, the medical staff asked for informed consent as noted in § 2.2. Before the patient signed the consent form the study physician had to exclude any other chronic disease or any existing or planned pregnancy and make sure that the participant understood the upcoming study well. After signing the informed consent, the screening checklist was filled in (see appendix). The HIV infection, pregnancy, BMI measurement, CD4 T cell count, and any medication taken were

verified. First, body weight and height were measured with subsequent calculation of BMI. If BMI was under 26 kg/m², the patient was invited to perform a CD4cell measurement. For inclusion at this stage, the count had to be higher than 350 cells/mm^3.

Inclusion criteria:

female, confirmed HIV infection, age 18 to 49 years, BMI below 26 kg/m², known medical history, and CD4 T cell count between 350 cells/mm^3 and 600 cells/mm^3.

Exclusion criteria were as follows: male, under any antiretroviral therapy, pregnant or planned pregnancy, breastfeeding, initiation of an antioxidant vitamin therapy, any opportunistic infection requiring intensive medical care, intractable diarrhoea (at least six liquid stools daily).

2.4 Randomization

Each study patient received an ID number composed of four digits and two letters. The first two numbers were based on the recruitment chronology; the last two numbers related to the year of birth, followed by the initials of first and family name.

For randomization of the pre-HAART patients, all ID numbers were entered into a MS Excel®-file in chronological order. Two equal population groups, A and B, were defined and put in front of a generated random number through the Excel® formula "=rand()". The decrease ordering of the random numbers resulted in the randomization list.

2.5 Blinding

The blinding was achieved by identical packaging of both supplemented products provided by greenValley GmbH, Berlin, Germany. The capsules were dark green, opaque, with no visible difference between the placebo and the *Asp* supplement. The patients and the medical personnel directly involved were blind about the products delivered to them. Pills were labelled A or B and distributed in blister packages to the participants. The blisters were labelled with 'A' or 'B'. For the open-labelled phase, pills were labelled with 'C'.

All supplements were kept in a dry dark room near the hospital at room temperature. Every morning, the bags were taken to the office where the distribution and data collection were conducted. During the intervention no breaking of the blinding occurred.

2.6 Intervention & Placebo Product

A five-gram supplement per day distributed in 10 pills containing 500 mg each is the dose considered adequate for study purposes. The pills were opaque green. The investigational product was 100% *Arthrospira platensis* powder provided by EARTHRISE® from California, USA. This producer applies ecological farming methods, highly automated harvesting, and respects strict hygiene regulations (ISO 9001:2008). The applied drying method through spraying *Asp* in a heat chamber guarantees the best possible preservation of all heat-sensitive nutrients, pigments, and enzymes without using preservatives or stabilizers (Earthrise, 2009). The *Asp* provided for the study was standardized on iron and phycocyanin.

For the randomized control study, a placebo was manufactured by HERBAMED Co. Euskirchen, Germany. The placebo had the same amount of protein and energy as the supplied *Asp*. The protein content was measured on the basis of a quantity of 500 mg of *Asp* Lot-No.14141. The energy calculation was based on 500 mg of *Asp* powder in 100 mg HPMC caps. The placebo was a mix of 362.57 mg of pea protein isolate Pisane F9 mix with 160 mg of Dextrans EMDEX. This results in 8.9 KJ or 2.1 Kcal per pill containing 310 mg protein.

TABLE 2.1 NUTRITIONAL COMPOSITION OF THE INTERVENTION AND PLACEBO PRODUCTS

Macronutrient composition	*Arthrospira p.* (500mg per pill)	Placebo (500mg per pill)
Energetic value	8.9 kj; 2.1 kcal	8.8 kj; 2.1 kcal
Protein	310 mg	310 mg
Carbohydrate	77.5 mg	186 mg
Lipid	35.5 mg	4 mg

The supplement or placebo was swallowed in the form of pills, distributed in one blister for a daily intake of 10 pills. It was recommended to ingest the pills after a meal, preferably five pills after breakfast and five pills after lunch.

2.7 Data Collection

Observations of each patient included an assessment made at the beginning, then monthly, and at the end of each supplementation period (see table 2.2).

Before the study began the screening group data was provided from the medical register, interviews, physical exam, and if required, a CD4 cell count was ordered at the HDJ

laboratory hospital. A blood sampling for the CD4 cell count was carried out for patients who could not present a CD4 count less than three months old at the screening. The method applied in the hospital laboratory was considered for HAART entry, and the results were valid as study inclusion criteria. Furthermore, diurnal variations are known to be found in CD4 counts, sometimes increasing during the day (Malone, Simms et al. 1990). A special focus was to set the sampling at similar times. The goal was to mitigate the diurnal cycle variation. Obtaining blood samples from fasting subjects would have been more appropriate for achieving higher reproducibility of the results. However, due to ethical reasons, the project decided to spare the participants the need to show up on an empty stomach.

During the intervention, at baseline, and after three and six months, anthropometric measurements and blood samples were taken. The monthly visit focused on observations, safety, distribution, and ingestion of supplements.

TABLE 2.2 ASSESSMENT TIMETABLE

	Screening	Baseline	RCT			Open-labelled		
Study month	-1	0	1	2	3	4	5	6
Informed consent	✓	✓						
Medical history	✓	✓	✓	✓	✓	✓	✓	✓
Physical exams	✓	✓	✓	✓	✓	✓	✓	✓
Effectiveness:								
Blood variable		✓	✓			✓		
Body weight		✓	✓	✓	✓	✓	✓	✓
24h recall		✓	✓	✓	✓	✓	✓	✓
QoL		✓	✓			✓		
Safety:								
Adverse events		✓	✓	✓	✓	✓	✓	✓

2.8 Measurement Methods

Outcome variables measured in the current study were anthropometric markers and blood variables of two groups: immuno-virological and biochemical markers.

- **Immuno-Virological Variables**

HIV infection is defined by the strong predictors of disease progression and outcome. The gold standard used as an inclusion criterion for the initiation of HAART is the CD4 cell count. Furthermore, the CD38 expressions on lymphocyte cells and the viral load were measured. The flow cytometer and viral load measurements were performed at the laboratories of virology of the CPC, under the supervision of Dr Kfutwah and conducted by Frank Winter, respecting good laboratory practice.

The FACS count from Becton Dickinson is the standard method in HIV monitoring of the CD4 cell count. The CPC is the reference laboratory in Cameroon. The samples were analysed according to protocol with the use of an electronically calibrated pipette of 50 µl from Beckman. The calibrated range is CD4: 1 to 2,000 cells/µl; CD8: 1 to 2,000 cells/µl; CD3: 1 to 3,500 cells/µl. The quality controls of sensitivity and precision were achieved by the standard IMMUNO-TROL and IMMUNO-TROL Low. The sensitivity is +/- 61 cells/mm^3 for CD3 cells for the low level, of +/- 36 cells/mm^3 for the CD4-cells, and +/- 21 cells/$mm^3$for CD8 cell.

CD38 expression is performed to describe the effect of an intervention or medication on immune activation that relates to infection evolution(Steel, John et al. 2008).Measurements of CD38 antigen expression were obtained with COULTER EPICS XL/XL-MCL®. The following antibody combination was used: PE-CD3 and PE/Cy5-CD4 from Biolegend®, Flurescein Iso Thio Cyanate, and FITC-CD38 from eBioscience®. The results were read with the CXP® software connected to the COULTER EPICS XL/XL-MCL®. This showed the result of the lymphocyte population in total blood. The results were expressed as %CD8, CD38 of CD8 lymphocytes (%CD38-CD8), and as %CD4, CD38 of CD4 lymphocytes (%CD38-CD4).

The **viral load** is a direct count of viral particles via one-step reverse transcriptase PCR in plasma. For the current study, plasma was stored at -80°C until the end of the intervention. To avoid methodical fluctuation, samples were handled chronologically for each patient during the same run. Viral RNA was extracted from the plasma using the QIAamp® Viral RNA

Mini extraction kit for medical diagnoses. The principle was a lysation to RNase's free condition. Then the RNA was isolated with an RNA carrier and purified with a silica gel-based membrane with selective RNA-binding properties. The purified RNA was free of protein, nucleases, and other contaminants or inhibitors. 500 µl of clear plasma was concentrated through a spin of 17,000 g for 30 min. 360 µl of supernatant was removed before adding the lysis buffer and transferring it into the column. The elution was in 60 µl QIAamp® buffer before storing the stable RNA at -20°C. The load-extracted RNA amount was 10 µl per qPCR reaction. The PCR was achieved during the 24 hours following the extraction by using a medical diagnosis kit from Biocentric, Generic HIV charge Viral®. The diagnosis kit was developed by the *Agence National de Recherche sur le SIDA* (ANRS) for a quantification of HIV-1. The absolute quantification was done with a standard curve of 5 points with a 1log dilution, implementing a one-step retro-transcription PCR master mix. The measurements were done on a 7500 Real-Time PCR System from Applied Biosystems©. The results were calculated in MS-EXCEL® and expressed in viral Log10 and copies/ml, regarding the validated diagnostic protocol of the Centre Pasteur. Non-detectable viral load at baseline, a virological serotyping, was performed with an ELISA test to determine the HIV type.

HIV serotyping is done through ELISA. The ELISA test is usually performed for patients not responding to HIV-1 quantification in order to confirm infection with the HIV virus by identifying an HIV-2 type. The ELISA test was developed by the clinical biology unit of CHU Rouen. The technique is based on the variation of two glycoprotein epitopes of the virus envelope, the region gp41/IDR (transmembrane) and gp120-V3 (external loop). These two epitopes allow one to discriminate type HIV-1 and HIV-2 and would also determine HIV-1 subtypes (M,N,O,P). For the current study, ELISA was performed by the trained personnel of the Centre Pasteur on a serum volume of 10µl.

- **Biochemical Outcomes**

C-reactive protein is the most accurate marker for the acute immune response phase. It is used to screen for infections and inflammatory injury/diseases. C-reactive protein was measured via a chemiluminescence-immunometric assay on an IMMULITE 2000®. The required volume was 5 µl serum. The results were available within four hours.

Total Antioxidant Capacity: The anticoagulant blood tubes were centrifuged and aliquoted. 300 µl serum were frozen at -80°C. To avoid handling and methodological fluctuation, samples were analysed chronologically per patient in duplicate in the same run. The mean of the two measures was taken as the value. Samples for each patient were put side by side from time 0, time 3, and time 6 months (if available) for meltdown. After the quantity needed had been sampled, the serum was conserved at -80°C as a serum library at the Centre Pasteur Cameroon for one year. The total antioxidant status (TAOS) was quantified by using the research ABTS assay kit CS790 from Sigma® for 96-wellplates. ABTS (2, 2'-azino-di-(3-ethylbenzthiazoline sulfonate) was incubated five minutes with metmyoglobin and hydrogen peroxide to produce the radical cation ABTS•+. It has a stable blue colour. Antioxidants added to the sample suppress colour production according to their concentration. The absorption was correlated with the trolox standard curve and expressed µM relative to the trolox standard. The measurement range was 45 –420 µM. Samples were diluted if needed by a factor of ½ with assay buffer. The sensitivity of the test was measured by the variation on the 5 trolox standard curve. The sensitivity is +/- 8µM. The serum reaction input was 10 µl of serum. The endpoint absorption was read at 405 nm on the micro plate reader, Dynex Technology® MRX Version 2.02, USA.

Further biochemical variables were measured in the biochemistry laboratory of the CPC on the sampling day from the same serum tube. The following analyses were performed with the biochemical analysis system Vitros 250® using the following methods:

Albumin is a un-glycosylated plasma protein playing a major role as a carrier and by maintaining osmotic pressure. The concentration was determined directly by building the albumin-bromocresol green complex. The measurement was achieved via spectrophotometry at 630 nm. The sample volume was 5.5 µl of serum. The method range was 10–60 g/l with an accuracy of 0.9 g/l.

Serum **iron** measures the transferrin-bound and free-circulating iron in the blood. About 30% of the iron in the body is bound to transferrin protein, and a small amount is solubilised in the serum. Iron concentration was determined by liberating iron from transferrin under acidic pH. The measurement was read at 600 nm. The sample volume was 10 µl of serum. The method range was 0.5– 14 mg/l with an accuracy of 0.5 mg/l.

Cholesterol is an important component in the hormonal systems of the body for the synthesis of bile, steroid hormones, and vitamin D. It plays a role in membrane fluidity. It can be synthesized by the body or comes from animal foods. Cholesterol concentration was determined by enzymatic catalysed hydrolysis of cholesterol through a final oxidation of a colorant. The measurement was analysed to be 540 nm. The sample volume was 10 µl of serum. The method range was 0.5– 3.25 g/l with an accuracy of 0.027 g/l.

Plasmatic **triglyceride** concentration is related to the balance of nutritional input and storage of the lipid in the body. The concentration was determined via enzymatic catalysed hydrolysis of triglyceride through a final oxidation of a colorant. The measurement was read at 540 nm. The sample volume was 10 µl of serum. The method range was 0.11– 5.93 g/l with an accuracy of 0.036 g/l.

Urea is synthesized in the urea cycle from the oxidation of amino acids. Urea is eliminated from the body through the kidney in the urine. Urea concentration was determined via hydrolysis of urea to ammoniac, which reacts with an ammoniac indicator. The measure was achieved at 670 nm. The sample volume was 10 µl of serum. The method range was 0.02–1.2 g/l with an accuracy of 0.007 g/l.

Creatinine is a breakdown product of creatine phosphate in muscles and is usually produced at a fairly constant rate by the body (depending on muscle mass). Creatinine concentration was determined via hydrolysis of creatinine through a final oxidation of a colorant, leucoderivative. The measure was established to be 670 nm. The sample volume was 10 µl of serum. The method range is 0.5– 1.4 g/dl with an accuracy of 0.5 g/dl.

- **Blood Cell Count**

Blood cell counts were handled on EDTA blood and analysed with the Sysmex XT-1800i NFS®. This instrument performs haematology analyses according to the hydrodynamic focusing (DC detection) flow cytometry method (using a semiconductor laser). Flow cytometry, using a semiconductor laser, analyses physiological and chemical characteristics of cells. The hydrodynamic focusing method improves blood count accuracy and reproducibility. Abnormal numbers of blood cells were observed under the microscope to exclude haematological malignancies. No malignancies were observed or detected.

Haemoglobin is measured via extinction.

- **Anthropometric Measurements**

Weight and height of the patients were measured after the interview. This procedure was carried out by the same medical student throughout the entire study. Patients with light clothes and without shoes were asked to stand on a standing-weighing machine, SECA® with a kilogram scale to the nearest 0.1 kilograms. The researcher read the weight appearing on the display and recorded it onto the chart of the patient. Respondents were asked to stand upright and in front of a standing board with a centimetre scale. The researcher used a ruler to equate the respective height and read the scale above the patient's head. Body height was measured to the nearest 0.5 cm. The measured weight and height of the patients were used to calculate their BMI, (kg/m^2). For that purpose, the patients' heights were converted into meters.

TABLE 2.3 MARKERS MEASURED IN THE STUDY

Variables	Method	Range; Accuracy	Normal values*
CD4	FACScount from Becton Dickinson	1–2,000 cells/mm^3; +/- 36 cells/mm^3	500–1,600 cells/mm^3
Viral load	RT-PCR	>50 copy/mm^3	<50 copy/mm^3
CD8-CD38	COULTER EPICS XL/XL-MCX	0–100%; +/- 1%	< 30%
CRP	Immunometric assay on an IMMULITE 2000®	0–400 mg/l; 0.1 mg/l	<6 mg/l
TAOS	ABTS ELISA	15–1,000 µM; +/- 8 µM	~1,000 µM
Albumin	Colorimetric measure with bromocresol green	10–60 g/l ; +/- 0.9 g/l	35–50 g/l
Haemoglobin	Photometric measure	0.0–30.0 g/dl; +/- 0.1 g/dl	11.1–16.8 g/dl
Erythrocyte	Discriminator	0.00–99.99 Tera/l; +/- 0.02 Tera/l	3.5–5.5 Tera/l
Iron	2 points enzymatic measure	0.5–14 mg/l ; +/- 0.5 mg/l	0.5–1.5 mg/l
Cholesterol	Colorimetric measure	0.5–3.25 g/l ; +/- 0.027 g/l	1.8–2.8 g/l
Triglyceride	Colorimetric measure	0.11–5.93 g/l ; +/- 0.036 g/l	< 1.5 g/l
Creatinine	2 points enzymatic measure	0.05–1.4 g/dl ; +/- 0.05 g/dl	0.5 g/dl- 1.4 g/dl
Urea	Colorimetric measure	0.02–1.2 g/l ; +/- 0.007 g/l	0.1–0.5 g/l

* Reference value from the CPC, Yaoundé, 2010

2.9 Concomitant Events & Therapy

During the monthly monitoring appointments, the patient was asked if any change had occurred during the past four intervention weeks. The discussion referred to a flyer filled out by the patient. The flyer mentioned the number of pills, concomitant events, and severity per day. The concomitant events were scaled as not at all, a little, as usual, a lot, and enormous. The proposed concomitant events were as follows: appetite, fatigue, nausea/vomiting, cough (divided into dry and spit), abdominal pain, and dejection (divided into diarrhoea and constipation). The physician filled the clinical register where the occurred concomitant events were registered and, if possible, diagnosed; the start and end dates were mentioned, and the action undertaken (view appendix).

The involvement in the intervention did not impose any medical restriction except for the intake of antiretroviral medication. A therapy affecting the main outcome could be the intake of steroids, cortisone, or dexamethasone. Immunosuppressive medications are not part of the standard therapy for people with an immunodeficiency syndrome. It is prescribed, for example, in cases of heavy malaria outbreak. Dexamethasone is usually taken at the beginning of the hospitalization to minimize the risk of encephalitis and to protect the blood-brain barrier from an acute inflammation that would lead to the parasite appearing in the brain (van de Beek, Farrar et al. 2010). Any patient taking this therapy was excluded, and it was mentioned as a co-medication. Anyone hospitalized due to an accident or tuberculosis was also noted as excluded for co-medication. The patients were also excluded from intervention in cases of pregnancy or the start of HAART. Other study dropouts were caused by the patient herself due to consensual withdrawal or travelling.

2.10 Compliance

A follow-up of the intervention compliance was implemented through daily self-reporting on the flyer by the patient. The patient was asked to document her daily intake of pills; general condition on that day; appetite; tiredness; events such as headaches, abdominal pains, and vomiting; and the dejection texture. There were 28 days between the meetings, and 30 daily portions were distributed. A compliance break was established by a delay of more than two days to the monthly monitoring meeting without reason. At each meeting, the patient and

the personnel in charge discussed the flyer. The number of pills was checked by counting the intake mentioned on the flyer and cross-checked by asking how many pills were left.

A 24/7 hotline ensured prompt management of complaints. In the case of severe complaints, the patient was invited to the office to see the medical staff. All information and measures were meant to build a trusting relationship between the medical personnel and the patient. No written protocols were made during the personal discussions or phone calls. Only medical data were summarized in the patient file, and the patient flyers were filed.

Furthermore, no blood sampling was done during an acute malaria crisis. In such a case, the patient was asked to come back one week later. Additional pills were given to the patient to cover the time gap.

2.11 Quality Control

The intervention was conducted by three persons, each of them having specific responsibilities. Francois Emakam coordinated the patient meetings, designed the questionnaires, and conducted the 24-hour recalls. He was the only person with access to the participant list with their names and phone numbers. Furthermore, he managed the hotline during the intervention. Dr Djenabou conducted all anthropometric measurements, filled in the medical history file on the basis of the hospital data, and conducted conversations with the patients. She handed out the supplement and the results of the blood analysis. She was in direct contact with the principal investigator, Dr Azabji, who was responsible for good clinical practice. The blood sampling was taken by a nurse of the HDJ under the supervision of Frank Winter. He was responsible for the analyses of the blood samples as well as the logistic part of the intervention.

The HDJ is 100 meters away from the CPC. The HCY and the CPC are in the so called ‘île de la santé’, or ‘health island’ in Yaoundé. In there the best possible electricity and water supplies were guaranteed.

The collected data complied with high-quality standards. The anthropometric measurements were performed with strict regard to the initial description by the same person throughout the entire intervention. The materials were used exclusively for this study and were stored as described. All laboratory analyses were done at the Centre Pasteur Cameroon in a controlled environment in accordance with good laboratory practice. Samples were analysed immediately after the laboratory had received them. If an automaton was in maintenance or

any reactive was missing, the blood sampling was not performed and was delayed until all tests could be processed. Tests results were collected a day later and controlled on likelihood. The laboratory results were printed in two copies, one for the study file and one that was handed out with an explanation to the patient by the clinic personnel. The patients were identified by their intervention ID number. This way the laboratory data could only be linked to the patient by the clinic personnel in charge. The data was managed by the same personnel from the beginning until the end of intervention. Data input was done in duplicate by an external study secretary using the software Epidata®. The database generated by Epidata® was controlled on plausibility by the statistician in charge of the study. No analyses were performed before the database was closed.

2.12 Data Analysis

The analysis of the collected data was done after the database had been checked and closed by the working group for medical statistics of the Department of Medical Information Technology in the Medical Faculty of Giessen. The double data entries were performed with Epidata® software. The two generated databases were compared. Discrepancies were corrected. The complete database was then transferred to SPSS® for analysis.

- **Efficacy Outcomes**

The main objective was to determine whether a three-month supplementation of five grams of *Arthrospira p.* is superior compared to a placebo with regard to the infection marker $CD4^+$lymphocyte and the viral load count. Furthermore, the blood immune activation, inflammatory, and nutritional markers of the two RCT groups were compared. The immune activation outcome was based on the CD38-CD8 cells. Inflammation was defined by the C-reactive protein concentration level. The ratio of concomitant events occurring over the intervention was documented. Outcomes related to nutrition were body weight, albumin, TAOS, anaemia status, iron, lipid status, and the elimination status. The outcome changes were interpreted through cut points as follows:

For the immuno-virology aspect, a stabilization or increase in **CD4 cells** and a lowering of the viral load combined with a CD4 cell increase can be regarded as a positive effect. Patients at these stages show a loss of CD4 cells bound to a high viral load (Mellors, Munoz et al. 1997; Lima, Fink et al. 2009). The endpoint is a decline in the effect of antiretroviral medication

with a gain of 100 CD4 cells/mm^3 in three months and a 1 log lowering of the viral load during the same time (Bourgeois, Laurent et al. 2005).

→ Supplementation of *Asp* results in an increase of CD4 cell count by 100 cells/mm^3

→ Supplementation of *Asp* results in a lowering of 1 log of the viral load

The immune system reacts to pathogensviaCD38 up regulation on CD8 lymphocytes. Research shows that an increased CD38 expression is related to an increased risk of AIDS (Mocroft, Bofill et al. 1997). At this stage, stabilization or lowering can be considered beneficial for the patient (Tilling, Kinloch et al. 2002).

→ Supplementation of *Asp* results in a lowering of the immune activation, CD38-CD8 T cell

Inflammatory aspect

The inflammatory marker depends on the presence of an inflammatory process going on in the body. **C-reactive protein** can also be a marker for an aggravation of the infection during the AIDS stage (Feldman, Goldwasser et al. 2003). This marker is not linear but exponential when inflammation is present. The CRP value is normally <6 mg/l in adult patients. For a better visualization, the inflammation status was dichotomised into yes or no.

→ Supplementation of *Asp* results in a CRP level under 6 mg/l

Concomitant events were documented by the medical register created for each patient. The rates of concomitant events were handled per symptom frequency shown during the period. The events were categorised into headache, gastrointestinal events, diarrhoea, malaria, opportunistic infection, respiratory infection.

→ Supplementation of *Asp* results in an absence of outbreak of concomitant events

Nutrition outcomes

The **bodyweight measurement** during the study was one indicator of nutritional intake and disease progression. The pre-HAART period is often characterized by a decrease in body weight (CDC 1987). A decrease can be due to intake problems and increased energy needs during the infection.

→ Supplementation with *Asp* and placebo results in body weight stabilization or increase

The **total antioxidant capacity of the serum (TAOS)** is related to the infection by a decrease, which is related to an increase in oxidative stress (Suresh, Annam et al. 2009). The oxidative balance is disturbed by infection progress. Pre-HAART patients are characterized by low TAOS (Coaccioli, Crapa et al. 2010). An increase in the antioxidative potential is to be considered a positive outcome.

→ Supplementation of *Asp* results in an increase in TAOS

The **serum albumin level** is considered a good predictor of the severity of the HIV disease in individuals who are not taking antiretroviral therapy and can also indicate the extent of a patient's response to HIV treatment (Graham, Baeten et al. 2007). Furthermore, the value gives information about the patient's nutritional and hydration status. Albumin is the most abundant protein in blood serum, normally ranging between 35 and 50 g /dl.

→ Supplementation of *Asp* results in stabilization of plasma albumin (at normal value)

Anaemia is defined as a condition in which red blood cells and consequently their oxygen-carrying molecule haemoglobin insufficiently meet the body's physiological needs (WHO 2008). Anaemia was defined by a **haemoglobin (Hb)** value lower than 11 g/dl (WHO 2011). Normal haemoglobin values in adult women ranges between 11 and 16.9 g/dl. The supplementation hypothesis on haemoglobin concentration is the following:

→ Supplementation of *Asp* results in a stable Hb concentration

Furthermore, **red blood cell count (RBC)** is used in the current study to measure the effect of the intervention on the cell's synthesis and erythropoiesis. RBC has a major influence on Hb and anaemia. The normal erythrocyte rang value is 3.5 to 5.5 Tera/l.

→Supplementation of *Asp* results in a stable amount or increase in erythrocyte cell count

The measure of **total serum iron** is related to the free iron in the body and is bound to dietary intake. It is also related to chronic inflammation and infection (Wessling-Resnick 2010). Normal values in adult women range between 0.5 to 1.5 mg/l.

→Supplementation with *Asp* improves serum iron concentration.

Lipid profile is defined in the study by **total cholesterol (TC) and triglycerides (TG),** which are important markers in the course of HIV infection. The interaction between the two markers is a TC decrease and a TG increase, which stands for HIV-related complications (El-Sadr, Mullin et al. 2005). Further disturbances in the lipid profile are outcomes of HAART medication. The pathology of lipodistrophy is related to an HIV infection and especially to HAART. The cut-off points used in the current study to determine lipodistrophy were TC≥2 g/l and TG ≥ 1.50 g/l (NCEP 2002). A lowering or stabilization of these variables is considered a good effect of the intervention. Normal cholesterol values for adults are between 1.8– 2.8 g/l; for triglyceride, normal values are under 1.5 g/l or 1.69 mmol/l; TG values over 2 g/l are considered elevated.

→ Supplementation of *Asp* has a stabilizing effect on cholesterol and triglyceride (at normal values)

Renal function is compromised due to an HIV infection, which can lead to nephropathy (HIVAN). **Urea and creatinine** serum are used to estimate renal function through the glomerular filtration rate (eGFR). The eGFR is estimated through the modification of diet renal disease (MDRD) formula chronickidney disease epidemiology collaboration published in May 2009 (Levey, Stevens et al. 2009). The formula is adjusted for black women and accurate to the serum creatinine concentration, blood urea concentration, and albumin concentration in the blood.

eGFR= 170 x serum creatinine $^{-0.999}$ x age$^{-0.176}$ x urea$^{-0.17}$ x albumin$^{0.318}$x 0.762 (woman) x 1.18 (black)

eGFR is expressed in ml/min

Renal failure is determined by eGFR<60ml/min and serum creatinine >2 g/dl. Furthermore, the serum concentrations are documented and analysed independently from each other and put in relation to the eGFR. The serum urea measurement is a common indicator for renal function, dehydration, and protein-rich food. Normal values for adult women are between 0.1– 0.5 g/l. Measuring serum creatinine is a common indicator for renal function. A rise in blood creatinine level is observed only in cases of marked damage to functioning nephrons.

→ Supplementation of *Asp* results in no change in the urea and creatinine presence in serum and a normal eGFR

- **Statistics**

The statistic approach defined first the populations involved in the intervention. The characteristics of the populations were age, height, DDS, marital status and education level. The populations' characteristics were compared for the pre-HAART at baseline between compliant and excluded. The intervention results start with a description of the compliance, where the exclusion reasons were summarized by population and over the intervention time.

The study efficiency outcomes were analysed in **three different approaches**.

- In the RCT intervention the outcomes were compared in terms of superiority of *Asp* compared to placebo.
- The long-term intervention focused on the evolution of the outcomes over six months of *Asp* supplementation.
- The crossover intervention profile analysed the difference between the placebo and *Asp* occurring in the same population.

The efficacy outcomes were analysed inside each group and compared to the other groups for each intervention approach. The focus was on the changes occurring during the intervention. To describe the evolution of each variable, the difference between the start and the end of the intervention was calculated. Accordingly the results for one outcome in the RCT intervention are defined by three values: a baseline, the three-month point, and the difference between baseline and three months. The six-month intervention follows the intention to document a long-term exposure to *Asp*. For this, the group was only defined by the baseline, three-month, and six-month values. A further purpose of the crossover intervention was to compare the change occurring during placebo supplementation to the change occurring during the *Asp* supplementation. The group was documented by two values, change under placebo and the change occurring during the *Asp*.

Further correlations were achieved in order to describe and relate the change in initial value that occurred during the intervention.

TABLE 2.4 STATISTICAL APPROACH PER INTERVENTION

	Baseline	Three months	Difference 3 months – baseline	Six months	Difference 6 months – 3 months	Main statistical test
Pilot-RCT	X	X	X			Mann Whitney
Long-term	X	X		X		Friedman ANOVA
Crossover	X		X		X	Wilcoxon rank

X stands for a description of the value as mentioned below.

All variables of the intervention groups were summarized and analysed with the patient set that finished the intervention, called the full analyses set (FAS). Variables were described as mean (standard deviation) and/or median (interquartile range; IQR) and minimum/maximum. For qualitative variables, the absolute and relative frequencies of the categories were computed as a percentage.

For the RCT intervention, the superiority of *Asp* to the placebo was checked by testing null hypotheses: "There was no difference in the variable level for patients treated with *Arthrospira p*. compared to the placebo: $\mu_{Asp} = \mu_{Placebo}$". The superiority of *Asp* to the placebo was demonstrated if the *p* value (2 sided) was less than 0.05 (significance level), and the 0.95confidence interval lay entirely to the right of (higher than) 0. The assumption of independence was achieved for the RCT with the trial construct. All analyses performed inside each group on the same population fulfilled the assumption of data dependence.

The choice of the statistical test was made in order to have a comprehensive and common analysis over the entire study. For this purpose, the same test form was chosen for all statistical analyses in the study. As the number of patients in each group finishing the study was less than 30, the non-parametric tests are to be considered more consistent (Kitchen 2009). The Mann-Whitney U test was used (comparison of two proportions) when the assumption of independence was valid. Wilcoxon ranks were used when the variables were analysed inside the same population, and the assumption of dependence was achieved. In the case of repeated measurements, as in the case of the long-term intervention, the analyses were performed with the Friedman one-way ANOVA. If the ANOVA result was

significant, a *post hoc* analysis was conducted based on three Wilcoxon rank tests between the three sampling points: baseline – three months, three months – six months, and baseline – six months. The correction for multiple testing was applied for *post hoc* tests through Bonferoni correction. The significance level was adjusted to the number of tests as follows: *p* value multiplied by the number of tests, in the present case three. The effect magnitude of all statistical tests performed in the study was reported by the effect size r. The variable 'r' was calculated with the following formula (z/√n). R shows the importance of an effect related to the observation population.

- **Analysis Population**

  - **Statistical Population Set**

The intervention population could be divided in three statistical population sets: the safety population, the full analysis set, and the per-protocol population. They were defined as follows:

The **full analysis set (FAS)** was a subset of the safety population including all randomized patients who received at least one supplementation dose and who had at least one assessment of the primary parameter after randomization, the baseline population.

The **per-protocol (PP)** population included all patients in the FAS without any major protocol violation. Patients were analysed according to the intervention they received. Major protocol violations were an intake of less than 504 caps during the 12 weeks (168x3; 28x6 = 168 because 6 caps a day represents the minimal doses). If the patient took less, she was included in the FAS. Moreover, between the sample time 0 and the sample time 12 weeks, the day limit should not be over 98 days (12x7 plus 14 days, since more caps were distributed for 6 extra days). If the intervention time was over this limit, the patient was included without adding a supplement in the FAS, not in the PP. The intake of immunosuppressive medication such as steroids, cortisol, and dexamethasone was also a criterion for exclusion from the PP.

- **Intervention Groups**

The intervention population was a per-protocol population. This population was involved in a three-month randomized control trial followed by an open-labelled intervention of three months, which could mean an intervention duration of six months. The randomization at baseline divided the pre-HAART population into two comparable groups, *Arthrospira* and placebo. The patients who wanted to continue the open-labelled intervention had to fulfil the baseline inclusion criteria and, especially, had to not be eligible for HAART (CD4 > 350 cells/mm^3). The randomization between the groups was abrogated for the open-labelled period. The groups achieving six months of intervention could not be compared to each other; comparisons could only be carried out within the individual OLI groups. The analyses were achieved on the population as follows:

The **randomized control intervention**, patients were given placebo for 12 weeks against *Arthrospira* for 12 weeks.

In the **long-term exposure group,** patients were supplemented for 24 weeks with *Arthrospira*.

The patients supplemented for 12 weeks with placebo and 12 weeks *Arthrospira* were part of the **crossover group** in the open-labelled analyses.

- **Software**

Data entry was achieved and controlled by Epidata. The database was imported to SPSS version 20.0 (SPSS, Chicago, IL) for statistical evaluation.

3 Results

3.1 Timeframe of the Trial

The randomized controlled study included a total of 73 participants after screening 516 pre-HAART patients between June and September 2010. The study was finished in December 2010 with 58 participants. Of those 58, 36 continued for the following three months of exclusive *Asp* supplementation in the open-label intervention until March 2011. Thirty patients accomplished the six-month intervention.

3.2 Recruitment Summary

The screening results on patient files were estimations. The percentage of patients' files not under HAART was between 8 and 16% of all files checked. The sex ratio was approximately one man to six women. The diagram of recruitment below shows the recruitment steps and the intervention with the patients effective.

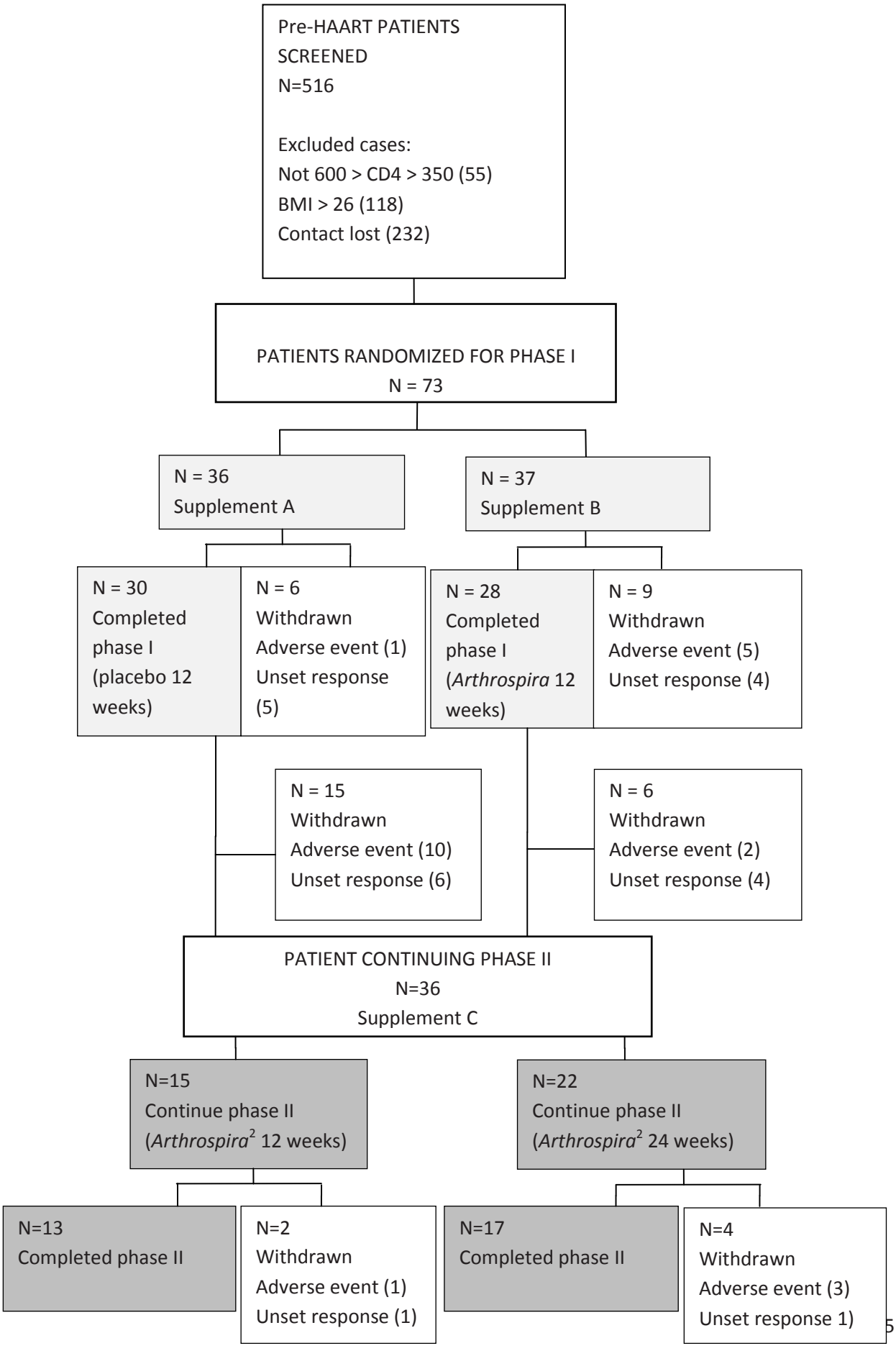
Pre-HAART PATIENTS
SCREENED
N=516
Excluded cases:
Not 600 > CD4 > 350 (55)
BMI > 26 (118)
Contact lost (232)
PATIENTS RANDOMIZED FOR PHASE I
N = 73
N = 36
Supplement A
N = 37
Supplement B
N = 30
Completed
phase I
(placebo 12
weeks)
N = 6
Withdrawn
Adverse event (1)
Unset response
(5)
N = 28
Completed
phase I
(*Arthrospira* 12
weeks)
N = 9
Withdrawn
Adverse event (5)
Unset response (4)
N = 15
Withdrawn
Adverse event (10)
Unset response (6)
N = 6
Withdrawn
Adverse event (2)
Unset response (4)
PATIENT CONTINUING PHASE II
N=36
Supplement C
N=15
Continue phase II
(*Arthrospira*^{2} 12 weeks)
N=22
Continue phase II
(*Arthrospira*^{2} 24 weeks)
N=13
Completed phase II
N=2
Withdrawn
Adverse event (1)
Unset response (1)
N=17
Completed phase II
N=4
Withdrawn
Adverse event (3)
Unset response 1)

FIG 3.1: THE DISPOSITION OF THE PATIENT GIVES AN OVERVIEW OF PATIENT HANDLING DURING THE STUDY
The data were collected during screening, baseline, after three months, and after six months. The dropouts were divided into two categories: health issues/adverse event and social events/unset response. The [2]indicates the supplementation groups during the open-labelled intervention.

The repartition in the statistical populations was achieved in the safety population, full analysis set, and per protocol.

- The safety population consisted of 73 pre-HAART patients grouped into two groups, 36 participants in the placebo group and 37 in the *Asp* group.
- The full analysis set included 30 participants of the placebo and 28 of the *Asp* group.
- The per-protocol population included 14 placebos of the placebo group and 17 of the *Asp* group.
- The open-labelled intervention (OLI) stands for the patients known to have taken at least three months of *Asp* supplement. The [2]indicates the pre-HAART groups during the open-labelled intervention.

3.3 Exclusion and Compliance Analysis

- **Exclusion Reasons**

Exclusion reasons were divided into five categories:

1. Reaching the endpoint of CD4 <350 cells/mm^3, thereby having to start HAART
2. Starting a relevant co-medication as heavy medication therapy, for example tuberculosis or a hospitalization
3. Withdrawal of consent
4. Lost to follow-up
5. Pregnancy

Reasons for exclusion varied between the placebo and treatment group during the RCT. Ten placebo patients were eligible for HAART compared to five out of the *Asp* group. The rate of withdrawal of consent and the lost to follow-up were similar in all groups. Over an

intervention time of six months, the same exclusion rate was reached in both pre-HAART groups. Twenty-three out of 36 patients in the placebo group did not complete six months of intervention, as with 19 out of 37 patients in the *Asp* group. That results in a dropout rate of 63% and 51% in the placebo and *Asp* group, respectively.

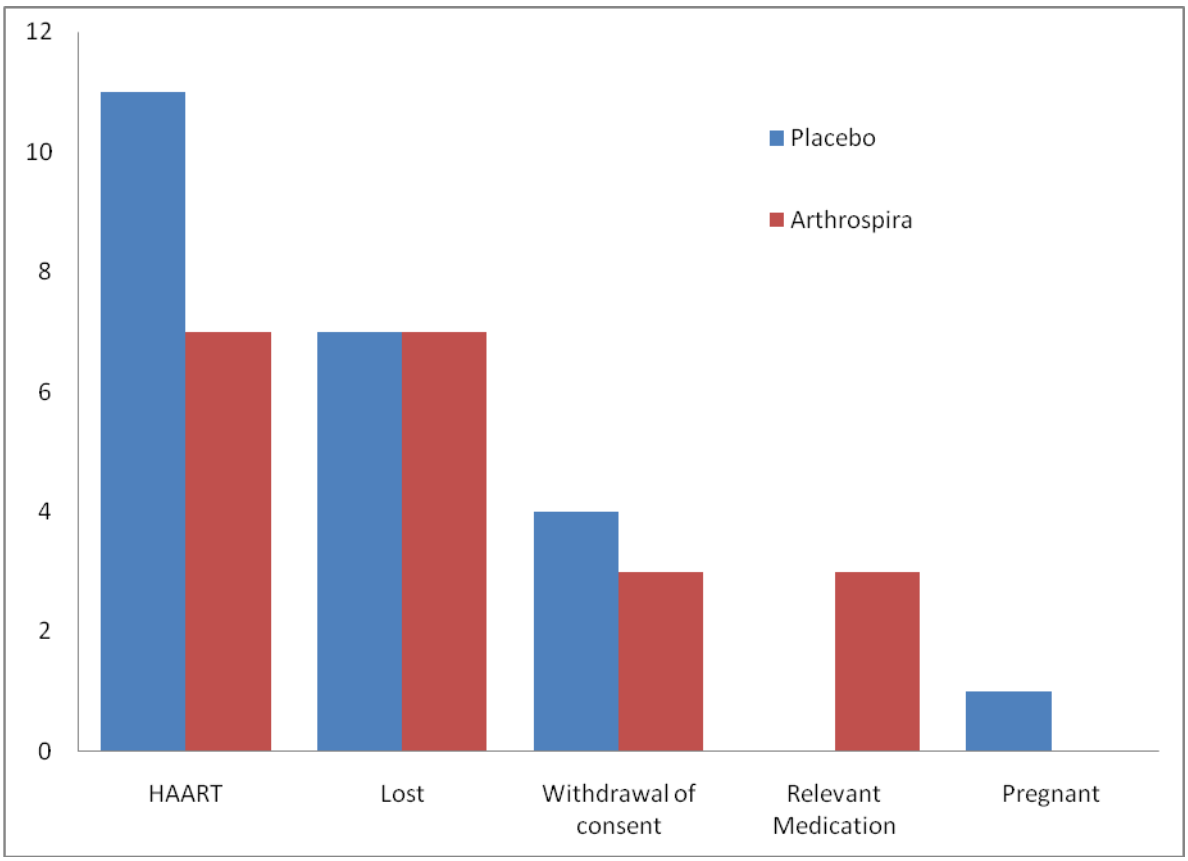

FIG 3.2 HISTOGRAM OF THE DROPOUT REASONS PER GROUP OVER THE STUDY
The histogram shows the dropout reasons by effective in each group, the pre-HAART with the mentioned placebo and *Arthrospira*.

The pre-HAART study population participated in a six-month intervention and was the subject of the RCT intervention in the first three months. The chronology was divided into three periods: the two intervention periods and the period in between.

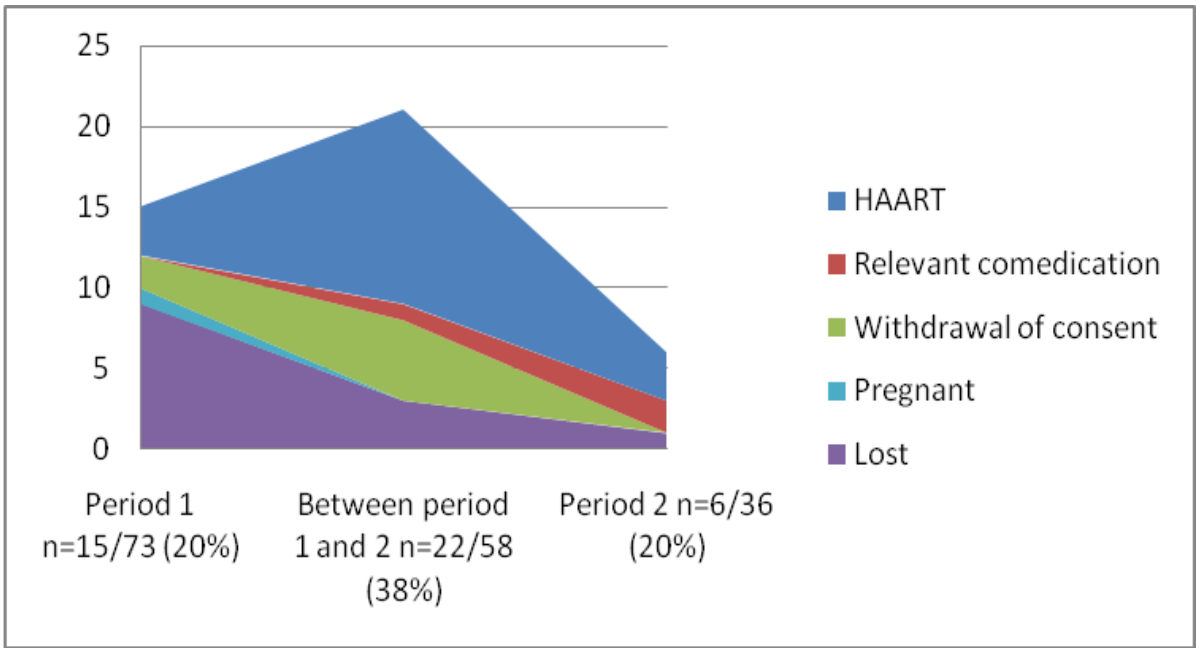

FIG. 3.3 REASONS FOR EXCLUSION AT DIFFERENT INTERVENTION PHASES

The diagram presents the exclusion reasons over the intervention. The dropout frequencies are represented by the coloured areas. The "n" stands for the total drop out on the total population given in percentage.

During the RCT, the exclusion rate reached 20%.The highest dropout (38%) was present, when participants finished the RCT and started with OLI. Here, the two major exclusion reasons were the start of a HAART therapy with 55% and withdrawal of consent with 27%. During OLI the dropout decreased to 16%. The main reasons were the start of HAART medication or relevant co-medication.

- **Variable Analyses**

The variable and patient information was analysed; those who were excluded during the intervention were compared to those who had completed a six-month intervention. These analyses included exclusively pre-HAART patients.

TABLE 3.1 MARITAL & EDUCATION LEVEL OF EXCLUDED AND COMPLIANT

Variables		Excluded n=43		Compliant n=30	
		Frequency (Patient)	Percent (%)	Frequency (Patient)	Percent (%)
Marital status	**single**	37	86	29	96.7
	married	6	14	1	3.3
Level of education	**primary & secondary**	39	90.7	23	76.7
	university & tertiary	4	9.3	7	23.3

TABLE 3.2 INFLAMMATION & ANAEMIA IN EXCLUDED & COMPLIANT

Variables		Excluded n=43		Compliant n=30	
		Frequency (Patient)	Percent (%)	Frequency (Patient)	Percent (%)
Inflammation	**normal**	37	86	26	87
	inflammation	6	14	4	13
Anaemia	**normal**	27	62	22	73
	anaemia	16	37	8	27

66% of the anaemic patients at baseline did not complete the intervention. The presence of an acute inflammation at baseline did not seem to have any relation to the exclusion. Furthermore, more highly educated persons completed the study than less educated persons. The same was true for unmarried women.

The variable comparison between the exclusion and six-month compliant patients was significantly different for RBC: r=.23, p=.041, and haemoglobin: r=.24, p=.037. An overall better health status could be found in the patients who concluded the intervention. The median immune activation, CRP, triglyceride, cholesterol, and urea were smaller in the compliant group than in the exclusion group. In contrast, BMI, CD4, TAOS, haemoglobin, iron, and albumin were higher.

3.4 Randomized Control Intervention

- **Study Population Characteristics**

The RCT deals with the pre-HAART patients who completed three months of supplementation. The following analyses were made with regard to the fully analysed population. Variables defining the population in terms of age, education, marital status, and anthropometric values are presented here.

TABLE 3.3 RANDOMIZED INTERVENTION GROUPS, CONSTANT VARIABLES

	Placebo n=30			*Arthrospira* n=28		
Variables	**Mean (SD)**	**Median (IQR)**	**Min-Max**	**Mean (SD)**	**Median (IQR)**	**Min-Max**
Age (year)	31.3 (8.0)	33 (25–36)	18–48	30.2 (7.9)	27 (25–36)	19–47
IDDS	4.8 (1.7)	5 (4–6)	1–8	5.3 (1.6)	5 (4–6.5)	2–8
Height (m)	1.62(0.06)	1.64(1.58–1.66)	1.48–1.75	1.63(0.06)	1.62(1.59–1.67)	1.53–1.73
Weight (kg)	57 (6)	57 (55–62)	46–70	58 (7)	57 (53–62)	48–73
BMI (kg/m^2)	22 (2)	22 (20–23)	17–26	22 (2)	22 (20–23)	18–26

The placebo group had a median age of 33 (25– 36) years compared to 27 (25– 36) years for the *Asp* group. The IQR were the same for both groups, and the median difference was not significant: r=.07, p=.513. Height was equal in the two groups, being approximately 1.63 (0.06) meters, r=.08, p=.944.

TABLE 3.4 MARITAL STATUS & EDUCATION LEVEL BETWEEN THE GROUPS

		PLACEBO		ARTHROSPIRA		TOTAL	
REASONS		FREQUENCY (PATIENT)	PERCENT (%)	FREQUENCY (PATIENT)	PERCENT (%)	FREQUENCY (PATIENT)	PERCENT (%)
MARITAL STATUS	SINGLE	21	70	24	86	45	77
	MARRIED	2	7	1	3	3	5
	DIVORCED & WIDOWED	7	23	3	11	10	17
	TOTAL	30	100	28	100	58	100
EDUCATION LEVEL	PRIMARY &SECONDARY	26	87	23	82	49	84
	TERTIARY &UNIVERSITY	4	13	5	18	9	16
	TOTAL	30	100	28	100	58	100

- **Primary Objective**

The primary objective was to assess the effect on the infection markersCD4 cells count and viral load.

➢ Lymphocyte CD4

The CD4 variables were not normally distributed. The applied tests were non-parametric. The CD4 cell count at baseline between placebo and *Asp* was not significantly different: r=.04, p=.744 (Appendix Table 10.5). After 12 weeks of intervention, the placebo and the *Asp* supplement did not differ significantly from each other: r=.01, p=.901. Within the two groups, the CD4 cells count decreased significantly, for placebo -52 (-112– -16) cells/mm^3: r=.67, p<.001, and for the treatment group -66 (-111–-20) cells/mm^3 for *Asp*: r=.61, p<.001.

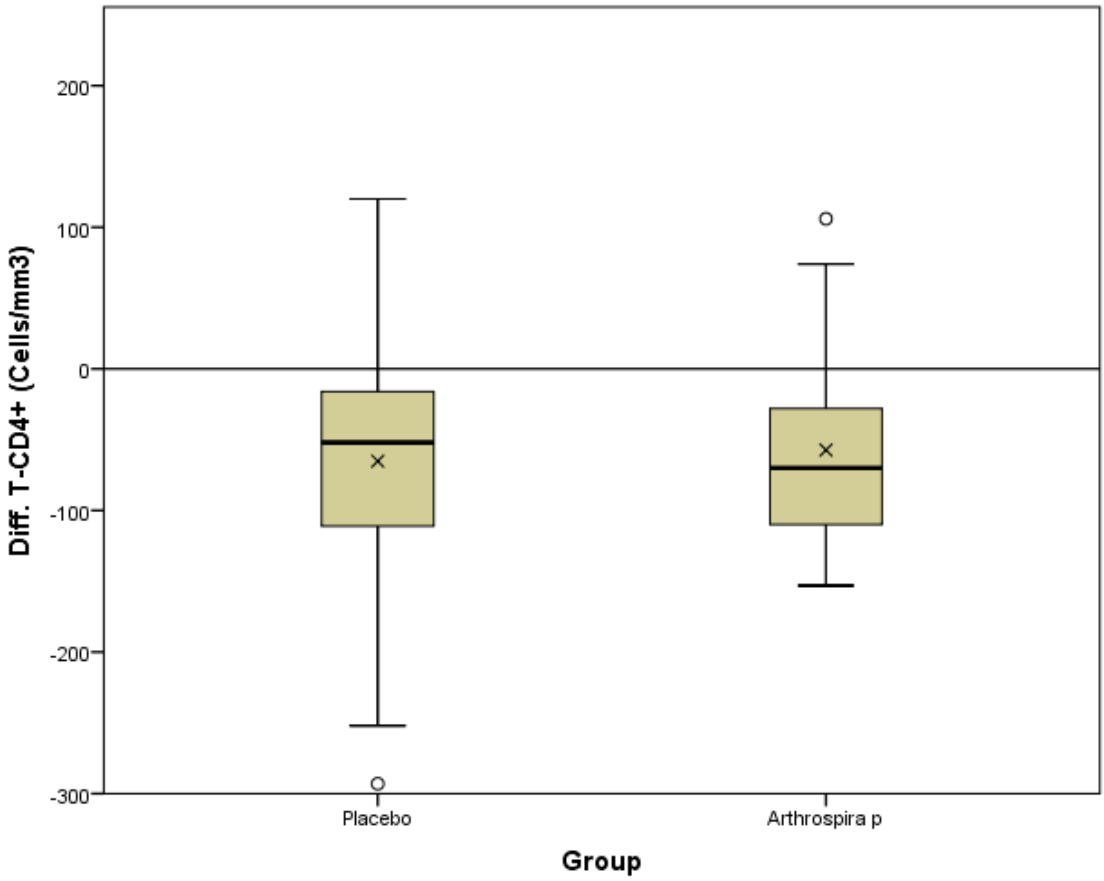

FIG 3.4 BOX PLOT OF T LYMPHOCYTE CD4 DIFFERENCE DURING THE RCT

The box plot represents the median and the IQR of the difference occurring during the 12 weeks of RCT. The zero line represents the baseline value. The crosses stand for the mean.

➢ Viral Load

For the serum viral load, no significant difference could be reported between the groups; at baseline: r=.14, p=.262 and after 12 weeks intervention: r=.03, p=.774. Within the groups, the changes were insignificant for placebo: r=.18, p=.329 and *Asp*: r=.13, p=.501. One patient in each group displayed had confirmed infection with HIV-2 subtype, not detectable by the applied quantification method. Furthermore, three patients, two in the placebo group and one in the *Asp* group, showed an undetectable viral load after three months (see table 10.6 in appendix).

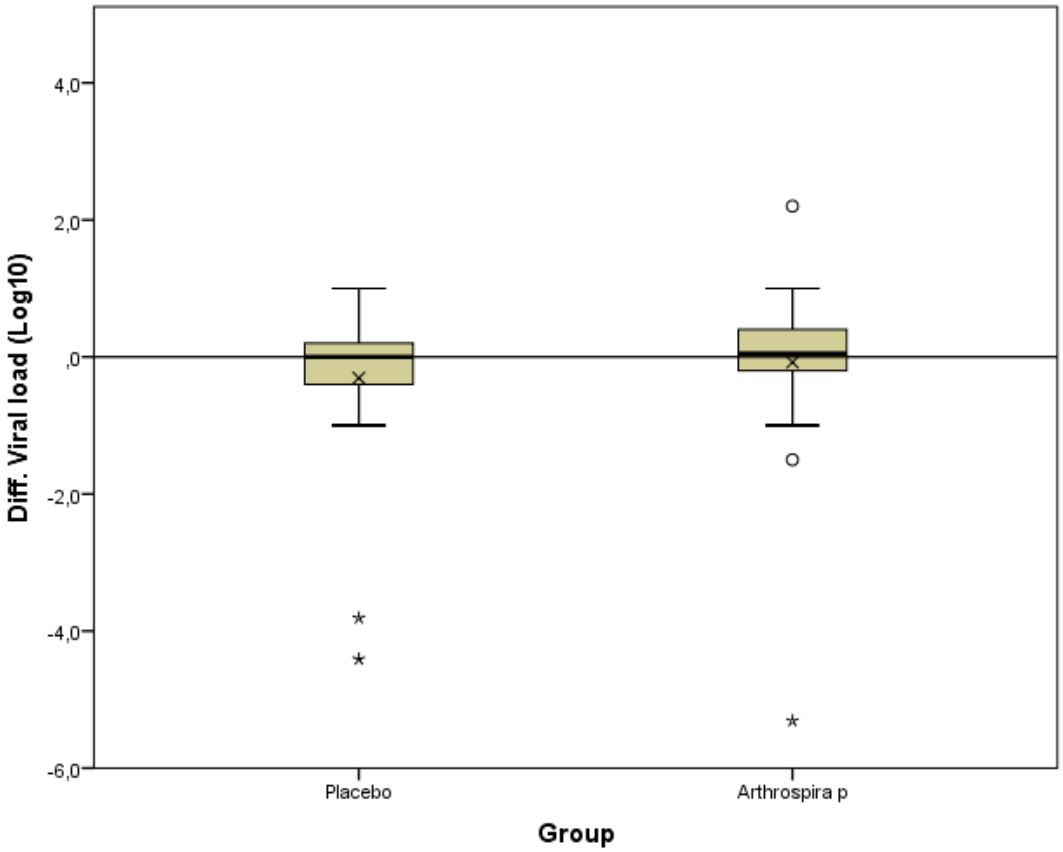

FIG 3.5 BOX PLOT OF VIRAL LOAD DIFFERENCE DURING THE RCT

The box plot represents the median and the IQR of the difference occurring during the 12 weeks of RCT in the placebo and *Asp* groups. The zero line represents the baseline value. The crosses stand for the mean.

- **Secondary Objective**

The immuno-inflammatory profile included the CD8 lymphocyte presenting the antigen CD38, the inflammatory marker CRP, and the adverse events recorded.

➢ Immune Activation

The assessment of the CD38-CD8 expression at baseline showed no significant difference between *Asp* and placebo: r=.08, p=.513. After the RCT period, the groups did not differ from each other: r=.04, p=.732. Within the groups, *Asp* median decreased by -1.3% (-5.8– 2.0) and -0.9% (-4.17– 1.56) for placebo. However, the decreases were not statistically significant, respectively *Asp*: r=.24, p=.185 and placebo: r=.18, p=.327. The effect sizes were small to very small for the immune activation decrease in placebo and *Asp*. The small decrease difference that occurred during the RCT correlated with the baseline CD8-CD38 value: Rho=-.325, p=.01 (see table 10.7 in appendix).

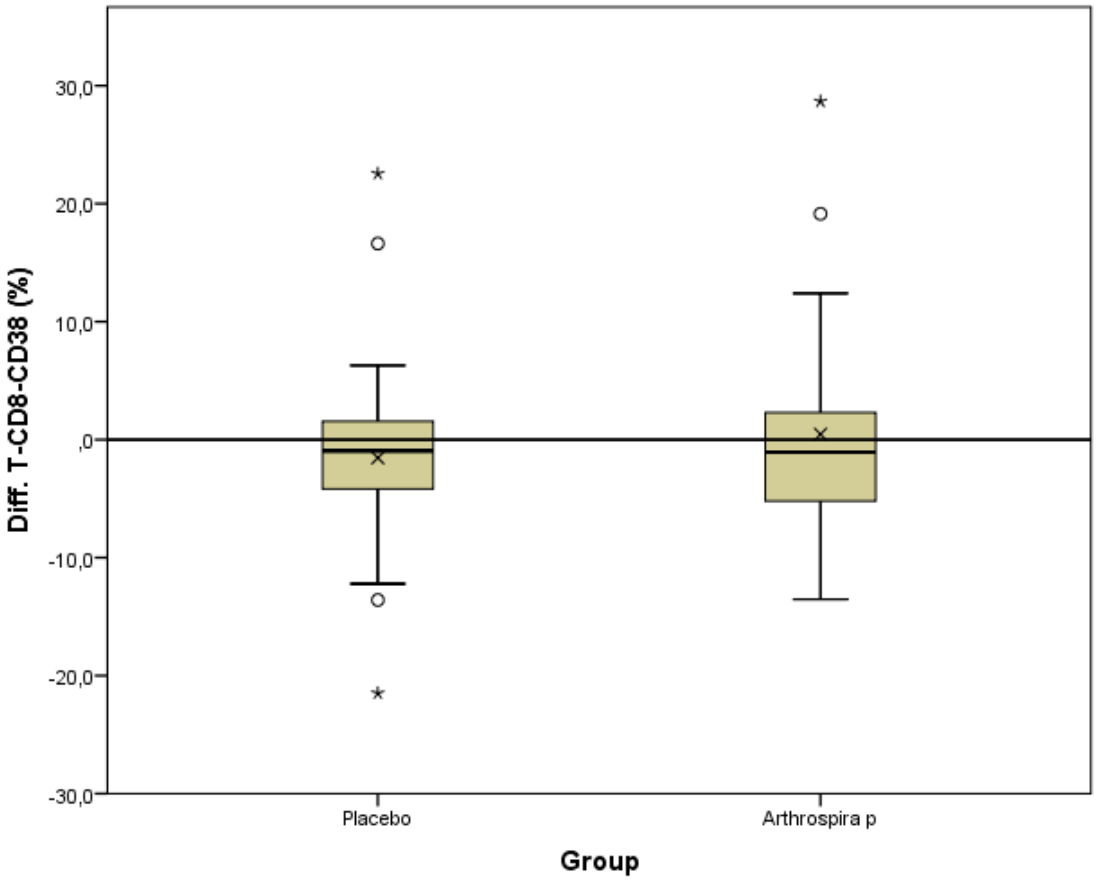

FIG 3.6 BOX PLOT OF CD8-CD38 DIFFERENCE DURING THE RCT

The box plot represents the difference occurring during the RCT in the placebo and *Asp* groups. The horizontal line stands for no change over the intervention. The cross represents the mean. Stars and dots stand for outlying patients.

➢ C-Reactive Protein

C-reactive protein has an exponential expression with high fluctuation. It occurs in strong outline influence (see graph below). CRP variable was dichotomized into active inflammation and no inflammation. At baseline, the clinical inflammation rate was 10% in both groups. After the intervention, the placebo group had an inflammation rate of 20% compared to 18% in the *Asp* group. The effect sizes of the CRP concentration variation in the groups were very small, only a few patients showed high fluctuation: inside the placebo group r=.18, p=.325 and r=.06, p=.374 inside the *Asp* group.

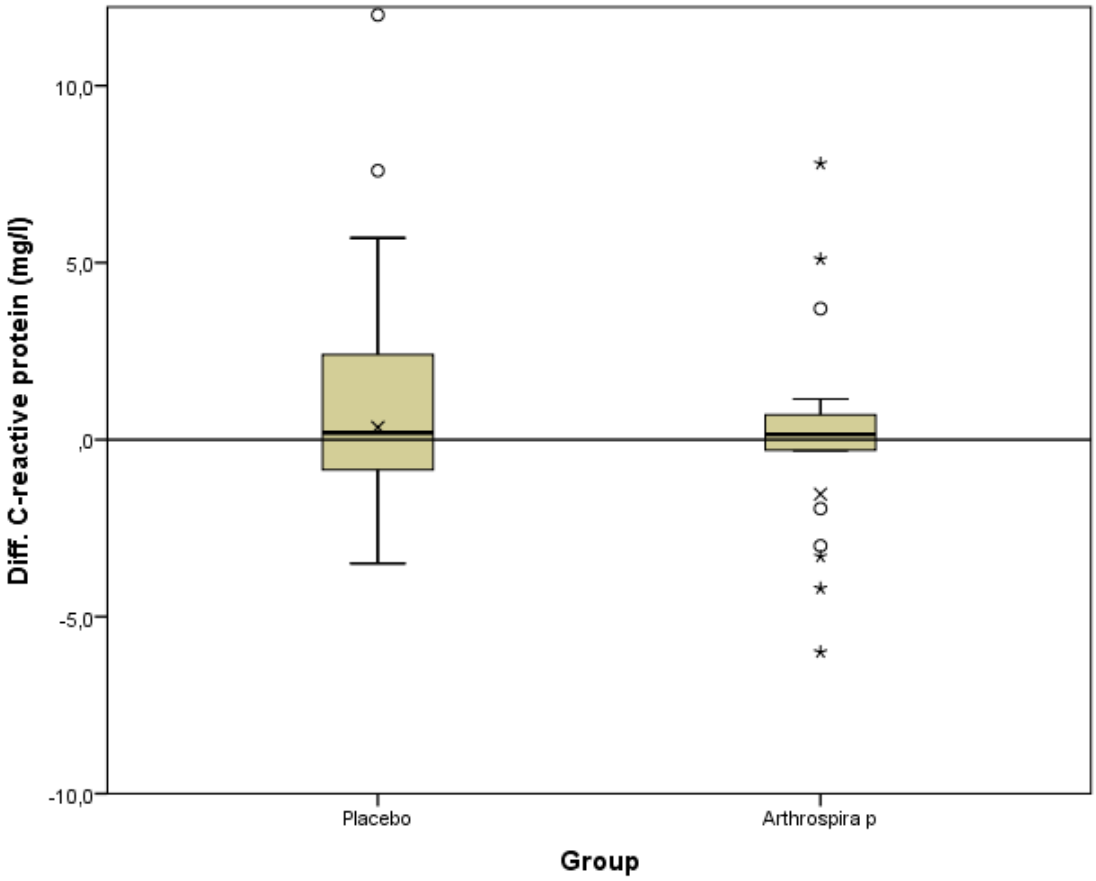

FIG 3.7 BOX PLOT OF C-REACTIVE PROTEIN DIFFERENCE DURING THE RCT

The box plot shows the difference occurring during the RCT in the groups. Three outlying placebo patients had an increase over 10 mg/l and are not shown, neither are two patients with a decrease over 40 mg/l. Two outlying patients from the *Asp* group had a decrease 40 mg/l and are not shown on the box plot.

In the following table the inflammation was dichotomized between normal and presenting an active inflammation. The inflammation status was described by a cut-off point for the CRP concentration over 6 mg/l.

TABLE 3.5 INFLAMMATION OUTBREAK DURING RCT

		Placebo n=30		*Arthrospira* n=28		Total	
Inflammation CRP		**n**	**%**	**n**	**%**	**n**	**%**
Baseline	Normal	27	90	25	89	52	90
	> 6 mg/l	3	10	3	11	6	10
After 12 weeks	Normal	24	80	23	82	47	81
	> 6 mg/l	6	20	5	18	11	19

➢ Concomitant Event

The concomitant event incidences were documented on the basis of the population that finished the RCT intervention, for the full analysis population of the RCT (see table 10.9 in appendix). Each placebo patient had a concomitant event incidence of 0.7. An *Asp* patient showed an incidence less than half of a concomitant event over the same time period. A total of 33 events occurred during the pilot RCT intervention, a third of which can be attributed to malaria.

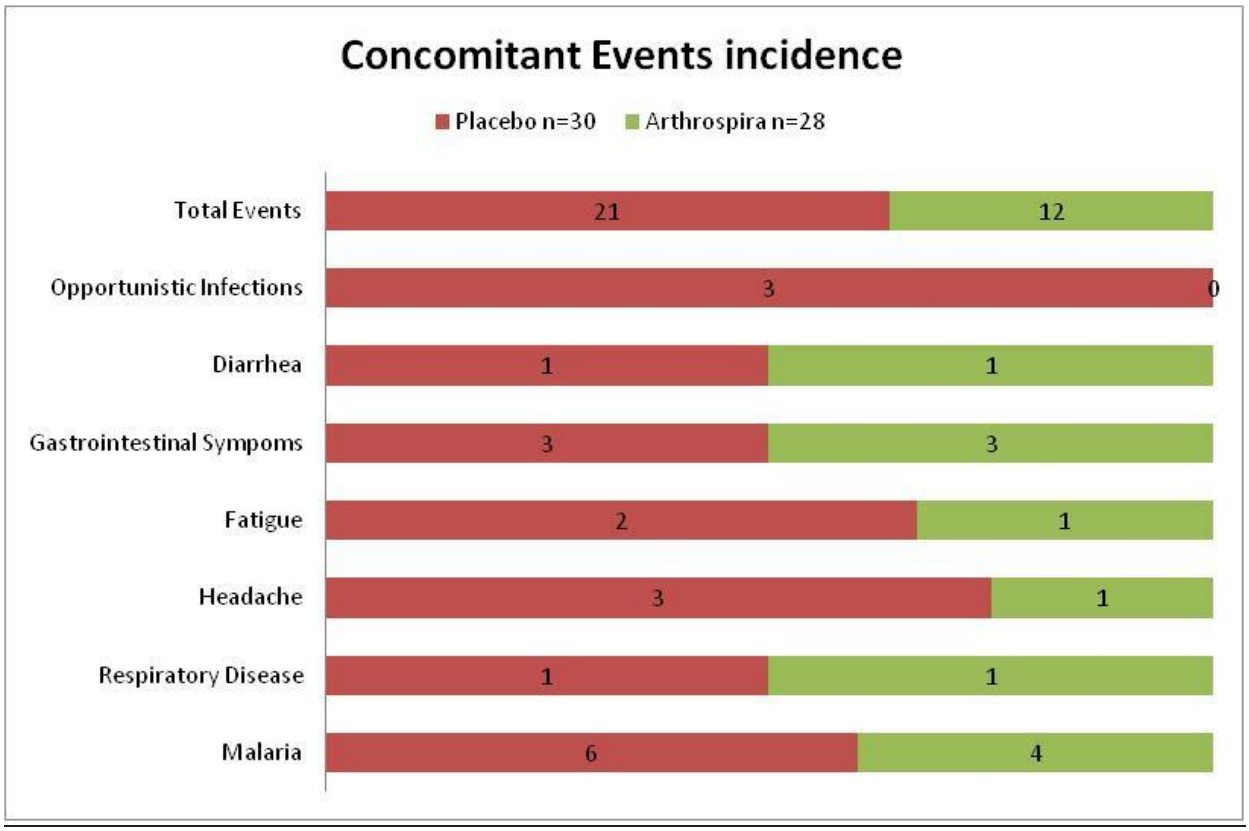

FIG 3.8 INCIDENCE OF THE CONCOMITANT EVENTS DURING THE PILOT RCT
Patient frequencies of the events are noted by the number.

- **Third Objective**

The variables described below were nutrition-related outcomes of the RCT intervention.

➢ Body Weight

Body weight was measured at baseline and after the RCT intervention (see table 10.10 in appendix). At baseline the groups were not significant: r=.02, p=.889. After 12 weeks of intervention, the placebo and the *Asp* supplement did not differ from each other: r=.05, p=.697. The differences occurring during the intervention in each group, placebo 0.6 (-0.1– 2.9) kg and *Asp* 0.5 (-0.3– 1.6) kg, were not significant: r=.21, p=.105. Body weight increased significantly in the placebo group: r=.52, p=.005 but not in the *Asp* group: r=.12, p=.517.

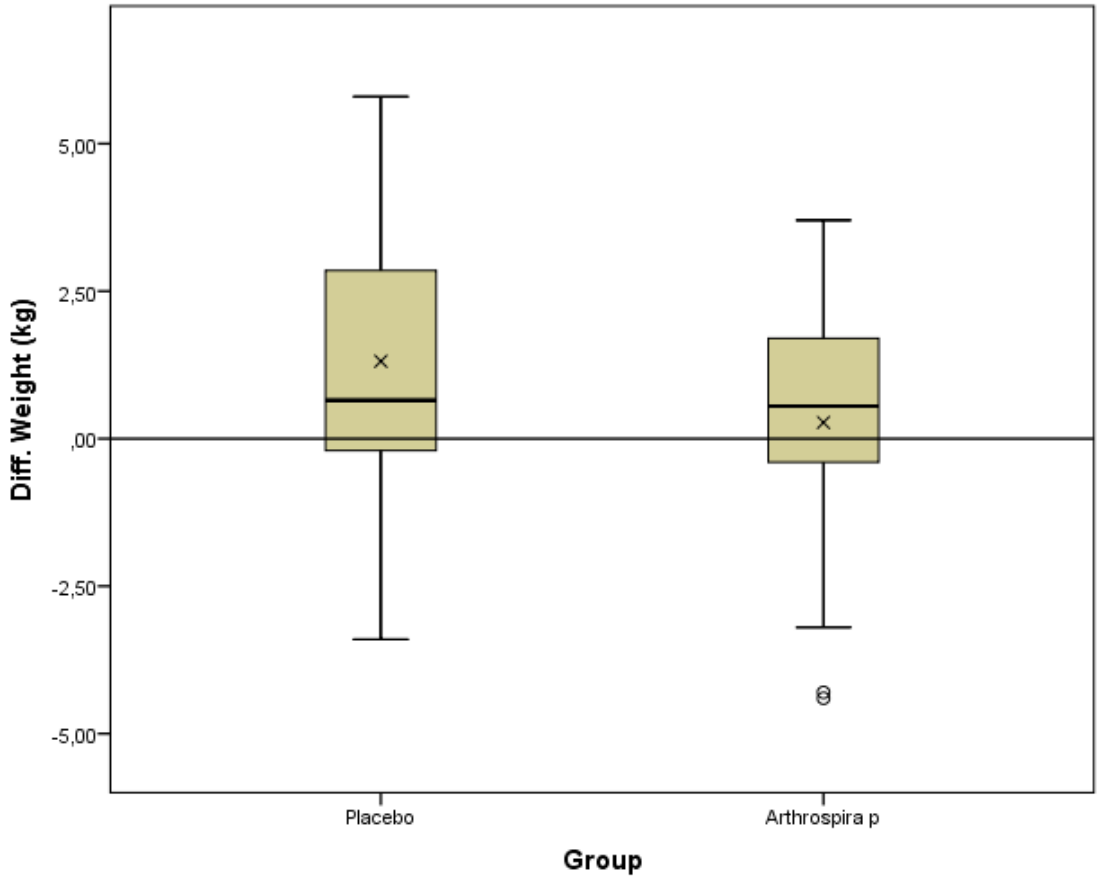

FIG 3.9 BOX PLOT OF BODY WEIGHT DIFFERENCE DURING THE RCT

The box plot represents the difference occurring during the 12 weeks of RCT in the placebo and *Asp* groups. The horizontal line stands for no difference between baseline and 12 weeks. The cross represents the mean.

➢ Total Antioxidant Status

Both groups had a similar TAOS value at baseline: r=.14, p=.269. After 12 weeks, the groups significantly differed: placebo 336 (275– 373) µM, *Asp* 387 (320– 430) µM: r=.30, p=.023. The difference occurring during the intervention between the groups was highly significant: placebo -22 (-64– 19) µM; *Asp* 56 (1– 98) µM: r=.51, p<.001. The change inside the groups was also significant, with an increase in the *Asp* group: r=.51, p=.007 and a decrease in the placebo group: r=.48 p=.008 (see table 10.11 in appendix).

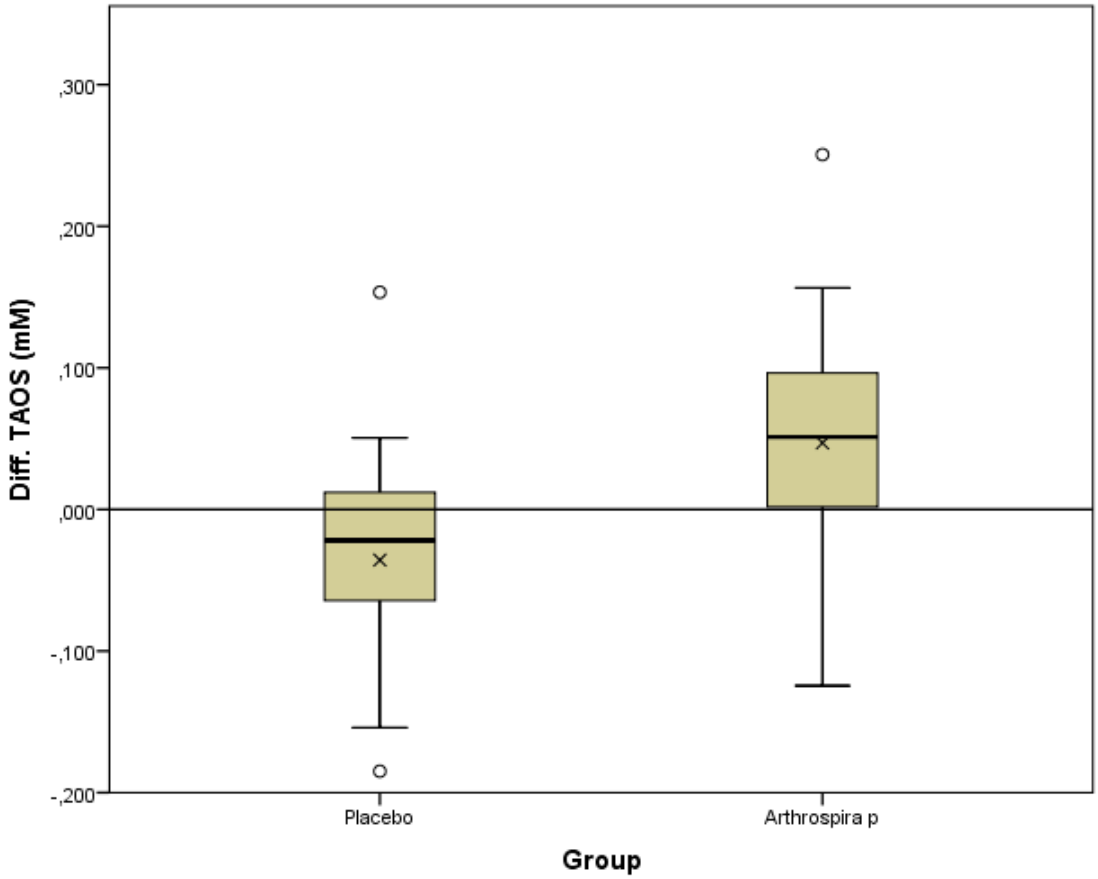

FIG 3.10 BOX PLOT OF TAOS DIFFERENCE DURING THE RCT

The box plot represents the difference that occurred inside the groups over three months, with the mean as a cross. The zero line stands for no difference between baseline and 12 weeks. The dots represent outlying patients.

➢ Albumin

Both groups had the same albumin concentration at baseline: r=.01, p=.938. After 12 weeks there was no significant difference between the groups: r=.03, p=.796. The difference that occurred during the intervention was also not significant between the groups: r=.07, p=.595. However, the decrease within the groups was statistically significant: placebo -1.5 (-4.0– 1.0) g/l: r=.39, p=.034 and *Asp* -3 (-4– 0) g/l: r=.42, p=.026. The effect size stood for a medium lowering in both groups (see table 10.12 in Appendix).

➢ Anaemia

The table below represents the anaemia incidence per group, at baseline and after the RCT intervention. The anaemia was defined as haemoglobin< 11 g/dl.

TABLE 3.6 ANAEMIA IN THE RCT GROUPS

		Placebo n=30		*Arthrospira* n=28		Total	
Anaemic, Hb		**Frequency (Patient)**	**Percent (%)**	**Frequency (Patient)**	**Percent (%)**	**Frequency (Patient)**	**Percent (%)**
Baseline	**Normal**	18	60	21	75	39	67
	Anaemic	12	40	7	25	19	33
After 12 weeks	**Normal**	15	50	16	57	31	53
	Anaemic	15	50	12	43	27	47

The absolute number of anaemic patients increased over the intervention in both groups from 12 to 15 patients in the placebo group and from 7 to 12 patients in the *Asp* group. The relative proportion of anaemic patients at baseline was 40% in the placebo compared to 25% in the *Asp* group. After the RCT intervention, the proportion was 50% in the placebo group compared to 43% in the *Asp* group.

➢ Haemoglobin

TABLE 3.7 HAEMOGLOBIN

	Placebo n=30			*Arthrospira* n=28			
Haemoglobin (g/dl)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	11.4 (1.9)	11.9(10.5–12.8)	5.7–14.6	11.9 (1.4)	11.9(11.1–12.9)	8.9–14.8	.3
After 12 weeks	10.8 (1.7)	11.1(9.1–12.2)	7.6– 14.5	11.6 (1.5)	11.8(10.6–12.5)	9.1–14.8	.07
Difference	-0.6 (1.2)	-0.8(-1.3– -0.2)	-2.7– 3	-0.3 (1.1)	-0.3(-0.7–0.0)	-4.2–2.5	.07
Wilcoxon (p=)		.003*			.031*		

*p<.05

Both groups had similar haemoglobin values at baseline: r=.12, p=.371. The groups did not differ significantly after 12 weeks of intervention: r=.24, p=.073. The difference occurring between the groups was not significant: placebo -0.8 (-1.3– -0.2) g/dl and *Asp* -0.3 (-0.7– 0.0) g/dl: r=.24, p=.071. However, the decreases within each group were significant for *Asp*: r=.40, p=.031 and for the placebo group: r=.54, p=.003.

➢ Erythrocyte Count

The randomized groups had the same count of erythrocytes at baseline, r=.04; p=.773 (see table 10.13 in appendix). The groups did not differ significantly from each other after 12 weeks of intervention: r=.06, p=.663. The change occurring during the intervention was not significant between the groups: r=.21, p=.109. The decrease within both groups was significant: placebo r=.68, p<.001 and *Asp*: r=.57, p<.002. The two groups had a large effect size for the decrease; the placebo group r=.68 was higher for a median decrease of -0.4 (-0.5– -0.2) tera/l, compared to the *Asp* at an effect size of r=.57 and a median decrease of -0.3 (-0.4– 0.0) tera/l.

➢ Iron

Both groups had a comparable iron concentration at baseline: r=.03, p=.795 (see table 10.14 in appendix). The groups did not differ significantly after 12 weeks intervention: r=.10, p=.415. The differences occurring during the intervention between the two groups were not significant: r=.11, p=.402. The decrease inside the *Asp* group was not significant: r=.01, p=.949, nor was the decrease in the placebo group: r=.13, p=.480.

➢ Lipid Profile

The variables below document the lipid profile of the patients involved in the intervention by the total cholesterol and the triglyceride concentrations (see tables 10.16 and 10.17).

- Cholesterol

The randomized groups had a comparable TC concentration at baseline: r=.05, p=.686. The groups differed but not significantly from each other after 12 weeks intervention: r=.21, p=.114. The group-internal difference over the intervention was not significant between the groups: r=.22, p=.101. The decrease within the groups was significant for *Asp*:-0.14 (-0.47– -0.04) g/l: r=.55, p=.004but not for the placebo median -0.07 (-0.20– 0.06) g/l: r=.28, p=.113. The mean decrease was -0.20 (0.36) g/l for *Asp* and -0.08 (0.30) g/l for the placebo group.

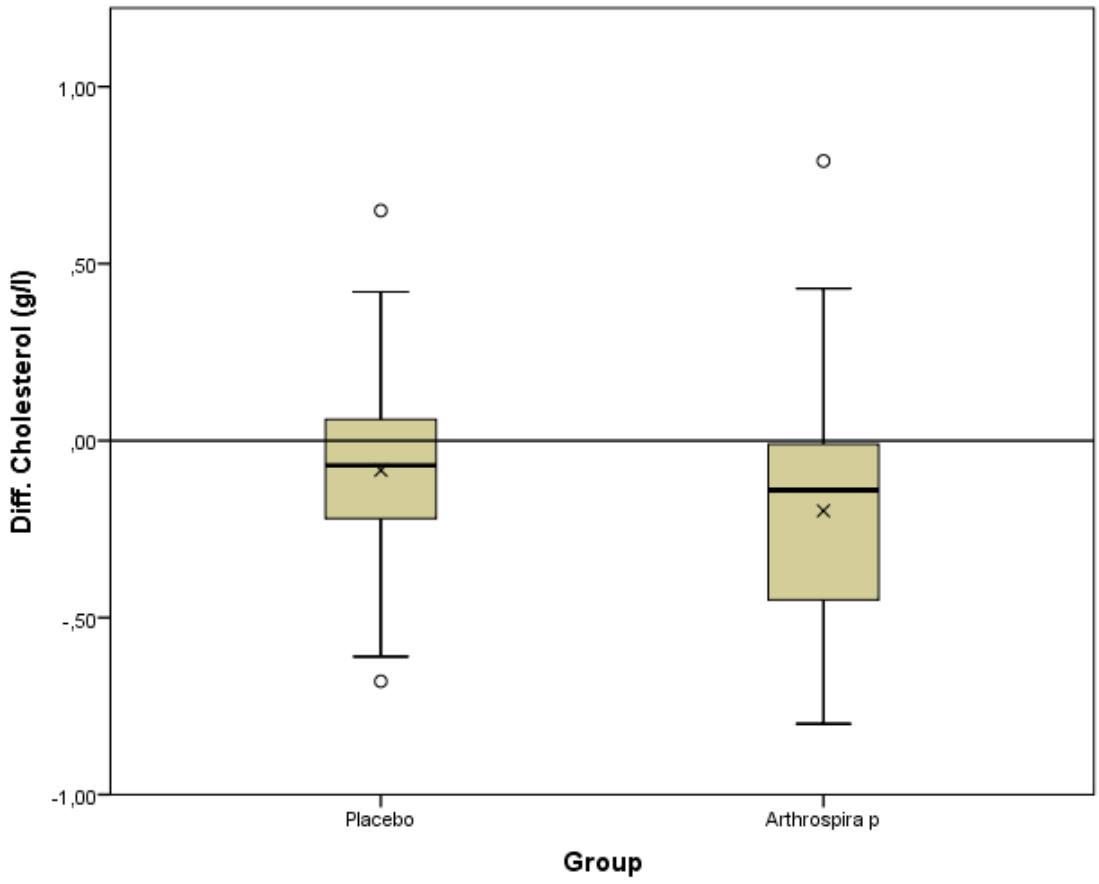

FIG. 3.11 BOX PLOT OF CHOLESTEROL DIFFERENCE DURING THE RCT

The box plot represents the difference that occurred within the placebo and *Asp* groups over the intervention. The line stands for no change between baseline and 12 weeks. The dots represent outlying patients.

- Triglyceride

The randomized groups had similar triglyceride concentrations at baseline: r=.05, p=.686. The groups differed but not significantly from each other after 12 weeks of intervention, r=.13; p=.286. The change that occurred inside the groups was not significantly different between the groups: placebo 0.07 (-0.16– 0.48) g/l, *Asp* 0.04 (-0.18– 0.2) g/l: r=.04, p=.72. The increase within the groups was not significant for *Asp*: r=.16, p=.407 and the placebo: r=.24, p=.188.

➢ Renal Function

The renal function in this study was defined by the serum creatinine, the serum urea concentration, and the calculated eGFR (see tables 10.-18. 10.19 and 10.20).

- Urea

Urea concentrations showed no significant difference at the beginning and over the intervention between the groups and within the groups. The randomized groups had similar urea values at baseline: placebo 0.19 (0.16–22) g/l and *Asp* 0.17 (0.13– 0.20) g/l: r=.20,p=.132. The groups did not differ significantly from each other after 12 weeks intervention: r=.13, p=.326. The changed urea concentration did not differ between the groups: r=.02, p=.882. The change within the groups was not significant for *Asp*: r=.03, p=.861 and the placebo: r=.02, p=.905.

- Creatinine

The randomized groups had identical creatinine concentration at baseline: r=.08, p=.527. The groups did not differ significantly from each other after 12 weeks intervention: r=.21, p=.097. The difference occurring over the intervention was significant between the groups: placebo 0.0 (-0.1– 0.1) g/dl, *Asp* 0.1 (0.0– 0.2) g/dl: r=.35, p=.008. The increase in the *Asp* group was significant: r=.59, p=.002. No change was observed in the placebo group: r=.12, p=.51.

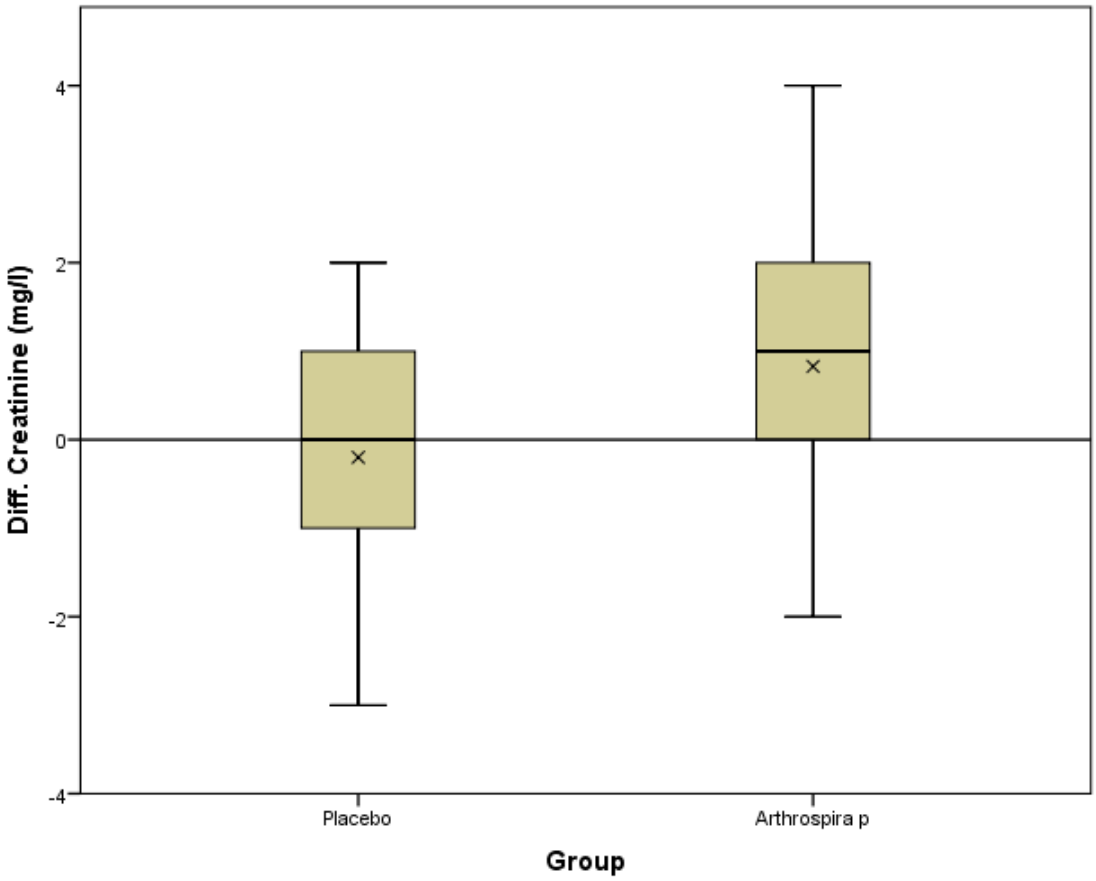

FIG 3.12 BOX PLOT OF CREATININE DIFFERENCE DURING THE RCT

The box plot represents the difference occurring during the RCT in the placebo and *Asp* groups in mg per litre. The zero line stands for no difference between baseline and 12 weeks. The crosses represent the mean.

- eGFR

The eGFR for the randomized groups did not differ significantly from each other at baseline: r=.12, p=.35. After 12 weeks the elimination ratio changed for placebo and *Asp* but not significantly between the groups: r=.15, p=.256. The differences occurring over the period were0.01 (-6.73– 4.62) ml/min for placebo: r=.04, p=.992 and -7.31 (-17.2– 0.95) ml/min for the *Asp* group. The groups differed significantly from each other: r=.32, p=.016. The change within the *Asp* group was significant with a large effect size: r=.55, p=.004.

3.5 Open Label Intervention

The OLI treatment had two separate interventions: the long-term intervention and the crossover for the pre-HAART population. The variables for population characteristics are presented together for both intervention arms. Additionally, the inflammation and anaemia ratio as the concomitant events in the involved intervention arms are compared to each other at the beginning of the chapter. The results are regrouped into tables, one table for immuno-virological markers and four tables for nutrition-related markers (weight, TAOS, albumin, anaemia outcomes, lipid profile, and renal function tables). The results are summarized by median, IRQ, and minimum-maximum. The variables are presented in absolute values at each sampling point for the long-term intervention. The crossover compares the difference during the two three-month supplementation periods.

- **Population Characteristics**

The crossover group had a median age of 34 (26– 36) years compared to 25 (23– 30) years of the long-term group. The IDDS was smaller in the crossover group, having a median of 5 (4– 6) index points, than in the long-term group, having a median of 6 (5– 6) index points. Height was equal in all groups with approximately 1.63 (1.59– 1.67) m.

TABLE 3.8 MARITAL STATUS & EDUCATION LEVEL DURING OLIL

		Crossover		Long-Term	
Variables		**Frequency (Patient)**	**Percent (%)**	**Frequency (Patient)**	**Percent (%)**
Marital status	**Single**	8	61	15	88
	Married	1	8	-	-
	Divorced & Widowed	4	31	2	12
	Total	13	100	17	100
Education level	**Primary &secondary**	9	69	14	82
	Tertiary &university	4	31	3	18
	None at all	-	-	-	-
	Total	13	100	17	100

- **Inflammation**

The inflammation status was dichotomized inactive inflammation with a CRP over 6 mg/l. Presented for each intervention arm at the three sampling time points.

TABLE 3.9 INFLAMMATION STATUS PER GROUP

		Crossover		Long-Term	
Inflammation CRP > 6 mg/l		**Frequency (Patient)**	**Percent (%)**	**Frequency (Patient)**	**Percent (%)**
Baseline	**Normal**	11	84.6	15	88.2
	Inflam.	2	15.4	2	11.8
Three months	**Normal**	12	92.3	14	82.4
	Inflam.	1	7.7	3	17.6
Six months	**Normal**	11	84.6	16	94.1
	Inflam.	2	15.4	1	5.9

- **Anaemia**

The Hb concentrations were dichotomized into anaemic and non-anaemic patient sat a cutoff point of Hb<11g/dl.

TABLE 3.10 ANAEMIA PREVALENCE DURING THE INTERVENTION

		Crossover		Long-Term	
Anaemia Prevalence		**Frequency (Patient)**	**Percent (%)**	**Frequency (Patient)**	**Percent (%)**
Baseline	**Normal**	9	69.2	13	76.5
	Anaemia	4	30.8	4	23.5
12 weeks	**Normal**	9	69.2	9	52.9
	Anaemia	4	30.8	8	47.1
24 weeks	**Normal**	8	61.5	9	52.9
	Anaemia	5	38.5	8	47.1

The table above shows an increase of the overall number of anaemic patients over the intervention. The crossover group had only an increase of one patient who became anaemic.

The number of anaemic patients in the long-term supplemented group doubled during the intervention from four to eight patients.

- **Long-Term Intervention**

The long-term group includes 16 patients who were on *Asp* supplementation for six months. The Friedman one way ANOVA test was applied to document the changes between baseline, 12, and 24 weeks. A *post hoc* test was applied and subsequently three Wilcoxon rank tests. A correction by Bonferroni through the multiplication of the p-value by the number of tests was achieved.

- **Immuno-Virological Marker**

The immuno-virological variables consisted of CD4 cell count, the viral load, and the CD38-CD8 expression.

TABLE 3.11 IMMUNO-VIRAL OUTCOMES OF THE LONG-TERM INTERVENTION

	CD4 (cells/mm^3)		Viral Load (log10)		CD38-CD8 Lymphocyte (%)	
Long-term n=17	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	440(427–559)	324–594	5.3 (4.8–5.8)	3.1–7.4	17.9(14.8–30.5)	11.5–46.9
After 12 weeks	436*(340–502)	227–540	5.2 (4.8–5.9)	3.7–6.5	23.5(14.1–30.3)	10.4–40.4
After 24 weeks	444(376–469)	261–586	5.3 (4.6–5.9)	2.8–6.5	18.7(14.6–27.3)	4.6–59.6

*p<.05

CD4 Lymphocyte

The CD4 count showed a significant change over six months, $X^2(2)= 6.1$, p=.046. Wilcoxon signed-rank tests were conducted to follow up the findings. CD4 count significantly decreased from baseline to 12 weeks, -64 (-104– -13) cells/mm³, r=.62, p=.03. It was followed by a non-significant increase between 12 and 24 weeks of 32 (-45–70) cells/mm^3, r=.21, p>.99. Nine patients showed an increase compared to eight decreases between 12 and24 weeks' intervention. The decrease observed between baseline and 24 weeks was attenuated and not significant, r=.52, p=.09.

Viral Load

Friedman's ANOVA detected no significant change over the six-month intervention: X^2 (2)=0.82; p=.664. No *post hoc* test was applied.

CD38-CD8 Lymphocyte

The ratio increased from baseline to the 12-week assessment point and decreased again at 24 weeks, from 18% (14.8– 30.5) to 23.5% (14.1– 30.3) after 12 weeks to end at 18.7% (14.6– 27.3). However, these changes were not statistically significant. The repeated ANOVA for non-parametric variables showed no significant change over the six-month intervention, X^2(2)=1.412; p=.494, no *post hoc* test was conducted to go further. However, the data showed a decrease in the maximal activation values over the entire intervention from 47% to 40.5% and finished at 38%.

- **Nutrition Outcome Variables**

The following variables are related to the nutritional status of the patients. First the body weight, TAOS, and albumin are presented in one table, followed by the anaemia status, lipid profile, and renal function at the end of the chapter.

➢ Body Weight, TAOS, Albumin

The following part presents the body weight, serum TAOS, and albumin (see table 10.23 in appendix).

Body Weight

According to Friedman ANOVA, body weight changed significantly over the course of six months, X^2(2)= 6.03, p=.049.During the first 12 weeks, median body weight increased by 1.2 (-0.1– 1.7) kg, r=.41, p=.24. Over the following period from 12 to 24 weeks, body weight increased by 0.7 (-0.9– 1.5) kg, r=.27, p=.76. Thus, the overall increase was 1.5 (0.3– 3) kg, r=.49, p=.13. The low Friedman ANOVA score results in the *post hoc* test are not significant.

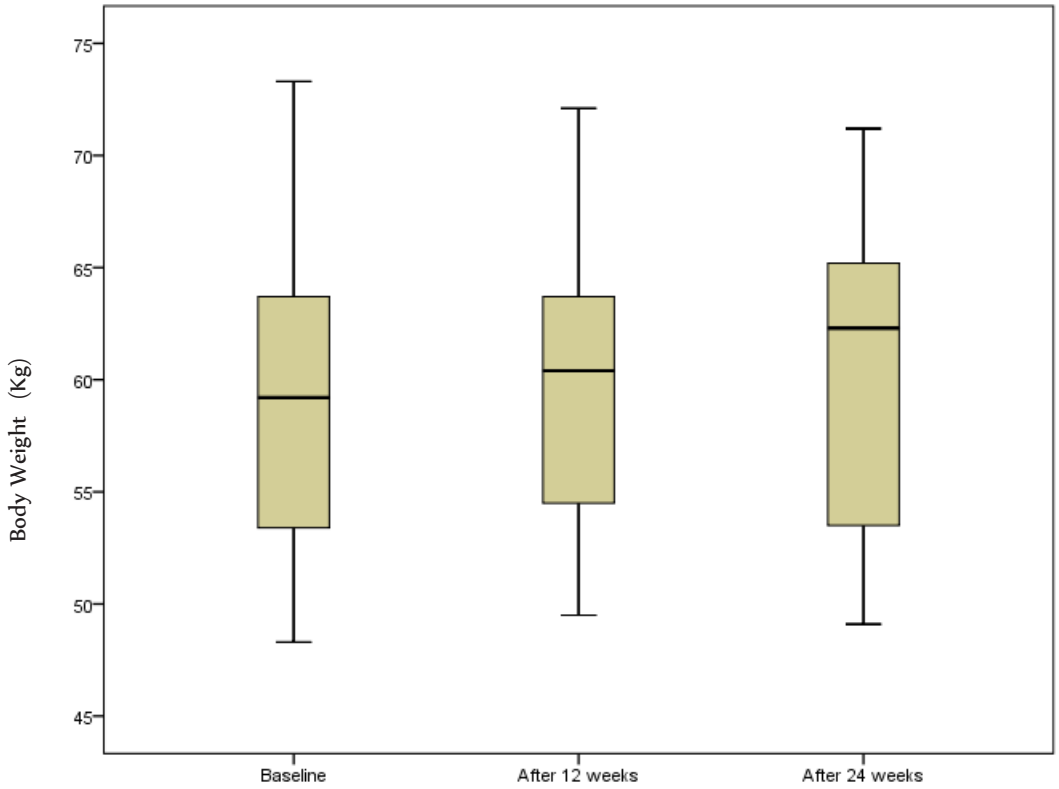

FIG 3.13BOX PLOT REPRESENTING BODY WEIGHT IN THE LONG-TERM INTERVENTION PER SAMPLING

Total Antioxidant Status

The TAOS outcome changed significantly over the six months, Friedman ANOVA, $X^2(2)= 21.5$; p<.001 (see graph below). After 12 weeks the median TAOS increased by 85 (9– 130) µM, r=.59, p=.045. In the second period, the increase was 107 (32– 133) µM,r=.77, p=.003. The overall effect of *Asp* supplementation had an elevated effect size for a total increase of 198 (88–254) µM, r=.85, p=.001. The correlation between the TAOS increase observed over the intervention and the baseline value was significant: Rho=-0.767, p<.001.

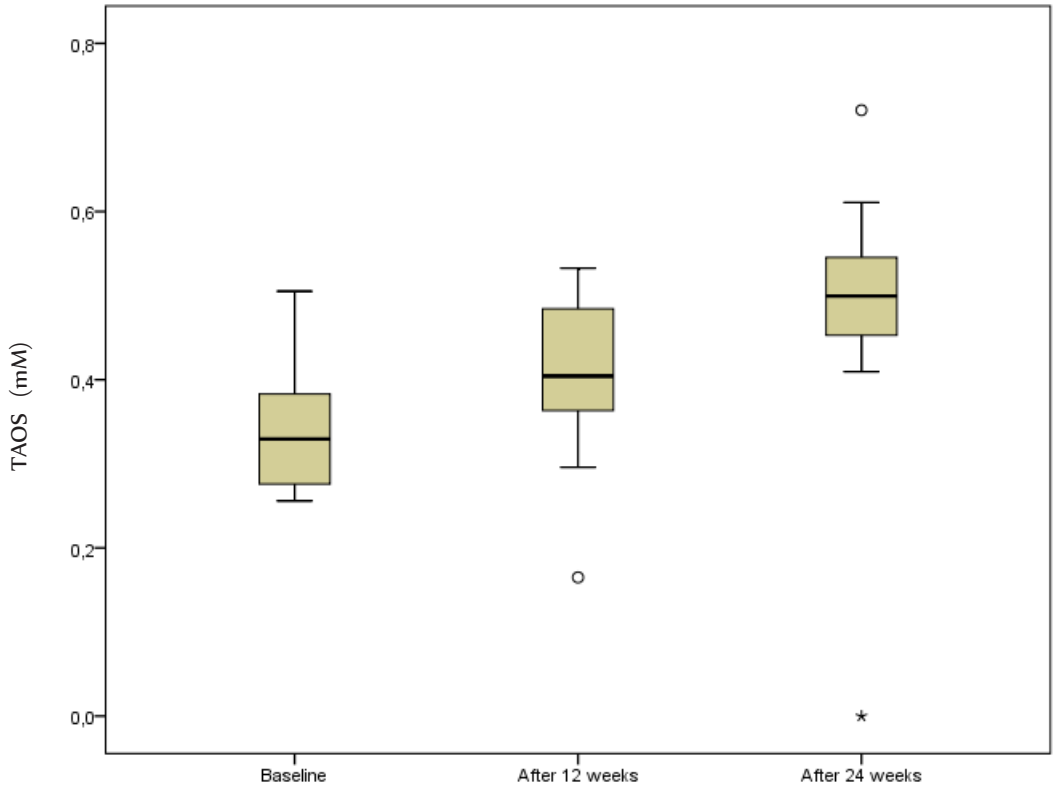

FIG 3.14 BOX PLOT REPRESENTING TAOS IN THE LONG-TERM INTERVENTION PER SAMPLING

Albumin

The *Asp* supplementation of 24 weeks significantly changed the albumin concentration, $X^2(2) =16$, p<.001. The albumin decrease was significant over the six-month intervention with a high effect size: -4 (-8– -2) g/l, r=.77, p=.003. Between the two periods, the decrease was significant between 12 and 24 weeks and not significant between baseline and 12 weeks:-5 (-6– 1) g/l, r=.58, p=.048 and -3 (-4– 0.0) g/l, r=.55, p=.069, respectively.

➢ Hb, RBC, and Iron

The following part presents the outcomes of haemoglobin concentration, red blood cell count, and iron concentration (see table 10.24 in appendix).

The long-term *Asp* supplementation showed a significant effect on Hb over the six-month intervention: $X^2(2)= 6.48$, p=.039. The applied *post hoc* test showed a significant decrease over the entire intervention over 24 weeks of -0.6 (-1.1– 0.0) g/dl, r=.63, p=.027. The decrease had a similar, non-significant extent during the two periods: for the first period-0.2 (-0.7– 0.0) g/dl, r=.27, p=.24, and for the second period -0.3 (-0.8– 0.3) g/dl, r=.42, p=.78.

Regarding the minimal values at the three sampling times t0= 8.9 < t12= 9.1 < t24= 9.6 g/dl, an absolute light increase in values was observed.

The RBC count showed also a significant change over the long-term supplementation: $X^2(2)$= 11.3, p=.003. The six-month decrease was significant: r=.80, p=.003. However, the main decrease occurred during the first intervention period of three months: -0.3 (-0.4– -0.1) tetra/l, r=.66, p=.028. The second period showed a slight increase for 7 out of 9 patients tested: 0.0 (-0.2– 0.2), r=.03, p> .99.

But, there was no significant change in the iron concentration over the six-month *Asp* intervention. The Friedman ANOVA resulted in significant results, $X^2(2)$= 0.222, p=.895. However, eight patients showed an increase, while seven had a decreased value. The median values showed an increase over the intervention from 0.7 to 0.9 mg/l.

➢ Lipid Profile

The following part presents the outcomes of the lipid profile, i.e. total cholesterol and triglyceride (see table 10.25 in appendix).

Cholesterol significantly changed during long-term *Asp* exposure $X^2(2)$= 7.731, p=.02. However, the *post hoc* test showed no significant change during the intervention with corrected *p* values. The highest effect size was registered during the first three months -0.13 (-0.22– 1.53) g/l, r=.53, p=.09. The second period, from 12 to 24 weeks, showed no change: 0.00 (-0.10– 0.23) g/l, r=.09, p>.99. The results of the overall intervention confirmed a small effect size: r=.26, p=.83 for a median decrease of -0.8 (-0.3– 0.11) g/l.

The long-term intervention presented no significant change of the triglyceride concentration over six months $X^2(2)$= 5.303, p=.07. In order to describe the variation, a corrected *post hoc* test was carried out. During the first three months, nine patients showed an increase: 0.02 (-0.19– 0.18) g/l, r=.27, p>.99, followed by a decrease in 12 patients during the second three-month period: -0.12 (-0.28– 0.0) g/l, r=.45, p=.193. The overall result was a decrease in 12 patients, r=.37, p=.37.

➢ Renal Function

The following part shows the assessed renal function variables creatinine and urea, with the subsequent calculated eGFR (see table 10.26 in appendix).

Creatinine

There was a significant change in creatinine concentration during the study, $X^2(2)= 17.8$; p<.001. The *post hoc* test confirmed a significant increase during the first period: 1 (0– 2) mg/l, r=.63, p=.027, followed by a non-significant increase between 12 and 24 weeks: 1 (0– 2) mg/l, r=.33, p=.519. The increase over the six-month intervention was significant with a high effect size: 2 (-1– 3) mg/l, r=.80, p=.003.

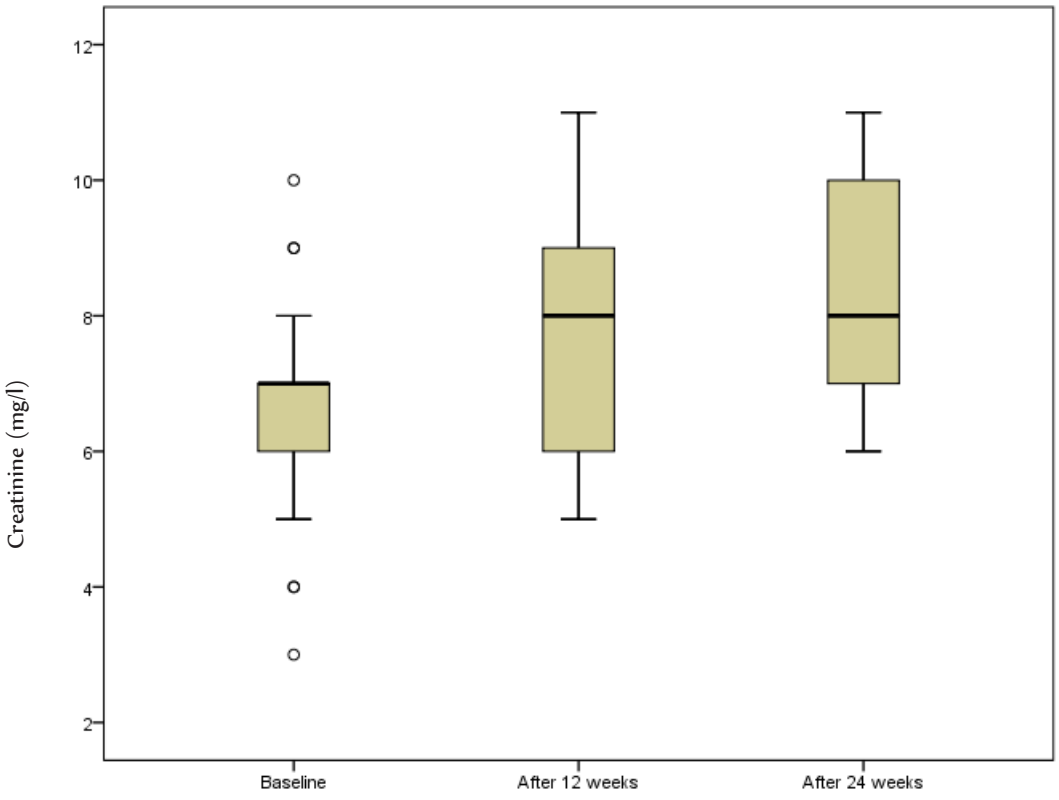

FIG 3.15 BOX PLOT REPRESENTING CREATININE IN THE LONG-TERM INTERVENTION PER SAMPLING

The long-term intervention had no significant effect of the urea over the six-month intervention: $X^2(2)= 0.215$, p=.898. The fluctuation was +/- 0.01 g/l of urea.

There was a significant change of the eGFR during the intervention: $X^2(2)= 13.765$, p=.001. The first period had a significant decrease of -0.1 (-20.6– -2.3) ml/min, r=.64, p=.024, where

as the second period had one of-6.4 (-9.5– 0.7) ml/min, r=.48, p=.150. Overall, there was a significant median decrease of -14.3 (-20.1– -6.81) ml/min, r=.84, p=.003.

- **Crossover Intervention**

The crossover group included patients who were on placebo for three months, followed by a three-month *Asp* supplementation. As the population is dependent, the differences occurring during each period were compared via the Wilcoxon rank test.

- **Immuno-Virological Marker**

The following marker describes the immunological status with CD4 lymphocytes, the CD38 expression on the CD8 lymphocytes, and the viral load see table 10.27).

CD4 Lymphocyte Count

The Wilcoxon rank test showed no significant difference in the CD4 median between the two periods: r=.20, p=.46. Both presented a decrease of -33 (-87– 14) cells/mm^3 during the placebo period compared to a decrease of -14 (-71– 131) cells/mm^3 during the *Asp* period. However, seven patients presented a positive and six a negative evolution of the CD4 after the implementation of *Asp*.

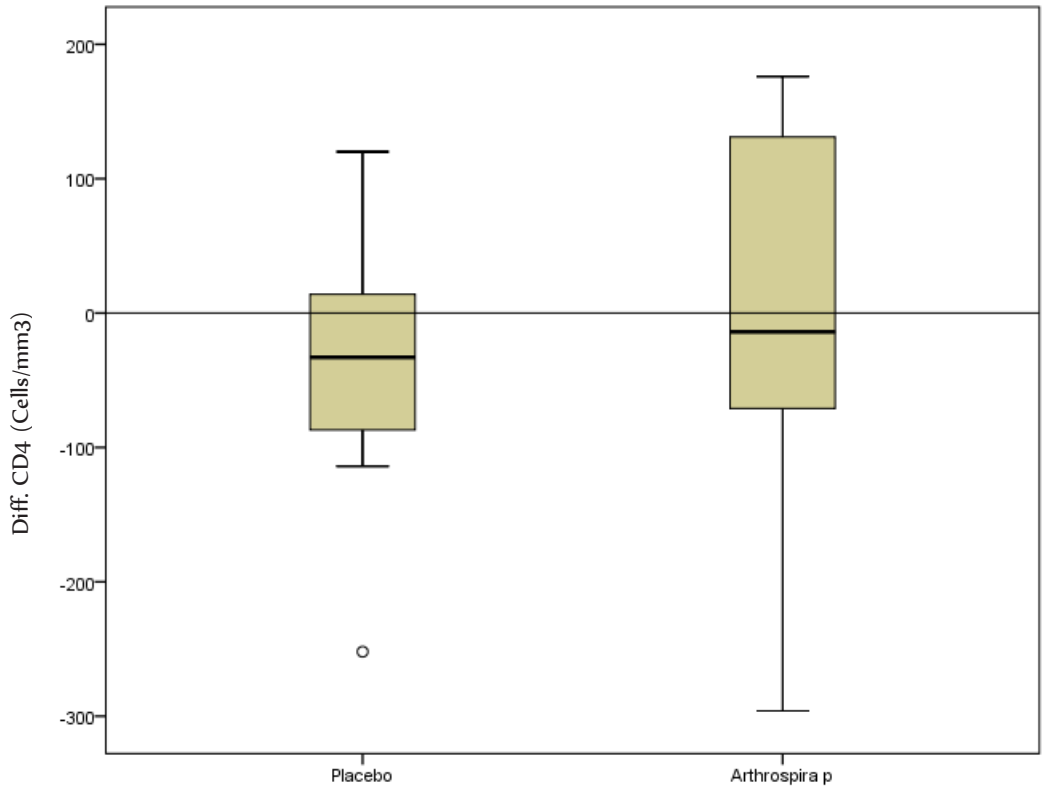

FIG. 3.16 BOX PLOT REPRESENTING THE CD4 DIFFERENCE OCCURRING IN THE CROSSOVER POPULATION DURING THE TWO SUPPLEMENTATION PERIODS: PLACEBO AND UNDER ASP

The VL decreased over the six-month intervention by -0.4 (-0.6– -0.2) log10. However, the difference between the two periods was not statistically significant: r=.13, p=.65. The tendency of a decrease seemed more accurate when *Asp* was given, placebo 0.0 (-0.4– 0.2) log10 and *Asp* -0.2(-0.4– 0.1) log10.

CD38-CD8 Lymphocyte

There was no significant difference between the placebo and *Asp* treatment period: r=.26, p=.35. The intervention yield with placebo was -2.4% (-11.3– 0.4) and *Asp* of 0.0% (-11.2– 3.5). However, the expression of CD38 on the lymphocytes tended to decrease over the entire intervention by -7.64 % (-16.1– 2.4).

- **Nutrition Outcome Variables**

The following variables are regrouped per outcomes or relationship.

➢ Body Weight, TAOS, Albumin

The following part presents body weight, TAOS serum variables, and albumin concentrations (see table 10.28 in appendix).

Weight

There was a significant difference in body weight change between both periods: r=.67, p=.015. The changes consisted of an increase of 0.4 (0.2– 2.5) kg during the placebo compared to a decrease of -1.1 (-2– 0.2) kg during the *Asp* period.

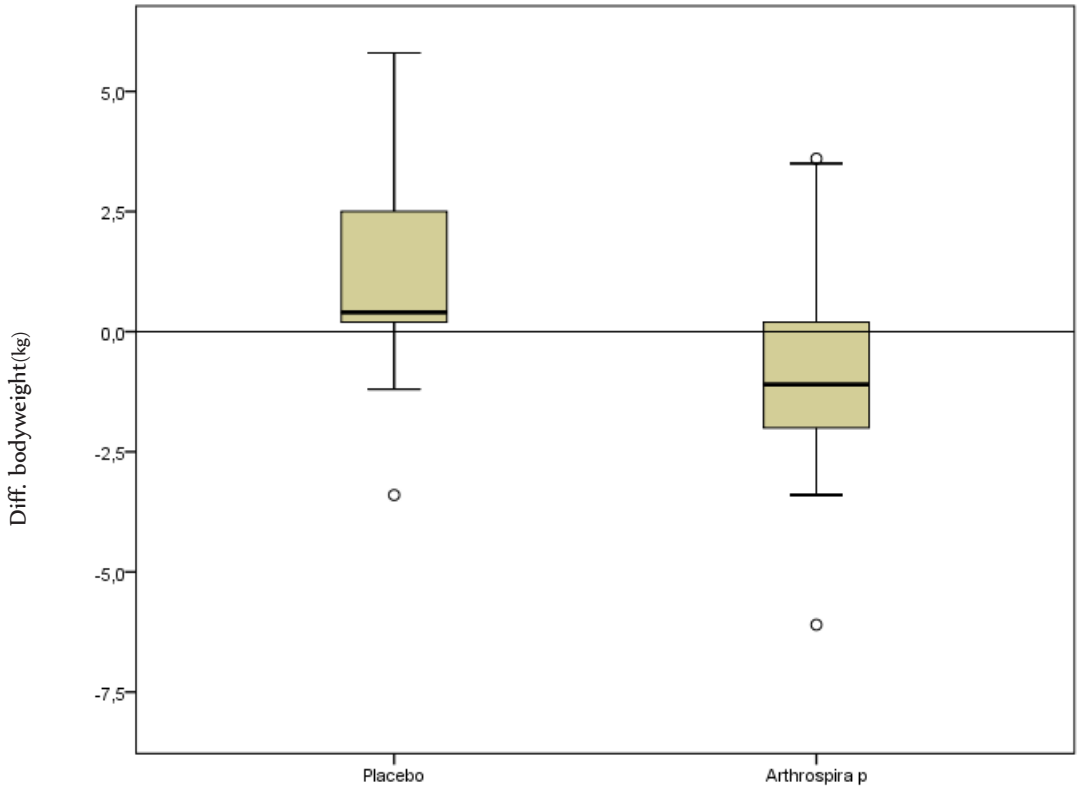

FIG. 3.17 BOX PLOT REPRESENTING THE BODY WEIGHT DIFFERENCE OCCURRING IN THE CROSSOVER POPULATION DURING THE TWO PERIODS: PLACEBO AND UNDER ASP

Total Antioxidant Status

The changes in TAOS values differed significantly between the two periods: r=.80, p=.004. During the placebo period, the median decreased by -54 (-103– 00) µM, compared to an increase of 67 (44– 243) µM after *Asp* implementation.

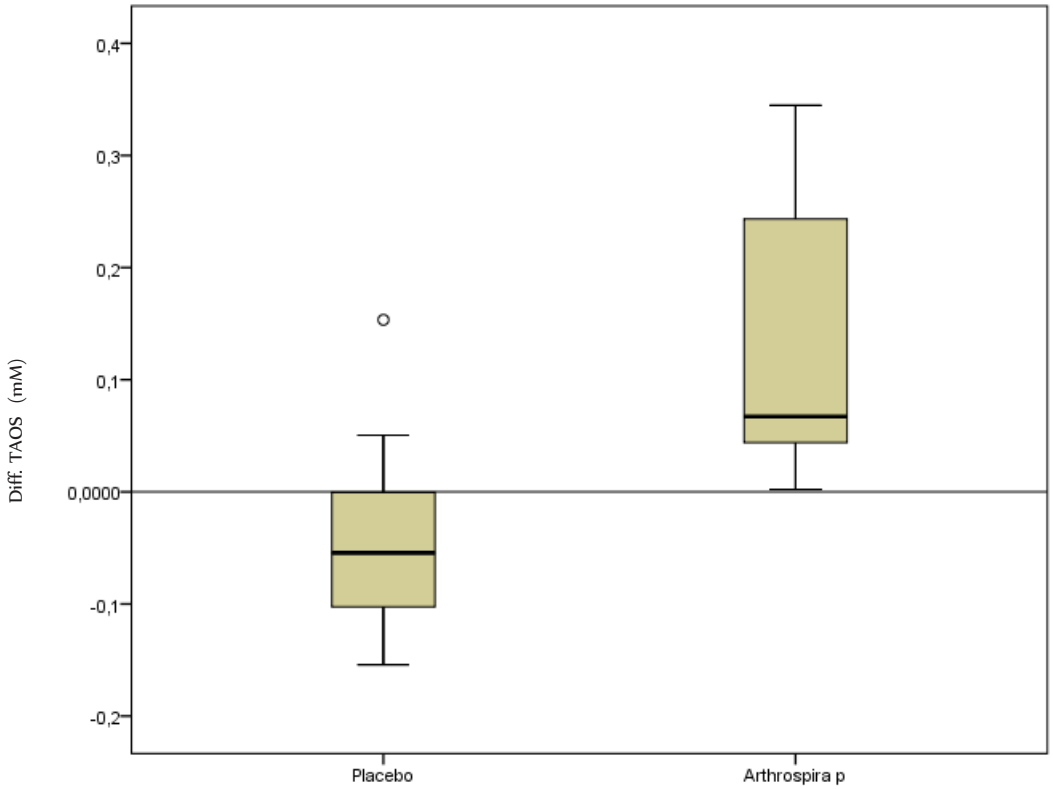

FIG. 3.18 BOX PLOT REPRESENTING THE TAOS DIFFERENCE OCCURRING IN THE CROSSOVER POPULATION DURING THE TWO PERIODS: PLACEBO AND UNDER ASP

Albumin

The occurring albumin changes between the two periods did not differ significantly from each other: r=.37, p=.484. The two periods showed the same tendency with median decreases of -2 (-5– 0) g/l during the placebo and 0 (-4– 1) g/l during the *Asp* period.

- Hb, RBC, Iron

The following part presents: haemoglobin concentration, red blood cells count, and iron concentration (see table 10.29).

There was a significant difference in the haemoglobin concentration between the placebo and *Asp* periods: r=.54, p=.05. There was a decrease of -1.1 (-1.3– -0.5) g/l during the placebo in contrast to an increase of 0.2 (-0.7– 1.0) g/l during the *Asp* period.

The change in RBC significantly differed between placebo and *Asp*: r=.82, p=.004. During the placebo period, the RBC decrease was -0.4 (-0.5–-0.3) tetra/l. In contrast, during the *Asp* supplementation, there was an increase of 0.3 (-0.2– 0.4) tetra/l.

The analyses of the iron difference registered during the two periods resulted in a non-significant difference between the periods: r=.09, p=.726. The data showed a larger spread for the placebo 0.0 (-0.3–0.3) mg/l and alight increase of 0.1 (-0.1– 0.2) mg/l during the *Asp* period.

➢ Lipid Profile

Cholesterol

There was no significant difference between the placebo and *Asp* period: r=.03, p=.917. Median TC decreased during the placebo period by -0.06 (-0.18– 0.0) g/l, and increased by0.05 (-0.15– 0.14) g/l in the *Asp* period (see table 10.25).

Triglyceride

Triglycerides did not differ significantly between the placebo and *Asp* group: r=.27, p=.328. However, there was an increase of 0.07 (-0.16–0.57) g/l during the placebo period compared to a decrease of -0.24 (-0.36–-0.08) g/l in the *Asp* period.

➢ Renal Function

The variables below define the renal function for the present intervention, i.e. by serum creatinine, serum urea concentration, and the calculated eGFR (see table 10.26).

The creatinine changes occurring during the two periods did not differ significantly from each other: r=.48, p=.083 (see figure below). However, creatinine remained the same during the placebo period compared to an increase after the *Asp* period: 0 (-2– 1) mg/l and 1 (0– 3) mg/l, respectively.

Creatinine

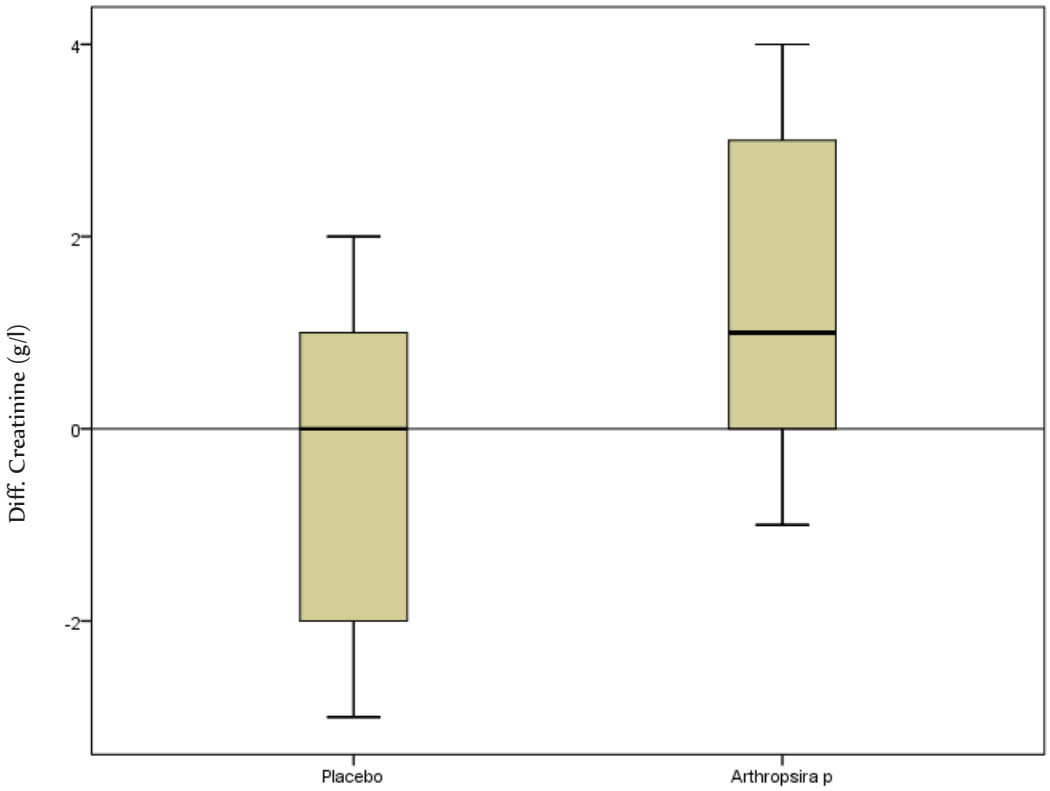

FIG. 3.19 BOX PLOT REPRESENTING THE CREATININE DIFFERENCE OCCURRING IN THE CROSSOVER POPULATION IN THE TWO PERIODS: PLACEBO AND UNDER ASP

Urea

There was not significant change in urea concentration in the two periods: r=.08, p=.263. The placebo period resulted in a decrease of -0.02 (-0.05– 0.01) g/l and the *Asp* supplementation period resulted in a low change of 0.0 (-0.02– 0.07) g/l.

The change of eGFR in both periods did not differ significantly but showed a high effect size in the periods: r=.51, p=.064. The placebo period resulted in an increase of 2.7 (-6.3– 4.6) ml/min. During *Asp* supplementation the eGFR decreased by -4.9 (-18.9– 0.1) ml/min.

3.6 Resumption of the Intervention

The different interventions were resumed by outcome for a better comparison. The effect size and the tendency have been summarized in the table below. The tendency was mentioned when it overstepped the variable method accuracy.

TABLE 3.12 TENDENCY AND EFFECT MAGNITUDE BY VARIABLE AND SUPPLEMENTATION

D = decrease; S = stable; I = increase; * = significant

	RCT		Long-term		Crossover		
Variable	**Plac1 n=28**	***Asp*1 n=30**	***Asp*1bis n=17**	***Asp*2 n=17**	**Plac1bis n=13**	***Asp*2bis n=13**	**Variable Accuracy**
CD4	D* r=.67	D* r=.61	D* r=.62	S r=.21	D r=.44	S r=.07	36 cells/mm^3
VL	S r=.18	S r=.13	S r=.18	S r=.00	S r=.22	D* r=.66	
CD38	S r=.18	D r=.24	I r=.23	S r=.05	D r=.45	D r=.24	+/- 1%
Weight	I* r=.52	I r=.12	I r=.41	I r=.27	I r=.42	D r=.33	+/- 0.1 kg
Alb	D* r=.39	D* r=.42	D r=.55	D* r=.58	D* r=.59	I r=.22	+/- 0.9 g/l
TAOS	D* r=.48	I* r=.51	I* r=.59	I* r=.77	D r=.49	I* r=.88	+/- 8 µM
Hb	D* r=.54	D* r=.40	D r=.27	D r=.42	D* r=.63	I r=.17	+/- 0.1 g/dl
RBC	D* r=.68	D* r=.57	D* r=.66	S r=.03	D* r=.88	I r=.48	+/- 0.02 Tera/l
Iron	S r=.13	S r=.01	S r=.16	S r=.19	S r=.13	S r=.19	+/- 0.5 mg/l
TC	D r=.28	D* r=.55	D r=.53	S r=.09	D r=.34	S r=.01	+/- 0.027 g/l
TG	I r=.24	S r=.16	I r=.27	D r=.45	I r=.30	D r=.48	+/- 0.036 g/l
Creat	S r=.12	I* r=.59	I* r=.63	I r=.33	S r=.24	I* r=.65	+/- 0.05 g/dl
Urea	S r=.02	S r=.03	S r=.03	S r=.11	D r=.38	S r=.17	+/- 0.007 g/l
eGFR	S r=.04	D* r=.55	D* r=.64	D r=.48	I r=.09	D* r=.67	

r = effect size

4 Discussion

This section is divided into three main parts. The first part debates the population involved and the findings of the intervention with regard to the main objectives, i.e. the effect on the immune virology outcomes, inflammation, and concomitant events frequency, as well as the effect on the nutrition markers. The discussion continues by comparing the findings of existing *Asp* supplementation among HIV patients to current studies on PLWHA in sub-Saharan Africa. In part three the general practice of supplementation will be discussed, followed by the conclusion and the outlook sections for the present study.

4.1 Debate of the Results

Spirulina is assumed to have antiretroviral and immunity-enhancing properties (Hayashi, Hayashi et al. 1996; Rechter, Konig et al. 2006; Teas and Irhimeh 2012). Therefore, the current study assessed the effect of an *Asp* supplementation on pre-HAART HIV-infected women. The main objective was to investigate the effect on CD4 lymphocyte count. Two groups, one *Asp* group and one placebo group, received five grams per day of supplement or placebo, respectively, for three months.

With regard to CD4 lymphocytes there was no significant difference between the groups before and after the supplementation. However, more than half of the patients showed a decrease of more than 50 cells/mm^3. An increase was observed in 8.6% of all patients involved in the RCT. An increase of 100 CD4 cells was achieved by one patient in each group. Those two patients did not mention having taken any antiretroviral medication. Thus, the assumption that *Arthrospira platensis* has an effect on CD4 lymphocytes could not be proved in the present randomized control trial over three months. Since spirulina is sold on the public market with the mention of therapeutic effects, we recommend avoiding this mention.

- **Target Population**

Cameroon was the focus of this study due to the high rate of HIV infection for the region and mainly because spirulina is consumed in the country. Furthermore, the country is becoming a growing market for functional food. For example, in the Cameroonian megacities Douala and Yaoundé, spirulina is already sold as nutraceutical along with local concoctions and

Chinese natural medicine. The focus is on women's response to the infection ratio inequity because two thirds of PLWHA in Yaoundé are women. But more important for the success of this pilot trial, women in low and middle income countries were described as having better health awareness (UNAIDS 2012).

Yaoundé, the country capital, is a diverse city in terms of ethnic and socio-economic groups. Recruitment was performed in four different health care centres. These were the modern hospital of "La Caisse" from the Cameroonian health insurance (CNPS), the public HIV care unit in the popular urban area of Biyem-Assi, the HIV care unit in the religious hospital of Djoungolo, and the largest HIV care unit of the city in the central hospital, the 'Hôpital du Jour'. The study intended to recruit a representative group of the female HIV-infected population being monitored in Yaoundé. The eligibility criteria mentioned in §2.3, specify that the patients be in a stable phase of their infection, with no specific ongoing or upcoming medication intervention. These criteria followed the purpose of minimizing interferences on the marker set measured in the study.

- **Protocol Deviation**

While elaborating the proposal, the health centre in Cameroon started a HAART whenever the CD4 cell count of the patients got below 250 cells/mm^3. At the beginning of 2010, the national council in Cameroon (CNLS 2010), in line with international guidelines, raised the HAART start-up point to 350 CD4 cell/mm^3 (WHO 2009). To maintain the CD4 cell window of 250 CD4 cell/mm^3 chosen for the study, an elevation of 100 from 500 to 600 CD4 cell/mm^3 was decided for the inclusion criteria.

During the recruitment screening, a total of 80 blood tests to document CD4 cell count were carried out at the HDJ hospital laboratory. Fourteen tests presented a CD4 cell count under 350 cells/mm^3, resulting in HAART eligibility. From the total of 80 blood tests, 41 were over the inclusion limit of 600 CD4 cells/mm^3. For the 25 recruited patients, the CD4 values were measured at different days in a time lapse of two weeks maximum between them and at two different laboratories, the hospital laboratory, and for the baseline at the CPC, those values did not significantly differ from each other, Wilcoxon rank test r= .02; p= .842 (see table below). Patients were included after their screening test results. In case the baseline CD4 results did not match the fixed inclusion criteria, the patient was classified in the FAS population analysis set.

TABLE 4.1 CD4 CELL COUNT OF THE RECRUITED PATIENTS AT SCREENING IN THE HDJ AND AT BASELINE

Pre-HAART n=73

CD4 Cells/mm^3	Mean (SD)	Median (IQR)	Min-Max
Screening, HDJ	455 (72)	446 (390–515)	350–595
Baseline; CPC	463 (119)	449 (397–558)	139–815

A further inclusion criterion measured was BMI. No person present at the screening showed signs of acute undernourishment, defined by a BMI <18.5 kg/m^2. The median BMI of the recruited population was 22 (20 – 23) kg/m^2. The screening data showed that approximately every second patient had a BMI over 23 and every fourth over 26 kg/m^2. Thus, the inclusion criterion for BMI was increased from 23 to 26 kg/m^2. The present study's inclusion criteria resulted in an exclusion of women with a BMI >26 kg/m^2, rejecting acute overweight and obesity.

- **Compliance**

The intervention was divided into two supplementation periods of three months each. The compliance was handled in three phases: the two intervention periods and the phase between.

During the first period the main exclusion reason with a rate of 53% was "lost to follow-up" out of an overall rate of 20%. At the end of the RCT period, 12 patients, representing 55% of the overall exclusion rate of 38% for this phase started with HAART. Starting with a HAART therapy remained the main exclusion criterion during the second intervention period (16% exclusion), with 41% of the exclusions. However, this dropout was part of the study design, related to the recruitment of pre-HAART patients with a CD4 cell count below 600 cells/mm^3.

With regard to social variables, only one of the seven married women completed the study. Furthermore, the education level was higher among women who completed the study than those who dropped out. A higher educational level and being unmarried seemed to have a positive effect on compliance. Possible explanations could be the quotidian pressure due to

time-consuming family management and/or the lack of acceptance by the husband. For example in the current study, one husband announced by phone that his wife would not travel anymore to the study site.

- **Intervention Population**

The intervention population involved all participants who completed at least three months of intervention. The study population is defined as being adult women living with HIV. The main focus of the study was on patients that had not started an antiretroviral therapy, presented a CD4 count lower than 600 cells/mm^3, and had a BMI below 26 kg/m^2. The pre-HAART patients were randomly distributed into two groups, the *Asp* and placebo groups, and were supplemented for three months (RCT). After the first three months of intervention, an additional three months of exclusive *Asp* supplementation followed (OLI). Here, one group continued to get the *Asp* supplement, thus representing the long-term *Asp* supplementation group, while the former placebo group was now given *Asp* supplementation as well, and changed its name to the crossover group.

Before starting the OLI, patients who met the criteria for starting HAART therapy based on their CD4 count were encouraged to start the therapy and stop the nutritional intervention. Patients who wanted to stop the nutritional intervention could do so at any time.

To interpret the data of the 36 patients who achieved the six-month OLI, the prerequisites are as follows: the population is to be considered responsive to the intervention, and the results of the OLI group can only be discussed as they relate to the previous outcome conclusion of the RCT.

In the present discussion, the RCT outcomes are related to the long-term intervention. At the end of the chapter the crossover group is discussed separately.

- **Variable Consideration for Spirulina**

The analyses were achieved by focusing on the first three months between the two groups in order to document the superiority of *Asp* against the placebo (*Asp* n=28, placebo n=30). It should be noted here that there were no significant differences in blood and immune markers between the two groups at baseline. The discussion handles each outcome individually.

➢ CD4 Lymphocyte

As already mentioned, the main outcome of the study is to document the CD4 lymphocyte count difference between the two groups during the RCT. The two groups did not differ and presented the same tendency. With regard to the effect size, a small non-significant difference between the groups placebo r=.67 versus *Asp* r=.61 can be illustrated by the mean difference, placebo -65.2 (86.2) cells/mm^3 versus -55.7 (71.4) cells/mm^3 for the *Asp*. An effect size over .5 stands for a large effect, and it has to be assumed that the decrease concerns most of the involved patients in both groups. As the difference was not significant, the interpretation is that the study showed no difference between the two products on this variable over the given time.

The long term *Asp* intervention (n=17), confirmed that CD4 cells decrease during the first three months with a large effect size of r=.62. However in the following period, nine patients registered an increase of their CD4 cells. This results in a median increase of 32 (-45– 70) cells/mm^3 defined by a small effect size of r=.21. The increase in the second period could not compensate for the precedent lowering as shown in the scatter plot below.

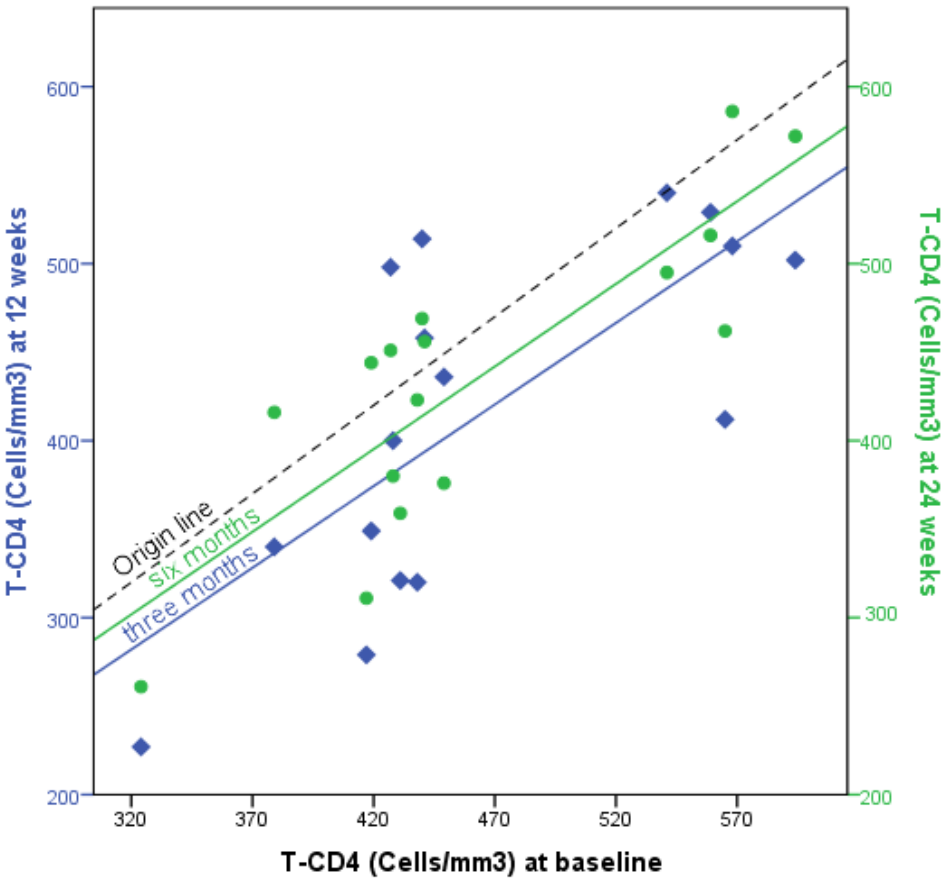

FIG.4.1 SCATTER PLOT WITH DOUBLE Y-AXIS SHOWING THE CD4 VALUES OF THE LONG-TERM GROUP

The hashed black line is the origin line, the blue lozenges and line represent the three-month intervention (y=0.86x+ 10.8) with an effect size of R^2=.52. The green dots and line represent the six-month intervention (y=0.69x+ 99.5) with an effect size of R^2=.69. Over the first three months, a median decrease was observed, illustrated by the blue line, followed by a slight increase after six months represented by the green line.

To interpret the intervention effect, it is important to consider the specificity of the long-term study population. As mentioned, this population has to be considered as pre-selected due to the fact that they had accomplished a six-month intervention. Moreover, the accuracy of the measuring method is 36 cells/mm^3. As the observed change stays within the method accuracy, the result is so hard to relate to the supplemented product. The assumption that a nutritional product such as *Asp* can affect the immunological progress was not proved. The interpretation of the CD4 lymphocyte count in relation to infection evolution and to the specific population will be done in "Discussion in Context" §4.2.

➢ Viral Load

An antiretroviral effect was documented by examining the blood's viral load. The presently used VL quantification method is restricted to the HIV-1 type virus. The study did not test for a double infection with both HIV-1 and HIV-2. However, two patients showed no VL value and were tested positive for HIV type 2, one in each group, and could not be considered for this outcome.

As expected, the VL at baseline was high with 5.5 (4.3– 6) log10, 315,000 (20,000–1,000,000) copy/ml. Such high values are common among pre-HAART patients with progressing immunodeficiency (Sabin, Devereux et al. 2000). The HIV-1 viral load did not significantly change over the RCT period, neither in the *Asp* nor in the placebo group (no difference between the group difference, r=.03, p=.774). Three outlier patients (over 2 standard deviations from the median) showing a VL decrease over the intervention indicated no medical intakes, but an intake cannot be excluded. The patients were asked about self-medication, but the use of street medicine made a proper recording very difficult.

The long-term intake of *Asp* did not present any significant change on VL value. The value seemed to decrease slightly, but the changes appeared to be within the methodological fluctuation range.

➢ Immune Activation

The immune activation profile is defined by the expression of the protein CD38 on CD4 and CD8 lymphocytes and on monocyte cells. In the current study, the expression showed strong correlations between the CD38 expression on CD4 and CD8 cells at baseline: Rho=.64, p<.001, and a correlation at baseline between CD38 expression and higher viral load: Rho= .47, p<.001, but no correlation to CD4 count. According to the literature, the activation is reported by the expression of CD38 antigen on the CD8 lymphocyte (Liu, Cumberland et al. 1997).

During the RCT, the ratio of CD8 cells expressing CD38 showed a slight decrease in both groups. However, it did not differ between the groups (r=.04, p=.732). The decreases inside the groups were non-significant but seemed to affect more patients in the *Asp* group (r=.24; p=.185) with a median decrease of -1.3% (-5.8– 2.0) as opposed to the placebo group (r=.18; p=.327) with -0.9% (-4.2– 1.6). An overall group analysis showed that the immune activation effects registered during the intervention were influenced by the initial activation level. The differences occurring correlated significantly with the absolute values at baseline: an initial high baseline value allowed a higher decrease (Rho= -.325, p= .010). Moreover, among seven patients presenting a CD38-CD8 expression over 40%, five had concomitant events. Due to the low number of patients with high immune activation and the lack of data on the vigorousness of the events, it is not possible to quantify the dependency between the two observations. In reverse, the concomitant events do not seem to be related to a high immune activation, as only five cases of concomitant events could be registered for a total of 24 patients with a high immune activity.

During the long-term intervention, the immune activation fluctuated from an initial baseline value of 18.0% (14.8– 30.5) to 18.7% (14.6– 27.3). However, the absolute percentage of CD38-CD8 lymphocytes for the patients stayed below 30%. The scatter plot below documents that no increase was observed. Regarding these results, it can be assumed that the *Asp* intervention did not increase the expression of CD38 on lymphocytes.

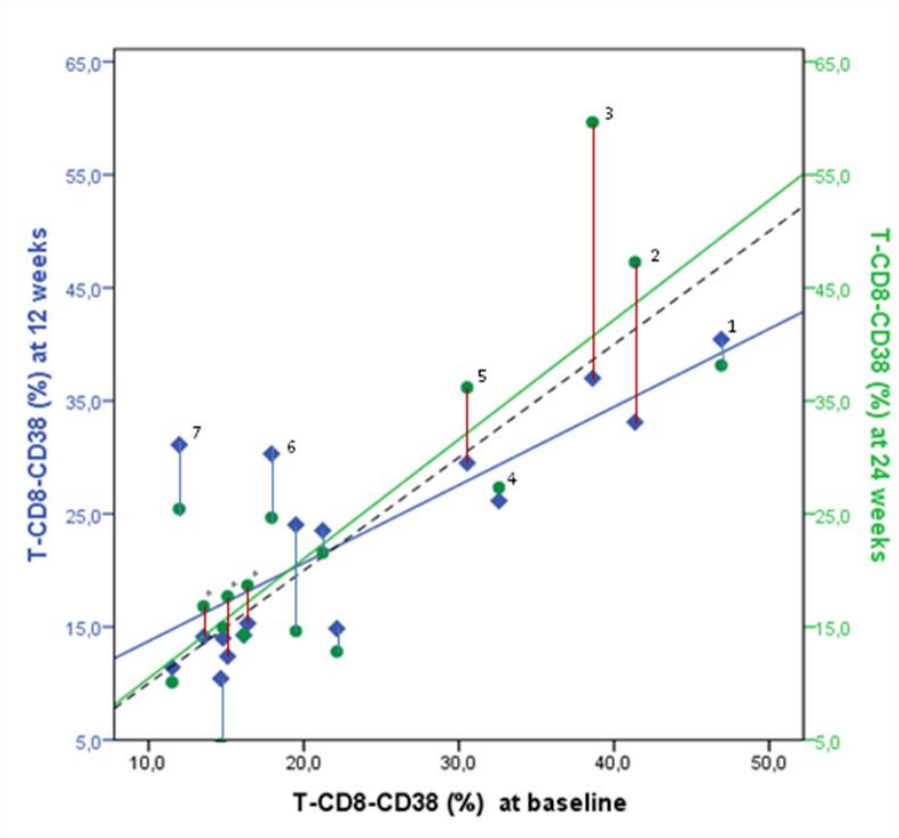

FIG 4.2 SCATTER PLOTOF T-CD8- CD38% WITH DOUBLE Y-AXIS PRESENTING THE LONG-TERM PATIENTS OVER THE SIX-MONTH ASP INTERVENTION.

The blue lozenges represent the first three-month supplementation summaries in the blue regression line (Y= 0.691X + 6.83), R^2=.61. The green dots stand for the value after an *Asp* supplementation of six months, summaries in the green regression line (Y= 1.057X -0.124), R^2=.68. The blue lines between the points stand for a decrease, and the red line that stands for an increase of the CD38 expression on CD8 cells. The numbers are resumed in the table below and stand for the patients over an activation of 30% CD38.

Number or * in graph	Concomitant events	Inflammation or an aemia status
1	Diarrhoea	Inflammation and Anaemia
2	Malaria in period 1	Normal
3	Fatigue in period 1	Anaemia
4	Fatigue in period 1	Anaemia
5	Malaria in period 2	Inflammation and anaemia
6	-	Anaemia during period 1
7	-	Inflammation at baseline
*	Malaria or fatigue	Normal

The graph shows that patients with immune activation increase were linked to concomitant events, inflammation, and/or anaemia.

➢ Concomitant Events

The outbreak of concomitant events can be an indicator for the patients' vulnerability. In the present study, concomitant events were documented during the monthly monitoring visits (see §3.9). The patient flyer was checked for recorded events and documented by the physician in the patient file. The documentation of events by the patients was not necessarily related to disease. The crossed concomitant events were cross-checked by the physician based on a consultation. Additionally, the physician recorded any medication taken.

The results showed a higher proportion of concomitant events in the placebo (70%) than in the *Asp* (43%) group. However, opportunistic infections such as candidoses and sona, were very low in both groups. One must consider that the appearance of severe concomitant events or the intake of some medication could lead to exclusion. The concomitant events must be considered in combination with the exclusion reasons. Infection-related exclusion reasons were: "start of HAART" and "start of relevant concomitant medication." Overlapping the AIDS-related concomitant events with AIDS-related exclusions at the end of the RCT summarizes the progression of AIDS status on the population over the first period. The placebo group had three cases of AIDS-related events and no related exclusion. The *Arthrospira p.* group had five exclusions due to starting HAART or starting co-medication but no AIDS-related events. The intervention products did not differ between the groups with regard to AIDS progression over the three-month intervention.

During the OLI, the three main events were malaria with a prevalence of 23%, gastrointestinal symptoms with 16%, and diarrhoea with 13%. The possible relationship between malaria and an *Asp* supplementation will be discussed in the context of iron supplementation in malaria-endemic areas in part §4.2. The prevalence of gastrointestinal symptoms and diarrhoea observed over the long-term intervention could be due to the digestive clash of *Asp*. However, a comparison of the event frequency between placebo and *Asp* in the RCT present the same rate for gastrointestinal symptoms and diarrhoea in both groups. Furthermore, the implementation of *Asp* in the crossover did not show an enhancement of those digestive events. No relationship seemed to exist between the

product and gastrointestinal symptoms or diarrhoea. However, due to the small occurrence of concomitant events, it is difficult to rule out or conclude a relationship between the supplement and events. It can be maintained that the study confirms the US safety analysis on the lack of relationships between the supplement and adverse events (Marles, Barrett et al. 2011).

➢ Inflammatory Outcomes

The inflammation marker CRP is an exponential variable, with a normal level under 6 mg/l, crossing this level in case of acute inflammation. The C-reactive protein concentration showed no significant increase in either of the two RCT groups. The effect can be described as follows: the data range condensed for the *Asp* (r=.06) while it spread out for the placebo group (r=.18). The absolute number of patients with acute inflammation increased from three in each group at baseline to six in the placebo and five patients in the *Asp* group after 12 weeks. The increase in the absolute number of patients presenting an inflammation status could be due to opportunistic and/or parasitic infections.

Overall, six out of eleven patients with inflammation suffered from anaemia. The scatter plot below shows the inflammation represented by the CRP on the Hb concentration with additionally reported malaria cases. *Plasmodium* is a red blood parasite exacerbating anaemia and the innate immune system (Perkins, Were et al. 2011). Seven out of eleven presenting an inflammation had malaria symptoms, and most of them were anaemic. The relationship will be brought into context in part 4.2.

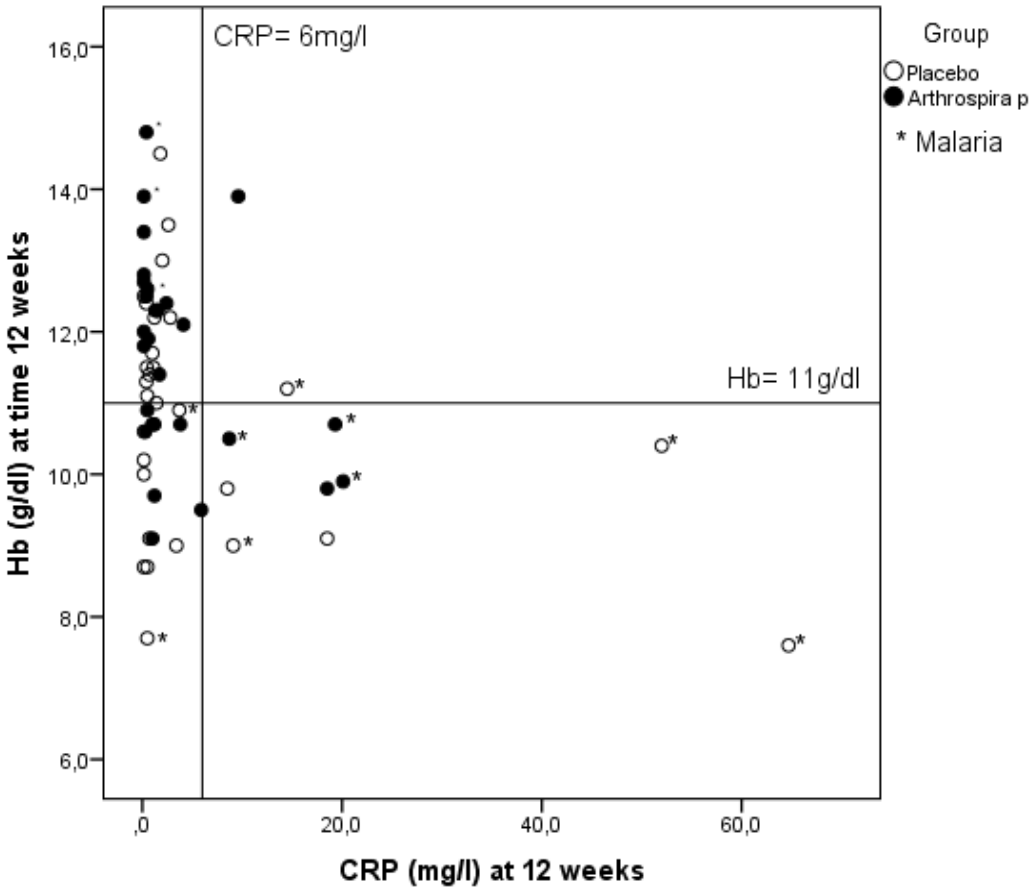

FIG. 4.3 SCATTER PLOT REPRESENTING HAEMOGLOBIN CONCENTRATION OF THE CRP CONCENTRATION AT 12 WEEKS OF INTERVENTION.

The vertical line represents the cut-off point CRP= 6 mg/l; all patients on the right hand side had an active inflammation, in total 11. The horizontal line stands for the anaemia cut-off point at Hb= 11 g/dl; 21 patients were below this line. The star shows patients who developed malaria over the period.

In the long-term intervention, one to two cases of active inflammation were observed per group. The result corroborates the assumption regarding the comparison of patients compliant with the six-month intervention with excluded patients, that the patients of the six-month intervention had a better health status.

➢ Nutritional Outcomes

The following markers are indicators of general health status by describing nutrition metabolic outcomes. Every metabolic variable stands in a multi-causal relationship to the body, the environment, and the infection. They are indirect markers of the infection and are assessed to document health complications associated with HIV infection. Some are postulated to become alternative clinical predictor markers of the infection course.

Weight

The intervention did not aim to increase body weight but to ameliorate the quality of proteins and implement anabolic nutrients. The energetic gain from a five-gram daily supplementation with *Asp* or placebo was negligible. The daily intervention added 21 calories. The total daily recommendation for an HIV-infected women is 2.4 kcal (Emmanuel 2006). The supplement thus represented 1% of total energy supply. The three grams of protein in capsules comprised 5-6% of the recommended 50 grams of protein intake per day. The hypothesis implies that the intervention would have a marginal effect on weight gain, independent of the product, *Asp*, or placebo.

The present intervention results showed a weight increase with large size effects inside both groups (placebo, r=0.5 and Asp, r=012). The groups showed no difference between them (r=.05, p=.697). The increase is not product related, and since it is not expected at this infection step, it seems to be related to involvement in the intervention. A more detailed explanation will be given in section 4.2.

The long-term *Asp* exposure group confirmed the weight effect with a constant significant increase of 1.5 (0.3– 3) kg over the six-month intervention (r=.49, p=.049). The major increase occurred during the first period with an effect size of r=.41. The registered weight evolutions over six months can be considered a stabilization of body shape.

Total Antioxidant Status

The TAOS measure represents the body's own antioxidant potential present in the blood serum (Mariken, Dallinga et al. 2004). The method used is a robust detection of the capacity of the sampled serum to reduce ABST. The measurement of total antioxidant serum capacity was reported to provide an integrated index as opposed to a simple summation of measurable antioxidants (Ghiselli, Serafini et al. 2000). The changes occurring in the two RCT

groups differed significantly: r=.51,p<.001. The placebo group presented a decrease of -22 (-64– 19) µM, which is consistent with the infection progression as described in the literature (Coaccioli, Crapa et al. 2010). The increase observed in the *Asp* group was 56 (1– 98) µM. The effect sizes registered for both trends were high with r=.51 for the increase in the *Asp* group and r=.48 for the decrease in the placebo group. Thus, *Asp* supplementation showed a positive effect on the antioxidative potential and seemed to be superior to the placebo. Furthermore, the increase shows a dependency on the initial TAOS baseline value. The increase in the *Asp*-supplemented group was negatively correlated with the baseline values Rho= -.436, p=.02. This dependence may support the effect of *Asp* that restores the antioxidant balance.

Long-term *Asp* exposure confirmed the results of the RCT. The TAOS value showed a continuous improvement. The increase was documented by a very large effect size between baseline and six months with a median increase of 198 (88– 254) µM, r=.85. The effect size was larger in the second period in comparison to the first one. It could be assumed that the supplementation effect increases with the intervention time. The correlation with the initial baseline value provided similar results to the RCT, namely a strong significant negative correlation, Rho= -.767. The scatter plot below presents the long-term progression of TAOS. The value of 450 µM where the regression lines cross each other may show the normal lower limit of total antioxidative capacity. However, the mechanism behind rehabilitation on TAOS of *Asp* remains unclear and demands further research.

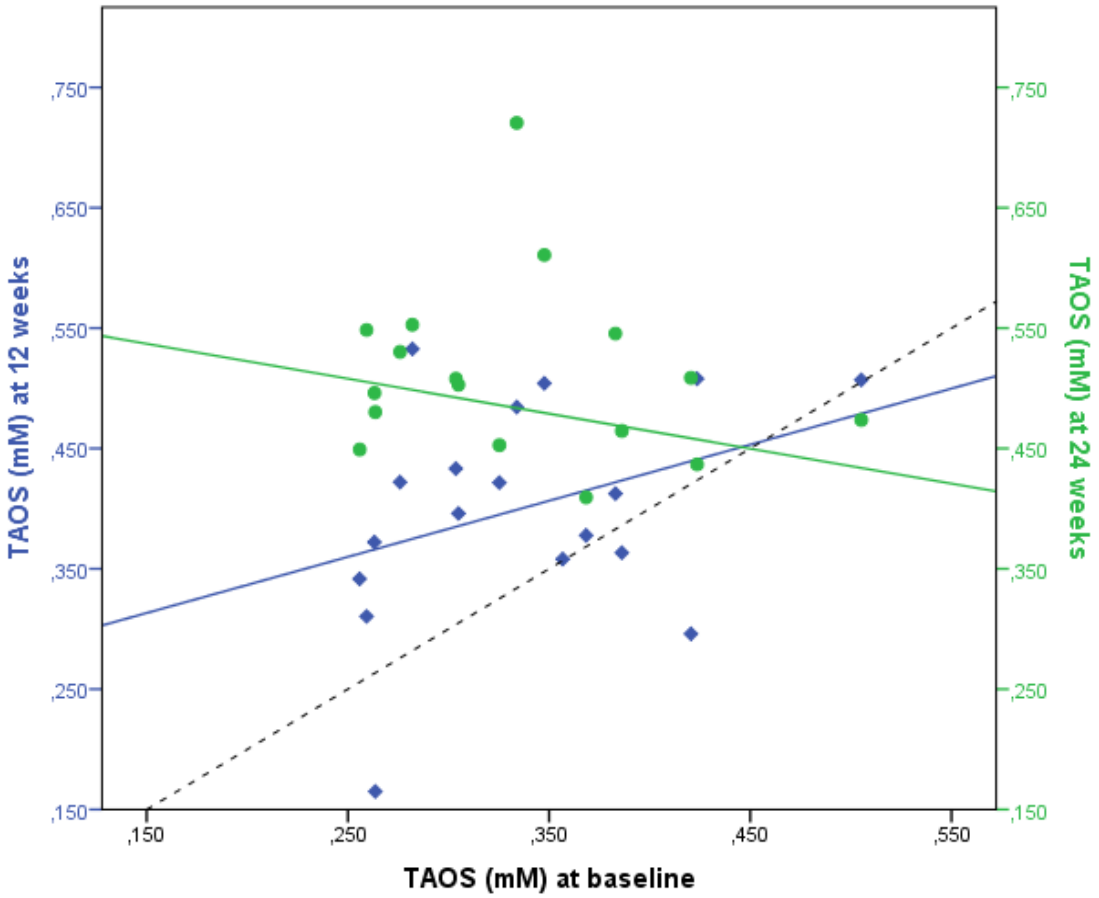

FIG. 4.4: SCATTER PLOT REPRESENTING TAOS WITH DOUBLE Y-AXIS PRESENTING THE LONG-TERM PATIENTS OVER THE SIX-MONTH ASP INTERVENTION.

The blue lozenges represent the first three-month supplementation, the green dots the value after six months of *Asp* supplementation. The regression lines are not representative, R^2 of .12 for the blue line and R^2= .021 for the green line, but resume a tendency that occurred during the periods.

Albumin

The blood marker albumin was studied as a prediction marker for an advancing infection resulting in AIDS; albumin is expected to slide below the normal value (Graham, Baeten et al. 2007). At baseline, the study observed a normal to high albumin concentration for almost all patients involved in the study: 50 (47– 52) g/l. Both groups had a significantly lower albumin level after three months: placebo r=.39, p=.034 and *Asp* r=.42, p=.026. In the long-term population, albumin decreased even more strongly in the second period over six months with -4 (-8– 0.2) g/l, with a magnitude of r=.77. The effect seemed to intensify with supplement duration. However, as the placebo presented the same tendency as the *Asp* group, the outcome can be ascribed to the environment and not related to the product (see table 10.1). One explanation could be that the baseline measurement took place during the

heavy rain season (mean rainfall 293 mm) when the patients maybe did not feel thirsty and were more likely to be dehydrated as opposed to the second sampling during the dry season (mean rainfall 19 mm), which could be a reason for a more accurate hydration at the end of the RCT. Differences in hydration between the two samplings could be responsible for the ensuing albumin reduction.

Haemoglobin/Erythrocyte

An abnormality in red blood cells and their oxygen-carrying protein, haemoglobin, is called anaemia (WHO 2011). The determination of anaemia through the Hb cut-off was adjusted to the study population. In the present study, Hb-relevant population factors were adult women, not pregnant, living at an altitude under 1,000 meters, non-smoking, and African (Sullivan, Zuguo Mei et al. 2008). Thus, the cut-off was Hb< 11 g/dl (WHO 2011). The CPC defines the normal haemoglobin range for adult women in Cameroon to be between 11 and 17 g/dl. A significant decrease was observed in both groups over the RCT. In the placebo group, the median difference was twice as high as in the *Asp* group. The effect magnitude showed significant Hb lowering for a large number of placebo patients r=.54, p=.003, compared to a medium effect size of r=.40, p=.03 in the *Asp* group. Regarding this result, an effect of *Asp* against Hb lowering could be presumed. There was a significant negative correlation for the study population between the baseline values and end assessment, Rho=-.288, p<.05. Thus, the higher the baseline value, the higher the decrease.

Parallel to the Hb decrease, RBC decrease also occurred in both groups (*Asp*: r=.57, p=.002 <placebo: r=.68, p=.001). The slight, non-significant difference between the two groups with regard to RBC was of 0.1 tetra/l (r=.21, p=.109). As haemoglobin is the major protein in RBC, the present results corroborate each other. An advanced explanation could be that the RBC count lowers and pulls down Hb concentration. With regards to haematocrit, the total RBC volume in the blood (see appendix), the two groups differed significantly from each other (r=.31, p=.039). The placebo RBC volume decrease was compared to a constant haematocrit value for the *Asp* group. The result is consistent with a study of senior patients in which the RBC count decreased while haematocrit and Hb increased (Selmi, Leung et al. 2011). The study concluded that spirulina may counteract anaemia in senescence.

The clinically relevant status of anaemic patients increased by five for *Asp* and three in the placebo groups. In the long-term intervention, the number of anaemic patients doubled from four at baseline to eight after the intervention. The increase in the absolute number of

anaemic patients can be explained in relation to inflammation, see §4.2. Furthermore, the Hb concentration decreased significantly over six months from 12 (11.4– 13.7) g/dl to 11.6 (10.4– 12.6) g/dl, p=.039, with a large effect size of r=.63.

Focusing on the outlier patients, the major decreases in Hb and RBC were related to malaria or AIDS symptoms. In contrast, the strongest Hb increases were related to medication as antibiotic therapy with Cotrim or Bactrim. These results strengthen the importance of a nutritional intervention as only an adjunct therapy in case of a major disease incidence.

Iron

In the current study, iron is an indicator of acute iron availability depending on diet and health state. The measurements of iron in the serum determine available iron in the blood (Chemistry 2013). During the RCT intervention, no significant changes were registered between and within the groups. The two groups presented the same median decreasing tendency of -0.1 mg/l (r=.11, p=.40). The effect sizes inside the groups were r=.01 for *Asp* and r=.13 for placebo. But the groups differed on the lower values. A low iron status at baseline seemed to limit a further decrease. Focusing on low iron concentration, an *Asp* versus placebo intervention seems to be better at preventing a large iron decrease. The number of patients did not permit us to run any statistical test besides a description of the value. The placebo had a 25%IQR value of -0.3 mg/l with a minimum of -0.9 mg/l, and *Asp* had a 25%IQR of -0.15 mg/l with a minimum of -0.5 mg/l.

Regarding the long-term supplementation, the increase in iron was not significant, but it showed a high effect size: r=.49. As mentioned before, the long-term population was a healthy selected population, which may explain why there was no further significant change in iron levels.

Lipid Profile

The two lipid markers, TG and TC, represent the main fatty acids stored in the blood (Cox and Garcia-Palmieri 1990). A comparison of the lipid profile at baseline between compliant and excluded patients showed that excluded patients tended to have higher TG and cholesterol values than compliant patients. With regard to this result, it could be supposed that low TG and TC are more common in a stable infection.

During the RCT, the total cholesterol concentration decreased significantly in the *Asp*-supplemented group: -0.14 (-0.47– -0.04) g/l, p=.004 with a large effect size of r=.55. In the

placebo group, cholesterol also decreased significantly with a small effect size for the variation: r=.28. The same effect of lowering TC was documented in both groups, but the affect was more accurate for patients in the *Asp* group than in the placebo group, (placebo r=.28 <*Asp* r=.55). The regression line in figure 4.5 shows that the decrease was higher among patients with initially high cholesterol levels.

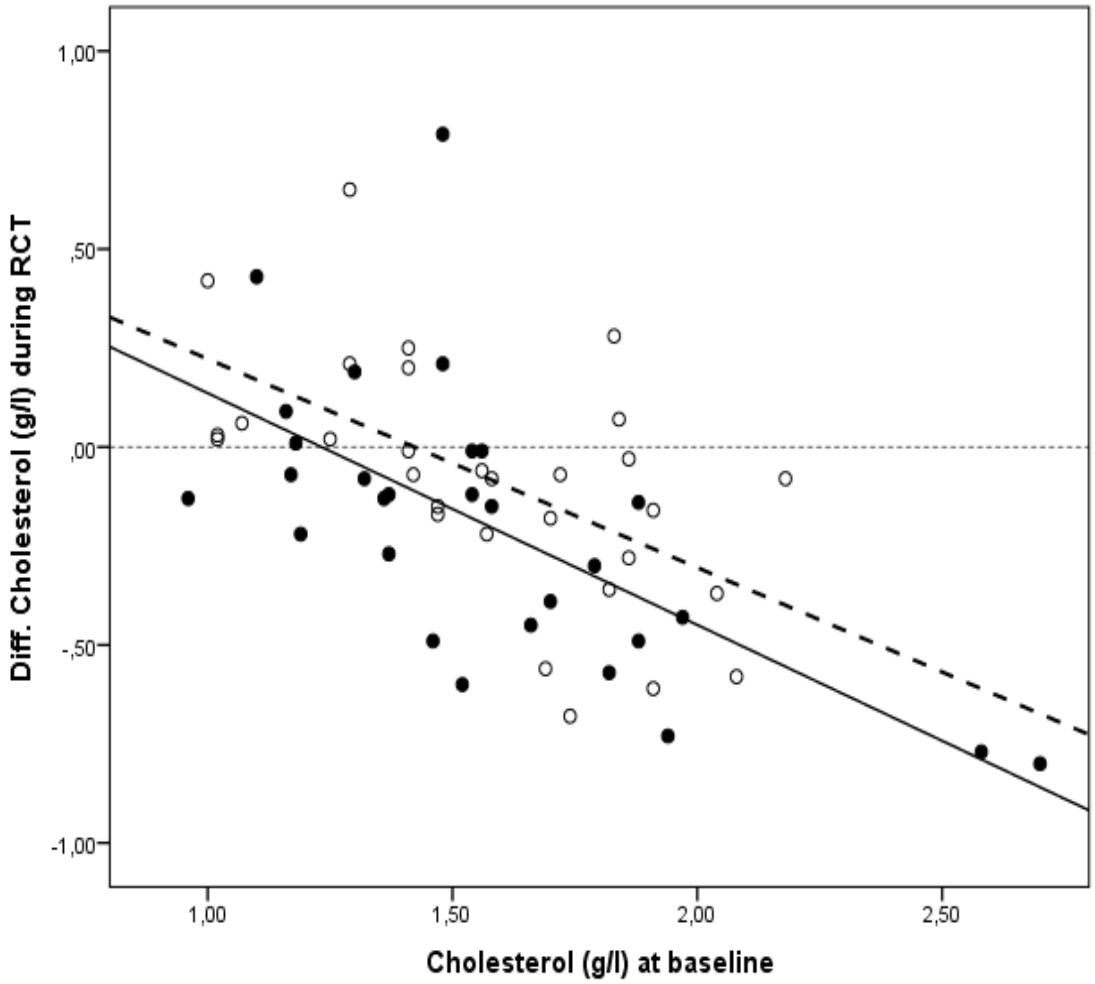

FIG 4.5 SCATTER PLOT OF THE TOTAL CHOLESTEROL DIFFERENCE IN THE BASELINE TOTAL CHOLESTEROL CONCENTRATION THAT OCCURRED DURING RCT

The black dots stand for the *Asp* group and the white for the placebo. The fat black line is the *Asp* regression line (y=-0.585x+0.721) R^2=.43. The fat hashed line is the placebo regression line (y=-0.527x+0.749) R^2=.39.

In the present study, a relationship between triglyceride increase and cholesterol decrease was observed. The tendency combining the two markers is resumed below in the scatter plot. Almost 82% of the *Asp* patients showed a decrease in TC as opposed to 60% among the placebo group. Most patients (60%) showed an increase in TG. The combination of lowering

TC and increasing TG is present in half of the patients (*Asp*: 57%; placebo: 40%). The two groups differ only in their cholesterol change. Resuming the changes in the lipid outcomes, for *Asp* a TC decrease with a stabilization of TG and for the placebo group a small decrease in TC and a small but non-significant TG increase could be established.

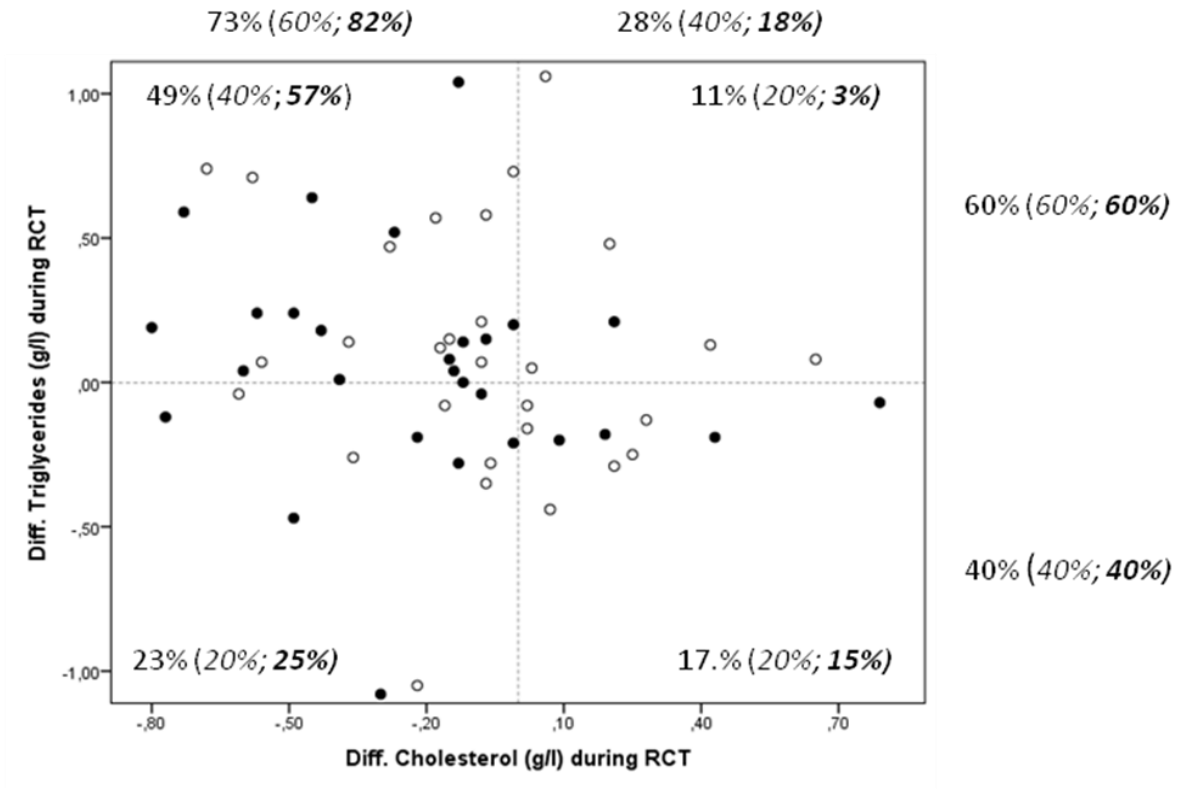

FIG 4.6 SCATTER PLOT OF THE CHANGE THAT OCCURRED DURING THE RCT IN CHOLESTEROL AND TRIGLYCERIDE AMOUNTS

The black dots stand for the *Asp* group and the white for the placebo. The patient distribution over the diagram is documented by the percent of cases expressing the tendency. The numbers are the total case tendency, in the parentheses the first number stands for the placebo group followed by the *Asp* percentage in bold.

The *Asp* effect on the lipid profile was consistent in the long-term intervention. In the first period, TG and TC decreased with high effect sizes: TC r=.53, TG r=.45. In the second period, TC stabilized at 1.28 (1.21– 1.51) g/l. The combination of the supplementation and ongoing infection may be one explanation for the lowering TC level. The meaning of a low TC will be discussed in context (see §4.2). Over the total intervention time of six months, 12 patients out of 17 registered a non-significant decrease in TG -0.06 (-0.26– 0.04) g/l, with a medium effect size of r=.37. The lack of a TG increase could present a beneficial effect during the course of an HIV infection.

Renal Function

The purpose of this measurement was to see if the intervention with an *Asp* supplement burdened renal function. Renal activity is measured indirectly by way of serum creatinine and urea concentration and is summarized by the estimated globular filtration rate.

Serum creatinine alone is a weak marker for renal function (Levey, Perrone et al. 1988). The serum values should be constant over time (Perrone, Madias et al. 1992). Factors that influence creatinine serum concentration are a rich protein dietary intake, and/or increased intense physical activity, and/or a change in filtration capacity of the kidneys (Perrone, Madias et al. 1992; King and Levey 1993). The normal value for the serum creatinine concentration for women is between 0.7– 1.2 g/dl. Above 1.5 g/dl, the serum creatinine concentration is considered abnormally elevated. During the RCT period, creatinine showed a significant increase in *Asp*-supplemented patients of 0.1 (0.0– 0.2) g/dl, with a high effect size of r=.59 as opposed to the placebo, where creatinine remained constant 0.0 (-0.1– 0.1) g/dl. The creatinine difference between the two protein-rich products was significant with an effect size of r=.35, p=.008. However, as both median values for the *Asp* group at baseline 0.7 g/dl and follow-up 0.75 g/dl were still below the cut-off of 1.5 g/dl, no clinically relevant action was taken.

Urea is an excretion product of protein ingestion (O'Connor and Summerill 1976). As both intervention products of the RCT are rich in protein, even if the absolute protein amount were supplemented, representing 5% of the daily requirement, urea concentration would not change within the two groups over time and would not differ. An additional five grams of protein per day had no effect on nitrogen metabolism and the elimination product urea.

The estimated GFR is calculated and summarizes renal activity including creatinine, albumin, and urea serum concentrations, as well as the age factor. A chronic renal disorder is reached at the cut-off point of eGFR <60 ml/min and a serum creatinine >2.0 g/dl. All patients participating in the RCT study had a low eGFR at baseline 57.6 (50.3– 69.0) ml/min, which can be related to a high albumin concentration. For the *Asp* patients, the eGFR was affected by an increase in creatinine and decrease in albumin that resulted in a low eGFR after the intervention. The eGFR value significantly decreased in *Asp*-supplemented patients by -7.3 (-17.2– 0.95) ml/min, with a large effect size of r=.55. There was no change in the placebo group. The renal burden seemed to be related to the *Asp* product.

For the patients involved in the long-term *Asp* supplementation, the creatinine concentration increase did not rise further after the first three months from a median baseline value of 0.7 (0.6– 0.7) g/dl to 0.8 (0.7– 1.0) g/dl. The serum creatinine was constant and equal to the creatinine production, representing 1-2% of the body's creatinine pool (Perrone, Madias et al. 1992). It can be assumed that the intake of *Asp* elevated the body's creatinine pool and thereby creatinine production and the serum creatinine level. An assessment of the fat-free mass of the patient via bioimpedence would have been helpful in describing the change in creatinine serum. Furthermore, measuring creatinine uraemia could have permitted the exclusion of filtration alteration by the nephron. A dose-dependent analysis is needed in order to explore the effect in more detail. In summary, an increase in creatinine was related to the *Asp* supplementation product, which was not the case for urea concentration.

- **Crossover Group**

There were only 13 patients in the crossover group. Thus, the interpretation of the results has to be done with caution. In the crossover group, patients first took the placebo for three months, followed by *Asp*. The presently named "crossover" group diverges from the usual appellation, as it includes a placebo control intervention on the same patients. This group should be considered selected because all participants could end the six-month intervention without starting HAART.

Focusing on the immune marker, the stable CD4 cell count over the first three months could be linked to the generally good health presented by compliant patients. There was no significant change between the two periods. However, the noted difference was an attenuation of the CD4 decrease from -33 (-87– 14) cells/mm^3 during the placebo to -14 (-71– 131) cells/mm^3 in the period under *Asp*. Half of the patients showed a positive development of their CD4 cell count after the implementation of *Asp*.

The viral load did not change significantly during the placebo supplementation period. However during the *Asp* one, the viral load tended not to decrease significantly, with a median of-0.2(-0.4– 0.1) log10.Since both changes occurring on the immuno-virological marker were not significantly different between the two periods, it has to be assumed that the measurements were marker variations and did not define a product-related tendency,

thereby confirming the lack of effect described in both of the other studies designed to run in parallel.

As to the immune activation, CD38-CD8, both periods showed tendencies to decrease. The overall decrease effect of -7.64% (-16.1– 2.4) was significant: r=.67, p=.048. The main decrease occurred during the first period under placebo: r=.45 > r=.24, compared to the *Asp* period. Furthermore, high CD38-CD8 levels at baseline may have been attributed to an adverse event such as malaria. The decrease in CD38-CD8 during the intervention can be due to *Asp* supplementation or the intake of antimalarial medication or antibiotics. No clear causal relationship could be drawn.

Nutrition Outcomes

In the crossover group, the change in body weight significantly differed between placebo and *Asp* periods: p=.015. In the placebo period, body weight increased by 0.4 (0.2– 2.5) kg compared to a decrease of -1.1 (-2– 0.2) kg in the *Asp* period. The switch between the two periods affected most of the patients, documented by a large size magnitude, r=.67. The implementation of *Asp* seemed to affect body weight, which was not the case in the other interventions. Events related to a weight decrease such as diarrhoea or gastrointestinal symptoms did not increase after the start of *Asp* supplementation. None of the patients mentioned any disturbance after changing from placebo to *Asp*.

Albumin tended to differ between the two periods. The albumin concentration in the crossover group continually decreased over six months from -2 (-5– 0) g/l during the placebo to 0 (-4– 1) g/l under *Asp*. As mentioned previously, the different seasons during which the samplings were conducted seemed to influence the hydration status of the patients and thus may have influenced albumin concentration. However, a clear relationship between change in body weight, albumin concentration, and placebo or *Asp* could not be drawn.

TAOS outcome documented well the switch between the two products: r=.80,p=.004. The decrease registered under placebo was followed by an increase in capacity under *Asp*. Furthermore, the effect on TAOS confirmed the previous results.

Regarding anaemia, the patients showed a median decrease in Hb concentration of -1.1 (-1.3– -0.5) g/dl during the placebo period against an increase of 0.2 (-0.7– 1.0) g/dl during *Asp* supplementation. The difference between the two intervention periods was significant, p=.05, with a medium effect size of r=.54. RBC had the same significant development as Hb, with a decrease during the placebo period followed by an increase during the *Asp*

supplementation period: r=.82, p=.004. These results were also seen in the RCT. In regards to the effect size, it can be assumed that the effect of *Asp* is higher on the erythrocytes than on haemoglobin (r=.82> r=.54).

Iron concentration did not significantly increase over the entire intervention in the crossover groups. *Asp* implementation did show a slight, non-significant improvement in iron concentration of 0.1 (-0.1– 0.2) mg/l. The effect is analogous to the long-term group, which showed a continuous improvement in iron median concentration of 0.1 mg/l every three months. The lack of significant change may be due to the small number of patients.

Total cholesterol values did not change significantly in either period. Triglyceride values tended to change differently between the two periods. The present intervention showed an increase of TG during the placebo of 0.07 (-0.16– 0.57) g/l and a decrease during the *Asp* period of -0.24 (-0.36– -0.08) g/l. The difference between the two periods was not significant p=.328. However, during the supplementation of *Asp*, eight out of 13 patients showed a decrease in TG.

The difference in serum creatinine between the two periods was not significant but presented a medium effect size of r=.48, p=.083 for an increase of 0.1 (0.0– 0.3) g/dl after the implementation of *Asp*. The urea concentration, as observed previously, did not differ between the two periods. As noted before, the creatinine clearance affected the eGFR value calculated. The difference between the two periods was not significant but had a large effect size of r=.51, p=.064. The eGFR decreased by -4.9 (-18.9– 0.1) ml/min during *Asp* supplementation.

4.2 Discussion in Context

- **Population Definition and Study Restriction**

The study population was defined by the inclusion criteria: HIV-infected females from 18– 49 years of age with a BMI <26 kg/m^3 and CD4 count below 600 cells/mm^3. The aforementioned criteria were chosen to focus on a homogenous population of women at reproductive age, without overweight and presenting a low immunity. The women were also distinct in that they were being treated at the health care centre in Yaoundé.

The last population census documented that women in Yaoundé were aware of supplementation and fortification practices; 45% of child-bearing women took vitamin A, 93% iron supplementation, and 96% consumed fortified food (National institute of Statistic 2012). Even if this awareness is essentially associated with pregnancy care, it has to be considered that the selected study population was sensitive to supplementation due to being women at childbearing age and in contact with the health centre.

Furthermore, the recruitment confirmed a higher proportion of women than men being registered in the health centre of the capital. This confirms the known difference in infection prevalence among gender, 8.8% for women compared to 3.3% among men. The gender prevalence discrepancy in case of young people between 20 to 24 years is even higher, being of 3.4% for women and 0.7% for men (National institute of Statistic 2011). One speculated reason for the gender difference may be the practice of “sponsoring,” where senior men offer to take care of young student girls in exchange for unprotected sexual intercourse (Abega and Magne 2006). An additional explanation for the predominance of women in health centres are the additional screening carried out in case or assumption of pregnancy, in addition to a voluntary screening. The opening of a registered file in an HIV health care unit is only possible with a confirmed positive HIV test and patient agreement.

The file ratio concerning pre-HAART patients was in the range of8 to 16% of all files checked in the health centres between 2008 and 2010. Furthermore, contact information of pre-HAART patients was often missing. The consultation and opening of a file must be paid by the patient, around 5,000 FCfa (7.50 euros). The half-yearly monitoring visit costs 3,000 FCfa and takes at least two to three days (Mbanya, Sama et al. 2008). Pre-HAART monitoring is very rarely done. An explanation could be the lack of symptoms at the early infection stage. The time between sero-conversion and the beginning of antiretroviral therapy can take

years. An estimation made among an urban African cohort resulted in a time of 7.6 years (Zwahlen 2008). For pre-HAART persons living with the virus who are otherwise healthy, it seems to be difficult to foresee the outcomes and consequences of their HIV infection. Furthermore, it is difficult to check the correctness of any personal data provided by the patient. The ratio of wrong phone numbers in the pre-selection was 41% of 474 patients' files. These facts help explain the high number of files screened, patients contacted, and low response rate.

The social outcomes of the recruitment undermine the specificity of the pre-HAART period in the HIV infection, especially for urban women. A low rate of patients in a relationship has been pointed out by Fabienne Marcellin. She studied delay reasons for the first consultation after HIV diagnosis in Cameroon. One significant reason for a woman to have a medical monitoring delay of at least six months was because she was in a relationship (Marcellin, Abe et al. 2009). Following the same argumentation, the care provided by the intake of a daily therapy such as HAART allows family planning and increases the rate of married women living with HIV (Myer, Carter et al. 2010). The period of time between infection and entering HAART care seems to be characterised by a low married rate, also shown in this present study.

A main consideration of this study is that the patients involved were all people with at least primary education. The result is related to a high level of literate people in the country (UNICEF 2010). Yaoundé has the lowest rate of female illiteracy in the country with 1.6% versus 33.9% for rural areas (National institute of Statistic 2012). The question of health access for illiterate women remains open. A selection based on access to health was a prerequisite for the present study.

A further concern for the implementation of the present results is the discrepancy between urban and rural women, which is also shown well in anaemia cases. The reported anaemia prevalence for Yaoundé is 46.9%, and it is 61.9% in rural areas (National institute of Statistic 2012). Usually urban areas have lower anaemia rates due to better access to food, health care centres, prevention, and treatment possibilities. Compared to that, the study population had a lower anaemia rate, namely 32.5%. The study population is assumed already to have an awareness of health concerns and also the capability to look after them.

This study also excluded an current development characterizing urban African areas: the transition to a Western lifestyle with a high fat and sugar-rich diet and low physical activity

(Popkin 2004). This nutrition transition is not considered in this study due to the exclusion of overweight patients. Median BMI of the study population was 22 (20– 23) kg/m^2 and did not reflect the BMI of women living with HIV in Yaoundé. The last population count in Yaoundé in 2011 presented a mean BMI of 25.5 kg/m^2 with 17.5% over a BMI of 30 kg/m^2 (National institute of Statistic 2012). The statistic showed that the more educated and wealthier the woman were, the higher their BMI was (National institute of Statistic 2012). In 2011 Lissock et al. described metabolic obesity in urban and rural Cameroonians and found an even higher obesity rate in the urban population, BMI 29.6 (5.86) kg/m^2 with high values in the lipid profile (Lissock, Sobngwi et al. 2011). The increased prevalence of obesity in African urban women has been shown to be recent and is becoming a severe public health problem for the Sub-Saharan countries (Villamor, Msamanga et al. 2006). At the time of the current study, no study documented an obesity problem among PLWHA in urban African areas. The addition of these new public health issues to chronic infection with HIV can lead to specific pathologies, such as lipodystrophy. No abnormal lipid profile was found in the study populations. Pathologies such as lipodystrophy are specific for advanced infection and HAART. The lack of this pathology could be related to the exclusion criteria of overweight. However, the health awareness presented by the patients willing to participate in a nutritional intervention could even be a stronger criterion.

The following discussion of the effects is largely influenced by the selected population and restricts the conclusion to this defined population of urban women present in the health care units of Yaoundé.

- **Immuno-viral outcomes**

The HIV type screening in the study population could not exclude any co-infection of the two HIV types, but only two patients presented an exclusive HIV-2 infection. The virus diversity had no effect on the study outcomes, as the variables were reviewed through relative changes occurring during the period of the study.

As documented in the current RCT, the two supplemented products (*Asp* and placebo) had the same effect, namely a decrease of over 50 CD4 cells. A precedent study associated a mean decrease of 55 cells/mm^3 in CD4 cells with an increase of 1 log10 in the viral load (Lima, Fink et al. 2009). The steady state in the study of a VL over 5 log10 corroborates the expectation of a high viral peak present in the terminal, asymptomatic HIV phase (Sabin,

Devereux et al. 2000). A result in the selected population is that over the time most of the patients reached the mark of 350 cells/mm^3 and started with HAART. The participant now could follow the intervention over six months and showed a non-significant CD4 cell increase. This may suggest an annihilation of the infection progression. The benefit of a nutritional supply can appear after a certain amount of time and individually depends on the body's enforcement and metabolization. The lack of a short-term outcome of *Asp* on direct infection markers such as the viral load and CD4 cells can be interpreted as an absence of therapeutic effects in acute infection progress, but it cannot exclude an enforcement effect in the long term. However, the goal to document immune fortification effects could not be reach in the present study. This could be due to the dose of 5 grams per day, the three-month timeframe, or the time point of the infection chosen with a high VL.

Additionally, the antiretroviral properties described *in vitro* were also not confirmed by the study. *In vitro* the antiretroviral effect has been related to the property of the polycarboxylate chain complex with the Ca^{2+} ion, which catches the virus and isolates it from further interaction with cells (Rechter, Konig et al. 2006). In the current study, this mode of action may have been affected by the digestion processes. However, studies implementing *Asp* water extract described contrasting results such as a CD4 increase and a reduction in VL (Oben, Enonchong et al. 2007). The focus on an antiretroviral active extract remains an important outlook for future research on *Asp*.

A further immune marker proposed to describe infection course could lead to a better understanding. Even if the literature mentioned more about the sensitivity analysis of CD8 cell activation by quantifying the expression of CD38 proteins on the cells, CD8-$CD38^{bright}$(Tuaillon, Al Tabaa et al. 2009), the present laboratory did not allow the implementation of the enumeration and calibration to determine $CD38^{bright}$. Only the percentage of CD38-CD8 cells was reported in the immune activation profile. The immune activation marker in this study corroborates the literature and presents an accurate correlation to the viral particle in the blood (Almeida, Cordero et al. 2007). Furthermore, the literature mentions that under HAART, immune activation seems negatively related to a CD4 recovery (Hunt, Martin et al. 2003). The relationship between immune activation and immune recovery through CD4 in patients under HAART is complex and multi-causal. For example, the literature presents a favourable prognosis for the expression of CD38 for children and in contrast to that, a negative one for adults (Savarino, Bottarel et al. 2000). In

the pre-HAART stage for adults, a stabilisation or lowering of immune activation can be considered beneficial (Tilling, Kinloch et al. 2002).

As all patients involved in the study present a stable immune activation, no relationship could be established between the supplements and immune activation. The restricted recruitment seems to be a more consistent reason for the stable immune activation registered for the participant. Furthermore, in regard to the OLI, all patients had a low initial immune activation, 22% (16– 40). CD38 expression did not vary significantly for patients involved over six months. The case description made in Fig 4.2 between high or increased immune activation levels and concomitant events help us understand the lack of change in this outcome. The reason seems to be that the recruited participants were in good condition in term of having no active immune reaction.

A future study on selected patients presenting a high immune activation level would also allow researchers to reach a conclusion on the effect *Asp* has on immune activation. For example, a dietary micronutrient intake study on stationary patients detected an influence of the intervention on the immune activation decrease; the baseline CD38-CD8 was 60% (Gil, Martınez et al. 2005). The current study level under 30% CD38-CD8 activation represents a low level and cannot respond to an intervention with the goal of lowering the activation level.

- **Antioxidant Balance**

It is assumed that a general infection increases oxidative stress in the human body (Pace and Leaf 1995). Oxidative stress is defined as a disturbance in equilibrium between pro- and antioxidant molecules in the body. Pro-oxidants are molecules that have an odd number of electrons and act as free radicals. An efficient immune system needs pro-oxidants and therefore depends on oxidative equilibrium (Shils and Shike 2006). The level of oxidative damage is directly influenced by the extent of oxidative stress and the activity of the body's antioxidant defence. This antioxidative defence line includes dietary and antioxidant enzymes, e.g. catalase and superoxide dismutase. These enzymes are over expressed during an HIV infection (Delmas-Beauvieux, Peuchant et al. 1996; Repetto, Reides et al. 1996).

Among other factors, the activity of antioxidant enzymes is influenced by the intake of nutrients. One example is glutathione peroxidase (GPX), which requires selenium as a co-factor for its activity and glutathione, a tripeptide, as a substrate (Stephensen, Marquis et al.

2007). In addition to its own antioxidant molecules, the animal body can assimilate nutritional antioxidant molecules such as carotenoid, tocopherol, and xanthin(Preziosi, Galan et al. 1998). The postulated nutrition model by Friis in 2006 for people suffering from acquired immunodeficiency syndrome relates nutrition to infection, as shown in the diagram below, but represents a more theoretical than practically proven concept (Friis 2006). One connection between nutrition and the immune system is the oxidative stress balance in the body. The preservation of this balance is a main issue for patients living with the virus.

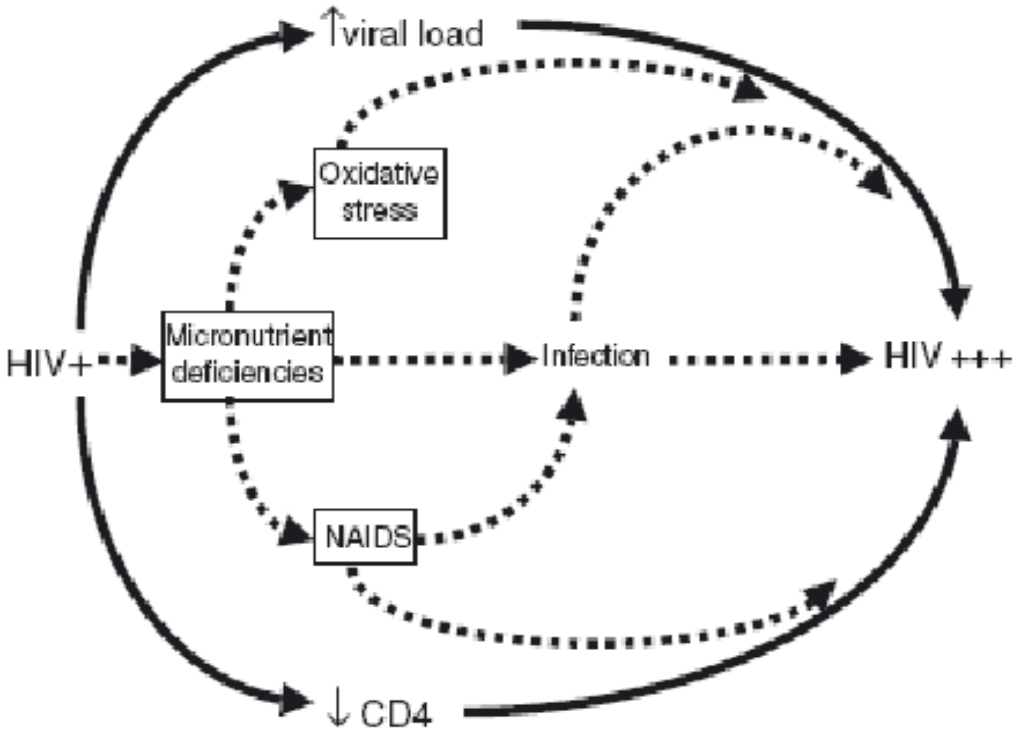

FIG. 4.7 NAIDS; NUTRITIONALLY ACQUIRED IMMUNODEFICIENCY SYNDROME FROM FRISS. MICRONUTRIENT INTERVENTIONS AND HIV INFECTION: A REVIEW OF CURRENT EVIDENCE
Henrik Friis; Tropical Medicine and International Health
Volume 11 no. 12 pp 1849–1857 December 2006

Beside the indirect cause of a chronic infection, a direct relationship mediated by the protein NF-$_K$B has been proposed between antioxidant status and HIV (Schreck, Rieber et al. 1991). The transcriptional factor NF-$_K$B is regulated by oxidative stress, an elevation in stress stimulates the release of the factor (Gloire and Piette 2009). The key regulator protein in the case of low oxidative stress is bound to an inhibitor, I-$_K$B, and forms a complex with NF-$_K$B in the cytoplasm. However, the present study could not document a relationship between an increase in antioxidant status and a decrease in viral particles in the blood. Furthermore, the total antioxidant potential showed no correlation with any other variable measured. The lack

of a relationship with any auxiliary marker may be due to the lack of the power of the study. A previous supplementation study with known antioxidant vitamins such as tocopherol at 800 mg/day plus ascorbic acid at 1 g/day has been shown to reduce oxidative stress and viral load of HIV-infected adults (Allard, Aghdassi et al. 1998).

However, the present intervention periods proved the capacity of *Asp* to restore the antioxidant capacity in the serums. The negative significant correlation between the change in TAOS during *Asp* intervention and the TAOS baseline value showed that *Asp* was especially beneficial among patients with low antioxidative capacity (Rho= -.436, p=.02). The present trial does not allow one to relate the increase in TAOS to a defined group of antioxidants, which could have been especially improved via supplementation with *Asp*. The enforcement of patients with low antioxidant capacity still permits the hypothesis of an action on the body's own antioxidative capacities such as the rehabilitation of the antioxidative enzymes. The enforcement of body serum micronutrients due to HAART is known (Jones, Tang et al. 2006). An examination of the antioxidative status of advanced HIV patients not under HAART showed a dramatic decrease of carotenoids during the ongoing infection (Favier, Sappey et al. 1994). The β-carotenoids in *Asp* could balance the carotenoid decrease and thus stabilize antioxidant capacity. This may not the only explanation for the stabilisation of the antioxidant status and would need to be further evaluated and differentiate between the multitudes of components acting in this multi-causal mechanism. The present measurements do not allow further interpretation but corroborate the antioxidant potential of *Asp*.

- **Inflammation & Anaemia**

As mentioned, the spreading of HIV affects the immune system and the body's overall health. This leads to an increased sensitivity to opportunistic infections. In the African context in particular, there is a large variety of opportunistic parasites: protozoa such as *Plasmodium falciparum*, bacteria such as *Mycobacterium tuberculosis*, fungi such as *Candida albicans,* and viruses such as the human cytomegalovirus. Focusing on Cameroon where the tropical forest represents a large part of the country, this serves as a storage basin for zoonotic diseases. According to literature, the Congo forest and its apes are the origin of a multitude of human parasites, for example for the most deadly malaria strain *Plasmodium falciparum,* which originated in West African gorillas, common in Cameroon (Liu, Li et al.

2010). Furthermore the African burden of malnutrition and low hygiene practices increases the co-infection rate and virulence of opportunistic diseases. Combinations of these factors amplify the spreading of HIV in the body and significantly reduce life expectancy and quality in infected people (Stillwaggon 2002).

The first outcome of any infection is an augmented and prolonged inflammation reaction. One such inflammation messenger is the C-reactive protein, which is released by the liver to target altered self and alien molecules and activates the immune system (Du Clos 2000). The expression of C-reactive protein increases very rapidly in response to an acute inflammation. In HIV infections, CRP has been presented as an inexpensive biomarker related to infection outcomes such as mortality (Feldman, Goldwasser et al. 2003; Noursadeghi and Miller 2004; Drain, Kupka et al. 2007). The relationship to HIV is not a direct one. The marker is considered a surrogate (Nixon and Landay 2010).The impact of the inflammation marker as such is hardly reliable, especially in a limited intervention with a small sample size and short duration time. The serum acute phase proteins may facilitate the interpretation of serum nutrient concentrations (Thurnham, Mburu et al. 2008). One result of an inflammation is a low iron concentration in the blood. The iron pathway is co-regulated by the immune system and the inflammation in order to keep the free serum iron level low (Wessling-Resnick 2010). The serum iron concentration depends on nutritional intake, the inflammation status, and the body's storage. Thus, under inflammatory conditions, there is a diversion of iron traffic from the blood circulation via the sequestration of the macrophages and enterocytes and a reduction of iron absorption (Cherayil 2011). The present study corroborates this with a significant negative correlation between CRP and iron concentration ($Rho=-.348$, $p<.05$). The regulatory mechanism by reducing the disposability of iron for opportunistic bacterial infections was observed by Weinberg and called "nutritional immunity" (Weinberg 1974). This phenomenon results in iron-restricted erythropoiesis and can lead to the development of anaemia, termed 'anaemia of chronic disease'(Weiss 2005). This mechanism may also influence the concentration of further essential metals such as zinc and manganese (Kehl-Fie and Skaar 2010).

The abnormality in the red blood cells and their oxygen-carrying protein haemoglobin is expressed as anaemia. Anaemia can have various reasons such as individual, infection, or nutritional reasons (WHO 2008). Those reasons can depend on the population, as in the genetic emergence of thalassemia, or be related to iron deficiency, malaria, and other

parasitic infections such as hookworms, ascaris, and schistosomiasis, as well as cancer, tuberculosis, and HIV. Further micronutrient deficiencies such as vitamin A and B12, foliate, riboflavin, and copper can also result in low Hb (Claster 2002).

The differentiation of anaemia types was not feasible within the context of this study. A common mutation of the RBC in Cameroon, thalassemia, raises the risk of anaemia. However, in the current study it remained constant, as the intervention results measured the change of Hb occurring over time. The present study showed no amelioration of Hb concentration or RBC count, but the variable decrease registered was less strong for the *Asp*-supplemented patients in comparison to the placebo. The literature mentions an erythropoiesis mechanism, which may be influenced by *Asp* phycocyanin, namely by a release of the erythropoietin hormone (Khan, Bhadouria et al. 2005). The stable haematocrit value in the *Asp* group in the present study may be an indicator of the effect on erythrocytes in the blood. To document an erythropoiesis action, a period of four months would have been more appropriate due to the RBC lifetime of 120 days; however, the long-term supplementation documented a significant decrease of the two variables RBC and Hb. Those results seemed to show that in this study, *Asp* does not act on Hb. The effect of the implemented *Asp* doses did not seem to be sufficient to show a reliable effect over the chosen periods of time.

The iron recommendation for the study population is a daily intake of 15 mg through the diet. The implemented *Asp* supplement contained 4.37 mg covering 1/3 of the RDA. Iron bioavailability of *Arthrospira* is poor and lies between egg and wheat (Kapoor and Mehta 1992). As shown, the *Asp* iron supply effect on Hb was not significant, but the long-term six-month exposure showed a non-significant improvement in iron concentration in the blood (r=.49).

Independently from the supplement, an absolute increase of anaemic patients in both groups was registered. The relationship between anaemia and HIV has been described as being a predictor of mortality for HIV-infected women (O'Brien, Kupka et al. 2005). As mentioned before, another mortality predictor is acute inflammation. The present data showed a significant association between acute inflammation and anaemia, Pearson chi-square $X^2(1)=7.08$, p=.008. Anaemic patients had a 7.08 higher chance of having an inflammation than patients with no anaemia. The relationship between anaemia and inflammation was absent at baseline $X^2(1)=0.69$, p=.404. An increase in anaemia over the

intervention time was probably due to inflammation and chronic disease. This increase seemed to represent the previously defined 'anaemia of chronic disease'. Patients with severe anaemia showed a beneficial evolution of their Hb after receiving medication. Furthermore, the intake of medications was very low in the study groups and could not been considered a cofounder in statistical analysis. Anaemia of chronic disease could not be compensated by the nutrition supplementation and had to be treated medically.

- **Weight**

Body weight is a major and easily measurable indicator of infection progression. The pre-HAART infection stage without medical or nutritional supply, according to the CDC's expectation, is related to a body weight decrease (CDC 1987). A 10% weight loss over a short period of time is considered to be a reason to start an antiretroviral therapy (WHO 2005). Weight loss can have a number of reasons such as health, socioeconomic, psychological, and/or metabolic reasons. A chronic infection increases the energy demand due to the constantly active immune system. If this demand is not covered by an increase in nutrition, the body starts a catabolic process to supply the energy, which then leads to body weight loss (Grinspoon and Mulligan 2003). HIV infection can affect the production of hormones such as glucagon, insulin, epinephrine (adrenaline) and cortisol, which are involved in carbohydrate, protein, and fat metabolism (Young 1997). Elevated levels of these hormones contribute to weight loss and to the wasting syndrome. In addition to the metabolic activity, a form of anorexia due to deglutition problems is frequent in HIV patients and is often the start of rapid health alterations.

In addition to an increased energy requirement, all essential nutrients have to be contained in a matching alimentation for infected people (WHO 2003). The macronutrients, especially protein with all essential amino acids, were present in both supplements. The difference between the two intervention products is the micronutrient content such as minerals, vitamins, and the secondary plant metabolites, which were present in *Asp* but not in the placebo. The intervention showed good results for the two products with regard to body weight stabilization and increase. The weight effect in the crossover group, where the implementation of *Asp* after the placebo showed a significant decrease between the two periods, has to be further investigated. The switch between the two products was not related to other markers, and the impacts on concomitant events or food intake were not

registered. Weight change during the intervention could also be due to new nutrition awareness created by involvement in the intervention. Furthermore, the presence of a nutritional counsellor in the HDJ health centre with the goal of creating awareness about a balanced diet has to be taken into consideration. Additional posters specifying country-based nutritional guidelines complemented the counselling (Emmanuel 2006). Even if the intervention participants did not have to go through the waiting hall or were actively instructed, an influence on the food quantity could not be excluded.

- **Albumin**

A particular trait of the patients' population involved in this study was a hyperalbuminaemia at baseline. A protein denaturation and hypoalbuminaemia would have been more likely expected in pre-HAART HIV patients (Salomon, de Truchis et al. 2002). The high albumin concentration over 50 g/l can have three main reasons: a high dietary protein intake, dehydration, or a retinol deficiency. After the RCT, both groups had a decreased albumin level, which was also true for patients of the long-term supplementation. The decrease was slightly more consistent in the *Asp* group during the RCT and continued over the long term. A possible reason may be an amelioration of a retinol deficiency by *Asp*. In this case, the supply of β-carotenoid from *Asp* ameliorates a low level of vitamin A and decreases the albumin concentration. Beta carotene is a precursor of retinol, which has a regulatory effect on albumin synthesis (Masaki, Matsuura et al. 2006). As the lowering effect was also present in the placebo group, a general explanation is needed. A possible reason related to all patients is probably a current dehydration state, which may be related to the high concentration at baseline. Dehydration is a known problem in relation to the disease. Some reasons for dehydration could be diarrhoea and/or no access to proper water. However, in the present study, there was a low rate of diarrhoea or having no access to water. The period during which the intervention was carried out coincided at the baseline with the rainy season and ended in the dry season for the RCT. The high albumin concentration registered could corroborate the state of dehydration at baseline. One assumption that would explain the general albumin level lowering over the intervention time is that light dehydration is more common during the rainy season than during the dry season. An argument would be that particularly in the dry season, attention given to proper hydration is more accurate than during the rainy season due to a stronger feeling of thirst in a warm environment. The intake

of water due to the ingestion of 10 caps a day can be an additional source of hydration that may have had an impact on the albumin concentration. However, no correlation between the absolute intake of pills and the albumin concentration was found: Rho=-.102, p=.469. Moreover, knowledge about the intake of protein-rich supplements and a sated feeling may also be able to influence the absolute protein intake not assessed in the intervention. The albumin evolution over the intervention can be multifactorial and due to the low power, no model could be tested in order to rank the effect of the different factors.

- **Lipid profile**

A burden related to a high lipid profile is coronary heart disease (CHD), which increases because of an industrial, processed diet and a sedentary lifestyle. Applying the NCEP cut-off point to detect an acute risk of CHD (TC >2.0 g/l and TG >1.5 g/l) (NCEP 2002), no patients involved in the study presented a risk of CHD. There was no elevated lipid profile among the participants. In another study among pre-HAART patients living with HIV in Yaoundé in 2010, subjects with CD4 >350 cells/mm^3 had a mean TC of 2.39 (0.8) g/l and a TG mean of 1.57 (0.12) g/l. This study by Nguema im did not mention the body weight of the patients (Nguemaim, Mbuagbaw et al. 2010). The absence of CHD risk in the *Asp* study is maybe related to the BMI of 22 (20– 23) kg/m^2, well documented by the general dependence between the lipid variables and the BMI (Carr, Emery et al. 2003).

The relationship between TC and TG has been described for advanced HIV infection in pre-HAART patients. Studies in Tanzania and Uganda showed that pre-HAART patients manifested an increase in TG and a decrease in TC (Buchacz, Weidle et al. 2008; Armstrong, Liu et al. 2011). An abnormal serum lipid profile described in pre-HAART patients, low cholesterol and high TG, seems to be related to CD4 values below 200 cells/mm^3 (El-Sadr, Mullin et al. 2005). Furthermore, for the ongoing infection in pre-HAART patients, the expected lipid changes are a TC decrease and a TG increase without antiretroviral treatment (Shor-Posner, Basit et al. 1993; Constans, Pellegrin et al. 1994). Only the increase in TG has been associated with an increased risk of mortality in the case of initiating HAART (Ngu, Heimburger et al. 2010). The two groups differ only in their cholesterol change. The infection progression documented by the CD4 cells does not presently correlate with the cholesterol.

The long-term *Asp* supplementation showed a stabilization of the TC value in the second period, which may show a regulatory effect of *Asp* on total blood cholesterol. The

cholesterol lowering registered in all *Asp*-supplemented groups has to be described further. However, more research with regard to the relationship between an *Asp* supplementation on cholesterol and infection progression on pre-HAART patients is needed. The comparison of the *Asp* effect to a well-documented cholesterol-lowering natural product such as bitter gourd could lead to a better description of efficiency.

- **Renal function**

The renal function documents the elimination mechanism of the body. The elimination molecules are creatinine and urea found in urine. Blood is filtered in the renal glomerulus. The estimation of the GFR is based on creatinine, urea, and albumin concentration in the blood. The renal unit nephron is affected by chronic infections and, in the case of HIV, can develop an associated nephropathy (HIVAN) (Herman and Klotman 2003). HIV infection impairs kidney function through a direct infection of the cells. The genetic mechanism and inclusion of the virus in the cells remains unclear, but some genetic predominance seems to play a role in the outbreak of HIVAN (Atta 2010). This pathology is the most common cause of chronic renal disease in HIV patients. A strong ethnic relationship has been documented; HIVAN mostly affects Africans (Bourgoignie, Ortiz-Interian et al. 1989). The screening of the present study population for nephropathy in adjusting the eGFR and creatinine value showed no case of HIVAN. In contrast, a study in Nigeria among 400 patients recorded a 38% prevalence of HIVAN. Renal disease was defined as serum creatinine above 1.5 g/dl. The mentioned risk factors were elevated age, low BMI, and low CD4 count (Emem, Arogundade et al. 2008). The lack of nephropathy in the current study has to be considered with regard to the inclusion criteria of an age between 18 to 49 years and the CD4 count over 350 cells/mm^3. A protective effect of these criteria against HIVAN could be the reason.

The present intervention with *Asp* caused a creatinine increase, which was clinically not relevant. A study using 10 grams of *Asp* on HIV patients in the Central African Republic also reported a serum creatinine increase (Yamani, Kaba-Mebri et al. 2009). The present increase of 0.1 g/dl seemed to stabilize over six months, which could be due to an adapted elimination balance. The increase in creatinine blood level was not associated with an increase in serum urea. It could be concluded that an additional five grams of protein-rich supplement per day had no effect on N-metabolism. However, creatinine level has to be studied dose dependently.

4.3 Discussion of Nutrition Practices in HIV

Benefits of nutrition supplementation, which delays the time to HAART and how it influences HAART, are currently discussed (Chandrasekhar and Gupta 2011). Furthermore, the new aging process of PLWHA is the result of the successful implementation of efficient HAART and needs innovative complementary medical management based on nutrition and physical activity (Somarriba, Neri et al. 2010). Nutrition, besides the disease, depends on multiple factors such as social, economic, environmental, psychological, and physiological ones. Each individual or group has special needs. Energy and nutrient requirements differ between men and women and vary with regards to age, the degree of physical activity, and climate conditions. According to these issues the American Dietetic Association highlights cross-cutting criteria to define the nutritional status of people living with HIV: sex, age, disease progression, and treatment (Fields-Gardner 2010). Furthermore, the HIV pandemic presents different backgrounds in each region and country in the world. As a result, the epidemic and the care gain a social character due to higher infection prevalence in marginal groups such as homosexuals, prostitutes, socially rejected people, intravenous drug users, or people having undergone blood transfusion but also groups of workers such as truckers and miners. These contexts have to be taken into account for the care support and in particular for the nutrition advice and/or supplementation proposed.

The African immunodeficiency context suffers from the prevailing burden of malnutrition. Pre-existing micronutrient deficiency has to be treated in addition to the infection burden (WHO 2005). Moreover, the burden of other infectious diseases such as malaria, hepatitis, and tuberculosis needs to be considered in the fight against immune deficiency (The Global Fund to fight AIDS 2001). The African HIV context also shows particular associated metabolic abnormalities such as iron deficiency anaemia, nephropathy, and lipodystrophy (O'Brien, Kupka et al. 2005; Manuthu, Joshi et al. 2008; Atta 2010). One main reason for iron deficiency in this region is related to malaria and other parasitic and bacterial infections (Weinberg and Moon 2009; Cherayil 2011). HIV-associated nephropathy (HIVAN), a collapse of the kidney function, is distinctive to African patients and patients of African descent (Bourgoignie, Ortiz-Interian et al. 1989). Finally, lipodystrophy is a redistribution of fat from subcutaneous regions to the visceral organs (Carr, Samaras et al. 1998). The syndrome is strongly related to the first HAART line and the use of protease inhibitors (Heath, Chan et al.

2002). A further burden on the lipid profile of the urban population in Africa is the transition to a fashionable Western lifestyle and nutritional behaviour (Pasquet, Temgoua et al. 2003). The major result is insulin-resistant diabetes related to nutrition transition and also appears as a complication in HIV infections and their HAART protease medication (Das 2011).

Nutrition is part of the care of HIV patients. In addition to the recommendation for better alimentation, the practice of supplementation takes up a large part of the help and care given to the patients. Nutritional societies encourage the implementation of evidence-based use of oral nutritional supplements to fight malnutrition in order to reduce health care costs and achieve economic benefits (Stratton and Elia 2010).However, in particular since beta-carotene and retinol efficiency trials with β-carotene, 30 mg/day, plus vitamin A, 25,000 IU, have shown to increase the risk of lung cancer among present and former smokers and workers exposed to asbestos (Omenn, Goodman et al. 1996),supplements have to be checked and validated in trials. The research has shown that a megadose of β-carotene increases serum levels of β-carotene 5 to 12 times higher than the normal physiological levels. At high doses, β-carotene has prooxidant effects (Palozza 1998).In humans,30 mg per day of β-carotene has been shown to decrease the activity of leukocyte superoxide dismutase and to lower levels of serum glutathione peroxidase, two important components of antioxidant defence (McGill, Green et al. 2003). Additionally, in the HIV context high vitamin A supplementation has been related to increased mother-to-child transmission, explained by an increased viral shedding in breast milk and the vagina (Villamor, Koulinska et al. 2010).

Highly concentrated micronutrient preparations can have a wide range of effects. It is known that the same micronutrients can act synergistically at moderate doses and antagonistically at high doses. Zinc, for example, is immunosuppressive at high doses but an essential mineral for the immune system (Chandra 1984). A statistic modelling found that at a high concentration, antioxidative supplements can have adverse effects, whereas at a moderate concentration, temporal supplementation can boost uninfected CD4 cells (van Gaalen and Wahl 2009). Vitamin C at high doses showed steady-state pharmacokinetics of the protease inhibitor indinavir in healthy volunteers (Slain, Amsden et al. 2005). Nonetheless in 2011, supplementation and implementation of enriched food have to be surveyed on HAART pharmacokinetics (Raiten 2011). Currently, a review of micronutrient supplementation

concluded that one should strictly follow the WHO 2003 recommendation, which endorses an intake at the recommended dietary allowance amounts (Forrester and Sztam 2011).

Iron supplements are the subject of large debates and special recommendations among regions in Africa. Iron plays a central role in the metabolism of protozoa, especially *Plasmodium spp.* (Wilson and Britigan 1998). The role of iron in the specific case of infection makes an iron supplement questionable (Clark and Semba 2001). Iron supplements have been shown to increase the incidence and morbidity of malaria in endemic areas (Prentice, Ghattas et al. 2007; Weinberg and Moon 2009). The international health agency refers to the dilemma of iron deficiency in malaria-endemic regions as a major public health problem (WHO 2007). The current recommendation is an iron supplement as a cure for identified iron anaemia. It warns against supplementation as prevention in malaria-endemic regions (Raiten, Namaste et al. 2011). The role of iron in humans and its interaction with infections, as is especially the case for HIV women in malaria-endemic regions, has increased attention on providing iron from natural products. The effect of natural products such as *Asp* could prevent patients from incurring an aggravation of iron deficiency and preserve them from an enhanced risk of malaria crises. Even if the study showed that a supplementation with *Asp* has no curative effect on anaemia status, *Asp* assures a minimal iron supply, which is vital for women at childbearing age. However, spirulina cannot replace an iron-balanced alimentation or iron supplementation in the case of severe anaemia. Nonetheless, *Asp* consumption could represent a sustainable solution to prevent severe iron deficiency anaemia without exacerbating malaria outbreaks.

Each individual has unique knowledge about food and access to it. The current solutions are educational programs, food distribution, or facilitated access to food. In special cases of acute disease phases or emergency situations when a direct, quick impact on the symptom is required, a highly concentrated supplementation or therapeutic ready-to-use food can be implemented. In these particular cases, medical monitoring must be present and deal with short-term objectives. Sustainable solutions regarding cultural specificity, economic potential, and the social and ecological burden are still needed, with attention to individual health outcomes.

Natural preparations can be implemented over longer periods of time due to their diversity and normal concentration of bioactive molecules. Important reasons for the study and implementation of herbal products are that they are mostly local, cultural, and socially non-

discriminating, as well as ecological. It also induces an empowerment of the people to find their appropriate individual preparation for an acceptable price. Animal source foods have been promoted by Friis for being richer in essential nutrients and specific vitamins, with a higher bioavailability of minerals in the management of malnourished children (Michaelsen, Hoppe et al. 2009). Herbal products and animal food have to be part of the set of answers given to a lifelong infection. The focus on plants that can be cultivated and prepared by PLWHA can represent a milestone in the achievement of a sustainable answer to the pandemic.

Each proposed solution has to answer the challenges of HIV nutrition intervention in a limited country setting. Those were summarized well by De Pee and Semba (De Pee and Semba 2010):

-Weight loss can be due to many factors. Its treatment and prevention have to address different factors simultaneously and take the specific circumstances of the individual patient into account.

-Management of weight and nutritional status becomes more and more complex as HIV infection progresses. Therefore, nutrition interventions should start as early as possible and should include nutrition assessment, education, and counselling, augmented with supplements and pharmaceutical preparations when required.

-HAART reduces the risk of weight loss and may increase appetite, but it also has side effects and long-term metabolic effects (dyslipidaemia, insulin resistance, and obesity) that require dietary management.

-Common sense dictates that a balanced diet containing all nutrients in the recommended amounts, including micronutrients, should be consumed, particularly by people who are vulnerable such as HIV-infected patients in order to support the body and immune system. In areas where micronutrient deficiencies are widely prevalent, HIV-infected people may need an intake somewhat above the RDA in order to correct these deficiencies in addition to meeting normal bodily needs.

-Products for vulnerable patients have to be chosen according to the following criteria:

The composition of the food variety, quality, and relative proportions of macro- and micronutrients; (higher energy density and lower content of a nutrients) and the use (less likely to be shared with other family members because it is ready to eat and more easily accepted as being a therapeutic food to be consumed exclusively by the patient).

4.4 Conclusion

The present research documented a supplementation of ten caps per day of *Arthrospira platensis,* a locally available food rich in nutrients that supposedly has effects on the immune system and antiretroviral activities among HIV patients. Even if the chosen product for the study was not a locally grown alga, due to standardization and purity requirements, the implementation of spirulina products is a current topic for Cameroon.

The study described an implementation of the natural product on a sensitive target population consisting of women between 18 and 49 years, not under HAART, presenting a low immunity, and it excluded patients with pregnancy, lactation, overweight, or currently suffering from a chronic disease. The exclusion criteria were required to keep a homogenous study population. The following conclusions are restricted to this defined urban female population, present in the health care units of Yaoundé, and on the amount and form of the product tested. The trial had two research objectives covering the therapeutic aspect versus a protein and energy equivalent placebo, and the nutritional aspect related to an HIV infection.

The *Asp* supplementation of five grams per day of dry powder had the same effect as the placebo on CD4 cells and the viral load among pre-HAART infected women over the duration of three months. A long-term action of *Asp* on the CD4 and VL stabilization cannot be excluded, as in the present study the long-term effects observed seem to be more related to the fact that involvement in the six-month intervention meant that no treatment had been started, which represents a selection of healthy patients.

Even if the immune marker measurement over the three-month RCT could not figure out whether *Asp* had a direct effect on the infection course, the start of therapy and the concomitant events frequency showed differences between the supplementation products. A main outcome of the RCT intervention was a non-significantly higher proportion of patients starting with HAART in the placebo versus the *Asp* group, ten against five patients, respectively, combined with a higher incidence of concomitant events, 70% in the placebo group versus 43% in the *Asp* group. These observations could not be deepened further but are useful for future studies that assess the properties of spirulina in relation to the time elapsed from seroconversion to initiation of HAART.

The nutritional aspect documented a positive effect of both intervention products on weight. No severe weight losses were observed, and most patients stabilized or increased their weight.

A main significant effect of spirulina intake among HAART-naïve HIV patients was documented by the increase in antioxidative capacity. This effect was stronger for patients with a poor antioxidative index at baseline, a rehabilitation effect could be proposed.

A further important outcome of the study is the decreasing effect on total cholesterol present among all study groups; however, it tended to be higher among *Asp* groups. This outcome confirms the cholesterol-lowering effect of spirulina documented in former studies. Lipid regulation, especially the TC-lowering effect has to be investigated for people receiving HAART.

In the current study, the active components of *Asp* did not seem to act as a curative element. Ingestion in other forms such as enriched *Asp* extracts or in larger amounts of raw powder mixed with already cooked food may be a more appropriate way to enhance the beneficial effect of *Asp* intake. Recommendations based on the present study could be to consider natural *Asp* as a nutrient with proven antioxidative potential and rich protein content. Natural products of *Asp* should not promise antiretroviral or immunotherapeutic activities. Furthermore, a stigmatization of the product with a special use for PLWHA has to be avoided. Therefore, *Asp* at a dose of five grams per day can only be recommended as support to antioxidative status.

4.5 Outlook

Clinical pilot studies with algae and spirulina suggested a stabilization or improvement for some patients in their HIV/AIDS outcomes (Teas and Irhimeh 2012). Dose-dependent studies with a sufficient sample size and with active extracts have to be carried out in order to provide a more detailed description of the long-term effects of *Asp* consumption. The measurement of the time lapse from seroconversion to the start of HAART could answer the question of whether spirulina has the benefit of enlarging the asymptomatic time lapse. A future study may assess the properties of spirulina by focusing on the time elapsed from seroconversion to HAART among HIV patients. One focus should be on the pharmacopeia effects of *Asp* on HAART. A result that needs to be looked into is the creatinine increase

related to the consumption of *Asp*. It has to be studied dose dependently in order to determine the burden of daily *Asp* consumption on the kidneys.

The research focuses on various molecules present in algae and, particularly in spirulina, sulphated polysaccharide, phlorotannins, lectins, and bioactive peptides, which could be key active molecules in the presumed antiviral activity (Vo and Kim 2010). The reviews of previous essays have attributed various nutritional and medicinal potencies to metabolites from spirulina (Nuhu 2013).

Table 4.2 *Arthrospira* active components promoting health

Molecule	Function
Phycocyanin	Antioxidant
	Lipid preoxidation inhibitor
	Hepatoprotection
	Anti-inflammation
	Anti-cancer
	Induce haematopoiesis
	Immunomodulation
	Antiviral
β-carotene	Source of vitamin A
	Anti-carcinogenic
	Antioxidant scavenging
Calcium-spirulina (sulphatedpolysaccharides)	Anti-viral (shielding off the positively charged amino acids in the V3 loops of the enlv glycoprotein gp120-->inhibition of the virus-cell binding)
	Anti-cancer
	Immunomodulation
GLA	Antioxidant
	Prostaglandin precursor
Immolina	Immunomodulation

Oral consumption of spirulina-polysaccharide can enhance components of the mucosal and systemic immune system (Balachandran, Pugh et al. 2006). Lectins, carbohydrate binding agents such as cyanovirin-N, could prevent transmission from infected cells to non-infected

cells (Huskens and Schols 2012). Immolina extracts from *Asp,* a braun-type lipoprotein, activate the immune system (Nielsen, Balachandran et al. 2010). A further substance-like phycocyanin presents a highly active antioxidant potential. The functions of major active molecules are described in the table below. The mechanisms of these molecules have to be described in relation to their action in the gut, and on the bioavailability of drugs and nutrients. Metabolism and their assimilation into the human body are key pieces of information that need to be collected.

Additionally the culture and transforming conditions have to be examined in terms of the enrichment of secondary metabolites such as phycocyanin, calcium polysaccharide, and lipids. For example, there are possibilities to enforce *Asp* with selenium and to combine it with a formula of ready-to-use food. As a microorganism, the growing conditions have to be studied in relation to the metabolite and their accumulation.

The quality and purity of products based on spirulina have to be controlled well. Furthermore, the origin of the supplement has to be clearly defined. The culture of *Asp* can be artisanal, semi-industrial and industrial, or naturally harvested. The growing conditions, quality of water, and correct drying are major factors influencing the safety of the product and its nutrient content. Spirulina is a cyanobacterium related to species producing cyanotoxine, therefore the purity of the product has to be guaranteed as well as good transformation of the product after harvesting wherever putrefaction can occur.

This study had the purpose of encouraging further research in the application field of *Arthrospira platensis* and supporting accurate monitoring of natural local products in their implement on patients suffering from chronic infection and diseases.

5 Abstract

Background: Supplements are often used to improve the nutritional status of people living with HIV. *Arthrospira platensis* (*Asp*) is an alga rich in proteins, minerals, and antioxidants. So far, there has been a paucity of data describing the immune-modulating activity and nutritional properties of *Asp*. This study describes the influence of *Asp* supplementation on the immune and nutritional status of HIV patients.

Objective: The focus of the study was on a sensitive population of pre-highly active anti-retroviral therapy (HAART) status and gender-oriented towards women. It aimed to assess the effects of a five-gram *Asp* supplementation on immune regulators and nutritional status.

Methods: The nutritional intervention was a pilot randomized placebo-controlled study (RCT) of three months, followed by a three-month open intervention with *Asp*. The use of a placebo mixture with the same protein calorie potential focused the observed effect on the micronutrient and secondary phytonutrient composition of the microorganism. An additional group of women undergoing a stable antiretroviral therapy was recruited. 73 HAART-naïve and 35 HIV-infected adult women under HAART with CD4 T cell count between 350 and 600 cells/mm^3 and a BMI ≤ 26 were recruited in Yaoundé between June and September 2010. The RCT groups were compared to each other. The six-month intervention groups were discussed separately as well as the *Asp* intervention on HAART patients. The disease predictors were CD4 cells, viral load, the immune activation marker CD38 expression, and concomitant events. Furthermore, common nutritional markers and the antioxidant marker total antioxidant status on the serum were measured. Statistical analyses were performed with non-parametric tests, and the effect size of each interaction was calculated to assure an adequate analysis of group variance and comparison.

Results: There were no significant differences in the immune inflammatory virological markers set during the RCT. In the placebo group, 21 of 30 patients (70%) developed concomitant events, while in the *Asp* group, only 12 of 28 patients (43%) did. The nutritional markers showed a beneficial aspect for the lipid profile, with a significant decrease in cholesterol of -0.14 (-0.47– -0.04) g/l, p<.004 and a slightly non-significant increase of triglyceride of 0.05 (-0.18– 0.20) g/l. Haemoglobinand erythrocytec ounts were not

significantly stimulated by *Asp*. Serum antioxidant capacities increased by 56 (1– 98) µM and were significantly different from the placebo p<.001 with a large effect size of r=.51. Creatinine showed a significant increase of 0.1 (0.0– 0.2) g/l, p=.002 with a large effect magnitude of r=.59 for the *Asp* group. The long-term exposure confirmed the tendency of the RCT. The HAART group showed a decrease in cholesterol but no other related effects.

Conclusions and Recommendations: The lack of an effect from a supplementation of ten pills per day on immune markers can be considered a lack of therapeutic activity of natural spirulina powder on HIV. However, the intervention seemed to reduce the incidence of concomitant events. The increase in antioxidant capacities could lead to a long-term effect. Furthermore, the decline in cholesterol especially for HAART patients should be the focus of additional research. The enhancing effect of *Asp* on creatinine should be investigated in a dose-dependent intervention. Moreover, further research on natural local products in terms of nutritional RCT intervention, during early HIV stages, and for patients under HAART on a larger population can be recommend, focusing on both urban and rural settings.

6 Zusammenfassung

Die Welt Gesundheitsorganisation schlug in ihrem Bericht von 2011 bereits vor, dass die bestehenden HIV Maßnahmen für Notsituationen durch längerfristige Maßnahmen für die Patientenfürsorge ersetzt werden müssen (WHO 2011).

Ein Problem stellen Nahrungsergänzungsmitteln in Bezug auf Vermarktung, Zugang und Gesundheitlichen Stellenwert dar. Die Gesundheitsministerien und internationalen Agenturen wurden bereits auf die Problematik aufmerksam und forderten 2005, Forschung, Ernährung und HIV in Afrika zu einem Schwerpunkt zu machen. Das Statement lautet wie folgt: "*there is a proliferation in the marketplace of unproven diets and dietary therapies, with exploitation of fears, raising of false hopes and further impoverishment of those infected and affected by HIV and AIDS*" (WHO 2005).

Am Beispiel von *Arthrospira platensis*, im allgemeinen Sprachgebrauch auch unter dem Namen Spirulina bekannt, wo aktuelle Forschungen auf der Basis von Zellkulturen und Tierversuchen gezeigt haben, dass das Cyanobakterium ein immunmodulatorische Funktionen (Hayashi, Katoh et al. 1994; Qureshi, Garlich et al. 1996; Mao, J et al. 2000) und antiretrovirale Wirkungen aufweist (Hayashi, Hayashi et al. 1996; Ayehunie, Belay et al.

1998; Rechter, Konig et al. 2006). Dieses Lebensmittel wird seit Jahrhunderten um den Tschad See von der Bevölkerungsgruppe der Kanembu konsumiert(FAO 2008). Obgleich aktuelle *in vitro* Forschungsergebnisse eine antiretrovirale Wirkung gezeigt haben, fehlen derzeit ausreichende Ergebnisse aus Humanstudien.

Die hier vorliegenden Studie untersucht die Wirkung von Spirulina bei Menschen die mit HIV Leben. Diese Studie ist eine Pilot-Studie, um Tendenzen in den Bereichen Immunologie, Virologie und Ernährung zu dokumentieren.

Die pilot-Studie ist eine klinische mit randomisiertem, Placebo kontrolliertem, doppel-blinden Studiendesign. Die Studie wurde am Zentralen Krankenhaus in Yaoundé, Kamerun im Zeitraum von 15.08.2010 bis 15.05.2011 durchgeführt. 73 Frauen die mit HIV leben und keine HAART bekommen, haben drei Monate lang täglich zehn Kapseln a 0,5 Gramm bekommen. Zu Beginn und am Ende der Intervention wurden venöse Blutproben entnommen T-CD4 und die Anzahl an viralen Partikel im Blutwurden gemessen. Blutparameter Eisen, Albumin, Kreatinin und Harnstoff wurden biochemisch bestimmt. Als Marker für Anämie wurden die Anzahl an Erythrozyten sowie die Hämoglobin Konzentration bestimmt. Außerdem wurde der Total Anti-Oxidant Status gemessen. Die Gruppen wurden wegen deren geringen Anzahl an probaten mittels robusten nicht parametrischen Test, Mann-Whitney und Wilcoxon rang Test auf signifikante Unterschiede getestet (p<0,05). Der Effekt Ausmaß würde für alle statistischen Test mittels der Effekt Größe, r ausgerechnet.

Resultate: Die Studie hat gezeigt, dass eine Anwendung von natürlicher Spirulina in Höhe von 5 gram pro Tag über drei Monate keine Auswirkung auf die hier untersuchten Infektionsmarker hat. Der immunologische und virologische Infektionsverlauf haben sich im Vergleich zu Kontrollgruppe nicht signifikant unterschieden. Die Zunahme und Stabilisierung des Gewichtes aller Patienten hat hingegen gezeigt, dass eine Zugabe an Protein und Energie einen positiven Effekt aufweist. Des Weiteren steht die Einnahme von Spirulina mit einer Erhöhung der antioxidativen Kapazität und der Blut Kreatinin-Spiegel in Verbindung.

Die vorliegend Studie ist hier ein Schritt in der direkten Beurteilung eines bereits verwendeten natürlichen Präparates bei gängiger Dose von einem hochwertigen reinen Produkt im Humanen Kontext. Die Ergebnisse haben gezeigt dass es keine direkte Auswirkung auf dem Infektionsverlauf gibt. Ein kurzfristiges einsetzen bei gängiger dose hat

keine Antiretrovirale oder immun verstärkende Wirkung. Die hier geführt Untersuchung lässt auf eine Langzeit Verstärkung über den Erhalt des Redox Gleichgewicht im Körper vermuten. Die Vorliegenden Ergebnisse müssen weitergeführt werden um eine bessere Einschätzung des Produkts auf verschiedene Populationen und Infektion Stadien zu dokumentieren.

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9 Declaration

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angeben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie inder "Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis" niedergelegt sind, eingehalten.

I declare: this dissertation submitted is a work of my own, written without any illegitimate help by any third party and only with materials indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At anytime during the investigations carried out by me and described in the dissertation, I followed the principles of good scientific practice as defined in the "Statues of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice."

Gießen, 15.07.15 Frank Winter

10 Appendix

- **Study Time**

The climatic conditions during the sampling periods, temperature and humidity, were taken at the airport in Yaoundé (see table 10.1).

TABLE 10.1 SAMPLING PERIOD IN RELATION TO THE CLIMATIC CONDITION

Sampling period	Temperature in°C	Mean rainfall in mm
June-September 2010	19.3 to 27.7	293
October-December 2010	19.5 to 28.5	19
March 2011	20.3 to 30.4	124

www.Worldweather.int

- **Exclusion Data**

TABLE 10.2 DROPOUT REASONS WITHIN THE GROUPS OVER THE INTERVENTION

	HAART Start		Co-medication		Withdrawal		Lost to follow-up		Pregnancy		Total	
Group	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Placebo-*Asp**	0/10/1	48	0/0/0	0	0/4/0	17	5/1/1	30	1/0/0	4	6/15/2=23	100
Asp-Asp*	3/2/2	36.8	0/1/2	15.8	2/1/0	15.8	4/2/0	31.6	0/0/0	0	9/6/4=19	100

*For the pre-HAART groups, the patients were divided into three time periods: during three months, continuing for six months, during six months.

TABLE 10.3 EXCLUSION REASONS PER PERIOD

Reasons	RCT n=15/73 (20%)		Between RCT and OLI n=22/58 (38%)		OLI n=6/36 (20%)	
	Frequency (Patient)	Percent (%)	Frequency (Patient)	Percent (%)	Frequency (Patient)	Percent (%)
HAART Start	3	20	12	55	3	-
Relevant co-medication	-	-	1	5	2	-
Withdrawal of consent	2	13	5	25	-	-
Lost to follow-up	9	60	3	15	1	-
Pregnant	1	7	-	-	-	-
Total	15	100	22	100	6	*

*Below 10 patients no percentage indication

- **Compliance**

TABLE 10.4 INTERVENTION VARIABLES BETWEEN EXCLUDED AND COMPLIANT TO THE INTERVENTION IN THE PRE-HAART POPULATION

Variable at Baseline	Excluded n=43		Compliant n=30	
	Median (IQR)	Min-Max	Median (IQR)	Min-Max
Age (year)	30 (26– 36)	18– 48	28 (24– 35)	19– 43
BMI (kg/m^2)	21 (20– 23)	17– 27	23 (21– 24)	18– 26
CD4 (cells/mm^3)	449 (364– 542)	139– 781	446 (427– 559)	321– 815
CD8-CD38(%)	24(16– 30)	7– 49	23 (16– 40)	11– 58
CRP (mg/l)	1.2 (0.4– 4.7)	0.2– 54.0	0.8 (0.4– 2.6)	0.2– 90.8
TAOS (µM)	357 (296– 400)	95– 533	365 (304– 424)	193– 509
Albumin (g/l)	49 (47– 52)	39– 55	50 (47– 52)	42– 58

Iron (mg/l))	0.8(0.7–1.0)	0.4– 1.4	0.9 (0.7– 1.0)	0.3– 1.2
Haemoglobin (g/dl)	11.8 (10.2– 12.5)*	5.7– 13.2	12.1 (11.0– 13.5)*	8.9– 14.8
Erythrocytes (tera/l)	4.4 (4.0– 5.0)*	2.5– 5.8	4.6 (4.4– 5.1)*	3.6– 6.4
Triglycerides (g/l)	0.87 (0.63– 1.14)	0.44– 5.05	0.76 (0.60– 0.96)	0.30– 1.35
Cholesterol (g/l)	1.65 (1.41– 1.88)	1.0– 2.70	1.54 (1.29– 1.72)	0.96– 2.18
Creatinine (mg/l)	7 (6– 7)	4–9	7 (6– 8)	3–16
Urea (g/l)	0.19 (0.15– 0.22)	0.11–0.30	0.18 (0.15– 0.22)	0.10–0.41
eGFR	57.5 (50.8– 66.8)	35.7– 109.3	57.6 (50.3– 69.0)	22.3– 143.2

* p<.05

- **Data from the RCT**

  - **CD4 Lymphocytes**

TABLE 10.5 LYMPHOCYTE CD4 DURING THE RCT

	Placebo n=30			***Arthrospira*n=28**			
CD4 (Cells/mm^3)	**Mean(SD)**	**Median(IQR)**	**Min-Max**	**Mean(SD)**	**Median(IQR)**	**Min-Max**	**U-test(p=)**
Baseline	467.0 (132.2)	462 (413–558)	139– 815	469.7 (95.9)	440 (415– 550)	324– 781	.7
After 12 weeks	401.8 (106.2)	417 (311– 486)	184– 595	414.0 (132.1)	406 (320– 499)	227– 887	.9
Difference	-65.2 (86.2)	-52 (-112– -16)	-293– 120	-55.7 (71.4)	-66 (-111– -20)	-153– 106	.9
Wilcoxon (p=)		<.001*			<.001*		

*p<.05

- **Viral Load**

TABLE 10.6 VIRAL LOAD DURING THE RCT

	Placebo n=30			*Arthrospira*n=28			
Viral load (Log10)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median(IQR)**	**Min-Max**	**U-test(p=)**
Baseline	5.3 (1.2)	5.6 (5.1–6)	1.4– 6.9	5.1 (1.3)	5.3(4.3–5.8)	1.4– 7.4	.2
After 12 weeks	5.0 (1.5)	5.5(4.7– 5.9)	1.4– 6.8	5.0 (1.3)	5.3(4.5–5.9)	1.4– 6.8	.7
Difference	-0.3 (1.1)	0.0(-0.4– 0.2)	-4.4– 1	-0.1 (1.2)	0.0(-0.2–0.4)	-5.3– 2.2	.2
Wilcoxon (p=)		.3			.5		

- **LymphocyteCD8-CD38**

TABLE 10.7 T LYMPHOCYTECD8-CD38 DURING THE RCT

	Placebo n=30			*Arthrospira*n=28			
CD8-CD38 (%)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	27.6 (13.3)	25.2(17.1– 37.1)	7– 58	24.6 (9.8)	22.4(16.2– 30.5)	11.5– 46.9	.5
After 12 weeks	26.1 (13.4)	21.2(15.5– 36.3)	3– 61.4	24.6 (11.3)	24.5(14.5– 29.9)	8.6– 52.4	.7
Difference	-1.5 (8.3)	-0.9(-4.17– 1.56)	-21.5– 22.5	0.1 (8.7)	-1.3(-5.8– 2.0)	-13.5– 28.7	.8
Wilcoxon (p=)		.18			.32		

- **C-Reactive Protein**

TABLE 10.8 C-REACTIVE PROTEIN ABSOLUTE VALUE DURING THE RCT

	Placebo n=30		*Arthrospira* n=28		
C-Reactive Protein (mg/l)	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	1.50 (0.40– 2.80)	0.20– 90.80	0.80 (0.17– 3.90)	0.20– 42.60	.5
After 12 weeks	1.15 (0.40– 3.40)	0.20– 64.70	0.35 (0.25– 0.85)	0.20– 20.10	.7
Difference	0.19 (-0.85– 2.40)	-90.4– 62.8	0.20 (-0.30– 0.77)	-42.50 - 19.30	.7
Wilcoxon (p=)	.32		.37		

- **Concomitant Events during the RCT**

TABLE 10.9 CONCOMITANT EVENTS DURING THE RCT

	Placebo n=30		*Arthrospira* n=28		Total n=58	
Events	**n**	**Percent (%)**	**n**	**Percent (%)**	**n**	**Percent (%)**
Headache	3	14	1	8	4	12
Fatigue	2	10	1	8	3	9
Malaria	6	29	4	33	10	30
Gastrointestinal symptoms	3	14	3	25	6	18
Diarrhoea	1	5	1	8	2	6
Opportunistic infections	3	14	-	-	3	9
Respiratory disease	1	5	1	8	2	6
Injuries	2	10	1	8	3	9
Total events occurred	21	70	12	43	33	57

- **Body Weight**

TABLE 10.10 BODY WEIGHT DURING THE RCT

	Placebo n=30			*Arthrospira* n=28			
Body Weight (kg)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	57 (6.5)	56.8(54.1 - 61.7)	45.9–69.7	57.9 (6.5)	57.3(53.2–62.3)	48.3–73.3	.9
After 12 weeks	58.4 (6.6)	58.4(54.9–63.1)	45.6–69.6	58.1 (6.5)	57.8(52.9–62.5)	46.6–72.1	.7
Difference	1.4 (2.2)	0.6(-0.1–2.9)	-3.4–5.8	0.2 (2.1)	0.5(-0.4–1.7)	-4.4–3.7	.1
Wilcoxon (p=)		.005*			.51		

*p<.05

- **Total Antioxidant Status**

TABLE 10.11 TOTAL ANTIOXIDANT STATUS DURING THE RCT

	Placebo n=30			*Arthrospira*n=28			
TAOS (µM)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	351 (101)	357(298– 424)	160– 509	331 (82)	330(275– 384)	95– 505	.2
After 12 weeks	315 (113)	336(275– 373)	7–498	379 (92)	387(320– 430)	165– 533	.02*
Difference	-36 (73)	-22(-64– 19)	-185– 153	48 (85)	56(1– 98)	-124–251	<.001*
Wilcoxon (p=)		.008*			.007*		

*p<.05

- **Albumin**

TABLE 10.12 ALBUMIN

	Placebo n=30			*Arthrospira*n=28			
Albumin (g/l)	**Mean(SD)**	**Median (IQR)**	**Min- Max**	**Mean(SD)**	**Median (IQR)**	**Min- Max**	**U- test (p=)**
Baseline	48.9 (4.0)	49.5(47– 52)	39 – 58	48.7 (3.7)	49 (47– 51)	40 – 54	.9
After 12 weeks	47.0 (4.1)	47.5(46 – 49)	39 – 55	47.1 (3.5)	47(44.5– 50)	40 – 53	.8
Difference	-1.9 (4.9)	-1.5(-4.0– 1.0)	-14 – 10	-1.5 (4.1)	-3.0(-4.0– 0.0)	-10 – 9	.6
Wilcoxon (p=)		.034*			.026*		

*p<.05

- **Erythrocyte Count**

TABLE 10.13 ERYTHROCYTE COUNT

	Placebo n=30			*Arthrospira* n=28			
RBC (tetra/l)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	4.6 (0.7)	4.5(4.2– 5.1)	2.5 – 6.4	4.5 (0.5)	4.5(4.1– 5.0)	3.4 – 5.4	.8
After 12 weeks	4.2 (0.6)	4.2(3.8– 4.7)	2.9 – 5.5	4.3 (0.5)	4.3(4.0 – 4.7)	3.2 – 5.2	.7
Difference	-0.3 (0.4)	-0.4(-0.5 - -0.2)	-1.5 – 1.1	-0.2 (0.5)	-0.3(-0.4–0.0)	-1.9 – 1.1	.1
Wilcoxon (p=)		<.001*			<.002*		

*p<.05

- **Iron**

TABLE 10.14 PLASMA IRON

	Placebo n=30			*Arthrospira*n=28			
Iron (mg/l)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test(p=)**
Baseline	0.8 (0.2)	0.9 (0.6– 1.0)	0.4– 1.2	0.8 (0.2)	0.8 (0.7– 1.0)	0.3– 1.4	.8
After 12 weeks	0.8 (0.3)	0.7 (0.6– 1.0)	0.3–1.4	0.8 (0.2)	0.8 (0.7– -0.9)	0.57 - 1.4	.4
Difference	-0.05 (0.36)	-0.1 (-0.3– 0.2)	-0.9– 1	0.01 (0.27)	-0.1 (-0.15– 0.2)	-0.5– 0.6	.4
Wilcoxon(p=)		.48			.95		

-

- **Haematocrit**

TABLE 10.15 HAEMATOCRIT

		Placebo n=30			Spirulinan=28		
Haematocrit (%)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	33.4 (4.5)	34.0 (31.5– 36.0)	20.0 – 41.0	34.5 (3.6)	34.5 (32.0– 37.0)	28.0 – 43.0	.304
After 12 weeks	32.6 (3.9)	33.0 (30.0– 35.0)	24.0 – 40.0	35.0 (3.6)	35.0 (32.0– 37.0)	30.0 – 43.0	.028*
Difference	-0.8 (2.9)	-1.5 (-2.0– 0.0)	-6.0 – 10.0	0.2 (3.1)	-0.0 (-1.0– 2.0)	-10.0 – 8.0	.039*
Wilcoxon (p=)		<.039*			<.645		

Baseline U-test (z=-1.027; r= .18; p=.304); U-test after 12 weeks (z=-2.200; r=.33; p=.028), Diff.U-test (z=-2.061; r=.31; p=.039); Placebo (z=-2.062; r=.31; p=.039); Asp (z=-0.461; r=.11; p=.645)

- **Cholesterol**

TABLE 10.16 CHOLESTEROL

	Placebo n=30			***Arthrospira*n=28**			
Cholesterol (g/l)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	1.58 (0.32)	1.57(1.41 – 1.84)	1.00 – 2.18	1.58 (0.39)	1.53(1.34 – 1.8)	0.96 – 2.7	.7
After 12 weeks	1.50 (0.29)	1.50(1.30 – 1.66)	1.04 – 2.11	1.38 (0.32)	1.35(1.20 – 1.53)	0.83 – 2.27	.1
Difference	-0.08 (0.30)	-0.07(-0.20– 0.06)	-0.68 – 0.65	-0.20 (0.36)	-0.14(-0.47- -0.04)	-0.80 – 0.79	.1
Wilcoxon (p=)		.113			.004*		

*p<.05

- **Triglyceride**

TABLE 10.17 TRIGLYCERIDE DURING RCT

	Placebo n=30			*Arthrospira*n=28			
Triglyceride (g/l)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	1.0 (0.8)	0.8(0.6 – 1.1)	0.4 – 5.1	0.8 (0.3)	0.8(0.6 – 1.0)	0.3 – 1.7	.7
After 12 weeks	1.1 (0.8)	0.8(0.7 – 1.4)	0.4 – 4.0	0.87 (0.33)	0.7(0.6 – 1)	0.5 – 1.6	.3
Difference	0.1 (0.5)	0.1(-0.2 – 0.5)	-1.1– 1.2	0.0 (0.4)	0.0(-0.2 – 0.2)	-2.1– 1.0	.7
Wilcoxon (p=)		.188			.407		

- **Creatinine**

TABLE 10.18 CREATININE DURING RCT

	Placebo n=30			*Arthrospira*n=28			
Creatinine (g/dl)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	0.7 (0.2)	0.7(0.6 – 0.8)	0.5 – 1.6	0.66 (0.2)	0.7(0.6 – 0.7)	0.3 – 0.1	.5
After 12 weeks	0.7 (0.2)	0.7(0.6 – 0.8)	0.4 – 1.4	0.8 (0.2)	0.8(0.6 – 0.9)	0.5 – 1.1	.09
Difference	-0.02 (0.14)	0.0(-0.1 –0.1)	-0.3 – 0.2	0.09 (0.14)	0.1(0.0 – 0.2)	-1 – 4	.008*
Wilcoxon (p=)		.51			.002*		

*p<.05

- **Urea**

TABLE 10.19 UREA

	Placebo n=30			*Arthrospira*n=28			
Urea (g/l)	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	0.20(0.05)	0.19(0.16– 0.22)	0.10–0.30	0.19 (0.07)	0.17(0.13– 0.20)	0.10– 0.41	.1
After 12 weeks	0.20(0.06)	0.18(0.15– 0.22)	0.12– 0.37	0.18 (0.06)	0.18(0.12– 0.22)	0.09– 0.28	.3
Difference	0.00(0.05)	0.00(-0.3 - 0.04)	-0.12 - 0.09	-0.01 (0.06)	0.00(-0.35– 0.03)	-0.19 - 0.07	.8
Wilcoxon		.905			.861		

- **eGFR**

TABLE 10.20 EGFR

	Placebo n=30			*Arthrospira* n=28			
eGFR ml/min	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**Mean(SD)**	**Median (IQR)**	**Min-Max**	**U-test (p=)**
Baseline	57.7 (13.0)	57.7(50.5– 61.1)	22.3– 88.8	64.7 (23.2)	59.8(51.2– 72)	38.2 - 143.2	.35
After 12 weeks	59.3 (16.0)	56.6(48.7– 70.5)	26.9 -103.1	55.2 (15.0)	51.3(43.0- 67.1)	34.0– 89.5	.25
Difference	1.6 (13.8)	0.0(-6.7– 4.6)	-18.1– 42.0	-9.5 (15.4)	-7.3(-17.2– 0.9)	-53.7– 14.4	.01*
Wilcoxon (p=)		.99			.004*		

*p<.05

- **Open-Label Intervention**

TABLE 10.21 CONSTANT VARIABLES OF OPEN-LABEL INTERVENTION POPULATIONS

	Crossover n=13		Long-Term n=17	
Variables	**Median(IQR)**	**Min-Max**	**Median(IQR)**	**Min-Max**
Age (year)	34 (26–36)	19– 41	25 (23– 30)	19– 43
IDDS	5 (4– 6)	2– 8	6 (5– 6)	3– 8
Height (m)	1.63 (1.60– 1.66)	1.47– 1.68	1.63 (1.59–1.67)	1.53–1.73
Weight (kg)	58 (56– 62)	50–68	59 (53–64)	48–73
BMI (kg/m^2)	22 (21– 23)	18– 26	23 (21–24)	18–26

TABLE 10.22CONCOMITANT EVENTS DURING THE OLI

	Crossover n=13*		Long-Term n=17*	
Events	**Frequency (Patient)**	**Percent (%)**	**Frequency (Patient)**	**Percent (%)**
Headache	1 / 2	7/13	4 / -	19/-
Fatigue	1 / 2	7/13	1 / 1	5 /5
Malaria	2 / 5	13 /33	5 / 2	24/10
Gastrointestinal symptoms	1 / 1	7/7	5 / 3	24/14
Diarrhoea	1 / 1	7/7	2 / 4	10/19
Opportunistic infections	2 / 1	13/7	1 / -	5/-
Respiratory disease	1 / 2	7/13	3 / 1	14/5
Other injuries	1 / 2	7/13	- / 1	-/5
Total	11/ 16	68/106	21/12	101/58

The incidence rate of concomitant events was lower in the crossover group compared to the long-term group. However, the long-term group showed a decrease in the incidence rate in the second three-month period. The main concomitant event was malaria with a roughly 20% clinical manifestation.

- **Long Term**

  - **Variable of the Long-Term Intervention**

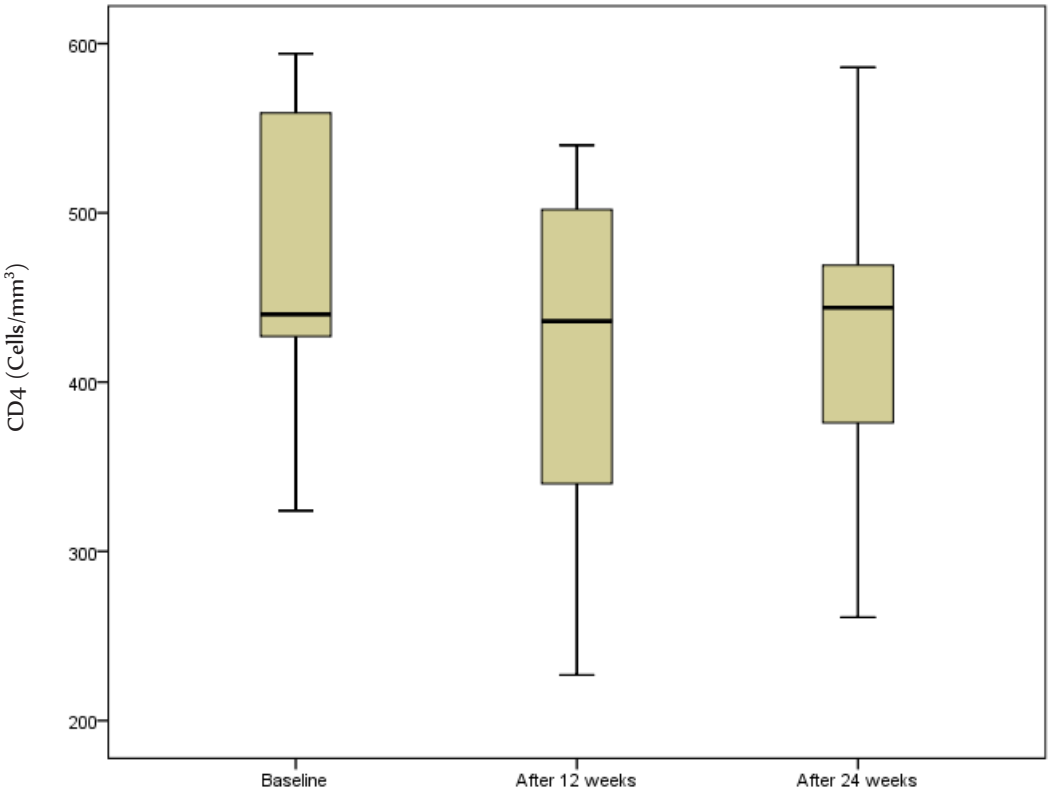


FIG 10.1 BOX PLOT REPRESENTING THE CD4 T LYMPHOCYTE COUNTS IN THE LONG-TERM INTERVENTION PER SAMPLING

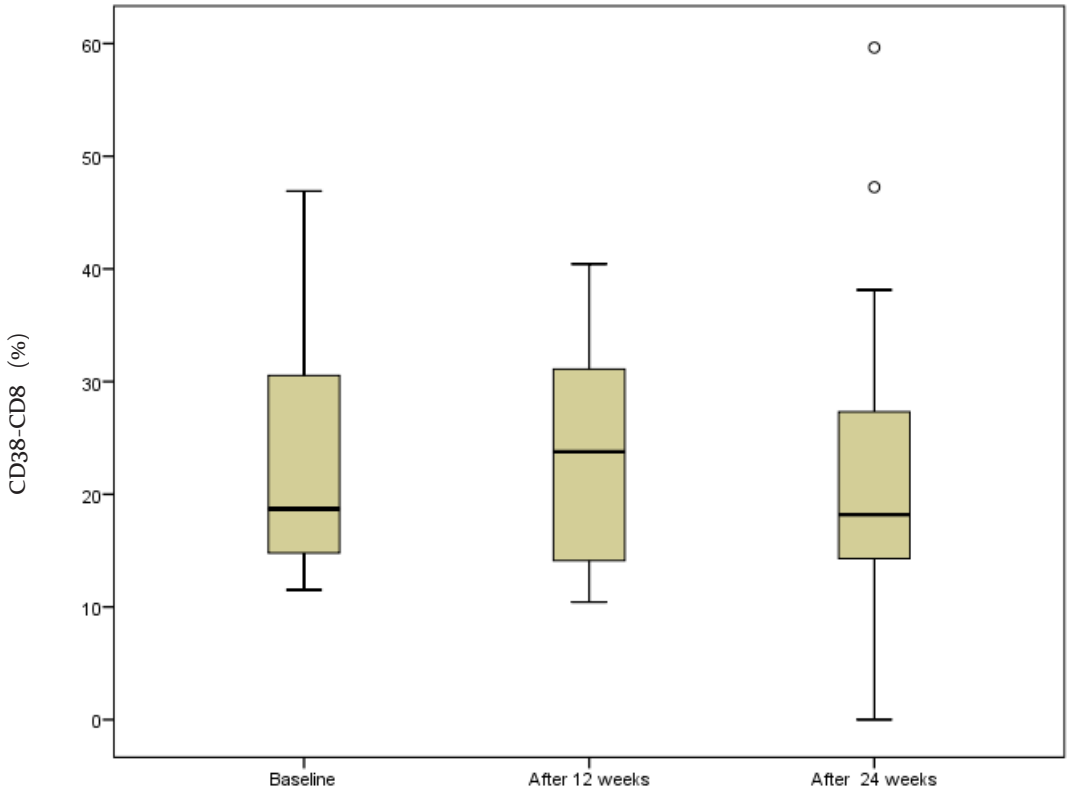


FIG 10.2 BOX PLOT REPRESENTING THE IMMUNE ACTIVATION MARKER CD38-CD8 IN THE LONG-TERM INTERVENTION PER SAMPLING

The CD8 lymphocytes expressing the CD38 antigen are presented at the three sampling points over the 24-week intervention.

TABLE 10.23 PRESENTING BODY WEIGHT, TAOS, AND ALBUMIN FOR THE LONG-TERM INTERVENTION

	Body Weight (kg)		TAOS (µM)		Albumin (g/l)	
Long-Term n=17	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	59.2 (53.4– 63.7)	48.3– 73.3	325(276– 383)	256– 505	50 (48– 52)	42– 54
12 weeks	60.4 (54.5– 63.7)	49.5– 72.1	412*(363 - 484)	165– 533	49 (46– 50)	40– 52
24 weeks	62.3 (53.5– 65.2)	49.1– 71.2	503*(464 - 545)	409– 721	44 (41– 47)	39– 53

*p<.05

Albumin

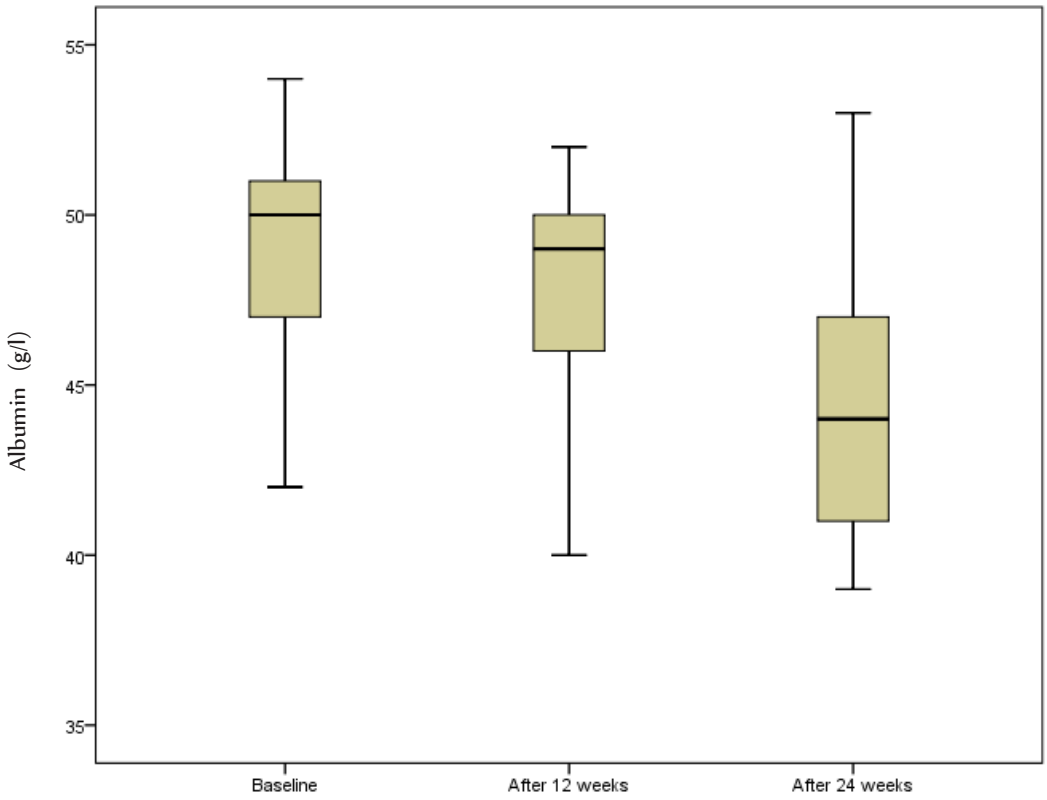


FIG 10.3 BOX PLOT REPRESENTING ALBUMIN IN THE LONG-TERM INTERVENTION PER SAMPLING

TABLE 10.24HAEMOGLOBIN, RED BLOOD CELL COUNT, AND IRON FOR THE LONG-TERM INTERVENTION

	Haemoglobin (g/dl)		RBC (tera/l)		Iron (mg/l)	
Long-Term n=17	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	12.0 (11.4– 13.7)	8.9– 14.8	4.9 (4.4– 5.1)	3.6– 5.4	0.7 (0.7– 1)	0.3–1.2
After 12 weeks	12.0 (10.7– 12.7)	9.1– 14.8	4.5 (4.1– 4.7)*	3.2– 5.2	0.8 (0.7 – 1)	0.5–1.4
After 24 weeks	11.6 (10.4– 12.6)*	6.9– 14.9	4.5 (4.0– 4.7)	2.6– 5.2	0.9 (0.7– 1.1)	0.5– 1.5

*p<.05

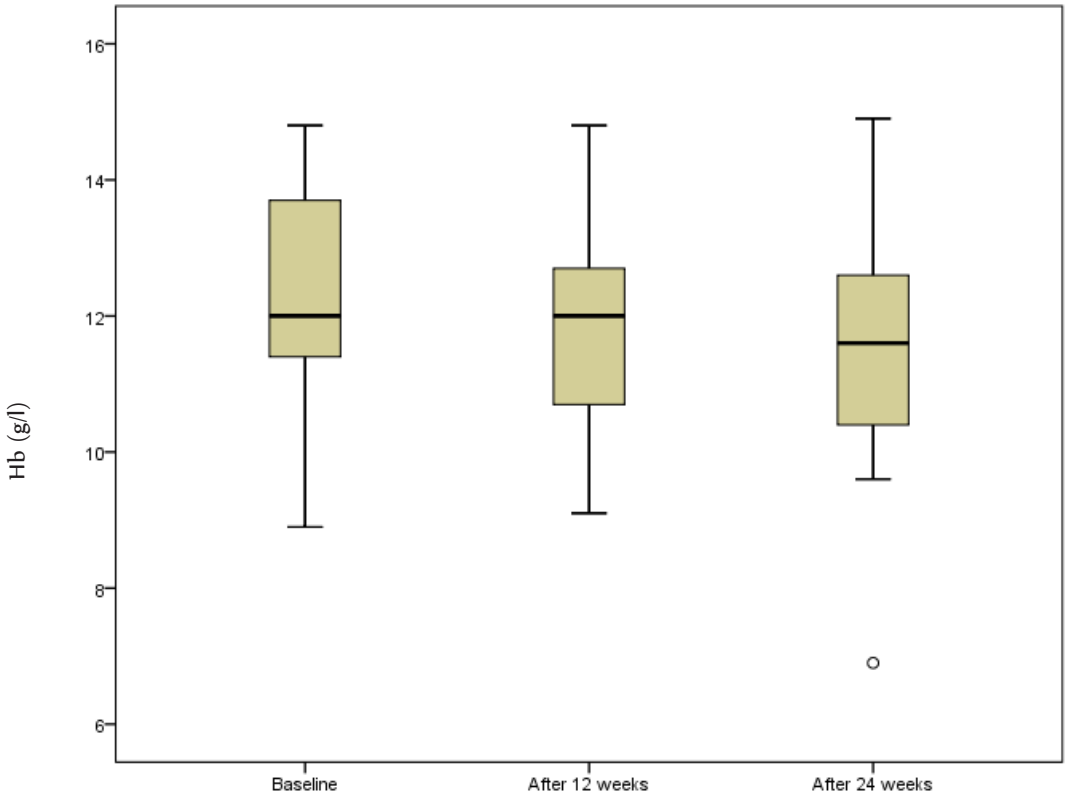


FIG C 10.4 BOX PLOT REPRESENTINGHB IN THE LONG-TERM INTERVENTION PER SAMPLING

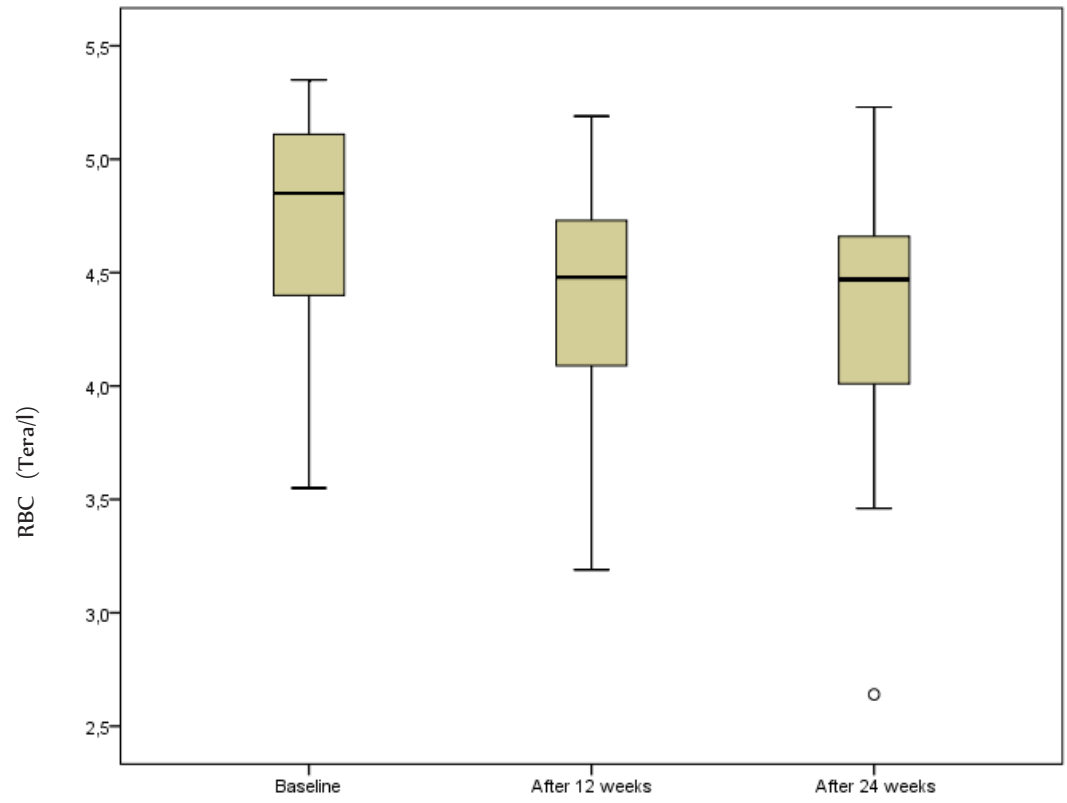


FIG 10.5 BOX PLOT REPRESENTING RBC COUNT IN THE LONG-TERM INTERVENTION PER SAMPLING

Iron

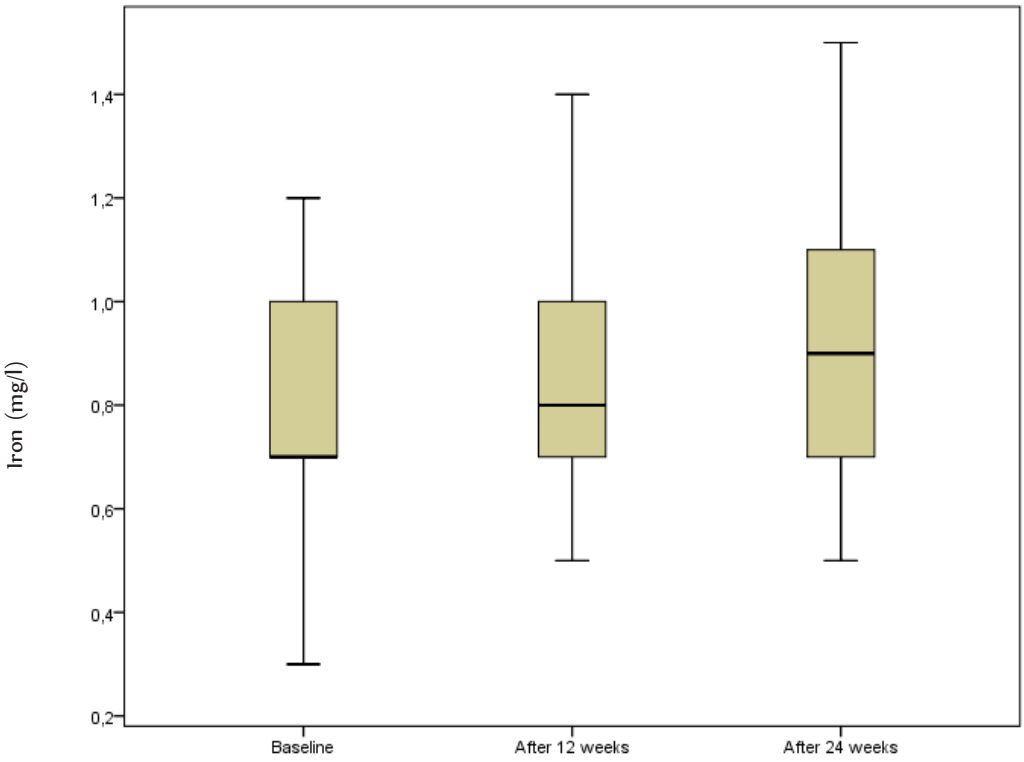


FIG 10.6 BOX PLOT REPRESENTING IRON IN THE LONG-TERM INTERVENTION PER SAMPLING

TABLE 10.25 PRESENTING TOTAL CHOLESTEROL AND TRIGLYCERIDES DURING THE LONG-TERM INTERVENTION

	Cholesterol (g/l)		Triglycerides (g/l)	
Long-Term n=17	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	1.46 (1.32– 1.56)	0.96– 1.97	0.72 (0.61–0.92)	0.3–1.08
After 12 Weeks	1.25 (1.10– 1.53)	0.83– 2.27	0.73 (0.60–1.0)	0.46–1.64
After 24 Weeks	1.28 (1.21– 1.54)	0.80– 2.25	0.61 (0.51–0.71)	0.4–1.24

*p<.05

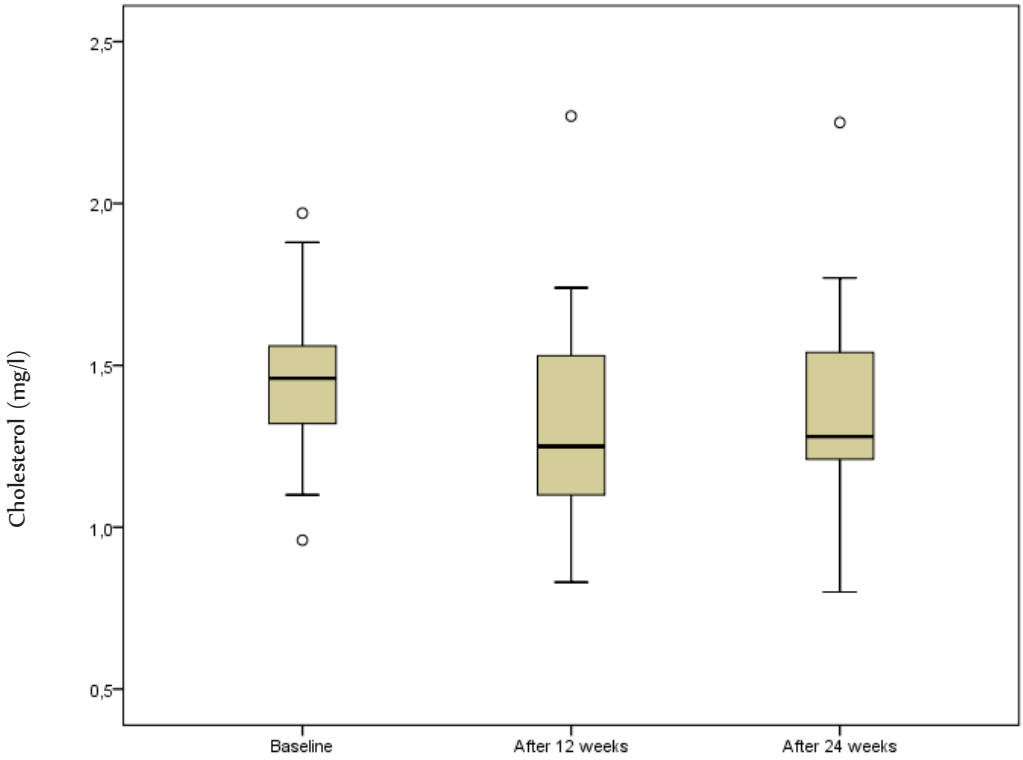


FIG10.7 BOX PLOT REPRESENTING CHOLESTEROL IN THE LONG-TERM INTERVENTION PER SAMPLING

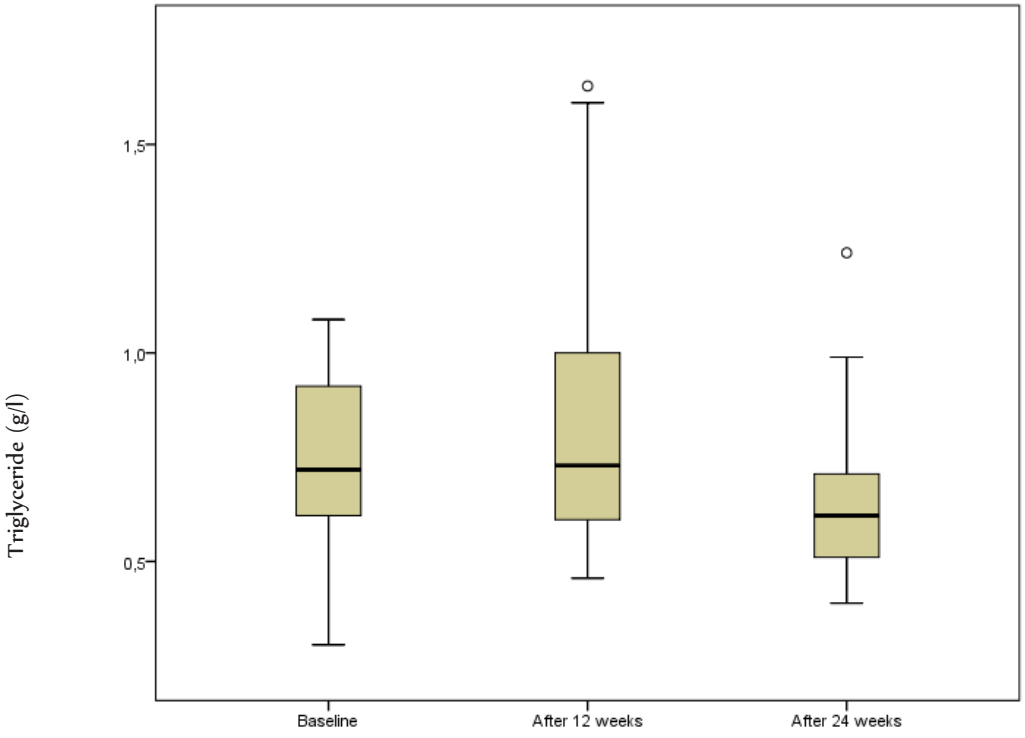


FIG 108 BOX PLOT REPRESENTING TRIGLYCERIDE IN THE LONG-TERM INTERVENTION PER SAMPLING

TABLE 10.26 RENAL FUNCTION DURING LONG TERM

	Creatinine (g/dl)		Urea (g/l)		eGFR (ml/min)	
Long-Term n=17	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	0.7 (0.6– 0.7)	0.3 - 1.0	0.18 (0.14 - 0.22)	0.1– 0.47	59.9 (51.5– 75.1)	38.2– 143.2
After 12 weeks	0.8 (0.6– 0.9)	0.5 - 1.1	0.20 (0.16 - 0.23)	0.09 - 0.28	48.4 (40.5– 67.8)	34.0– 89.5
After 24 weeks	0.8 (0.7– 1.0)*	0.6 - 1.1	0.17 (0.15 - 0.22)	0.11 - 0.33	47.2 (35.1– 59.1)	30.8– 69.2

*p<.05

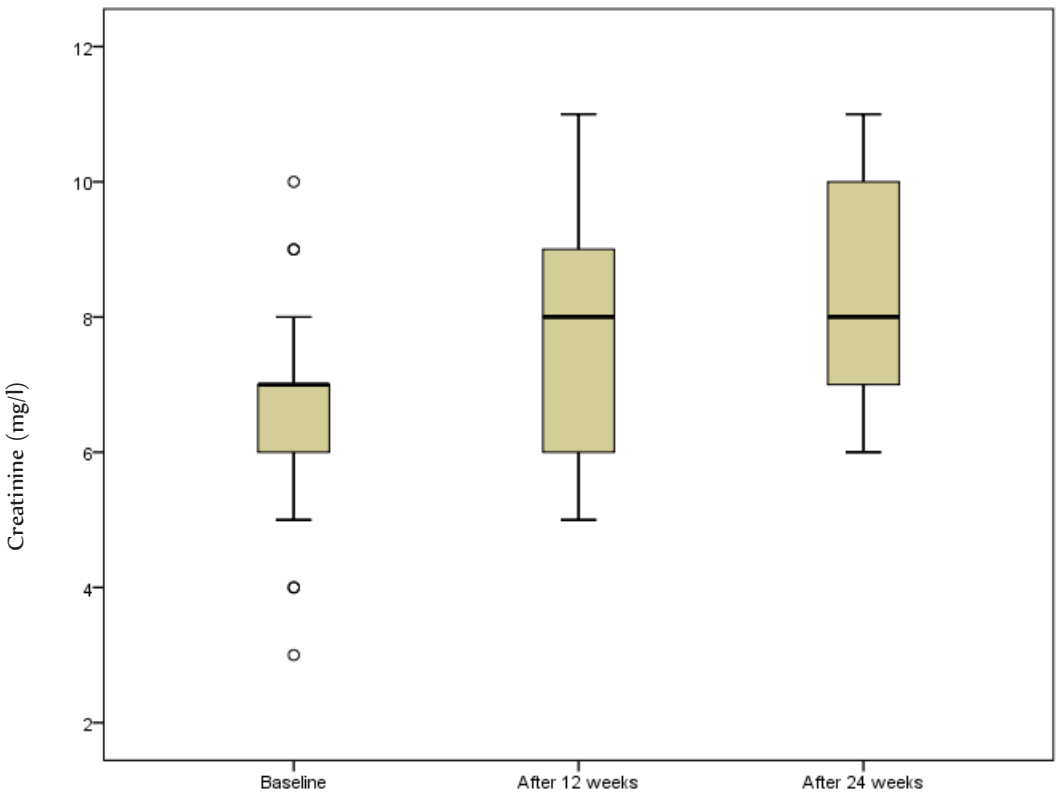


FIG 10.9 BOX PLOT REPRESENTING CREATININE IN THE LONG-TERM INTERVENTION PER SAMPLING

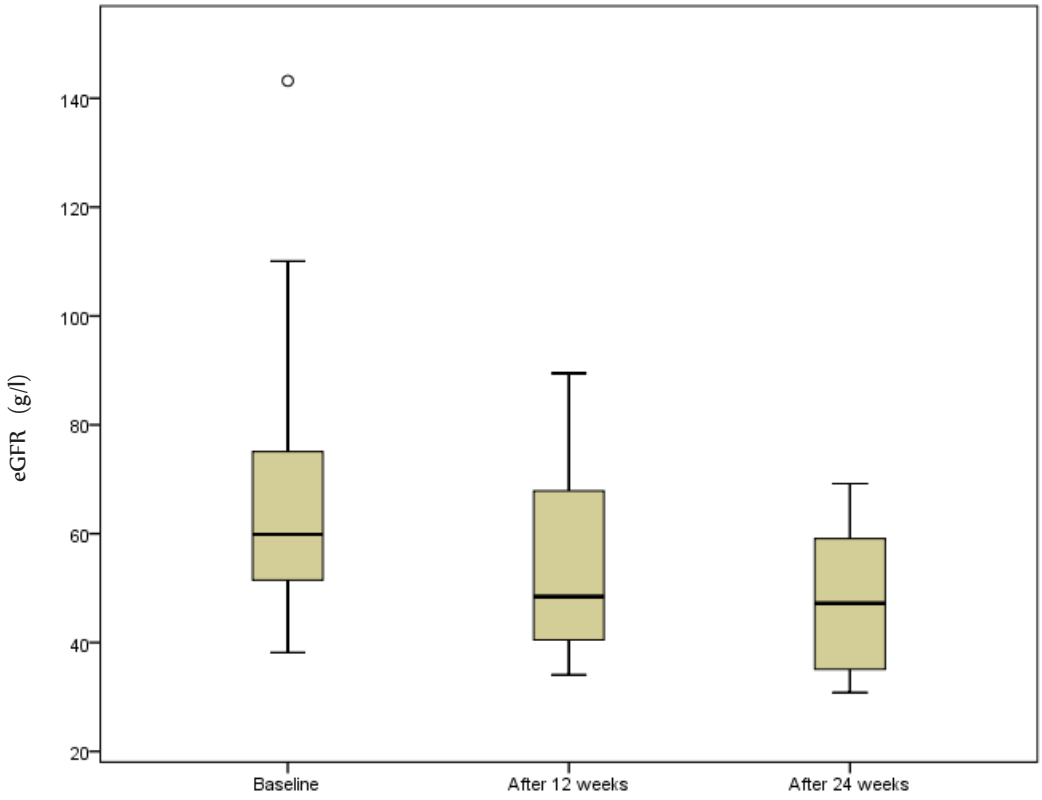


FIG .10.10 BOX PLOT REPRESENTING EGFR IN THE LONG-TERM INTERVENTION PER SAMPLING

- **Cross over Intervention**

TABLE 10.27 IMMUNO-VIRAL OUTCOMES OF THE CROSSOVER INTERVENTION

	CD4 (cells/mm^3)		Viral Load (log10)		CD38-CD8 (%)	
Crossover n=13	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	469(435–558)	321– 815	5.9 (5.6– 6.2)	3.1–6.7	26.6(23.3 -44.8)	15.5–58
Placebo Period	-33 (-87– 14)	-252 - 120	0.0 (-0.4 - 0.2)	-1.0–0.6	-2.4(-11.3– 0.4)	-21.5–16.6
***Asp* Period**	-14(-71–131)	296– 176	-0.2(-0.4 - 0.1)	-1.4–0.6	0.0(-11.2– 3.5)	-23.9–4.7

Viral load

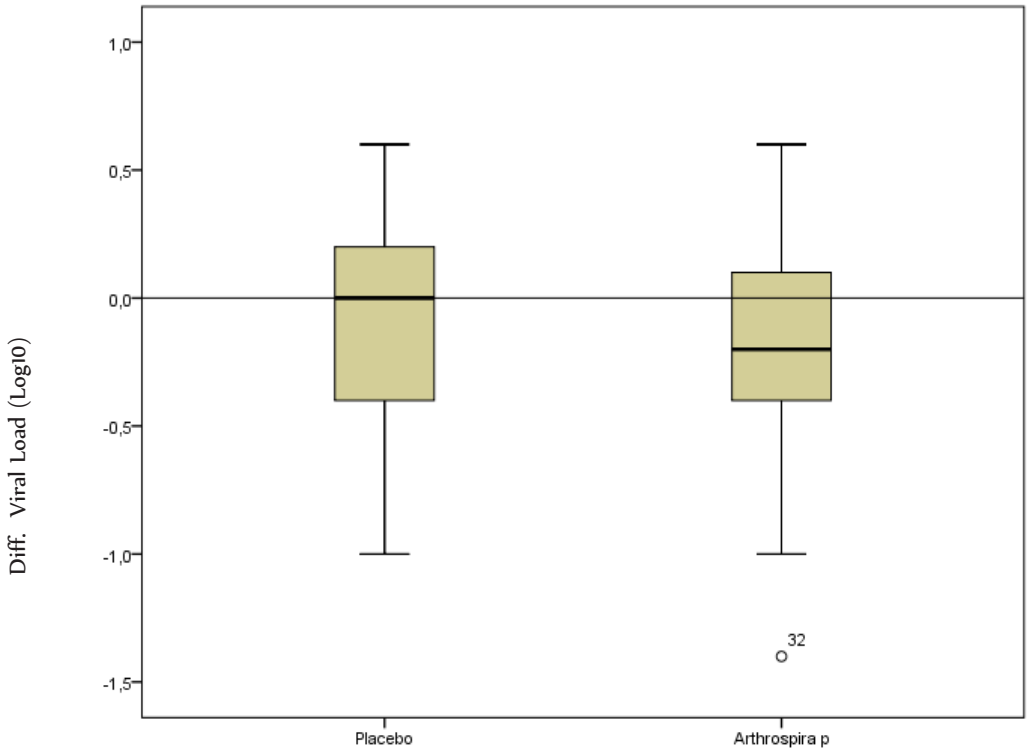


FIG. 10.11 BOX PLOT REPRESENTING THE VL DIFFERENCE OCCURRING IN THE VL IN THE CROSSOVER POPULATION DURING THE TWO PERIODS, PLACEBO AND UNDER ASP

TABLE 10.28 BODY WEIGHT, TAOS, AND ALBUMIN VARIABLES

	Weight (kg)		TAOS (µM)		Albumin (g/l)	
Crossover n=13	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	57.9 (55.8– 62.3)	49.7– 68	408 (361– 460)	193– 509	50 (47– 51)	43– 58
Placebo Period	0.4 (0.2– 2.5)	-3.4– 5.8	-54 (-103– -00)*	-154– 153	-2 (-5– 0)	-12– 5
***Asp*Period**	-1.1 (-2.0– 0.2)	-6.1– 3.6	67 (44– 243)*	2– 345	0 (-4– 1)	-7– 5.2

*p<.05

TABLE 10.29 HAEMOGLOBIN, RED BLOOD CELLS, AND IRON

	Haemoglobin (g/dl)		RBC (tera/l)		Iron (mg/l)	
Crossover n=13	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	12.5 (10.7– 12.8)	9– 14.6	4.5 (4.4– 4.9)	4.1– 6.4	1 (0.9–1.1)	0.4–1.2
Placebo Period	-1.1 (-1.3– -0.5)*	-2.2– 2.5	-0.4 (-0.5– -0.3)*	-1.5– -0.1	0.0 (-0.3–0.3)	-0.9–1.0
***Asp* Period**	0.2 (-0.7– 1.0)*	-1.7– 3.4	0.3 (-0.2– 0.4)*	-0.3– 0.9	0.1 (-0.1– 0.2)	-0.6–0.4

*p<.05

Haemoglobin

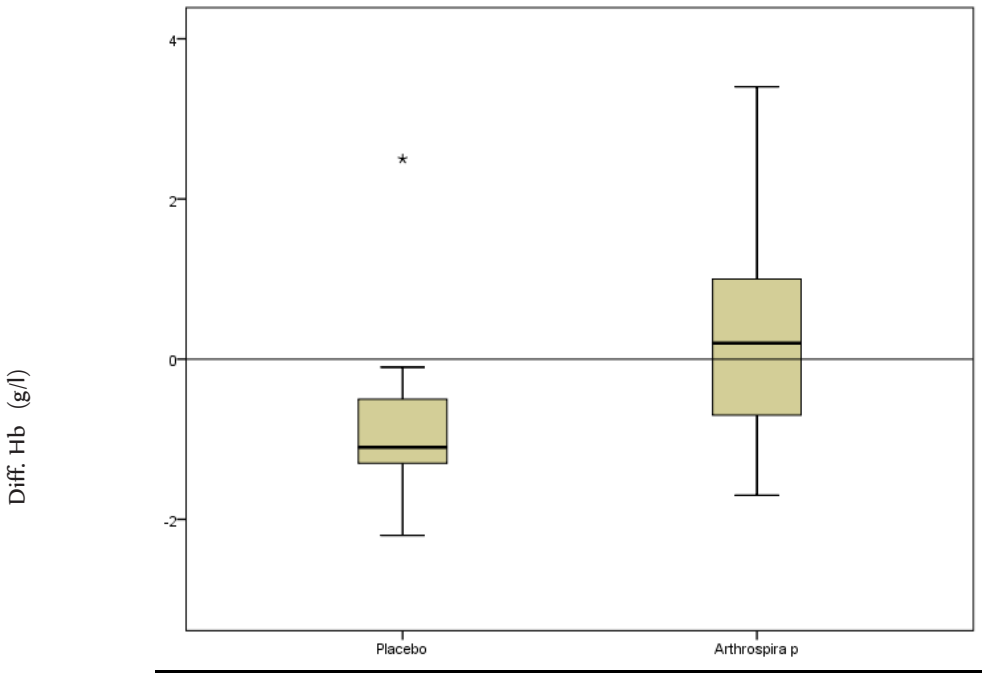


FIG. 10.12 BOX PLOT REPRESENTING THE HB DIFFERENCE OCCURRING IN THE CROSSOVER POPULATION DURING THE TWO PERIODS, PLACEBO AND UNDER ASP

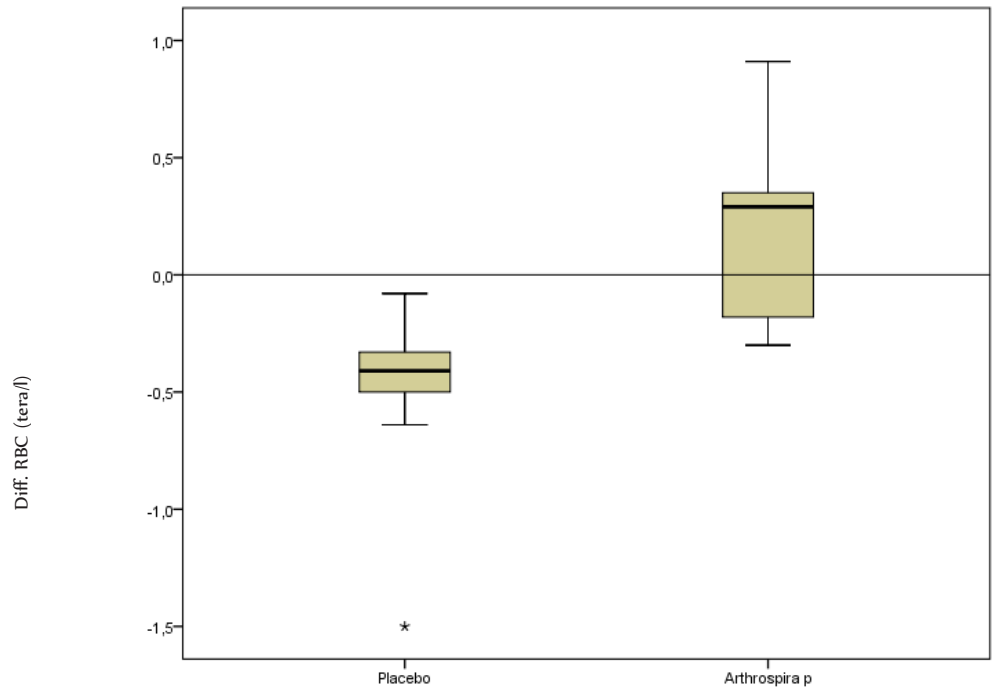


FIG. 10.13 BOX PLOT REPRESENTING THE RBC DIFFERENCE OCCURRING IN THE CROSSOVER POPULATION DURING THE TWO PERIODS, PLACEBO AND UNDER ASP

Iron

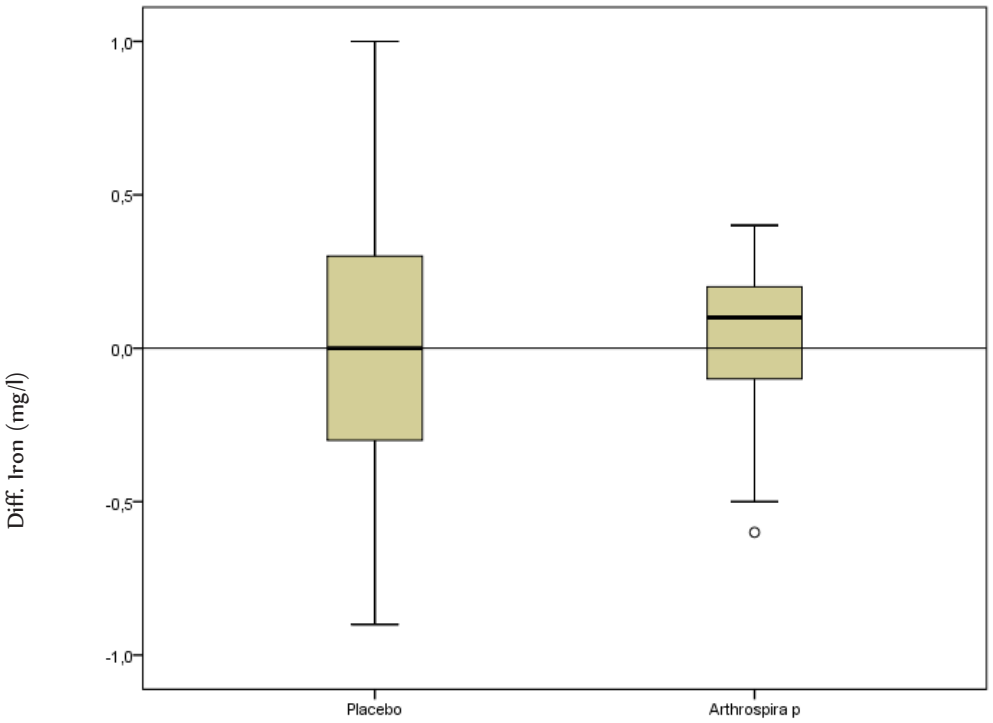


FIG. 10.14 BOX PLOT REPRESENTING THE IRON DIFFERENCE OCCURRING IN THE CROSSOVER POPULATION DURING THE TWO PERIODS, PLACEBO AND UNDER ASP

TABLE 10.30 TOTAL CHOLESTEROL AND TRIGLYCERIDES IN THE CROSSOVER GROUP

	Cholesterol (g/l)		Triglycerides (g/l)	
Crossover n=13	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	1.70(1.29– 1.84)	1.00– 2.18	0.79(0.60–0.97)	0.39–1.35
Placebo Period	-0.06 (-0.18– 0.03)	-0.68– 0.42	0.07 (-0.16–0.57)	-0.44– 1.22
***Asp*Period**	0.05 (-0.15– 0.17)	-0.72– 0.45	-0.24 (-0.36–-0.08)	-1.10–0.26

TABLE 10.31 RENAL FUNCTION

	Creatinine (g/dl)		Urea (g/l)		eGFR (ml/min)	
Crossover n=13	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**
Baseline	0.7 (0.6– 0.8)	0.5– 1.6	0.21 (0.17– 0.24)	0.10– 0.30	51.2 (49.3– 61.1)	22.3– 77.1
Placebo Period	0.0 (-0.2– 0.1)	-0.3– 0.2	-0.02 (-0.05– 0.01)	-0.12– 0.09	2.7 (-6.3– 4.6)	-18.1– 42.0
***Asp* Period**	0.1 (0.0– 0.3)	-0.1– 0.4	0.0 (-0.02– 0.07)	-0.19– 0.14	-4.9 (-18.9– 0.1)	-44.1– 1.6

eGFR

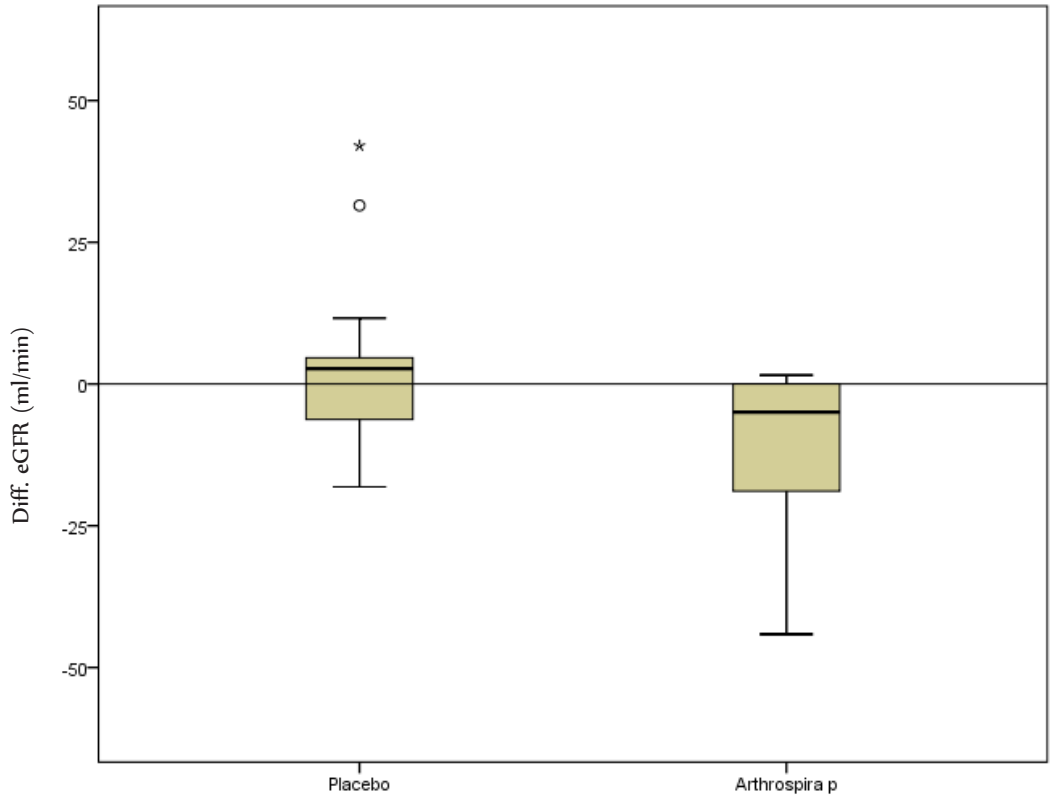


FIG. 10.15 BOX PLOT REPRESENTING THE EGFR DIFFERENCE OCCURRING IN THE CROSSOVER POPULATION DURING THE TWO PERIODS, PLACEBO AND UNDER ASP

Arthrospira Platensis **comme supplément nutritionnel chez les adultes vivant avec le VIH/SIDA à Yaoundé au Cameroun**

Fiche d´information du Participant

Les déficits nutritionnels sont en général un problème de santé publique dans les pays en voie de développement .La malnutrition ne signifie pas seulement une perte de poids, (malnutrition quantitative) mais aussi une carence en éléments essentiels comme les vitamines, les sels minéraux (malnutrition qualitative).
La nutrition représente une composante essentielle de la prise en charge globale des personnes infectées par le VIH. Cependant, l'aspect nutritionnel n'est pas encore suffisamment intégré dans la prise en charge des personnes vivant avec le VIH/SIDA, malgré l'effet bénéfique d'une nutrition adéquate sur l'évolution de la maladie d'une part et sur la prévention de la transmission mère-enfant d'autre part.
Prévenir la survenue des déficits nutritionnel chez les personnes vivants avec le VIH/SIDA par une supplémentation des repas est très important, et surtout un supplément adapté á l´alimentation locale et peu couteux.

La spiruline scientifiquement appelé « *Athrospira Platensis* » est une algue de la famille des Cyanobactérie. Elle est consommée traditionnellement depuis des décennies par les habitants du Kanem pourtour du lac Tchad et certaines populations en Amérique centrale. De nombreuses études scientifiques réalisées dans diverses universités dans le monde et grandes institutions internationales ont établi que la spiruline est très riche en protéine et micronutriment (les vitamines et oligo-éléments).
Même si le nombre d'études sur l'homme reste encore insuffisant, l'effet positif sur la santé de la Spiruline ne fait plus aucun doute.
Ces effets sont particulièrement intéressants dans la régulation du système immunitaire, dans la prévention de certains cancers, dans les infections virales et dans la réduction du taux de cholestérol.
Ceci est d'autant plus intéressant que la culture de la Spiruline est bien maîtrisée et qu'elle a fait la preuve de sa parfaite innocuité. En Afrique, une étude scientifique menée au Burkina-Faso a introduit la spiruline avec succès dans le programme de réhabilitation des enfants malnutris vivant avec le VIH/SIDA Plusieurs pays africain ont commencé la production locale de la spiruline (RDC, Mali, Burkina-Faso, Mauritanie, etc…).

Investigateurs	E-mail	Organisation	Téléphones
Dr. Azabdji Kenfack Marcel (Medecin)		Faculté des sciences et biomédicale de l´université de Yaoundé I	
Prof.Dr.Michael krawinkel (Medecin-nutritioniste)		Justus-Liebig-Universität Gießen: Institut internationale de Nutrition	
Francois Emakam (Biologue)			
Frank Winter (Biologue)			
Dr. Abena Foe Jean louis (medecin)			
Dr. Tsague Dongo (medecin)			
Dr. Sobngwi Eugéne (medecin)			

But de l´Etude

Vous êtes appelé à participer volontairement á une étude qui consiste á évaluer l´impact bénéfique qu´un aliment á base de spiruline peut avoir notamment chez les personnes adultes vivant avec le VIH/SIDA et sujette á un déficit nutritionnel.

Plusieurs composés extraits de plantes ou d'algues possèdent un effet bactéricide et virucide. Les extraits totaux de plantes contiennent souvent plusieurs composants qui agissent en synergie et permettent d'augmenter cet effet tout en diminuant le risque de résistance. Par ailleurs, comme déjà mentionné plus haut, certains produits naturels ont également un effet modulateur sur le système immunitaire, ce qui permet d'augmenter l'effet protecteur contre les virus et bactéries.

Les études réalisées montrent une activité antivirale de la Spiruline dans divers modèles in vitro et chez les animaux. La Spiruline peut donc avoir un rôle intéressant pour le patient porteur du virus HIV. Elle aura non seulement une action sur le virus lui-même, mais également sur les autres infections virales ou bactériennes présentes chez le malade porteur du virus HIV.La Spiruline permet d'apporter une quantité importante d'antioxydants nécessaires à notre organisme : des caroténoïdes comme le béta-carotène et la zeaxanthine, des enzymes comme la superoxyde-dismutase ou encore de la phycocyanine.

Notre But est d´évaluer les bénéfices apporté par la spiruline, au vue de son prix dérisoire, compte tenue du pouvoir d´achat des Camerounais. Pour notre étude nous utiliserons une spiruline fournie gracieusement par une ONG…….. dont la qualité est officiellement admise selon les standards requis par les autorités alimentaires européennes.

Risques et préjudices possible.

Il n´y n'a aucun préjudice connu associé à la participation de cette étude. Aucun dommage spécifique non plus n´est envisageable. La réhabilitation nutritionnelle par les aliments connus ne pose pas de risque particulier. La Spiruline peut être consommée sans aucun danger. Son innocuité en tant que nourriture a été établie par des siècles d'utilisation humaine ainsi que par des études toxicologiques rigoureuses. Elle reçut en 2003 de l´administration Américaine (US Food and Drug-administration) de sa biomasse sèche, le statut de Aliment conventionnel (GRAS generally regarded as Safe).

Bienfaits possible pour le Patient

L´établissement et la validation de l´impact bénéfique de la réhabilitation nutritionnelle des personnes vivant avec le VIH/SIDA pourrait constituer une amélioration des soins á cette catégorie de personne, avec une base de production nationale d´une source protéique(Spiruline) de haute qualité comme c´est déjà le cas dans certains pays de l´Afrique de l´ouest.

Nous attendons une amélioration de la qualité de vie des patients et participant ainsi aux efforts de lutte contre la pauvreté á l´échelle nationale.

Le principal bénéfice pour le patient est le suivi biologique aux normes du plan nationale de lutte contre le SIDA, mais financé par l´étude, pendant la durée de l'inclusion. En plus de ce qui est prévu dans le plan national de lutte contre le SIDA, le participant aura un complément de suivi biologique du point de vue de la charge virale. Evaluation scientifique complète du statut nutritionnel avec plan de renutrition taillé-sur –mesure pour chaque participant (habituellement très difficile dans la pratique quotidienne).

Description de l´étude

Cette étude est étalée sur une période de six mois. A trois reprises les échantillons de votre sang seront prélevés pour analyse des éléments nutritionnels, immunitaires et infectieux. Au début et á la fin de l´étude un médecin vous examinera, en dehors des rendez-vous usuels avec votre médecin-traitant, ou avec le psychologue. Vous passerez une interview de 10 á 20 minutes. Vous aurez à consommer la spiruline pendant la durée d´inclusion.

Des séances seront organisées au fur et á mesure de l´avancement de l´étude dans le but d´apporter des réponses á vos réponses éventuelles futures questions

Confidentialité :

La confidentialité sera respectée. Aucune information révélant l'identité du sujet ne sera diffusée ou publiée sans consentement à moins d'en être astreint par la loi. En dehors de l´interview médicale et les examens physiques, votre identité est anonyme.

Consentement :

En signant ce formulaire, j´atteste que :

- Toutes les explications m´ont été donnée concernant l´étude.
- Toutes mes questions ont étés répondues.

- Les préjudices et les malaises possible ainsi que les bienfaits de cette étude m´ont été expliqués
- J´ai le choix de ne pas répondre á toute questions particulière.
- Je suis libre de poser des questions, maintenant et á l´avenir concernant l´étude.
- J´ai été informé de la confidentialité de mon information personnel
- Aucun renseignement pouvant m´identifier ne sera diffusé ou imprimé sans mon consentement.
- Je recevrai une copie signée due présent formulaire de consentement.

Par la présente, je consens á Participer á l´étude :

Nom :……. Age :……Date :……

Numéro de Téléphone :……

Etabli par :…… Tel.Nr……

- CPC Tube Distribution

Marker	Used Blood	Blood fraction	Quantite need	Probe storage	Mesurement	Departement
Charge viral	EDTA	Plasma	1ml			Viro
Protein C		serum				Viro
CD4+ T-Cell	EDTA	Whole blood		2.5 ml of Whole EDTA blood	Cytometrie	Viro
CD8+ T-Cell	EDTA	Whole blood		-	Cytometrie	Viro
CD38/CD8+ T-Cell	EDTA	Whole blood		-	Cytometrie	Viro
total T-Cell	EDTA	Whole blood		-	Cytometrie	Viro
CMV	EDTA	Whole blood		-	PCR	Viro
TAOS	heparin	Plasma		Plasma in cup at -70°C (Hep. aliquot)	Spectro, 600nm	Biochemi
Albumin	heparin	serum	10 µl	Heparin tube 0.5 ml serum aliquot	Spectro, 628nm	Biochemi
Creatinin	heparin	serum	100 µl	-	492nm	Biochemi
Fer	tube sec	serum	20 µl	-		Biochemi
Ca	heparin	serum	20 µl	-	570nm	Biochemi
Zn			20 µl	-		Biochemi
Uree	tube sec, pas d´heparin	serum	10 µl	-	578nm	Biochemi
Protein total	tube sec			-		Biochemi
cholesterol	heparin ou EDTA	serum	10 µl	-	500nm	Biochemi
Bilirubin	heparin	serum	100 µl	-	546nm	Biochemi

UNIVERSITY OF YAOUNDÉ I
Faculty of Medicine and Biomedical Sciences
Department of Physiologie and Biochemistry

UNIVERSITE DE YAOUNDE I
Faculté de Médecine et de Sciences Biomédical
Département de Physiologie et Biochimie

Fiche de Donnée :

Étude Intervention Nutritionnelle sur des Patientes de l'Hôpital du Jours, Yaoundé

Nr de la patiente dans l´étude :
Groupe d'intervention :
Code anonyme (Nr étude, année de naissance, deux lettres du nom, lettre de group): _____._____._____.__

Echéancier des visites prévues dans le protocole pour la patiente :

N°	Echéancier	RDV prévu	Vu le	Commentaire
1	**J0** Visite générale			
2	J30 ou S4			
3	J60 ou S8			
4	**J90** ou **S12** visite générale			
5	J 120 ou S16			
6	J150 ou S20			
7	**J180** ou **S24** visite de fin d'intervention			
8	J 210 après l'intervention			

Dossier contient :

-Consentement éclaire signer du patient
-les fiches résultats Laboratoire du CPC (3 fiches)
-Fiche Vérification des critères d'inclusion dans l'intervention
-Fiche Consultation General (3 fiches)
-Fiche Clinique (nombre : ___)
-Fiche Biologie (3 fiches)
-Fiche Consultation Suivie (4 fiches)
-Fiche Dernière Visite

Phase de l'Etude : _____ Code Patient : ___.____.___.__

-Questionnaire Patient (4 questionnaires)

-Dépliant Patient (6 dépliants)

VERIFICATION DES CRITERES D'INCLUSION DANS L'INTERVENTION	**Oui**	**Non**
- Femme	❑	❑
- Age supérieur ou égal à 18 ans, inferieur à 49 ans	❑	❑
- Infection VIH documentée	❑	❑
- BMI<26	❑	❑
- CD4 >350 mm^3/l	❑	❑
- ARV naif.	❑	❑
- Absence d'infection opportuniste en cours non stabilisée ou toute pathologie grave ou évolutive	❑	❑
- Patient en mesure de comprendre l'information qui lui est délivrée et de participer au protocole, acceptant l'intervention et le suivi pendant au moins 24 semaines et acceptant de ne prendre aucun Supplément nutritif associé pendant la durée de l'essai sans en aviser l'investigateur.	❑	❑
- Test de grossesse négatif pour les femmes en âge de procréer, n'allaitant pas et acceptant une contraception mécanique.	❑	❑
- Critères Clinique en pré-inclusion.		
. Diabètes ……	❑	❑
. Cirrhose du foie ……	❑	❑
. Fumeuse active ……	❑	❑
. dysfonctionnement rénal ……	❑	❑
.Diarrhée persistante ……	❑	❑
Signature du consentement éclairé : Date de signature |__|__| |__|__| |__|__|	❑	❑

COMMENTAIRES concernant les critères d'éligibilité ?

……

En conclusion : ❑ **patiente non éligible** **Raison** : ……

❑ **patiente éligible** **Stade OMS :** ……..

Date prévue pour l'inclusion (J0) : |___|___| |___|___| |___|___|

Je certifie l'exactitude des critères d'inclusion ci-dessus

Nom investigateur : Signature :

…… Date de recueil : _____

Phase de l'Etude : _____ Code Patient : ___.____.___.__

La patiente est-il venu en consultation ? Oui ❑ **DATE DE LA CONSULTATION PREVU** ……..

Non ❑ Raison : ……..

Consultation General N°

Nom :	Nationalité :	
Age date de naissance:	Religion :	
Quartier à Yaoundé :		
Tel. de la Patiente :		
Tel. 2 :		
Tel. 3 :		
Nr de la patiente dans l´étude :		
Groupe d'intervention :		
Code anonyme (Nr dans l'étude, deux derniers chiffres de l'année de naissance, deux dernières lettres du nom, lettre de group): ____.____.____.__		

Donnée Clinique :

	Poids :_____ g	Taille :_____cm	IMC :_____g/cm	
	Température : _____,___°C			
	Pression artérielle, systolique/diastolique : _____/_____mm.Hg *(après 10 minutes de repos allongé)*			
	Tour de taille _____ cm ; Tour de hanche _____ cm ; Tour de cuisse _____ cm (debout, à l'endroit le plus étroit à mi-distance de la dernière côte et de la crête iliaque) (à l'endroit le plus large des hanches) (à 15 cm du bord supérieur de la rotule)			

Test de grosse :

	Test sanguin β hCG	**Oui** ☐ **Non** ☐ **Fait le : _____ Résultat :_____**	

Historique des CD4 et charges virales :

Année de Test :_____ Année d´infection :_____

Sérologie VIH (ci-connu) : VIH Type _____

Valeur Virologique :

		Prélèvement le : _____		
	CD4	/ mm ^{3}		
	CD8	/ mm ^{3}		
	CV	Cp/ ml		

Phase de l'Etude : _____ Code Patient : ___.____.___.__

FICHE CLINIQUE N°

Antécédents Clinique :

Noter les **antécédents cliniques importants** portés à votre connaissance (maladie chronique, cardio-vasculaire, diabète, allergie, etc…). **et** les évènements cliniques que présente le patient **actuellement**.

	Nature de l'événement (***diagnostic si possible, sinon symptômes)***	**classement OMS** Oui	Non	**Degré (a)**	**Date de début**	**Date de fin**	**Degré max (a)**	**Action (b)**	
		❑	❑						
		❑	❑						
		❑	❑						
		❑	❑						
		❑	❑						
		❑	❑						
		❑	❑						
		❑	❑						
		❑	❑						

(a) Degré ou degré max : **1** = Léger **2** = Modéré **3** = Sévère **4** = Menace vitale

(b) Action : **0** = Aucune **1** = Traitement ou chirurgie **2** = Traitement ou chirurgie avec Hospitalisation

Traitements associes :

Noter les traitements **ayant été pris en prophylaxie** ou les traitements **pris actuellement**

	Traitement	**Posologie** dose/j mg ou cp	**Prophylaxie**	**Curatif**	**Indication (cf. évènements cliniques)**	**Date de début**	
			❑	❑			
			❑	❑			
			❑	❑			
			❑	❑			
			❑	❑			
			❑	❑			

Phase de l'Etude : _____ Code Patient : ___.____.___.__

Antécédents cliniques lies au VIH :

	Antécédent clinique nature de l´événement, symptôme	oui	Degré de sévérité	
	OMS Stade 1			
	◆ Asymptomatique			
	◆ Adénopathies généralisées persistantes			
	OMS Stade 2			
	◆ Chéilite angulaire (perlèches)			
	◆ Dermite séborrhéique			
	◆ Eruption papulaire prurigineuse (prurigo)			
	◆ Zona			
	◆ Infections des voies aériennes supérieures récurrentes			
	◆ Mycose unguéale			
	◆ Perte de poids inexpliquée < 10%			
	◆ Ulcérations orales récurrentes			
	OMS Stade 3			
	◆ Anémie (<8g/dl) ou neutropénie (<500/mm3) ou thrombocytopénie (<50 000/mm3) inexpliquée			
	◆ Candidose persistante orale			
	◆ Diarrhée inexpliquée > 1 mois			
	◆ Fièvre constante ou intermittente inexpliquée >1 mois			
	◆ Infections bactériennes sévères (pneumonie inclue)			
	◆ Leucoplasie orale chevelue			
	◆ Perte de poids > 10 %, inexpliquée			
	◆ Stomatite/gingivite/desmodontite ulcérative aigue nécrosante			
	◆ Tuberculose pulmonaire			
	OMS Stade 4			
	◆ Candidose de l'oesophage, trachéobronchique ou pulmonaire			
	◆ Carcinome cervical invasif			
	◆ Cardiomyopathie liée au VIH			
	◆ Cryptococcose extra-pulmonaire (méningite incluse)			
	◆ Cryptosporidiose avec diarrhée > 1 mois			
	◆ Cytomégalovirus, infection			
	◆ Encéphalopathie liée au VIH			
	◆ Herpes cutanéo-muqueux > 1 mois ou viscéral			
	◆ Isosporose avec diarrhée > 1 mois			
	◆ Leishmaniose viscérale			
	◆ Leucoencéphalopathie multifocale progressive			
	◆ Lymphome cérébral ou non-Hodgkinien à cellules B			
	◆ Mycobactériose atypique disséminée			
	◆ Mycose disséminée (e.g. histoplasmose, penicilliose, coccidioïdomycose)			
	◆ Néphropathie liée au VIH			
	◆ Pneumonie bactérienne récurrente			
	◆ Pneumonie à Pneumocystis carinii			
	◆ Sarcome de Kaposi			
	◆ Septicémie récurrente à salmonelle non-typhique			
	◆ Syndrome cachectique lié au VIH(perte de poids >10% et soit >1 mois diarrhée ou >1 mois fièvre)			
	◆ Tuberculose extra-pulmonaire ou disséminée (eg Tuberculose ganglionnaire abdominale)			
	◆ Toxoplasmose cérébrale			

*) 1 = Leger ; 2 = Modéré ; 3 = sévère ; 4 =menace vitale

Classification de l'infection VIH aujourd'hui : T=_____ OMS

Phase de l'Etude : _____ Code Patient : ___.____.___.__

Fiche Biologie N°

Bilans biologique :

(Voire résultat fiche CPC, reporter dans le tableau ci-dessus)

	Test	Valeur	
	NFS		
	Hémoglobine		
	Leucocytes		
	Polynucléaires		
	neutrophiles		
	éosinophiles		
	basophiles		
	Lymphocytes		
	Monocytes		
	VGM		
	Plaquette		
	Biochimie		
	Albumine		
	Bilirubine		
	Cholestérol		
	Créatinine		
	Urée		
	Fer		
	Marqueur antioxydant		
	TAOS		
	MDA		

Test malaria :

	Goutte épaisse		

Commentaire :……
…… ……
……
……
……

Date /signature :

Phase de l'Etude : _____ Code Patient : ___.____.___.__

La patiente est-il venu en consultation ? Oui ❑ **DATE DE LA CONSULTATION PREVU** ……

Non ❑ Raison :…….

Consultation de suivie N°

	Date de consultation Effectuer		
	Code Patiente		
	Nouveau Tel.		

Bonne réception du dépliant Patiente : Oui ☐ Non ☐

	La patiente présente t´il de nouveau évènement clinique et/ou évolution d´événement déjà rapporté ? **Oui ☐ Non ☐**	
	Ci oui, (pour des symptômes et traitement nouveau remplir une fiche Clinique)	

Donnée Clinique :

	Poids :_____ g	Taille :_____cm	IMC :_____g/cm	
	Température : _____,___°C			
	Pression artérielles, systolique/diastolique : _____/_____mm.Hg *(après 10 minutes de repos allongé)*			
	Tour de taille _____ cm ; Tour de hanche _____ cm ; Tour de cuisse _____ cm (debout, à l'endroit le plus étroit à mi-distance de la dernière côte et de la crête iliaque) (à l'endroit le plus large des hanches) (à 15 cm du bord supérieur de la rotule)			

Date/ Signature :

Phase de l'Etude : _____ Code Patient : ___.____.___.__

DERNIERE VISITE DE LA PATIENTE DANS LE PROTOCOLE

Date du suivi : _____

RAISON DE FIN D'ESSAI

Indiquer la raison principale :

- ❑ Fin conforme au protocole
- ❑ Patiente sous ARV

  Date de début de traitement : _____
- ❑ Abandon avec retrait du consentement

  Date du retrait : _____
- ❑ Patiente perdu de vue

  Date du dernier contact : _____

Commentaires : ....

- ❑ Patiente décédé

  Date du décès :_____

Cause :..... ....

- ❑ Autre (préciser) : ....

.....

COMMENTAIRES

.....

.....

.....

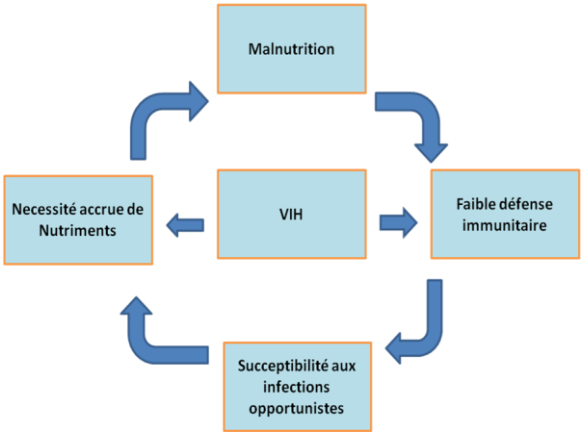
Date / Signature :

Quels sont les buts de l´étude?

Notre étude vise á évaluer l´impacte qu´une supplémentation á base de *Arthrospira platensis* peut avoir chez les P + et sujette á un déficit alimentaire

Cercle vicieux Maladie et Malnutrition

Les P + ont un besoin accrus en nutriment. Cet apport est dû à l´activité permanente du system immunitaire. En plus l´augmentation de l´apport en nutriment devra compenser les pertes dues aux perturbations lors de l`assimilation de ces même nutriment dans le corps. Le supplément nutritif est censé compléter votre alimentation ordinaire (Vitamines, sels minéraux, acides gras etc..).



Quel bénéfice pour les Personnes ?

Suivi personnalisé aux normes du plan de lutte. Le patient recevra un complément de suivi biologique, á chaque visite une fiche sur son poids, sont statut immunitaire, ainsi qu´une évaluation scientifique complète du statut nutritionnel avec plan de renutrition á la fin de l´étude. Il lui est garantie de recevoir au cours de l´étude un supplément nutritif

Vous vous engagez á vous présenter á la **visite mensuelle**. ICI, votre boite de capsule ainsi que ce dépliant avec le tableau dument remplie, vous seront remplacés par un nouveau dépliant ainsi que les capsules pour un mois.

Votre prochaine visite est prévu pour

le : A :

Nous nous engageons á vous donner toutes les informations sur votre état de santé dans le cadre de cette étude. Ainsi qu´une fiche patient documentant de votre poids (BMI), proportion de gras et statut immunitaire.

Vous vous engagez á prendre 5 gr. de supplément par jour soit 10 capsules. Ceci pendant les heures de repas.

Vous vous engagez á nous informer de tout changement vous concernent pendant la durée de l´étude (adresse, santé..), ainsi que de remplir le tableau au verso.

Nous sommes á votre disposition 7jours/7 á tout moment. Sous le numéro de téléphone:

Dr :

Etude clinique sur l´effet d´un supplément alimentaire chez les P. plus à Yaoundé

Consentement éclairé pour le patient

Le médecin m´a fourni les explications orales et les informations écrites sur l´objet de l´étude, l´intervention, les effets attendus, avantages et inconvénients éventuels.
Par ma participation, je confirme avoir été bien informé du déroulement de l´étude et je m´engage á fournir les informations correcte.

Je peux retirer á tout moment mon consentement á participer à l´étude sans avoir á fournir de raison et sans que mes soins médicaux futurs soient affectés.

Tableau de suivie journalier; Prise du Supplément et des petits malaises rencontrés.

Nom et prénoms du Patient :_____ Nr de téléphone :_____ Prochain Rendez-vous :_____ Heure :_____

Date		**Semaine Nr ._____ du_____/_____**							**Semaine Nr.____ _du_____/_____**							**Semaine Nr ._____du_____ /_____**							**Semaine Nr ._____du_____ /_____**						
Jours de la semaine*		**Lu**	**Ma**	**Me**	**Je**	**Ve**	**Sa**	**Di**	**Lu**	**Ma**	**Me**	**Je**	**Ve**	**Sa**	**Di**	**Lu**	**Ma**	**Me**	**Je**	**Ve**	**Sa**	**Di**	**Lu**	**Ma**	**Me**	**Je**	**Ve**	**Sa**	**Di**
Prise des 10 Capsules ; Oui/Non = O/N																													
Documentez votre santé le soir pour la journee passée		*Remplir le Tableau á l´aide des nombres,* ***1=pas du tout ; 2= un petit peu ; 3= moyennement ; 4= beaucoup ; 5= énormément***																											
Appétit																													
Fatigue																													
Nausée/Vomissement																													
Toux	Sèche																												
	Crachat																												
Douleurs abdominales																													
Selles	Diarrhée																												
	Constipation																												
Jours de la semaine*																													

Remplir le Tableau á l´aide des nombres, **1=pas du tout ; 2= un petit peu ; 3= moyennement ; 4= beaucoup ; 5= énormément.**

*lundi=Lu, Mardi=Ma, Mercredi=Me, Jeudi=Je, Vendredi=Ve, Samedi=Sa, Dimanche=Di.

Remarque :_____

_____

_____

_____

_____

_____