THE EFFECT OF *LACTOBACILLUS REUTERI* SUPPLEMENTATION ON ANTHROPOMETRIC MEASUREMENTS, LUNG FUNCTION AND LUNG INFECTIONS IN A CYSTIC FIBROSIS POPULATION IN KWAZULU-NATAL

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ABSTRACT

BACKGROUND: Cystic fibrosis (CF) patients grow poorly and tend to be malnourished. They frequently suffer from lung infections necessitating the repeated use of antibiotics.

AIM: This study was conducted to determine whether supplementation with a probiotic *Lactobacillus reuteri* (*L. reuteri*) could reduce the incidence and duration of lung infections, and whether this would impact on their anthropometric data. The secondary purpose was to compare the nutritional status of the CF patients attending CF clinics in Kwazulu-Natal (KZN) with CF patients attending CF clinics in Cape Town (CT).

METHODS: Twenty three CF patients 6-31 years of age from 2 CF clinics in Kwazulu-Natal started the study although only 16 patients completed it. The study was a randomized, double blind, placebo controlled crossover trial with six months on placebo and six months on probiotic. Weight, height, mid arm circumference (MAC), triceps skin fold thickness (TSF), forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were measured, sputum collected and a symptom diary completed over the 12 month period. Anthropometric data of CF patients attending CF clinics in CT was obtained from the publication by Westwood & Saitowitz (1999).

RESULTS: Compliance with taking the *L. reuteri* was poor. Most took only 50% of the required daily dose. Probiotic supplementation showed a slight (non significant) trend to improve FEV_1 and FVC, while no significant difference could be seen in the number and duration of the lung infections. Sputum analysis showed a non significant trend towards the probiotic reducing the number of bacteria in the sputum. There was a significant reduction of symptoms for fever, running nose, sore throat and ear ache while on placebo. There was a significant increase in weight gained off probiotic compared to the probiotic period. The changes in height, weight for age (WFA) percentiles, height for age (HFA) percentiles, WFA and HFA Z-scores, percentage expected weight for age and percentage expected height for age all showed no difference whether on or off probiotic. Over half the CF children in the KZN clinics were underweight for their actual height compared to one third in the CT clinics with a higher number of subjects below the 5^{th} percentile for MAC and TSF readings compared to CT.

CONCLUSION: Due to a small sample size and poor compliance no firm conclusions could be drawn. However a slight (non significant) improvement could be seen in favour of the probiotic for $FEV_{1,}$ FVC, and sputum analysis. Although all other findings were not significantly different it would be of benefit to carry out further investigation with improved compliance with the probiotic to see if the parameters set out above could be improved. The KZN and CT CF groups were comparable and the nutritional status of CF patients on KZN was well below that of the CT CF clinics and further monitoring would need to be carried out.

DECLARATION

I hereby declare that the work presented in this thesis is of my own investigation. Where use was made of the work of others, this has been duly acknowledged in the text.

AJP Read **June 2007**

As supervisor, I agree to submission of this thesis for examination:

Prof. E Maunder **June 2007**

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PREFACE

This study was conducted at St Augustine's Hospital and Addington Hospital in Durban Kwazulu-Natal. It was a master's project carried out in the School of Agricultural Sciences and Agribusiness, University of Kwazulu-Natal, Pietermartizburg from October 2002 to October 2007 under the supervision of Professor Eleni Maunder.

This study represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of work of others it is duly acknowledged in the text.

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CHAPTER 1: INTRODUCTION, THE PROBLEM AND ITS SETTING

1.1 IMPORTANCE OF THE STUDY

Cystic fibrosis (CF) was first identified by Andersen 1938 who published a paper describing the characteristics of the disease (Wikipedia citing Andersen 1938). Although in some countries such as Asia the existence of CF is not well recognized (Wikipedia 2006), the prevalence in first world countries is reasonably well established (World Health Organization WHO 2007). In 2000 the CF registry of the United States of America (USA) had approximately 22303 patients registered (Zhang & Lai 2004). In Britain currently there are 7500 registered CF patients and it is considered the most common life threatening inherited disease in the United Kingdom (UK) and Europe (UK CF association 2007, CF association Ireland 2007, European CF association 2007). Thirty thousand patients are currently registered in Europe (European CF association 2007). It is more common in those from European descent with the Jewish population being the most effected (Wikipedia citing Kerem, Chiba-Falek & Kerem 1997). In a similar situation to Asia, the prevalence of CF in South Africa (SA) is thought to be under diagnosed particularly in the non caucasian population (South African Cystic Fibrosis Consensus Document 2007). In SA CF is thought to effect 1 in 27 caucasians, 1 in 50 coloureds and 1 in 90 blacks (South African Cystic Fibrosis Consensus Document 2007).

At the time of Andersen's publication (1938) CF patients were often misdiagnosed as suffering from kwashiorkor. This highlights the well recognised association between malnutrition and CF (Pencharz & Durie 2000). In those early days, failure to thrive in infancy was common and their maximum life span was 10 to 15 years (Elbourn & Bell 1996). In more recent years the vastly improved medical and nutritional therapy has resulted in a significant decrease in the incidence of malnutrition and consequently CF's survive on average 37 years, with some reaching their late forties (BUPA 2006). Malnutrition however still appears to be a significant problem among the CF population.

Anthropometric data supplied by the different CF registries (America, Canada, United Kingdom and Europe) has been difficult to compare as different countries have reported the prevalence of malnutrition using different growth standards. In the USA in spite of improved therapy, CF children still experienced subnormal growth at all ages (Lai, Corey, Fitzsimmons, Kosorok & Farrell 1999) and malnutrition was particularly pronounced in infants, adolescents and patients with newly diagnosed CF (Lai, Kosorok, Sondel, Chen, FitzSimmons, Green, Shen, Walker & Farrell 1998). According to Shaw & Lawson (2007, p 179) malnutrition amongst the UK CF patients is still a significant issue as in Spain where approximately one fifth suffer from malnutrition (Molina, Bozano, Oses & Allue, 2001). In SA, anthropometric data on CF patients is limited as the only published information is from the Cape Town cystic fibrosis clinic (Westwood & Ireland 2000). In their study Westwood & Ireland (2000) showed that malnutrition, especially growth failure, is also a significant problem in CF patients in Cape Town.

Malnutrition increases susceptibility to infections (Shaw & Lawson 2007, p179) which is particularly detrimental in CF patients as lung infections are the major cause of death (Anthony, Paxton, Catto-Smith & Phelan 1999; Chandra 1997; Calder & Kew 2002).

Pseudomonas aeruginosa is one of the most important pathogens in lung infections in CF patients and has the greatest impact on their life expectancy (Hart & Winstanley 2002). The treatment options are extremely limited as very few antibiotics are effective against *P. aeruginosa* and the development of resistance is a very real issue. In SA approximately two thirds of the patients attending Groote Schuur Hospital (Cape Town) CF clinic were colonized with *P. aeruginosa* and of those one third were resistant to the antibiotic fluoroquinolone (Ventre, Wilcox & Roditt 2000). In light of the limited treatment options, other strategies to enhance the CF patient's resistance to infection are crucial.

Supplementation with probiotics has been shown to stimulate the systemic immune system (Bourlioux, Koletzko, Guarner & Braesco 2003). In healthy children with a normal population of colonic microflora, supplementation with probiotics has been

shown to reduce the incidence of respiratory tract infections (Hatakka, Savilahti, Ponka, Meurman, Poussa, Nase, Saxelin & Korpela 2001). This effect could be more pronounced in CF patients as they probably have an abnormal population of colonic microflora as a consequence of the frequent use of antibiotics, as well as the unusual substrates entering the colon due to food malabsorption (Walters & Littlewood 1998). A disturbance in both the population type and number of the colonic bacteria could suppress the immune response. There are several criteria for the use of a probiotic that will be discussed in the literature review. A single probiotic needed to be used and *Lactobacillus reuteri* was chosen because it is of human origin, is acid and bile stable, colonizes the human gastro intestinal tract (GIT), adheres to human intestinal cells, produces antimicrobial substances, is antagonistic to pathogenic bacteria, has clinically validated and documented health effects, is safe and has a guaranteed viable shelf life and as it meets all the criteria of a successful probiotic making it a suitable choice for the trial. *L. reuteri,* has the potential to stimulate the immune system, possibly reducing the incidence and shortening the duration of lung infections while maintaining or preventing deterioration in lung function. A reduction in the number of lung infections would decrease both the nutritional requirements and nutrient losses of patients with CF while increasing food intake. This would improve nutritional status, which would enhance resistance to lung infections in turn interrupting the malnutrition/infection cycle and reducing mortality.

1.2 PURPOSE OF THE STUDY

The primary purpose of the study was to determine whether supplementation with a probiotic (*L. reuteri*) would improve anthropometric measurements, reduce the incidence and shorten the duration of lung infections and would maintain or prevent deterioration in lung function in CF patients attending the CF KwaZulu-Natal (KZN) clinics in Durban. The secondary purpose of the study was to compare the nutritional status at the end of the study of CF patients attending CF KZN clinics in Durban with CF patients attending CF clinics in Cape Town from the published data by Westwood & Saitowitz (1999).

1.3 STUDY DESIGN

This was a randomized, double blind, placebo controlled crossover trial run over the period of a year.

Figure 1 is included in order to clarify the study design. This figure is repeated in Chapter 3 alongside the objectives indicating exactly the timing and the measurements taken for each objective.

1.4 OBJECTIVES AND HYPOTHESES

1.4.1 Weight

1.4.1 1 To determine weight at baseline[1], start of the probiotic and end of probiotic for the group as a whole as well as by age and gender and to compare weight gained over the six months prior to and while taking the probiotic.

The hypothesis was that all subjects' weights would improve more while taking the probiotic.

1.4.2 Height

1.4.2.1 To determine height at baseline, start of the probiotic and end of probiotic for the group as a whole as well as by age and gender and to compare height gained over the six months prior to and while taking the probiotic.

The hypothesis was that all subjects 20 years and younger would improve in height more while taking the probiotic.

[1] Baseline was six months before the start of the probiotic

1.4.3 Comparison of anthropometric status of cystic fibrosis patients in Durban and Cape Town

1.4.3.1 To compare the percentage expected weight for height (%EWFH), mid arm circumference (MAC) and triceps skinfold thickness (TSF) in CF patients in Durban and Cape Town. For the comparison to the Cape Town patients this data was obtained from the publication by Westwood & Saitowitz (1999).

1.4.4 Incidence and duration of lung infections

1.4.4.1 To determine and compare the incidence and duration of lung infections while taking the probiotic and while taking the placebo.

The hypothesis was that supplementation with *L. reuteri* would reduce both the incidence and duration of lung infections in CF patients.

1.4.5 Lung function

1.4.5.1 To determine and compare the mean change in lung function while taking the probiotic and while taking the placebo.

The hypothesis was that CF patients while taking *L. reuteri* would experience an improvement in lung function.

1.4.6 Sputum analysis

1.4.6.1 To determine the number of CF patients in the study colonized with *P. aeruginosa.*

1.4.6.2 To determine and compare the change in the presence and quantity of *P. aeruginosa* (dry and mucoid), *S. aurens* and *B. cepacia* in the sputum while on the probiotic and while on the placebo.

The hypothesis was that CF patients while taking *L. reuteri* would experience a decrease in the count of *P. aeruginosa* (dry and mucoid), *S. aurens* and *B. cepacia* in the sputum.

1.4.7 Symptoms

1.4.7.1 To determine and compare the number of episodes of symptoms (coughing, wheezing, thrush, diarrhea, constipation, vomiting, stomach ache, fever, runny nose, sore throat and ear ache) while on the probiotic and of the probiotic.

The hypothesis was that CF patients while taking *L. reuteri* would experience a decrease in the number of episodes of symptoms.

1.4.8 Food frequency questionnaire regarding pre and probiotic use

A food frequency questionnaire was used to determine the frequency of use of pre and probiotics.

1.5 STUDY PARAMETERS

The study included all CF patients, 6 years and older, who attended either the Private CF clinic at St Augustine's hospital or the State CF clinic at Addington hospital both in Durban, KZN and who had attended either clinic for longer than one year. Those who had regularly been supplementing with *L. reuteri* and/or other probiotics and/or prebiotics or patients with a Shwachman score[2] of less than 40 were excluded from the study. For

[2] The Shwachman score was used to measure the severity of lung disease. The questionnaire consists of four sections, each with a rating from 0-25. The four sections included are general activity, physical examination, nutrition and chest x-ray. A score of 86-100 is excellent, 71-85 is good, 56-70 is mild, 41 -55 is moderate and 0-40 indicates severe lung disease.

the comparison of Durban patients to the Cape Town patients, the Cape Town data was obtained from the publication by Westwood & Saitowitz (1999).

1.6 DEFINITION OF TERMS

Age groups

The age groups were divided into 6 to 9.99 year olds, the 10 years to 12.99 year olds, the 13 to 19.99 year olds and the ≥20 year olds. Non adolescents were defined as being ≤ 12.99 years, the adolescents as between 13 to 19.99 years old and the adult as ≥ 20 years old. As the study ran for a 12 month period two subjects started in the ≤ 19.99 year old group but moved into the ≥ 20 year old group during this time frame.

Anthropometric measurements

For the purpose of this study anthropometric measurements refers to the measurements of body size and composition, including height, weight, body circumference measurements (mid arm circumference) and skinfold thickness (triceps skinfold thickness) that was collected in this study.

Baseline

Baseline referred to the first set of measurements that was taken six months prior to starting the probiotic. Patients were divided into two groups. Group A that started the probiotic first these measurements were taken from the patient's medical record. This was six months before the trial started. For group B, the group that started the placebo first, these measurements were taken at the start of the study (that is start of placebo) as this was six months before the start of the probiotic.

Cystic fibrosis

Patients were included in the study if their diagnosis had been made on the basis of two positive sweat tests where the sweat chloride exceeded 60 mmol/l in children and 70 mmol/l in adults and/or the presence of CF mutations had been established by DNA testing.

Duration of lung infections

The duration was measured as the number of days of prescribed antibiotic treatment required to treat the lung infection as ordered by the doctor.

Incidence of lung infections

The incidence was the number of new lung infections experienced over the 12 month study period for which the doctor wrote up antibiotic treatment. Continuous antibiotic treatment was not included as a new infection. The 12 month study period included both placebo and probiotic treatment for both groups.

Lung function

Lung function was measured by the forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC). FEV_1 was defined as the maximal amount of air that can be exhaled in one second after taking a deep breath. FVC was defined as the total amount of air that can be exhaled until no more air can be exhaled.

Microbiological culture

Microbiological culture is a method of growing microbial organisms to determine what type and the number present in a sputum sample being tested.

Nutritional Status

For the purpose of this study height for age, weight for age, weight for height, %EWFH, MAC and TSF percentiles were used to establish the nutritional status of the CF patients in the Durban clinics. This was done to compare to the Cape Town CF clinic.

Prebiotic

A prebiotic is the substrate (a non digestible carbohydrate) that favours the growth of the probiotics.

Probiotic

Is a beneficial bacteria that has health properties in that it protects the host and prevents disease.

Probiotic used in this study

Biopro's *Lactobacillus reuteri* straws were used. Each straw contained a guaranteed dose of 1 x 10^8 colony forming units (cfu). The straws were to be used to drink 100 ml to 250 ml of any liquid, which was at room temperature or cold once a day (for example water or juice).

Placebo used in this study

Biopro provided a straw which was similar to the product but which did not contain *L. reuteri*.

Severity of Lung Disease (Shwachman score)

The Shwachman score was used to measure the severity of lung disease. The questionnaire consists of four sections, each with a rating from 0-25. The four sections included are general activity, physical examination, nutrition and chest x-ray. A score of 86-100 is excellent, 71-85 is good, 56-70 is mild, 41 -55 is moderate and 0-40 indicates severe lung disease. Patients scoring less than 40 out of 100 were excluded from the trial.

1.7 ABBREVIATIONS

Abbreviations used in this thesis are shown in Table 1.

Table 1 Abbreviations

Abbreviation	**Meaning**
ANOVA	Analysis of variance
B. cepacia	*Burkholderia cepacia*
BMI	Body mass index
CDC	Center for Disease Control
CF	Cystic Fibrosis
Cfu	Colony forming units
CHO	Carbohydrate
CT	Cape Town
ED	Eating disorders
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GI	Gastrointestinal
GIT	Gastro intestinal tract
H. influenzae	*Haemophilus influenzae*
HFA	Height for age
IQR	Inter quartile range
KZN	KwaZulu-Natal
L. acidophilus	*Lactobacillus acidophilus*
L. casei	*Lactobacillus casei*
L. reuteri	*Lactobacillus reuteri*
LGG	*Lactobacillus rhamnosus* Gorbach and Goldin
MAC	Mid arm circumference
NE	Neutrophil elastase
P. aeruginosa	*Pseudomonas aeruginosa*
PPB	Potentially pathogenic bacteria
S. aureus	*Staphylococcus aureus*
S. boulardii	*Saccharomyces boulardii*
SA	South Africa
TSF	Triceps skinfold thickness
WFA	Weight for age
%EHFA	Percentage expected height for age
%EWFA	Percentage expected weight for age
%EWFH	Percentage expected weight for height
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
Wht	Weight

1.8 ASSUMPTIONS

1.8.1 That the local sweat tests and genetic testing was accurate and that patients attending the CF clinic actually have CF.

1.8.2 That the sputum tests were both accurate and reproducible and that patients could produce sputum.

1.8.3 The *L. reuteri* straws did contain the dose stated by the manufacturer of 1×10^8 viable cfu.

1.8.4 That the presence of lung infections was detectable – making the assumption that the length of antibiotic prescribed reflected the severity of the infection.

1.8.5 That the participants were compliant with enzyme therapy, daily physiotherapy, nebulisation, reporting infections in time and taking their antibiotics as prescribed.

1.8.6 That the CF patients would be interested in testing a new treatment and that they would be compliant with taking the *L. reuteri* straws or placebo as instructed and would record and report information in the symptom diary accurately.

1.8.7 That the CF patients would return the unused straws after use and not just open the straws and say that they had been used.

1.9 SUMMARY

Both malnutrition and antibiotic resistance are significant concerns in the CF population in SA. If supplementation with *L. reuteri* could be shown to stimulate the immune system, possibly reducing the incidence and shortening the duration of lung infections while maintaining or preventing deterioration in lung function then a reduction in the number of lung infections would decrease both nutritional requirements and nutrient losses of the patients with CF while increasing food intake. This would improve nutritional status, which would enhance resistance to lung infections in turn interrupting the malnutrition/infection cycle and reducing mortality. Data is scarce concerning the prevalence of malnutrition in local CF patients in KZN. It is important to ascertain the prevalence of malnutrition locally as well as to determine if supplementation with a probiotic can influence the incidence and duration of lung infections and if so whether this impacts on their anthropometric data.

CHAPTER 2: REVIEW OF RELATED LITERATURE

2.1 INTRODUCTION

Cystic fibrosis was identified as early as 1938. As the condition was not well described in the literature, these patients were mistakenly identified as suffering from kwashiorkor demonstrating the close association between malnutrition and CF. Since 1938, improved care has resulted in a significant decrease in the prevalence of malnutrition in CF patients although approximately one fifth still present with frank malnutrition (Molina *et al* 2001). Malnutrition is particularly detrimental to the CF patient as this predisposes them to infections and recurrent lung infections are the major cause of death in the CF patient (Anthony *et al* 1999; Chandra 1997; Calder & Kew 2002).

The prevention and treatment of lung infections in CF patients is particularly challenging as *P. aeruginosa*, the most problematic pathogen responds to a limited number of antibiotics (Bengmark 1998). In a SA study by Ventre *et al* (2000) they demonstrated that one third of there CF population were resistant to an antibiotic which is used to treat CF patients. The issue of resistance is especially important as the treatment regime in CF patients is demanding and non-compliance to therapy in any chronic disease is well described (Dodd & Webb 2000). The reliance on these antibiotics might be reduced by the use of probiotics. These non-pathogenic bacteria have been shown to stimulate the systemic immune response and there is some evidence showing that they may reduce the incidence and duration of respiratory tract infections in healthy children (Hatakka *et al* 2001).

This review of the literature will consider the prevalence of CF in South Africa, the prevalence of malnutrition in CF patients in SA, whether compliance is an issue in the treatment of CF patients, whether probiotics could reduce the incidence of lung infections and which probiotic would be suitable for use.

2.2 PREVALENCE OF CYSTIC FIBROSIS

Although a CF data base is being developed in SA, the current statistics on the prevalence are imprecise. It is thought that there are approximately 600 people suffering from CF in SA - of these 42 are known to reside in Kwazulu-Natal and most attend either of the two CF clinics.

The CF clinic in Cape Town, situated at the Red Cross War Memorial Children's Hospital, has published the only studies on the incidence of malnutrition in CF patients in SA.

2.3 PREVALENCE OF MALNUTRITION IN CYSTIC FIBROSIS PATIENTS IN SOUTH AFRICA

A study done by Hill, MacDonald, Bowie & Ireland (1988) reported that 36% of the CF population of the Red Cross Hospital were below the 3rd percentile for weight and 24% were below the 3rd percentile for height (Hill *et al* 1988) indicating that a significant percent suffered from severe malnutrition.

Westwood & Saitowitz (1999) reassessed the nutritional status of the CF population at the Red Cross Hospital in 1996. Their study on 45 patients included the entire population of this CF clinic minus those under 2 years old, those with very severe lung disease and those who lived more than 60 km away. As 7 did not complete the 3 day weighed food record (18.4%), a final total of 38 patients were included. They assessed malnutrition using weight for height (WFH), TSF and MAC. No comment was made on the number remaining below the 3rd percentile for weight. When looking at the weight for height a total of 13.5% were moderately or severely malnourished (Table 2). An interesting occurrence is the prevalence of overweight. Westwood & Saitowitz (1999) did not comment of this. As 16.2% were below the 5th percentile for height this is also an improvement on the 1988 figures where 24% were below the 3rd percentile. These authors found the mean height was 96% of expected. They found that 38.1% and 28.6%

were below the 5th percentile for TSF and MAC respectively. These results could have been skewed by the exclusion of those with serious lung disease however.

Table 2 Anthropometric indices of cystic fibrosis at the Red Cross Hospital in 1996 (Westwood & Saitowitz 1999)

	Frequency (%)
WFH > 110% (overweight)	5.4
WFH 90-110% (normal)	62.2
WFH 85-89% (underweight)	5.4
WFH 80-84% (mild malnutrition)	13.5
WFH 75-79% (moderate malnutrition)	5.4
WFH < 75% (severe malnutrition)	8.1
HFA < 5th percentile (n=21)	16.2
MAC < 5th percentile (n=21)	38.1
TSF < 5th percentile (n=21)	28.6
WFH = weight for height, HFA = height for age, MAC = mid-arm circumference, TSF = triceps skinfold thickness	

Westwood & Saitowitz (1999) found that the prevalence of malnutrition was related to age. In the above 10 years old group 47% were malnourished versus 14.3% in the 10 years and under group. This seemed to coincide with the decrease in compliance often seen in adolescents. They concluded that young SA children with CF are growing adequately but more attention needs to be paid to the older children and adults.

Westwood & Ireland (2000) in a later publication compared the growth of CF patients who were regularly attending the clinic at the Red Cross Hospital for the first 6 months of

1986 (Group 1, n = 49) compared to the first 6 months of 1996 (Group 2, n = 63). The study methodology was similar to their previous study. Their results showed no difference in weight for age (WFA) between the 2 groups. Insufficient height measurements were available from Group 1 so no conclusions could be drawn for height for age and weight for height. They concluded that there was a significant improvement in growth from 1986 as indicated by a 14.3% improvement in WFA in 5 to 10 year old children (10^{th} to just below the 50^{th} percentile).

Westwood & Ireland (2000) commented that in spite of the reductions in health services and support in SA for those with uncommon diseases requiring specialized health care, the nutritional status of CF patients seemed to be improving. They attributed the improvement to the development of improved enzymes in combination with the high energy, high fat diet, the early use of nutritional supplementation plus regular meetings with the whole health team to encourage compliance (Westwood & Ireland 2000, Westwood & Saitowitz 1999). Westwood & Ireland (2000) concluded that although there appears to have been an improvement in the prevalence, malnutrition especially growth failure, is still a significant problem in the over 10 year olds which is probably due to the progression of lung disease and a decreased compliance associated with adolescence.

2.4 COMPLIANCE IN CYSTIC FIBROSIS PATIENTS

Compliance of CF patients internationally, includes eating adequately to maintain growth and weight, taking enzymes and drugs, daily physiotherapy as well as regular visits to the CF clinic (Pendleton & David 2000). Clinic visits occur either once a month or every 2, 3 or 6 months. It has been demonstrated however that CF patients that attend CF clinics regularly have higher growth patterns and improved respiratory function (Anthony *et al* 1999). Clearly the treatment regime is very time consuming requiring a high degree of compliance.

2.4.1 Measurement of compliance

Adherence to clinic visits is easy to establish but compliance in other areas such as food and medication is more difficult to ascertain.

Compliance can be measured either subjectively or objectively (Dodd & Webb 2000). Subjective measures include questionnaires, symptom diaries or chart type observation. Objective measures include both direct measures as in lung function readings, heights and weights and indirect measures of counts of antibiotics and enzymes remaining after treatment.

Dodd & Webb (2000) commented that most studies on compliance in CF rely on patients using a subjective self reporting type questionnaire which usually overestimate compliance when compared to objective tests. Often there is a huge difference between the patient's and the medical team's perception of compliance (Pendleton & David 2000). Patients and families who lack the skills and confidence to implement treatments may tell the medical team what they think they want to hear rather than what they are actually doing as they may be afraid of being reprimanded (Anthony *et al* 1999).

2.4.2 Compliance in children

CF patients comprise of infants through to adults. The treatment adherence of the infant and child 10 years old and under largely depends on the commitment of their primary care givers. As many adults feel that a low fat intake is healthy, some experience difficulty in believing that a high fat intake is actually appropriate for a CF child. Powers, Patton, Byars, Mitchell, Jelalian, Mulvihill, Hovell & Stark (2002) showed that CF infants and toddlers only took in 34% of energy from fat and not the 40% as recommended. In addition he went on to say that these age groups (n=35) were not meeting the CF energy recommendation of 120% of the RDA. In the SA studies by (Westwood & Saitowitz 1999; Westwood & Ireland 2000) compliance seemed better in the younger children.

2.4.3 Compliance in adolescents

Adolescence was found to be the worst period of compliance (Anthony *et al* 1999) as indicated by the increasing malnutrition in CF patients over 10 years old. Teenagers with CF face the same psychosocial problems as their healthy counterparts. Of particular concern is the preoccupation with figure shape, body weight, dieting and "healthy eating" prevalent amongst teenagers, particularly females. The CF patient's high fat diet is the opposite of the current health message promoting a reduction in the intake of fat to attempt to maintain health. This could make them feel isolated and "not trendy or cool". Eating disorders (ED), which are a high risk in this age group, may be an additional challenge as disease treatments which focus on control and food are suspected to predispose to ED (Stark, Mulvihill, Jelalian, Bowen, Powers, Tao, Creveling, Passero, Harwood, Light, Lapey & Hovell 1997).

2.4.4 Compliance in adults

Due to the improved health care CF patients are living longer. This means that the older CF patients are studying, securing full time employment, marrying and starting families. All this requires time allowing less time for compliance to the CF patients daily treatment requirements (Dodd & Webb 2000). The longest living CF patient in the KZN clinic is currently in her late forties. Usually the older the CF patient the more the infections, which means more treatment time, more time off work and less time with their family and to relax. Not many studies have addressed the issue of compliance in CF adults. It is interesting to note that male CF patients will have a 5 year survival advantage to females (Anthony *et al* 1999) and male CF generally do better than there female counterparts. The reason for this has not yet been established but it is thought that the poorer nutritional status of the females has a negative impact (Anthony *et al* 1999).

2.4.5 Reasons for non-compliance

Three basic causes of non-compliance in CF patients have been identified as inadequate knowledge, psychosocial resistance and educated non-compliance (Koocher, McGrath & Gudas 1990). Abbott & Gee (1998) grouped reasons for non-compliance in CF patients slightly differently into 4 categories which included health, time, social and emotional reasons. They found that health and time were the major reasons for non-compliance (Table 3).

Table 3 Reasons for non-compliance (Abbott & Gee 1998)

Category	**Reasons given**
Health	'I feel well without treatment' 'I'm not as serious as others' 'I don't feel any benefit'
Time	'not enough time' 'I am too busy' 'I forget'
Social	'interferes with my social life' 'embarrassing taking enzymes in public or doing physiotherapy'
Emotional	'I resent it' 'I can't be bothered'

Dodd & Webb (2000) claim that in addition in CF the actual treatment determines compliance. They found that where treatments offered an immediate benefit there was 84% compliance whereas with treatments that offered a long term but no immediate benefit the compliance dropped to between 41 to 65%. For example, there was an 80% compliance with enzymes, insulin and exercise and only a 50% compliance with chest clearance (Conway, Pond, Hamnett & Watson 1996, Abbott, Dodd, Bilton & Webb 1994).

Interestingly regardless of age Gudas, Koocher & Wypij (1991) established that optimistic CF patients were more compliant than pessimistic CF patients. Pendleton & David (2000) suggested that this type patient may have found a way of coping with CF rather than developing a rebellious attitude towards their medical care.

A lack of compliance in CF patients with regard to an adequate food intake predisposes to malnutrition which predisposes to lung infections. Non-compliance with medication, physiotherapy and exercise also predisposes to lung infections.

2.5 PREDISPOSITION TO LUNG INFECTIONS IN CYSTIC FIBROSIS

Because of the mutation of the gene in CF patients, the normal transport of electrolytes across epithelial membranes is disturbed causing the mucus in their airways to be thick, sulphated and easily able to form aggregates (Bals, Weiner & Wilson 1999). Bacteria that are carried by air into healthy lungs are trapped by the mucus layer and moved upwards towards the pharynx by the "mucociliary escalator" as shown in Figure 2 (Williams 2002). These are then either swallowed or expectorated. In spite of the fact that the airways above the vocal cords are heavily contaminated with bacteria, the lower airways remain sterile because of the action of the "mucociliary escalator" (Hart & Winstanley 2002).

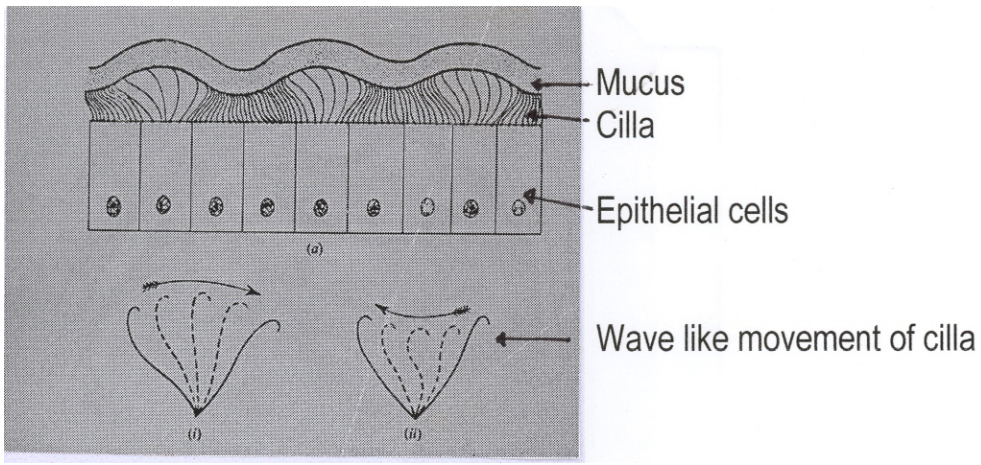

Figure 2 Mucocillary escalator (Williams 2002).

As the mucus in the CF lungs is much thicker, the bacteria are trapped but cannot be transported out of the lower airway resulting in the frequent lung infections. The most problematic pathogen in the CF lungs is *P. aeruginosa* (dry and mucoid) followed by *Staphylococcus aureus* and *Burkholderia cepacia* (West, Zeng, Lee, Kosorok, Laxova, Rock, Splaingard & Farrell 2002, Hart & Winstanley 2002). There are few treatment options as only a limited number of antibiotics, such as fluoroquinolone, are effective against *P. aeruginosa.* Resistance to fluoroquinolone was shown in one third of CF patients in SA (Ventre *et al* 2000).

In addition, extracellular fluid cultured from normal airways has been shown to have a bacteriocidal effect but this has not been shown for the extracellular fluid from the CF lungs (Smith, Travis, Greenberg & Welsh 1996). The prolonged microbial colonization and infection may be exacerbated by a defective non-specific immune system (Hart & Winstanley 2002).

Chronic bacterial lung infections in CF patients cause the immune system to respond by producing specific antibodies, forming immune complexes, rapidly recruiting neutrophils from the blood stream, and producing cytokines. This is associated with the release of lysosomal enzymes particularly neutrophil elastase (NE). NE interferes with ciliary beating, encourages mucus production and further facilitates *P. aeruginosa* adherence. This in turn could result in acquired immune suppression (Memoranda 1994).

An alternative treatment option which could reduce both the reliance on antibiotics and stimulate the immune system is an interesting concept. Bengmark (1998) suggested that supplementation with non pathogenic bacteria such as probiotics could stimulate the immune system thus reducing or negating the need for antibiotics.

2.5.1 Discovery of probiotics

In the 1900, the Nobel Prize was awarded to Elie Metchnikoff for his hypothesis “that the long, healthy lives of Bulgarian peasants were the result of their consumption of

fermented dairy products" (Kopp-Hoolihan 2001). This eventually resulted in the hypothesis that micro organisms in the gastro intestinal tract (GIT) could beneficially influence health not only in the GIT but in other areas of the body. In turn this lead to the concept of probiotics, the term probiotics being coined from 'pro-life' (Reid, Jass, Sebulsky & McComick 2003).

2.5.2 Definition of a probiotic

A probiotic is a live micro organism which, when consumed by either animals or humans, alters their indigenous microflora resulting in health benefits to the host (Stanton, Gardiner, Meehan, Collins, Fitzgerald, Lynch & Ross 2001).

2.5.3 Indigenous microflora

The indigenous intestinal microflora of healthy people consists of a static resident population and a transient population. Both the static and transient bacterial flora plays an important role in maintaining health. Potentially pathogenic bacteria (PPB) such as *P. aeruginosa* and pathogenic bacteria are a natural part of this bacterial flora. They are normally kept in check by the non pathogenic bacteria, the gut-associated lymphoid tissue as well as non-specific defence factors (Van der Waaij 1999). For example *Lactobacillus,* found throughout the human GIT, protects the enterocytes, controls metabolic and immunologic processes and prevent pathogens from colonizing (Levy 2000).

The composition of the diet, especially the carbohydrate (CHO) content, is important to maintain the balance of healthy microflora as this provides their nutrients (Walters & Littlewood 1998). As the resident microflora is dictated by the food substrates in the GIT as well as the use of medication such as antibiotics, a disturbance in the microflora's food supply and/or the use of antibiotics (Levy 2000) would result in a derangement in the normal microflora which in turn would imply a negative impact on health.

According to Walters & Littlewood (1998) the faeces of CF patients contain excessive amounts of CHO, bile acids, fat and nitrogen in spite of the use of pancreatic enzymes. These authors hypothesised that the microflora in the colon were unable to metabolise high concentrations of CHO residues resulting in GIT discomfort and an overfilled colon which is common CF patients. They suggested that this resulted in a predominance of aerobic organisms which were unable to break down primary bile salts to secondary bile salts. High concentrations of bile acids would preclude the existence of bacteria that were not resistant to bile acids further altering the Gastrointestinal (GI) microflora.

Only one study was found that attempted to establish the composition of the "normal" microflora in CF patients. This controlled study on 29 CF children investigated their faecal microflora (Roy, Delage, Fontaine, Robitaille, Chartrand, Weber & Morin 1979) but unfortunately looked at the amounts of anaerobic versus aerobic bacteria rather than the presence and quantity of individual species.

In Roy's study the children were divided into 3 groups consisting of 7 off antibiotics, 10 on oral Cloxacillin and 6 on intravenous triple therapy composed of Cloxacillin, Gentamycin and Carbenicillin. They ate a moderately low fat diet (50 to 75g per day) and all took pancreatic supplementation. The control group consisted of 7 healthy children with no gastrointestinal problems, hepatic or pancreatic disease and who ate a normal diet.

A reduction in the total microflora was shown in the CF children off antibiotics and those on triple therapy. CF children on Cloxacillin did not show a reduced microflora. The composition of the microflora was altered in all groups. Aerobes were increased in CF children off antibiotics versus controls. The triple therapy group had more aerobes than those off antibiotics but less than the Cloxacillin group. Fifty percent of those on triple therapy had no strict anaerobes. Faecal *P. aeruginosa* was not found in the controls but was found in 1 of the 7 off antibiotics. Forty percent of the Cloxacillin group and 33% of the triple therapy group were positive for faecal *P. aeruginosa* (Roy *et al*, 1979). This

study indicates that there is an alteration in the composition of the indigenous microflora in CF children both on and off antibiotic therapy.

The presence of excessive bile acids and excessive CHO in the colon as well as the frequent use of antibiotics would have a negative impact on both the total amount and the composition of the GIT microflora. This reduction of and alteration in the GI microflora could feasibly suppress the immune system predisposing the CF patient to lung infections. Supplementation with probiotics may help normalise the GI microflora increasing resistance to lung infections.

2.6 THE USE OF PROBIOTICS IN THE PREVENTION OF LUNG INFECTIONS

According to Gorbach (2002) there is mounting evidence of a potential role of probiotics in the prevention of acute respiratory infections among children in day care centres.

2.6.1 Use of probiotics in the prevention of respiratory infections in healthy children.

Gorbach (2002) cited a recent placebo controlled study in Brazil (authors not mentioned) where probiotics (type not mentioned) given in milk to children in day care centres showed a significant prevention of respiratory infections.

Hatakka *et al* (2001) conducted a randomised, double blind, placebo controlled study in Finland for a period of 7 months. Their objective was to establish whether milk fortified with *Lactobacillus rhamnosus Gorbach & Goldin* (LGG) reduced the occurrence of both GI and respiratory infections in children aged 1 to 6 years attending day care centres. The study was done in winter when there is a high incidence of infections.

Hatakka's study group consisted of 282 healthy children and the control group consisted of 289 healthy children. The average daily consumption of milk in both groups was 260 ml. The *Lactobacillus* milk contained 5-10 x 10^5 cfu of LGG. A daily symptom diary was filled in by the parents documenting respiratory symptoms, fever, runny nose, sore

throat, cough, chest wheezes, ear ache as well as GI symptoms such as diarrhoea, vomiting and stomach ache. Absences from the day care centre were recorded. Stool samples were taken at the start, midway and conclusion of the study. The recovery of LGG was assessed in a random sample of 100 stools to confirm compliance. Fifty eight children dropped out the study - no reason was given. In the study group there were significantly fewer days absent and significantly fewer children were prescribed antibiotics for respiratory infections. However 15% of the children in the control group were found to be positive for LGG at the end of the study probably because LGG products are widely available in Finland. This could have concealed a greater effect of LGG in the prevention of respiratory infections as some of the control group were inadvertently supplementing with LGG.

2.6.2 The use of probiotics in the prevention of respiratory infections caused by *Pseudomonas aeruginosa.*

Alvarez, Herrero, Bru & Perdigon (2001) studied the effect of *Lactobacillus casei* or yogurt on 3 week old mice (number unknown, design of trial unknown) to establish whether either one reduced the incidence of lung infections from *P. aeruginosa*. Their results showed that the clearance from the lung of *P. aeruginosa* improved (significance not stated) and that the phagocytic activity of the alveolar macrophages increased in a dose-dependant manner (significance and dose not stated) with both *L. casei* and yoghurt. The white blood cell differential counts remained unchanged. These normally increase in the presence of infection.

Guarino (2002) used LGG supplementation in 30 CF children in Italy in a randomised, blinded, cross over trial (pending publication). The children had a mean age of 9 years (SD±5). Twenty out of 30 were colonised with *P. aeruginosa*. They were given an oral rehydration solution with or without LGG for a period of 6 months and then crossed over for another 6 months. The dose of LGG was not stated. The results showed a reduction in the incidence of respiratory infections in all groups but particularly in the *P. aeruginosa* group (Figure 3). The duration of pulmonary exacerbations decreased in the

P. aeruginosa group (Figure 4). A reduction in abdominal pain was experienced in 7 out of 9 of the children (Figure 5). In addition there were improvements in both steatorrhea and weight gain in the groups taking the LGG (Figure 6). The significance was not stated.

The limited trials on mice and children have indicated that probiotics (specifically LGG and *L. casei*) may play a role in the prevention of lung infections in both healthy people and those with CF and would therefore be a suitable choice for a local trial involving CF children.

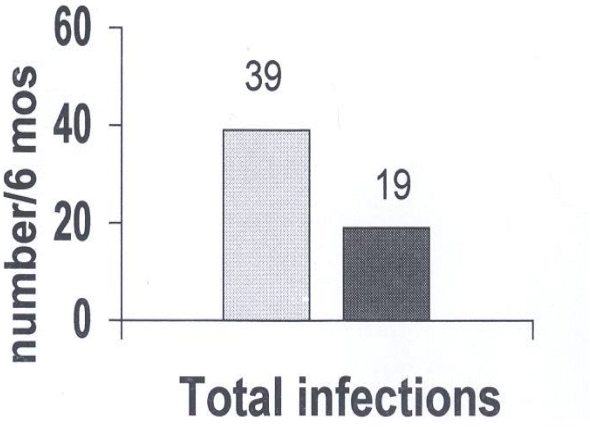

Figure 3 The effect of *Lactobacillus rhamnosus Gorbach and Goldin* in cystic fibrosis children and total number of infections (Guarino 2002)

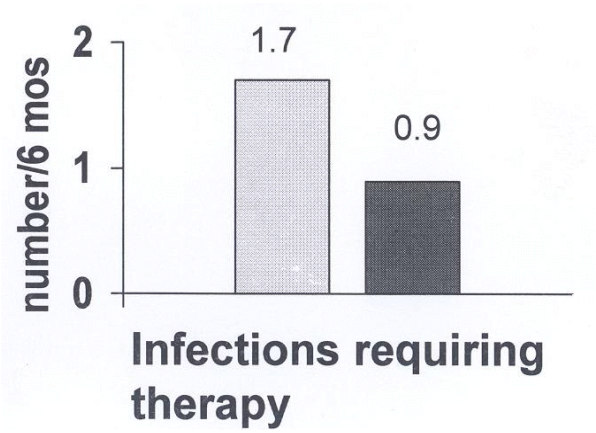

Figure 4 The effect of *Lactobacillus rhamnosus Gorbach and Goldin* in cystic fibrosis children and number of infections requiring therapy (Guarino 2002)

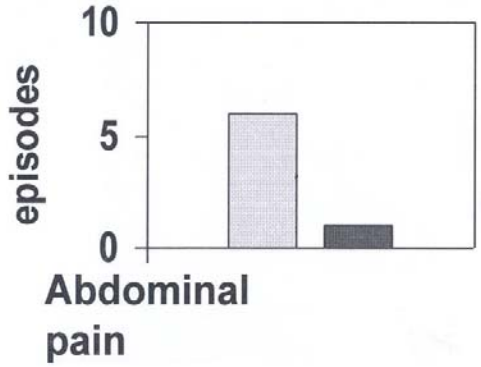

Figure 5 The effect of *Lactobacillus rhamnosus Gorbach and Goldin* in cystic fibrosis children and episodes of abdominal pain (Guarino 2002)

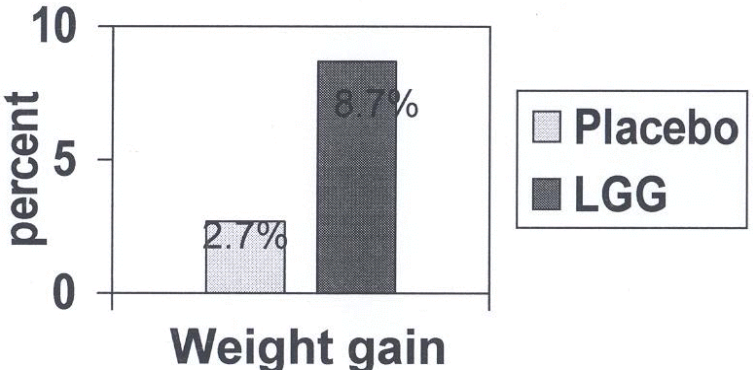

Figure 6 The effect of *Lactobacillus rhamnosus Gorbach and Goldin* in cystic fibrosis children and percent weight gain (Guarino 2002)

2.7 CHOOSING A PROBIOTIC

The most clinically tested probiotic to date is LGG (Goldin, Gorbach, Saxelin, Barakat, Gualtieri & Salminen 1992). However LGG is only available in SA as part of a probiotic "cocktail". Bioflora (Bioforce SA) contains *Lactobacillus rhamnosus* as well as *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Culturelle (Pharma Dynamics) contains *Lactobacillus rhamnosus* and *Bifidobacterium longum* in combination with a prebiotic (fructo-oligosaccharide). For the purposes of research it would be more informative to test the effect of a single species rather than a mix of species as probiotics seem to have differing effects and potencies. This means that LGG would need to be imported for the trial which would be costly. In addition if the clinical trial proved to be of significant benefit, the local CF patients would not be able to continue with LGG after the trial.

L. casei is available in a yoghurt sold in the Gauteng area of SA. The concentration of bacteria in SA yogurt varies greatly (Louren, Viljoen & Jooste 2000). As with any other yogurt the concentration of *L. casei* would not be constant in the yoghurt as it would vary according to shelf temperature and length of storage time (Lourens, Viljoen & Jooste 2000). The *L. casei* in yoghurt also has a short shelf life versus the longer shelf life of probiotics marketed as capsules or tablets. The yoghurt would also not be easily accessible for the trial or for use by the local CF patients after the trial.

As neither of the 2 probiotics investigated in the treatment of lung infections is available in SA, an alternative which meets the criteria for a successful probiotic needs to be selected. According to Gorbach (2000) criteria for a successful probiotic were drawn up in 1985 (Table 4). It is important that all of these criteria be met.

Table 4 The major criteria for a successful probiotic (Gorbach 2000, & Bengmark 2000)

Human origin
Acid and bile stable
Colonize the human intestinal tract
Adhere to human intestinal cells
Produce antimicrobial substances
Antagonistic to pathogenic bacteria
Clinically validated and documented health effects
Safety
Technical properties

Potential single probiotic supplements currently available in South Africa include *L. reuteri* and *Saccharomyces boulardii* (Interflora).

To offer a health benefit the bacteria needs to be of human origin (Salminen 2001) as there are species dependant health effects. As *S. boulardii* is a yeast of non-human origin it does not meet the criteria for a successful probiotic and was therefore not considered suitable for use in the study reported in this dissertation on CF patients in KZN. *L. reuteri* is of human origin as it is a normal inhabitant of the GIT of healthy humans (Wolf, Wheeler, Ataya & Garleb 1998) and is frequently identified in the faecal samples of healthy volunteers (Jacobsen, Rosenfeldt Nielsen, Hayford, Moller, Michaelsen, Paerregaard, Sandstorm, Tvede & Jakobsen 1999).

The probiotic needs to be acid stable to ensure survival through the stomach. According to Bengmark (1998) *Lactobacillus* are acid loving. The probiotic needs to be bile stable

to ensure survival through the intestines and to maintain adhesiveness (Bengmark 1998). This is particularly important in CF patients as they have much higher concentrations of bile acids in the colon. *L. reuteri* is not only bile resistant but also releases bile salt hydrolase which enhances bile salt digestion and appear to decrease the bioavailability of the harmful bile salts (De Boever, Wouters, Verschaeve, Berckmans, Schoeters & Verstraete 2000).

It has been clearly established that *L. reuteri* colonizes the human GIT. Shornikova, Casas, Mykkanen, Salo & Vesikari (1997) established that supplementation with *L. reuteri* at doses of 10^7 to 10^{10} cfu per day established colonisation in children aged 6 to 36 months suffering from rotavirus diarrhoea. Salminen (2001) showed that *L. reuteri* colonised in the human GIT.

After colonization of the GIT by the probiotic, adhesion is important. The complex adhesion studies performed by Jacobsen *et al* (1999) showed that *L. reuteri* adhered strongly to the Caco-2 human epithelial cell lines in vitro.

The production of antimicrobial substances is important for pathogen inactivation in the GIT as well as for normalisation of the GIT flora (Jacobsen *et al* 1999; Gorbach 2000). *Lactobacilli* in general are very efficient at counteracting gram negative bacteria (Bengmark 1998). Jacobsen *et al* (1999) showed that *L. reuteri* broadly inhibits pathogenic bacteria. According to Thornball (2000) *L. reuteri* produces reuterin and reutericyclin which appear to be effective antimicrobial agents (Figure 7). It was clearly demonstrated at the European Society for Parenteral and Enteral Nutrition Congress by Sinkiewicz (2000) cited by Thornball (2000) that *L. reuteri* inhibited the growth of both *Salmonella* and *S. aureus*. Nollet, Pereira & Verstraete (1999) showed that the antimicrobial range of reuterin includes both gram positive and gram negative bacteria, yeasts, fungi, protozoa and viruses. They commented that *L. reuteri* from a human was more effective against *Salmonella* from a human, while *L. reuteri* from a rat was more effective against *Salmonella* from a rat demonstrating the species dependent effect.

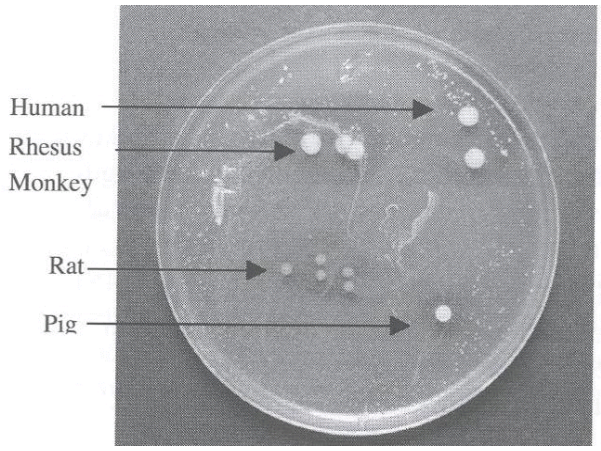

Figure 7 Different strains of *Lactobacillus reuteri* showing an inhibiting effect on the growth of *Salmonella* – the dark zones around the colony show areas where the pathogens can not grow (Thornball 2000).

Although *L. reuteri* has not been used in any trials studying its effect on lung infections, there are clinically validated and documented health effects, particularly in the treatment of rotavirus induced diarrhoea. Shornikova *et al* (1997) showed a significant improvement in watery diarrhoea in hospitalised children. The children were divided into 3 groups who received either *L. reuteri* at 10^7 cfu or *L. reuteri* at 10^{10} cfu or placebo for five days. Both the *L. reuteri* groups showed a significantly reduced duration of watery diarrhoea. The duration of diarrhoea was 2.5 days in the placebo group, 1.9 days in the 10^7 *L. reuteri* group and 1.5 days in the10^{10} *L. reuteri* group. Ruiz-Palacios, Tuz, Arteaga, Lourdes Guerrero, Dohnalek & Hilty (1996) studied 408 healthy bottle fed children to assess whether probiotics could reduce the incidence of community-acquired diarrhoea. The first group (placebo) received milk as part of their diet. The second group received milk with added *L. reuteri, Lactobacillus acidophilus* and *Bifidobacteria infantis*. The third group received milk with *L. acidophilus* and *Bifidobacteria animalis*. Both groups receiving the probiotics had a significantly reduced risk of diarrhoea.

Thornball (2000) stated that it is generally well established in the literature that the use of *Lactobacillus* is safe for human consumption and *Lactobacillus* has been classified as Generally Recognised As Safe (GRAS). Salminen, Von Wright, Morelli, Marteau, Brassart, de Vos, Fonden, Saxelin, Collins, Morgensen, Birkeland & Mattila-Sandholm

(1998) stated that the best test for food safety is a well documented history of safe human consumption. If this criteria is used *L. reuteri*, which has been on the market since 1980, has no adverse reports.

There has been some concern that probiotic bacteria may translocate and become pathogenic in immune suppressed individuals. Thornball (2000) showed some bacterial translocation of *L. acidophilus, L. casei* and LGG in immune suppressed mice but there was no translocation of *L. reuteri*.

With high doses of *L. reuteri* (10^{11} cfu) mild flatulence was the only GI symptom noted in 30 healthy male adults over a period of 21 days (Wolf, Garleb, Ataya & Casas 1995). No other side effects have been reported in the scientific literature.

In summary the literature shows that *L. reuteri* meets all the criteria of a successful probiotic making it a suitable choice for the trial as it is of human origin, is acid and bile stable. It colonizes the human GIT, adheres to human intestinal cells, produces antimicrobial substances, is antagonistic to pathogenic bacteria, has been clinically validated and has documented health effects. It is also safe and has a guaranteed viable shelf life.

2.8 DOSE OF PROBIOTIC

Ruiz-Palacios *et al* (1996) classified 10^6 cfu per ml as a low dose, 10^8 cfu per ml as a medium dose and 10^{10} cfu per ml as a high dose of *L. reuteri*. When using probiotics therapeutically a medium to high dose is usually prescribed. Different disease conditions however may need different therapeutic doses to exert a positive effect (Van der Waaij 1999). Wolf *et al* (1998) gave *L. reuteri* or placebo to 39 HIV-positive patients, aged 18 to 60 years, in a dose of 10^{10} cfu per ml per day for 21 days. It was established that the faecal levels of *Lactobacillus* was much lower in HIV-positive group compared to healthy subjects even after consumption of 1×10^{10} cfu/day, implying that high doses may be necessary in certain disease conditions.

As no studies have been done using *L. reuteri* in CF patients the optimal dose is unknown. The study investigating the effect of LGG on the incidence of respiratory infections in healthy children used a low dose of between 5 to 10×10^5 cfu per day (Hatakka *et al* 2001). Although it may be appropriate to use a high dose in CF patients due to the abnormal substrates entering the colon and the frequent use of antibiotics, safety has not been established in CF patients and it would appear prudent to use a more conventional dose of 1×10^8 cfu per day.

L. reuteri is available in a dose of 1×10^8 cfu in both tablet and straw form. The recommended dose is 1×10^8 cfu per day (that is one straw a day).

2.9 CONCLUSION

In spite of the improvement in treatment, malnutrition in CF patients, especially in those over 10 years of age, is still a significant problem locally if the studies done at the Red Cross Hospital are representative of the South African CF population as a whole. As the CF patient reaches adolescence, compliance to treatment decreases resulting in an increase in the severity of malnutrition and the risk of developing drug resistance to the few effective antibiotics available.

Increasing evidence is indicating that the indigenous microflora plus supplementation with probiotics may have a significant positive impact on the immune system. The use of probiotics may be even more beneficial in CF patient as their indigenous microflora is likely to be deranged from the abnormal food substrates entering the colon as well as the routine use of antibiotics. Limited studies on the use of LGG as a probiotic in the treatment of respiratory infections in both healthy children and those with CF have shown a significant decrease in the incidence of infections. This in turn would decrease the use of antibiotics reducing the risk of resistance (Gorbach 2002, Hatakka *et al* 2001, Guarino 2002).

Although LGG is the most commonly used and most commonly researched probiotic it is not available in SA as a single strain. *L. reuteri* which is available appears to be a suitable alternative as it meets all the criteria of a potential probiotic. A moderate dose of 1×10^8 cfu per day appears prudent as the use of probiotics and their safety has not been established in CF patients.

CHAPTER 3: METHODOLOGY

The primary purpose of the study was to determine whether supplementation with a probiotic (*L. reuteri*) would improve anthropometric measurements, reduce the incidence and shorten the duration of lung infections and would maintain or prevent deterioration in lung function in CF patients attending the CF clinics in KZN Durban. The secondary purpose of the study was to compare the nutritional status, at the end of the study, as determined by anthropometry (percentage weight for height) of CF patients attending CF clinics in KZN Durban with CF patients attending CF clinics in Cape Town from the published data by Westwood & Saitowitz (1999).

3.1 DURBAN STUDY

3.1.1 Study Design

This study was carried out in two parts. The primary study purpose was experimentally determined by a study in Durban that was a randomized, double blind, placebo controlled cross over trial run over the period of a year. The patients were randomly allocated to either placebo or probiotic for the first 6 months and then crossed over. The group that received the probiotic first was group A while group B received the placebo in the first six months.

The study started in January 2005. After the study had begun it was pointed out by Lawson (2006) that the normal growth patterns of children should have been established before intervention with the probiotic. For group B the normal growth patterns could be established as they were on the placebo for the first six months before intervention with the probiotic for the second 6 months. To establish normal growth patterns the height and weight measurements for group A who were on the probiotic in the first six months (group A) was taken retrospectively from the medical records of the patients. This data was available as the same two dieticians who had taken the measurements at the start of the study take all the anthropometric measurements at the CF clinics. See Figure 8 for

flow diagram. This is why data is presented in the results as the measurement eg weight taken six months before probiotic to start of probiotic (1st six months before intervention that is at baseline) and start of probiotic to the end of probiotic (2nd six months intervention with the probiotic). The difference between the six months before probiotic to start of probiotic (changes in weight while on placebo) and start of probiotic to end of probiotic (changes in weight while on probiotic) were shown in the tables in chapter four. All the other measurements were taken at the start of the study (January 2005), middle (June 2005) and end of the study (December 2005).

Mid arm circumference and TSF measurements were taken at the start and end of the study only. Unfortunately these measurements were not retrospectively available. All other measurements that is incidence and duration of lung infection, duration on antibiotics, lung function, sputum analysis and symptom diaries were taken at the actual start of the study (Jan 2005), at crossover six months later and at the end of the study. Data presented in the results chapter were for group A on probiotic January to June and for group B on probiotic July to December. Group A of probiotic July to December and group B January to June.

The secondary purpose of the study was to compare the nutritional status of the patients attending the CF clinics in KZN Durban (at the end of the study) with the patients attending the CF clinics in Cape Town (data collected in 1996). The data from the Cape Town clinics was obtained from the publication by Westwood & Saitowitz (1999). This is the only anthropometric data published for CF patients in SA. Westwood & Saitowitz (1999) reassessed the nutritional status of the CF population at the Red Cross Hospital in 1996. Their study on 45 patients included the entire population of this CF clinic minus those under 2 years old, those with very severe lung disease and those who lived more than 60 km away. As 7 did not complete the 3 day weighed food record (18.4%), a final total of 38 patients were included.

The studies had a similar number of patients and exclusion criteria so were comparable. As Westwood & Saitowitz (1999) used a modification of the Waterlow and Rutishauser classification for growth measurements (Ramsey *et al* (1992) based on percentage expected weight for height (% EWFH) the Durban data was converted to %EWFH to enable the comparison. The weight, height, MAC and TSF readings from the end of the study (Dec 2005) were used as this was the most recent data from Durban.

3.1.2 Sample population

All the CF patients who had attended either the private CF clinic held at St Augustine's Hospital or the state clinic held at Addington Hospital (both in Durban) for at least 1 year were asked to take part in the study.

The secretary of the KwaZulu-Natal Cystic Fibrosis Committee provided a list of contact details of all known CF patients in the province. Dr Egner, the CF pediatrician at St Augustine's Hospital, supplied the details of any additional CF patients in his practice that were not on the list. In total number 42 patients with CF were located. Six were excluded from the study at this stage as they were below six years of age and were too young to perform the lung function test. Another exclusion criterion was a Shwachman score[3] of less than 40. One patient was excluded who had a score below 40. This left 35 patients suitable for inclusion in the Durban study.

Three weeks before the start of the Durban study a letter of introduction was sent to these 35 CF patients explaining the purpose of the study, what would be required of them and asking them to phone the CF clinic dietician to confirm participation (Appendix A). A week before the January clinics all patients that had not responded were reached by telephone and asked to participate.

[3] The Shwachman score was used to measure the severity of lung disease. The questionnaire consists of four sections, each with a rating from 0-25. The four sections included are general activity, physical examination, nutrition and chest x-ray. A score of 86-100 is excellent, 71-85 is good, 56-70 is mild, 41 -55 is moderate and 0-40 indicates severe lung disease.

As it was the holiday season a few were still on holiday so it was decided that these patients could start the study in February. These patients were phoned again a week before the February clinic to remind them about the study.

A total of twenty-three of the thirty five (66%) patients consented to be involved in the study. Reasons given by either the CF patients themselves or by the parents of minors as why they did not want to participate in the study are listed in Table 5.

Table 5 Reasons for not participating in the study

'too busy' and 'no time'
'do not attend the clinics' regularly'
'too inconvenient to be part of the study'
'appreciating & admiring what you are doing but not for me'
'too much effort to be part of the study'

3.1.3 Randomization and blinding

In the Durban study the two study groups were matched for both sex and age. Firstly the participants (n=23) were grouped into males and females as males live longer than females and seem to have less severe lung infections. Each group of males and females were then ranked in order of birth date because there is deterioration of lung function with age. The pharmacist then allocated every second person in the group of males and every second person in the group of females to Group A (n=11). Those remaining formed Group B (n=12). Group A received *L. reuteri* for the first 6 months and then placebo for the last 6 months and Group B received placebo for the first 6 months and then *L. reuteri* for the last 6 months. The dose of *L. reuteri* was of 1×10^8 cfu per straw per day This was done by the pharmacist to ensure that the process was double blinded for both the researcher and CF patients. The pharmacist kept a record of which patients were in each study group until blinding was broken at the end of the 12 month study period. As the study was a crossover no control was necessary as the patients acted as their own controls.

3.1.4 Intervention

The intervention of the study was that patients were to take one straw a day either probiotic (1×10^8 cfu dose) or placebo. It was decided to use the straw as it was felt that in this population group they would be less likely to be compliant if they were required to take yet another tablet. Placebo straws were available for use at the time but no placebo tablet.

The technical properties of *L. reuteri* are good as the tablet has a shelf life of 18 months while the *L. reuteri* containing straw has a shelf life of 12 months. Viable concentrations are guaranteed up until the expiry date if stored in a refrigerator (Shornikova, Casas, Mykkanen, Salo & Vesikari 1997).

To ensure blinding the straws had been packed into the bags by the same pharmacist who randomized the groups. Sufficient straws were issued for 6 months (180 straws). In an attempt to monitor compliance patients were asked to keep the used straws and to bring back both the used and unused straws for the 6 month period. Straws were issued for a 6 month period rather than on a monthly basis as the CF patients varied in their timing of clinic visits.

3.1.5 Pilot study

No formal pilot study was done as this masters study was the pilot study for a multicentre trial.

3.2 STUDY METHODS AND MATERIALS

The following variables were obtained; weight, height, midarm circumference, triceps skinfold thickness, lung function, lung infection rate, sputum samples, a food frequency

questionnaire regarding pre and probiotic use was asked and a symptom diary was collected.

3.2.1 Measurement techniques

The dieticians measured the weight, height, MAC and TSF. One dietician took a set of readings on each patient. The other dietician then repeated the measurements on the same patient to confirm that the readings were consistent. There was no significant difference between the two dietician's readings for weight, height, MAC and TSF for each patient for the first period and for the second period with the p value are given in each section. This was done in an attempt to validate the readings between the two dieticians. Repeatability studies were not conducted in this pilot study. The same equipment was used during the 12 month trial period. As there was only one physiotherapist for the 12 months of the study and she used the same equipment MicroLAB 2000 3300 (Micro Medical) for the entire length of the study validity and reliability studies were not undertaken. The food frequency questionnaire regarding pre and probiotic use was piloted on ten non CF subjects before the trial began to ensure that the format and questions were understandable. No changes needed to be made to the questionnaire.

3.2.1.1 Weight

Weight was measured using a portable Masskot scale which weighed a maximum of 150 kg and a minimum of 50g. The scale was calibrated by the Discipline of Dietetics and Human Nutrition Pietermarizburg before the study began. The same scale was used throughout the study. The patient was weighed in light clothing, barefoot and after voiding. They were asked to step onto the center of the scale, with their weight evenly distributed on both feet, and to look up and directly ahead. Their weight was then recorded (Norton & Olds 1996). The second dietician repeated the weight measurement. If the readings differed by a 100 g a third reading was taken. The average of the two similar readings was used. The time of day that the weight was measured was also noted.

There was no significant difference between the two dietician's readings for weight for each patient for the first period (p=0.669) and for the second period (p=0.326). The mean of the 2 weights was used.

3.2.1.2 Height

Height was measured using a portable stadiometer (as approved by ISAK, the International Society for the Advancement of Kinanthropometry). The same stadiometer was used throughout the study.

The free-standing stature method described by Norton & Olds (1996) was used. The patient was asked to take off their shoes and then stand on the base of the stadiometer with their back against the stadiometer. Their heels, buttock, upper part of the back and back of the head was in contact with the stadiometer. They were asked to stand erect with their heels together and arms hung naturally by the sides. The subjects head was placed in the Frankfort plane. They were asked to take a deep breath while the headpiece was lowered onto their vertex and a reading was taken during inhalation and recorded. The second dietitian repeated this measurement. If the readings differed by 2 mm a third reading was taken. The average of the two similar readings was used. The time of day that the height was measured was also noted.

There was no significant difference between the two dietician's readings for height for the first period (p=0.278) and for the second period (p=0.108).

3.2.1.3 Mid arm circumference

The patient was asked to stand in a relaxed position with arms hanging loosely. All land marks were done on the right side of the patient as described by (Norton & Olds 1996). First the acromial point was located at the superior and lateral border of the acromion process. This was then marked midway between the anterior and posterior borders of the

deltoid muscle when viewed from the side. The head of the radius was then located by using the left thumb or index finger, palpating downwards in the lower portion of the lateral dimple of the elbow. A line was drawn between the acromial and radiale horizontal to the long axis of the humerus at the mid-acromiale-radiale. The distance between these two points was measured using an anthropometric tape and the mid point was marked. The other dietician checked that the tape was straight. The mid arm circumference was measured at the mid point using an anthropometric tape and recorded. The second dietician repeated this measurement. If the measurements differed by 2 mm they were repeated. The average of the two similar measurements was used. There was no significant difference between the two dietician's readings for MAC for the first period ($p=0.347$) and for the second period ($p=0.110$).

3.2.1.4 Triceps skinfold thickness

At the mid point the horizontal line was extended to the posterior surface of the arm, where a vertical line at the most posterior surface was marked to intersect with the horizontal line. This was the point where the triceps skinfold was measured (Norton & Olds 1996).

The skinfold was raised with the left thumb and index finger on the marked posterior mid-acromiale-radiale line. The fold was vertical and parallel to the line of the upper arm. The skinfold was taken on the most posterior surface of the arm over the triceps muscle when viewed from the side. For the measurement, the arm was relaxed with the shoulder joint slightly externally rotated and the elbow extended by the side of the body. The skinfold was raised on the landmark. The calipers were placed 1 cm below the landmark, after a two seconds lapse the first reading was taken and recorded. The second dietician took the second reading after a minimum of 5 minutes had passed to allow the skinfold to return to normal. If the readings differed by 2 mm they were repeated. An average of the 2 similar readings was used. There was no significant difference between the two dietician's readings for the TSF readings for the first period ($p=0.080$) and for the second period ($p=0.609$).

3.2.1.5 Lung infection rate

The incidence of lung infection was the number of new lung infections experienced over the 12 month study period for which the doctor wrote up antibiotic treatment. Continuous antibiotic treatment was not included as a new infection. Five patients received continuous antibiotics for the whole 12 months of the study (probiotic and placebo) while one patient received continuous antibiotics during the second six month period while on placebo.

3.2.1.6 Lung function

Forced expiratory volume in 1 second and FVC were measured by the same physiotherapist. One or 2 readings were taken and the better of the two readings were used in the study. If the CF patient only managed 1 FEV_1 and FVC reading this result was used. The equipment used by the physiotherapist was a MicroLAB 2000 3300 (Micro Medical). The patient stood in front of the machine, inhaled a full breath, then closed their lips around the mouthpiece and blew out as hard and fast as possible until they had expelled all the air in their lungs. The inspiration was full and unhurried and the expiration was continued without pause. The patient was verbally encouraged to blow as hard and fast as possible by the physiotherapist. Two readings were taken if the patient was able to do two. Occasionally the patient was only able to do the test once – in this case this was the only reading used.

3.2.1.7 Sputum

The sputum was assessed quantitatively for pathogenic bacteria (Table 6). The laboratories were unwilling to release the methodology as they claimed it was confidential. The results were placed in the CF patients file before the next clinic visits.

Table 6 Analysis of bacterial type in sputum

Gram stain Final
Pus cells
Epithelial cells
Gram negative bacilli
Gram positive cocci
Mixed oropharyngeal flora
Ps aeruginosa (dry)
Ps aeruginosa (mucoid)
Yeast, not Candida alblicans
S. aureus
B. cepacia
H. influenzae

The sputum score at baseline, at the end of first period and at the end of second period was calculated as the sum of responses (on a scale of 0 to 4) for *P. aeruginosa* (dry and muciod), *S. aurens*, and *B. cepacia*. These three were selected as they are the most serious and problematic for CF patient (Hart & Winstanley 2002). The higher the score, the more bacteria were present.

3.2.1.8 Food frequency questionnaire regarding pre and probiotic use

Questions regarding food supplements and foods (particularly yogurts) where asked of the CF patients. This was not a diet history as the diet/food intake of the subjects in the study was not looked at. To ensure standardization, the questionnaire was administered and recorded by the same dietician. Questions were asked as to the use and frequency of food supplementation and consumption of yogurt, as well as vitamin and mineral supplementation. Subjects were asked if they were brand specific when buying yogurt so that the type of probiotic found in specific brands could be identified (Appendix E). The

questionnaire used to establish whether the CF patients consumed significant amounts of either pre or probiotics.

3.2.1.9 Symptom Diary

To ensure standardization the purpose of the symptom diary and how to fill it in was explained to the patient by the same dietician. Patients were asked to record daily whether they had experienced fever, runny nose, sore throat, cough, chest wheeze, earache, thrush, diarrhea, constipation, vomiting and stomach ache Appendix C. The scoring system was one tick for mild symptoms, two ticks for moderate and three ticks for severe symptoms. The total number of ticks was recorded for each symptom per month.

3.2.1.10 Shwachman Score

The Shwachman Score was used to measure the severity of lung disease. The questionnaire consists of four sections, each with a rating from 0-25. The four sections included are general activity, physical examination, nutrition and chest x-ray. A score of 86-100 is excellent, 71-85 is good, 56-70 is mild, 41 -55 is moderate and 0-40 indicates severe lung disease.

3.2.2 Data collection

3.2.2.1 Prior to the start of the study

A meeting was held with the doctors, the physiotherapist and the dieticians involved in the KZN Durban CF clinics to advise every one of the standards and definitions required for the study. The collation sheets (see Appendix B) that the doctors were required to fill in during the study were explained to them and any questions answered regarding the filling in of these collation sheets. They were asked to record the type of treatment and

duration of treatment of lung infections at each visit or hospital admission on the collation sheets (Appendix B). As antibiotics would destroy the probiotics, information regarding the type and amount of antibiotics was requested. They were asked not to script probiotics during the course of the study.

3.2.2.2 Start of the study

On arrival at the clinic before signing the consent form, each participant had the study explained to both them and their family in detail. They were shown the straws containing the probiotic, how to use them and the symptom diary (see Appendix C) that they needed to fill in daily. They were asked if they had any questions regarding the study and whether they were willing to sign the consent forms (see Appendix D1 & D2). Only once the consent forms were signed the measurements (described below) were taken. See Figure 8 page 39.

The physiotherapist did 2 FEV_1 and FVC readings on each CF patient. In some cases however the CF patient could only do 1 FEV_1 and FVC reading. These results were recorded in Appendix J. At the end of the FEV_1 and FVC tests the CF patient was requested to produce a sputum sample which was then sent to the laboratory for microbial analysis. Not all patients were able to produce sputum at the start of the trial. In this case the sputum sample collected at the next clinic was used.

The dieticians measured the height, weight, MAC and TSF. Because of the potential carry over effect of the probiotic on the patient's growth it was necessary to determine the weights and heights of those that took the probiotic first (Group A), for six months prior to the start of the probiotic. Baseline referred to the first set of measurements that was taken six months prior to starting the probiotic. Group A started the probiotic first and these baseline measurements were taken from the patient's medical record. This was six months before the trial started. For group B, the group that started the placebo first, these measurements were taken at the start of the study (that is start of placebo in January 2005) as this was six months before the start of the probiotic. This data for group A was

taken retrospectively from the patients medical records at the end of the study after blinding had been broken so that their growth could be assessed independently from the effects of the probiotic. All other data (lung function, infection rate) was taken from the starting point of the study - the first group that was on probiotic and then placebo for the 12 months (group A) and the second (group B) that received placebo then probiotic.

A food frequency questionnaire (Appendix E) was asked regarding the consumption of pre and probiotics. This was not a diet history as information was needed to establish the use (if any) of a pre and probiotic consumption by the CF patients. The questionnaire was completed by the interviewer. The participants were asked not to take any new supplements during the trial without informing the dietician.

The purpose of the symptom diary (see Appendix C) was explained as well as how to fill it in. Patients were also asked to contact the dietician if they consulted a doctor other than one of the CF doctors during the trial for treatment of a lung infection. If they did consult a doctor that was not at the KZN Durban clinics a letter (Appendix F) was faxed to the other treating doctor. This letter informed the doctor about the study and requested information about the reasons for the CF patient's visit and the medications prescribed. They were also requested not to prescribe a probiotic. The Shwachman score was filled in by the doctor, physiotherapist and dietician (Appendix G). The straws were then issued to the patients in a brown paper bag labeled with their name.

3.2.2.3 During the first six months of the trial

The CF doctors filled in the collation sheets each time they prescribed any antibiotic treatment for the CF patient. The dieticians collected these. The physiotherapist and dieticians encouraged the CF patients to use their straws and fill in their symptom diary. Just before the 6 month clinic the CF patients were sent a letter reminding them to attend and to bring their straws and symptom diary (Appendix H).

3.2.2.4 At the 6 month crossover period

After 6 months the patients returned their straws and symptom diary. The straws were counted by the dietician and a record was kept of the used and unused straws per patient. If both the straws and the diary were returned they received R75 – they were not paid if either the straws or diary was not returned. They were then crossed over to receive the other straws. Again a 6 months supply of straws was issued to each patient in a brown paper bag labeled with their name.

The same physiotherapist repeated the lung function measurements and collected a sputum sample for microbial analysis. The same dieticians repeated the weights and heights.

3.2.2.5 During the second six months of the trial

The CF doctors continued to fill in the collation sheets each time they prescribed any antibiotic treatment for the CF patient. The physiotherapist and dieticians attempted to encourage compliance. Three weeks before the end of the trial, a reminder was sent out asking each patient to bring their used and unused straws and symptom diary to the clinic (Appendix I).

3.2.2.6 At the end of the 12 month study period

At the clinic the same physiotherapist took the better of 2 FEV_1 and FVC readings and collected sputum. The same dieticians repeated the height, weight, MAC and TSF on each patient, using the same equipment and techniques as in the beginning of the study. The data was recorded on a form (Appendix J).

Both the symptom diary and the used and unused straws were collected. Each participant was then thanked for their cooperation and again paid R75 and informed that they would receive a copy of the results of the study as soon as the data was processed.

The collation sheets were collected from the doctors. The straws from the second six month period were counted and recorded by the dietician. The data was entered into the database.

3.3 DATA ANALYSIS

The data was analysed in two different ways. Measurements relating to growth (heights and weights) were taken at baseline, which was 6 months before the start of probiotic, taken 6 months later at the start of the probiotic, and at the end of the 6 months on probiotic. This group consisted of half of the study population (n=8) and was labeled Group A. The other half of the study population (n=8) had the first set of growth measurement taken at the beginning of the study in January/February while starting on placebo for 6 months. The next set of measurements were taken at the start of the probiotic, and the last set were done after taking the probiotic for 6 months. This has been labeled baseline, start of probiotic and end probiotic in the tables that follow. For the results and discussion the 'on placebo' time period refers to the 6 months not on the probiotic. Measurements pertaining to lung infection and duration, lung function, antibiotic use, and the symptom diary was taken at the start of the study (January/February), at the cross over (June/July) and at the end of the study in December/January. Group A started the probiotic first and then took the placebo while Group B started placebo first and then took the probiotic (Figure 8 page 39).

Analysis of food frequency questionnaire

The frequency of use for food supplements and yogurt plus the types consumed was calculated from the data.

Height for age

The height for age (HFA) percentile for those 20 years and below was read off the Centre of Disease Control Charts (CDC) 2000. Using the correct height-for-age percentiles chart for sex, the 50^{th} percentile for height for chronological age was found (this is the expected height for age EHFA), and the actual height was expressed as a percentage of this.

$$\text{Percentage expected height for age} = \frac{\text{Actual height for age}}{\text{Expected height for age}} \times 100$$

For example if the actual height of a 10 year old girl is 126.8 cm and the 50th percentile for height and age is 138 cm then her %EHFA would be 126.8 cm divided by 138 cm and multiplied by 100 which equals 92%.

Weight for age

The weight for age (WFA) percentile for those 20 years and below was read off the CDC chart (2000). Using the correct weight-for-age percentiles chart for sex, the 50th percentile for weight for chronological age was found (this is the expected weight for age EWFA), and the actual weight was expressed as a percentage of this.

$$\text{Percentage expected weight for age} = \frac{\text{Actual weight for age}}{\text{Expected weight for age}} \times 100$$

For example if the actual weight of a 10 year old girl is 23.6 kg and the 50th percentile for weight for age is 33 kg then her %EWFA would be 23.6 divided by 33 multiplied by 100 which equals 72%.

Weight for height

The weight for height was calculated using Epi Info ^{TM} for those below 126 cm in height. This was only one subject (the youngest subject whose height was below 126 cm). A file was generated for this subject. Height, weight, MAC and TSF were then entered into this file for the starting point and 6 months later for this subject. Epi Info ^{TM} then calculated the weight for height and Z-scores for this subject. Percentage expected weight for height were then used for the rest of the subjects.

Percentage Expected Weight for height

The expected weight for height (EWFH) was calculated for those 20 years or below by reading off the CDC charts (2000) (FAO 1990). Using the height-for-age and weight-for-

age percentiles chart for the sex, the actual height of the subject was located on the chart. A line was then drawn from the actual height across until it intersected with the 50th percentile for height. A line was then taken from the 50th percentile vertically down the age line. Where this line intersected the 50th percentile for weight a horizontal line was then drawn back and the corresponding weight was recorded. This was the EWFH.

To work out the percentage expected weight for height (%EWFH) the actual weight of the child was taken and divided by the EWFH and then multiplied by 100

$$\text{Percentage expected weight for height} = \frac{\text{Actual weight}}{\text{EWFH}} \times 100$$

For example the actual weight of a 10 year old girl was 23.6 kg. This is divided by EWFH which was 25 kg and then multiplied by 100 to equal 94% EWFH

Percentiles

The CDC 2000 percentile charts, weight for age and stature for age, were used to plot the growth over the 12 months for each patient under 20 years of age (CDC growth charts 2000).

Z-scores

Z-scores describe the distance that an individual measurement is away from the mean or 50th percentile measured in terms of standard deviations (FAO 1990). A Z-score equal to zero means that the measurement is on the 50th percentile, a positive (+) Z-score indicates the number of standard deviations above the 50th percentile and a negative (-) Z-score indicates the number of standard deviations below the 50th percentile. Z-scores are used as indicators for malnutrition. A height for age Z-score of <-1.0 to -1.99 indicates mild stunting, <-2.0 to -2.99 moderate stunting and <-3.0 to -3.99 severe stunting. A weight for age Z-scores of <-1.0 to -1.99 indicates mild underweight, <-2.0 to -2.99 moderated underweight and <-3.0 to -3.99 severe underweight and a weight for height Z-score of, <-1.0 indicates mild wasting, <-2.0 moderate wasting and <-3.0 severe wasting (Dibley, Staehling, Nieburg & Trowbridge 1987). The data for each subject was entered into Epi Info ™ so the Z-scores could be calculated.

Body Mass Index (BMI)

The BMI is a relationship between weight and height. It is calculated by taking the weight (kg) and dividing by the height (m) squared.

$$BMI = \frac{Weight\ (kg)}{Height\ (m^2)}$$

For example a 21 year old male has a height of 1.8 m and weighs 60 kg, his BMI would be 18.8.

Body Mass Index Z-scores (BMIZ)

BMIZ were also calculated by Epi Info ™. Being a Z-score this is a reading that is the number of standard deviation away from the 50th percentile for BMI. Epi Info ™ version 3.3.2 was used to calculate BMI and BMI for age Z-score. Epi Info ™ uses the 2000 CDC-WHO growth charts.

The data was captured into SPSS version 11.5 (SPSS Inc, Chicago, Ill, USA) and analysed. Epi Info ™ version 3.3.2 was also used to capture the anthropometric data and analysed. As the numbers for the study were small an intention to treat analysis was used. The 95% confidence intervals for rate estimates were calculated in EpiCalc 2000 (version 1.02, Joe Gilman and Mark Myatt, Brixton Books). The objectives, data collections and statistical analysis used in the study have been tabulated in Table 7.

3.4 ETHICAL CONSIDERATIONS

Ethics approval (Ref.: E048/04) was granted by the Nelson R Mandela School of Medicine on the 24th of November 2004 (Appendix K1). Permission to use the State and private hospitals can be seen in Appendix K2. Written consent forms from participants can be seen in Appendix D1 and D2.

3.5 CAPE TOWN STUDY

To show validity for comparison. The secondary purpose of the study was to compare the nutritional status of the patients attending the CF clinics in KZN Durban (n=35) with the patients attending the CF clinics in Cape Town (n=38). The data from the Cape Town clinics was obtained from the publication by Westwood & Saitowitz (1999). This is the only anthropometric data published for CF patients in SA. The studies had a similar number of patients and exclusion criteria so were comparable. For the Cape Town study by Westwood & Saitowitz (1999) their CF population at the Red Cross Hospital in 1996 consisted of 45 patients as the entire population. They excluded those under 2 years old, those with very severe lung disease and those who lived more than 60 km away. As 7 did not complete the 3 day weighed food record (18.4%), a final total of 38 patients were included in that paper. As Westwood & Saitowitz (1999) used the classification for malnutrition from the consensus committee of the CF Foundation (Ramsay *et al* 1992) based on percentage expected weight for height (% EWFH) the Durban data was converted to %EWFH to enable the comparison. The weight, height, MAC and TSF readings from the end of the study (Dec 2005) were used as this was the most recent data from Durban.

Table 7 Objectives, data[4] collection and analysis

Objective	**Data Collection**	**Data Analysis**
To determine weight at baseline, start of the probiotic and end of probiotic for the group as a whole as well as by age and gender and to compare weight gained over the six months prior to and while taking the probiotic.	Weight Age Gender	Descriptive statistics and paired t test Calculation of anthropometric indices: WFA percentiles, WFA Z-scores, % EWFA.

[4] All data was collected in Durban expect where data from Cape Town reported in the literature is mentioned

Table 7 Objectives, data collection and analysis continued

To determine height at baseline, start of the probiotic and end of probiotic for the group as a whole as well as by age and gender and to compare height gained over the six months prior to and while taking the probiotic.	Height Age Gender	Descriptive statistics and paired t test Calculation of anthropometric indices: WFH percentiles, WFH Z-scores, HFA, % EHFA. BMI for age percentiles and BMI Z-scores
To compare the percentage expected weight for height (%EWFH), mid arm circumference (MAC) and triceps skinfold thickness (TSF) in CF patients in Durban and Cape Town. For the comparison to the Cape Town patients this data was obtained from the publication by Westwood and Saitowitz (1999)	Weight Height Height for age and weight for age percentile charts for sex. MAC readings TSF readings Publication of Westwood & Saitowitz (1999)	Descriptive statistics (mean and standard deviation)
To determine and compare the incidence and duration of lung infections while taking the probiotic and while taking the placebo.	Number of lung infections filled in by the KZN Durban CF doctors at each clinic visit	Incidence rate and 95% confidence interval Descriptive statistics (mean and standard deviation)
To determine and compare the mean change in lung function while taking the probiotic and while taking the placebo	FEV_1 & FVC readings	Descriptive statistics

Table 7 Objectives, data collection and analysis continued

To determine the number of CF patients in the study colonized with *P. aeruginosa*	Sputum results	Descriptive statistics
To determine and compare the change in the presence and quantity of *P aeruginosa* (dry and mucoid), *S. aurens* and *B. cepacia* in the sputum while on the probiotic and while on the placebo.in KZN Durban	Sputum analysis reports	Repeated ANOVA analysis Descriptive statistics for change in sputum score
To determine and compare the number of episodes of symptoms (coughing, wheezing, thrush, diarrhea, constipation, vomiting, stomach ache, fever, runny nose, sore throat and ear ache) while on the probiotic and while on the placebo in KZN Durban	Symptom diaries	Paired t-tests

CHAPTER 4: RESULTS

Introduction

The primary purpose of the study was to determine whether supplementation with a probiotic (*L. reuteri*) would improve anthropometric measurements, reduce the incidence and shorten the duration of lung infections and would maintain or prevent deterioration in lung function in CF patients Kwazulu-Natal (KZN) attending the CF clinics in Durban. The secondary purpose of the study was to compare the nutritional status at the end of the study of CF patients attending CF clinics in KZN Durban with CF patients attending CF clinics in Cape Town from the published data by Westwood & Saitowitz (1999). Data reported from the Durban study included weights, heights, MAC, TSF, lung functions, sputum analysis, a food frequency questionnaire to assess whether the CF patient was consuming other probiotics and a symptom diary recording the incidence of eleven different symptoms.

4.1 SAMPLE CHARACTERISTICS DURBAN STUDY

Twenty three patients started the study. Of these 1 withdrew after the first month having claimed that the probiotic gave him diarrhoea. Five patients were excluded at the 6 month crossover as they did not return either their straws or their symptom diary. The reasons are listed in Table 8. One was excluded at the end of probiotic of the study as they did not attend the last clinic for their final measurements. A total of 16 patients completed the study, 8 were in group A that received the probiotic first and then the placebo and 8 were in group B that received the placebo first and then the probiotic.

Table 8 Reasons for not returning straws

'left straws in Durban when going back to Cape Town for last 6 months'
'straws & symptom diary got lost while moving house'
'Maid threw straws and symptom diary away'
'straws and symptom diary got lost'
'left them at home'

As the final sample sizes were small the exclusions could have affected randomization therefore baseline data was checked for incomplete randomization. Since at baseline there were no significant differences in mean age, proportion of males and females, anthropometry, lung function measurements and mean sputum scores between the 2 groups, the groups were comparable. Any changes which occurred over time therefore could be attributed to the treatment received rather than to baseline differences.

In addition to the small sample size there was a large degree of non compliance both with taking the straws and with keeping the symptom diary. Patients were required to take 360 straws over the 12 month study period (1 per day). Table 9 shows the compliance rate in each 6 month period. Sixty nine percent of the group took under 75 % of the required number of straws for the study period for the first 6 month period. This improved slightly for the second period dropping to 56 %.

There was no difference between the proportions of compliant participants per group in either of the 6 month periods (Fisher's exact p=1.000). Because non compliance was the same in each group, an intention to treat analysis was subsequently used[5]. Across the year the 3 most compliant patients were two 14 year olds and one 20 year old.

[5] The rationale for an intention to treat analysis analyses placebo and treatment groups as per initial allocation regardless of compliance levels in the treatment groups.

Table 9 Compliance rate of straw use

Number of straws used	Compliance rate	First 6 months n=16	Second 6 months n=16
0-90	0 to 50 %	7	4
91 to 135	50 to 74%	4	5
136 to 161	75 to 89%	2	4
162 to 180	90% to 100%	3	3

Most of the adult patients mentioned that by using the straw they did not feel like they were taking medication and that they would have preferred to have taken a tablet which they perceived to be more efficient.

4.1.1 Age and Gender

All participants in the study were of Caucasian origin. As there was a wide range in age for this study (8 to 31 years) the age groups were divided into non-adolescents which were 6 to 12.99 years old (n=4), adolescents from 13 to 19.99 years of age (n=8) and adults ≥ 20 years (n=4). The study ran for 12 months and 2 male subjects moved from the under 19.99 years to the ≥ 20 year old group after the first six months. The groups were further divided into those 6 to 9.99 years of age (n=2), 10 to 12.99 years (n=2), 13 to 19.99 years (n=8) and ≥ 20 years of age (n=4). These age divisions were done so that the data could be compared to other studies that had looked at the ≤ 9.99 year olds and the adolescence years. The mean age was 17.5 years (SD 6.4 range 8-31). The majority (n=6) were adolescents (Table 10), 4 were ≤12.99 years and 6 were ≥ 20 years. There were 9 males and 7 females (Table 10).

Table 10 The age and gender distribution of the patients who completed the study

Age group	No. of patients	No. of Males	No. of Females
6 to 9.99 years Non-adolescent	2	1	1
10 to 12.99 years Non-adolescent	2	1	1
13 to 19.99 years Adolescent	6	2	4
≥ 20 years	6	5	1
Total	**16**	**9**	**7**

4.1.2 Shwachman Score

The Shwachman score was used to measure the severity of lung disease. The questionnaire consists of four sections, each with a rating from 0-25. The four sections included are general activity, physical examination, nutrition and chest x-ray. A score of 86-100 is excellent, 71-85 is good, 56-70 is mild, 41 -55 is moderate and 0-40 indicates severe lung disease. The mean score for the group was 71.06 (SD 15.24 range 41 to 90). Two patients scored in the moderate range, 6 in the mild range, 4 in the good range and 4 in the excellent range.

4.2 WEIGHT

The mean weight at baseline (which was 6 months before they took the probiotic) was 45.3 kg (Table 11). On starting the probiotic 6 months later[6] the mean weight was 47.3 kg which was an increase of 2 kg. Six months after that at the end of probiotic it was 48.5 kg which was an increase of 1.2 kg. This was a total weight gain of 3.2 kg across the 12 month period which was significant (p=0.010). The expected weight gain over a

[6] Weights for the placebo group were the weights six months before start of probiotic ie when off probiotic.

six month time period would have been between 1.5 to 3 kg readings off the CDC 2000 growth charts.

There was a significant difference in the weight at baseline compared to end of placebo ($p=0.006$) but no difference in weight at the start of the probiotic compared to the end probiotic ($p=0.166$). This meant that there was a lower weight gain while on probiotic. Statistically however there was no significant difference between the treatment groups for weight gain ($p=0.304$). See summary Table 12.

There was a non significant gain in weight in all age groups over the 12 months period. The 13 to 19.99 year olds were the only group to non significantly increase their weight more on the probiotic versus off the probiotic. However the amount of weight gained off probiotic was not significantly different to the amount of weight gained on probiotic for all age groups except the $\geq$ 20 years who significantly lost weight while on the probiotic ($p=0.006$) (Table 11, Table 12).

Table 12 Summary of weight changes across the study

Comparison of weight change from start to end of the study					
	Group in total	**≤ 9.99 years**	**10 to 12.99 years**	**13 to 19.99 years**	**≥ 20 years**
Group as a whole	> S (p=0.010) n=16	> NS (p=0.170) n=2	> NS (p=0.300) n=2	> NS (p=0.087) n=6	> NS (p=0.506) n=6
Males	> NS (p=0.114)	> ? S	> ? S	> NS (p=0.301)	> NS (p=0.680)
Females	> S (p=0.041)	> ? S	> ? S	> NS (p=0.200)	> ? S
Comparison of weight change on probiotic with weight change off probiotic					
	Group in total	**≤9.99 years**	**10 to 12.99 years**	**13 to 19.99 years**	**≥ 20 years**
Group as a whole	<NS (p=0.304)	<NS (p=0.205)	<NS (p=0.742)	> NS (p=0.320)	<S lost wht (p=0.006)
Males	<NS (p=0.412)	<? S	<? S lost wht	> NS (p=0.131)	<S lost wht (p=0.027)
Females	<NS (p=0.592)	<? S	> ? S	<NS (p=0.921)	<? S

> increase < decrease S significant NS non-significant ? S Query Sig (n=1) wht weight

The mean weight of the males at baseline was 49.6 kg (Table 13). Their weight before starting the probiotic was 51.6 kg which was an increase of 2 kg. At the end of the probiotic, six months later it was 52.4 kg which was an increase of 0.8 kg. This was a total weight gain of 2.8 kg across the 12 month period which was not significant (p=0.114). Although they appeared to gain more weight off probiotic this finding was not significant (p=0.412) possibly due to the small sample size. There was an improvement in weight among the males in all the age groups however as (n=1) in some of the groups significance could not be calculated. There was no significant difference in weight gain whether on or off probiotic in all age groups except for the ≥ 20 year olds males (p=0.027) who significantly lost weight. In addition one male subject in the 10 to12.99 age group lost weight on the probiotic (Table 12).

The mean weight of the females at baseline was 39.8 kg (Table 14). Their weight before the start of probiotic was 41.8 kg which was an increase of 2.0 kg. At the end of probiotic it was 43.3 kg which was a weight increase of 1.5 kg. This was a total weight gain of 3.5 kg across the 12 month period which was significant (p=0.041). Although they appeared to gain slightly more weight off probiotic the finding was not significant (p=0.592). There was an increase in weight among the females in all age groups except the ≥ 20 year olds who lost weight while on probiotic however as n=1 in some of the groups significance could not be calculated (Table 12, Table 14). The 10 to 12.99 age group was the only age group to gain a non significant amount of weight on the probiotic. There was no significant difference in weight gain in females in all age groups when on or off probiotic (p=0.592).

The males gained 2.8 kg compared to the females 3.5 kg over the 12 months which was not significant (p=0.377).

The CDC tables for WFA were used to calculate the percentiles of those ≤ 20 years (Table 15, Figure 9, and Figure 10).

When considering the patients individually, at baseline 3 males and 3 females were below the 3rd percentile and therefore wasted. Of these one was 10 years, one was 13 years, three were 14 years and one was 20 years old. At the end of the probiotic 2 males and 3 females were below the 3rd percentile. The 20 year old male had managed to gain weight to below the 25th percentile.

Table 15 Weight for age percentiles for those less than and equal to 20 years by gender at baseline, start of probiotic and end of probiotic

Percentile	Number of males n=6			Number of females n=6		
	Base line	Start of probiotic	End of probiotic	Base line	Start of probiotic	End of probiotic
On and below 95th	1	0	0	0	0	0
On and below 90th	0	1	1	1	0	0
On and below 75th	0	0	0	0	1	1
On and below 50th	0	0	0	1	2	2
On and below 25th	1	1	2	1	0	0
On and below 10th	1	1	1	0	0	0
On and below 5th	0	0	2	0	0	1
On and below 3rd	3	3	0	3	3	2

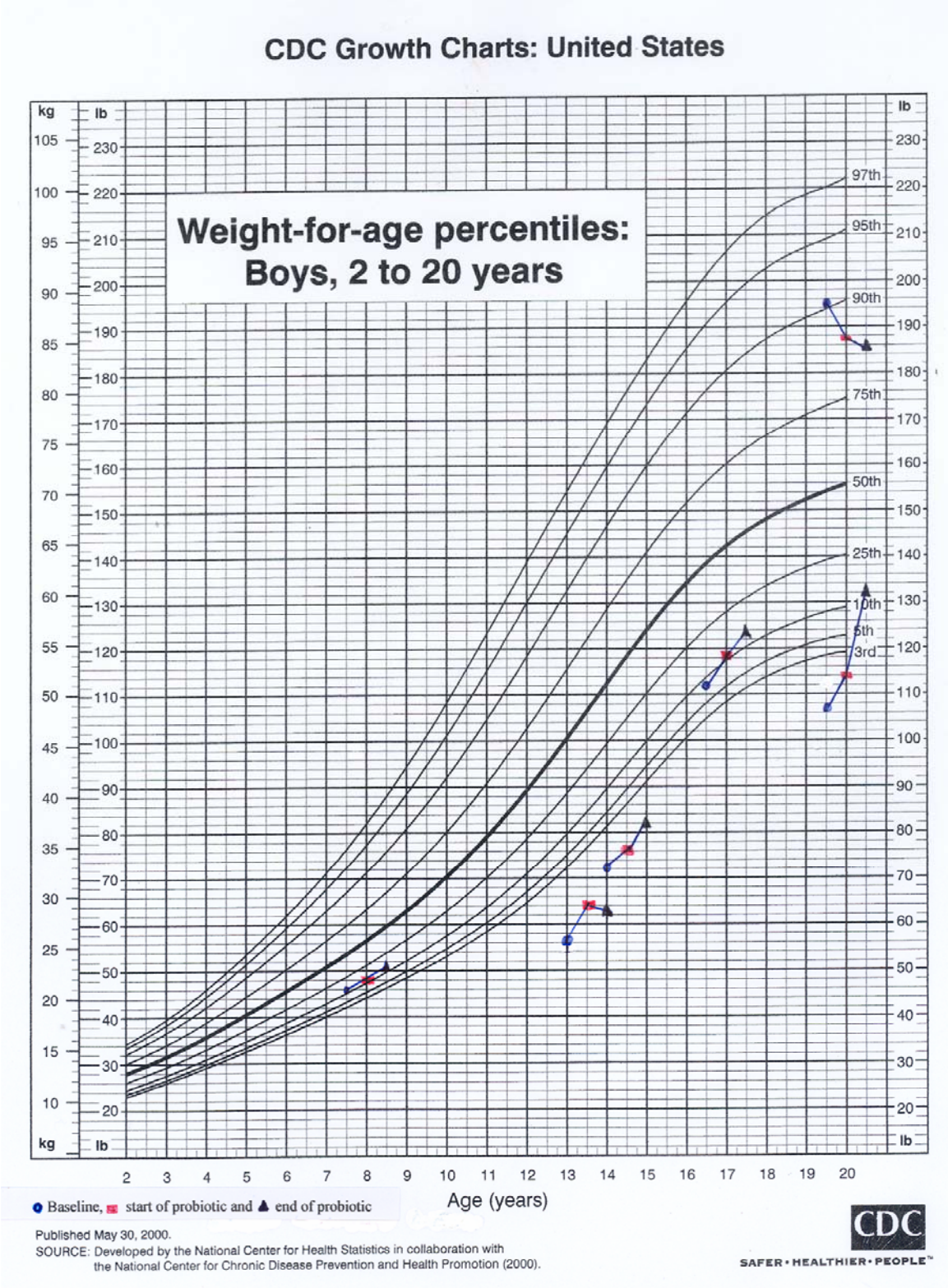

Figure 9 Weight for age percentiles for six males at baseline, start of probiotic and end of probiotic

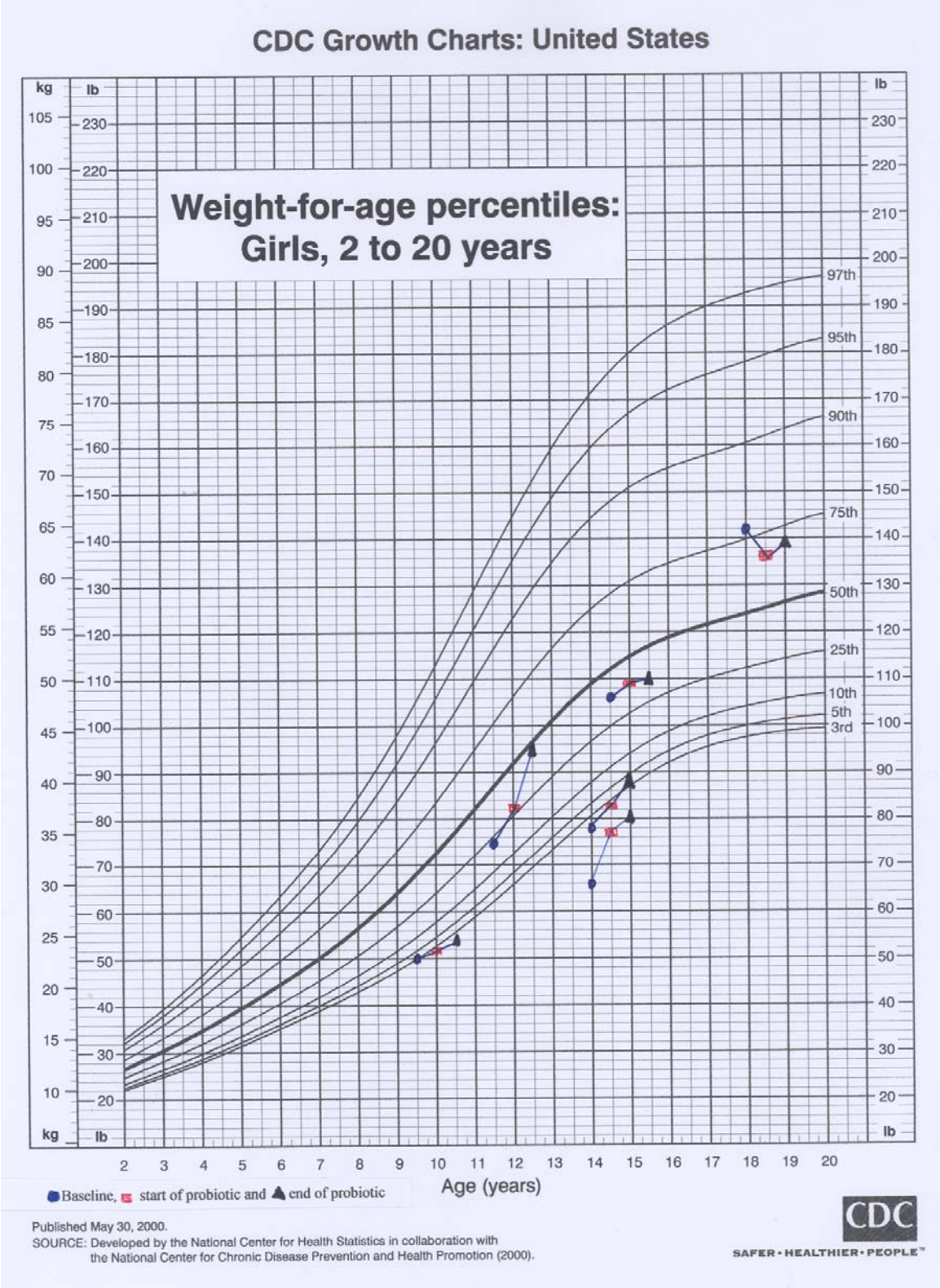

Figure 10 Weight for age percentiles for six females at baseline, start of probiotic and end of probiotic

The weight data was also analysed using the following indictors: Weight for age (WFA) Z-scores (Table 16) and %EWFA (Table 17). Although the WFA Z scores seemed to improve slightly off probiotic and regress while on probiotic this change was very small and not significant (p=0.742). There was no significant difference between the treatment groups in %EWFA (p=0.765). Weight for age, %EWFA and WFA Z scores all followed a similar trend to all the other weight categories that have been discussed.

4.3 HEIGHT

The mean height was 156.22 cm at baseline, 158.14 cm at the start of probiotic and 160.17 cm at the end of probiotic (Table 18). The mean heights improved significantly from the baseline to the end of the study by 3.95 cm (p=0.001). There was no difference in their increase in height whether they were off probiotic or on probiotic (p=0.838).

When looking at the increase height over the year by age group the only group to reach significance was the 13 to 19.99 year olds (p=0.006) (Table 18). The height for the ≥20 year olds remained the same over the year. While on probiotic the group as a whole, the 9.99 years olds and the 13 to 19.99 year olds increased more in stature than off probiotic but this did not reach significance (Table 19).

The mean height of the males at the beginning of the study was 160.94 cm, at the start of probiotic was 162.82 cm and at the end of probiotic was 164.47 cm (Table 20). From six months before the start of the probiotic to the end of probiotic the males height increased significantly by 3.3 cm (p=0.010). The mean height gained for males in the group off probiotic was 1.90 cm and on probiotic was 1.66 cm. There was no significant difference in their increase in height whether they were off probiotic or on probiotic for each age group (Table 19). The male aged 10 to 12.99 years, was the only group to increase their height more while on probiotic than off probiotic but the difference was not significant (Table 19).

Table 19 Summary of height changes across the study

Comparison of change in height from start to end of the study					
	Group in total	**≤ 9.99 years**	**10 to 12.99 years**	**13 to 19.99 years**	**≥ 20 years**
Group as a whole	> S (p=0.001) n=16	> ? S n=2	> NS (p=0.300) n=2	> S (p=0.006) n=6	Same NS (p=0.190) n=6
Males	> S (p=0.010)	> ? S	> ? S	> NS (p=0.580)	Same NS (p=0.225)
Females	> S (p=0.022)	> ? S	> ? S	> NS (p=0.128)	Same NS
Comparison of change in height on probiotic with height change off probiotic					
	Group in total	**≤ 9.99 years**	**10 to 12.99 years**	**13 to 19.99 years**	**≥ 20 years**
Group as a whole	> NS (p=0.838)	<? S (p=0.795)	> NS (p=0.897)	> NS (p=0.704)	Same NS (p=0.476)
Males	<NS (p=0.616)	<? S	> ? S	< NS (p=0.430)	<NS (p=0.510)
Females	> NS (p=0.577)	>? S	< ? S	>NS (p=0.343)	Same ? S

> increase < decrease S significant NS non-significant ? S Query Sig (n=1) wht weight

The mean height of the females at the beginning of the study was 150.17 cm, at the start of probiotic was 152.14 cm and at the end of probiotic was 154.63 cm (Table 21). The females height increased significantly by 4.46 cm (p=0.022) (Table 21). There was a non significant increase in height in all age groups except for the ≥ 20 year olds whose height remained the same. Although the females appeared to grow 1.13 cm more than the males the difference was non significant (p=0.549).

The mean height gained for females off probiotic was 1.97 cm and on probiotic was 2.49 cm. This was a non significant finding of p=0.577. All age groups appeared to grow more while on probiotic except the 10 to 12.99 year olds (Table 19) although the findings were non significant.

On average the males were 10.77 cm taller than the females six months before the start of the probiotic and 9.64 cm taller at the end of probiotic. This difference was not significant (p=0.295).

The CDC tables for HFA were used to calculate the percentiles of those ≤ 20 years (Table 22, Figure 11, Figure 12)[7].

When looking at the data on an individual basis, three boys (13, 14 and 20 years) were under the 5th percentile for height six months before start of probiotic. Two remained below the 5th and the 20 year old improved to below the 25th percentile by the end of probiotic. Two girls (9 ½ and 14 years) were below the 5th percentile six months before start of probiotic and were still under the 5th percentile at the end of probiotic.

[7] CDC tables range from 2 to 20 years

Table 22 Height for age percentiles for those less than and equal to 20 years by gender at baseline, start of probiotic and end of probiotic

Percentile	Number of males n=6			Number of females n=6		
	Base line	Start of probiotic	End of probiotic	Base line	Start of probiotic	End of probiotic
On and below 90th	0	0	0	0	0	0
On and below 75th	1	2	2	1	2	3
On and below 50th	1	0	0	2	1	0
On and below 25th	0	0	1	1	1	1
On and below 10th	1	1	1	0	0	0
On and below 5th	3	3	2	2	2	2

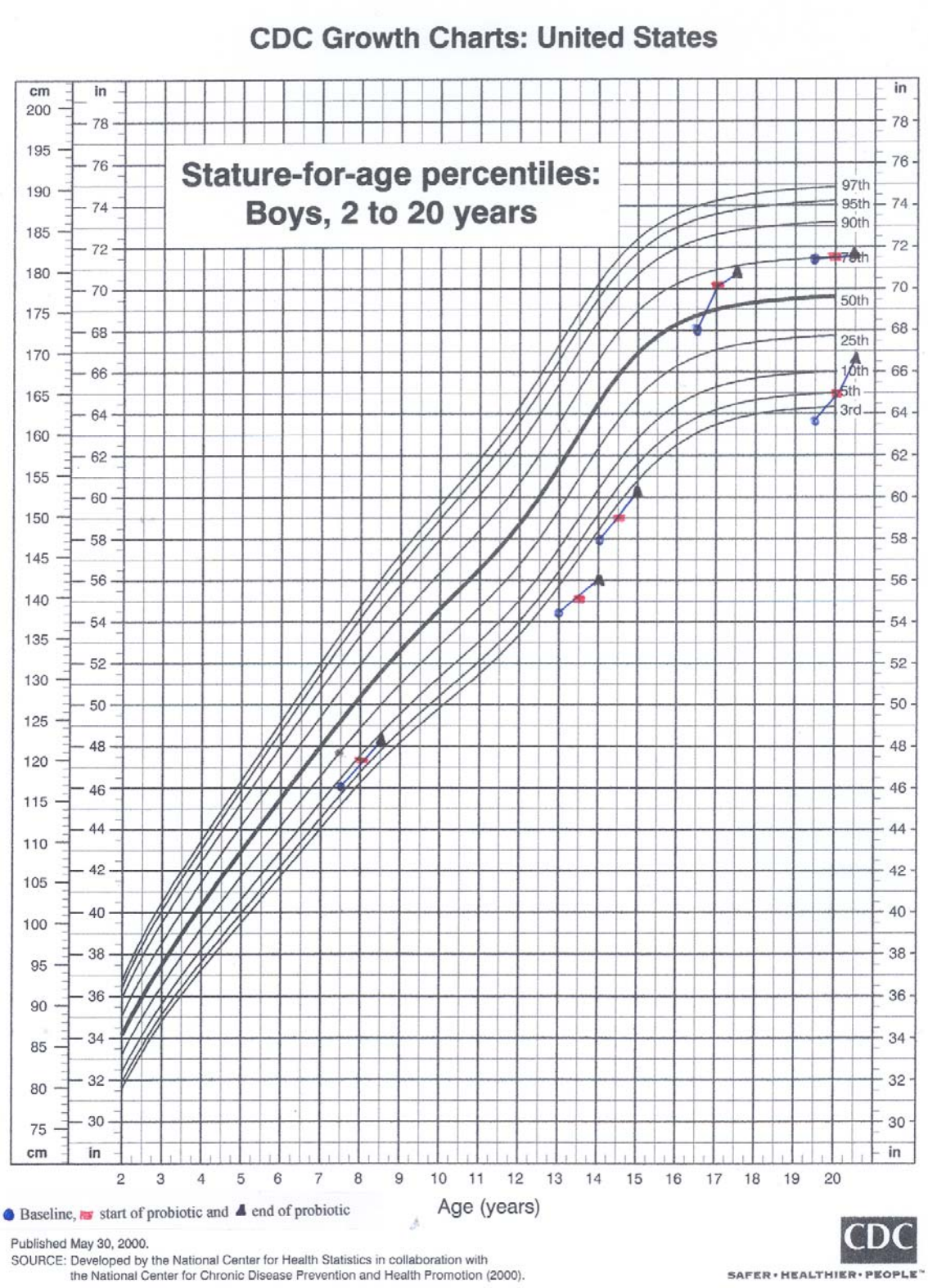

Figure 11 Height for age percentiles for six males at baseline, start of probiotic and end of probiotic

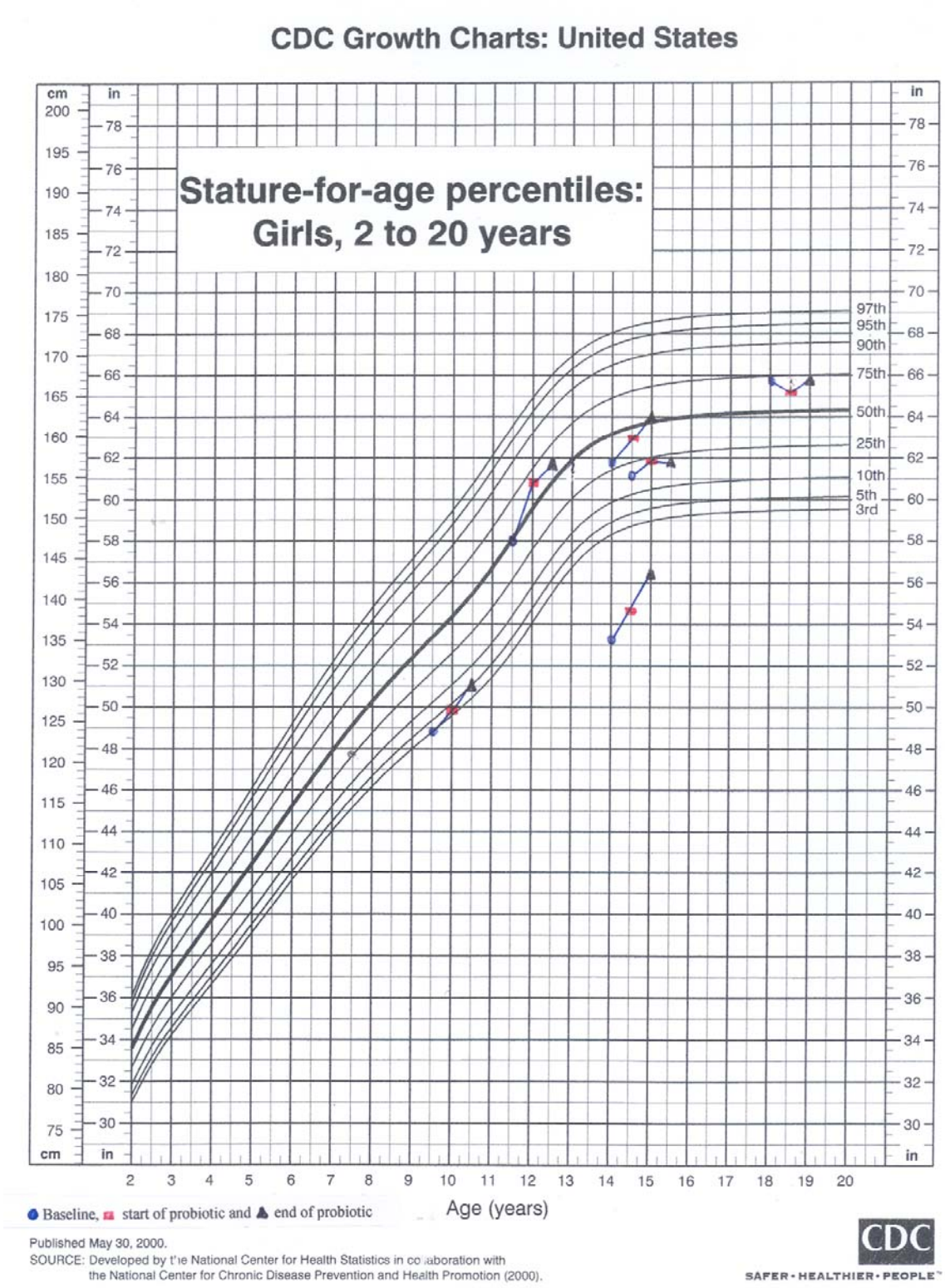

Figure 12 Height for age percentiles for six females six months before start of probiotic, start of probiotic and end of probiotic

Summary data for Z-scores for HFA can be seen in Table 23. There was no significant difference in the Z-scores HFA for the group as a whole, whether off probiotic or on probiotic for both males (p=0.989) and females (p=0.460). Summary data for percentage expected height for age can be seen in Table 24. There was no significant difference between the treatment groups (p=0.254).

Midarm circumference and TSF measurements were not taken at the crossover point of the study but only at the start and end of the study. The readings have been shown in Appendix L. The measurements were used to compare with the published data of Westwood & Saitowitz (1999).

4.4 COMPARISON OF ANTHROPOMETRIC STATUS OF CYSTIC FIBROSIS PATIENTS IN DURBAN AND CAPE TOWN

Anthropometric data namely weight, height, MAC and TSF measurements were collected to compare the nutritional status of CF patients attending the 2 KZN Durban clinics to that of the CF patients attending the Cape Town clinics. Westwood & Saitowitz (1999) used a modification of the Waterlow and Rutishauser classification for growth measurements (Ramsey *et al* (1992) based on percentage expected weight for height (% EWFH) the Durban data was converted to %EWFH to enable the comparison (Table 25). Only 50% of the Durban patients had a normal %EWFH versus 62.2% of the Red Cross patents. A higher percentage of the Durban patients (50%) were suffering from underweight/mild malnutrition versus the Red Cross patients (32.4%). At the Red Cross clinic however 13.5% suffered from either severe or moderate malnutrition which was slightly higher than the 12.5% moderate malnutrition found in the Durban clinics where no severe malnutrition as defined by %EWFH <75% was recorded.

Table 25 Comparison of the percentage expected weight for height at the end of the study of the Durban Clinics (2005) to those attending the CF clinic at Red Cross Hospital in 1996 (Westwood & Saitowitz 1999)

	Red Cross CT	Durban Clinics
	Frequency (%) (n=21)	Frequency (%) (n=16)
%EWFH >110% (overweight)	5.4	0.0
%EWFH 90-110% (normal)	62.2	50.0
%EWFH 85-89% (underweight)	5.4	18.8
%EWFH 80-84% (mild malnutrition)	13.5	18.8
%EWFH 75-79% (moderate malnutrition)	5.4	12.5
%EWFH < 75% (severe malnutrition)	8.1	0.0
MAC < 5th percentile	38.1	56
TSF < 5th percentile	28.6	38

Fifty six percent of the study group was below the 5th percentile for MAC readings at the end of the study (Appendix L). These figures are much higher than the Cape Town (n=21) who had only 38 % below the 5th percentile.

Thirty eight percent of the study population in Durban had TSF below the 5th percentile compared to 29% of the Cape Town clinic (n=21).

4.5 INCIDENCE AND DURATION OF LUNG INFECTION

Incidence rate was defined as the number of new episodes of lung infection divided by person-months at risk. Person-month at risk was calculated using the number of persons (n=16) multiplied by the number of months (n=6). While on probiotic there were 38 infections in total out of 96 person-months at risk (Table 26). This is an incidence rate of 39.6 per 100 person-months (95 % CI 27.0 to 51.2). While off probiotic there were 28 lung infections in total out of the 96 month period, resulting in an incidence rate of 29.2 per 100 person-months (95% CI 18.4 to 39.9) (Table 26).

Table 26 Episodes of lung infections by treatment group

No of infections	Probiotic Jan to June (n=8)	Placebo July to Dec (n=8)
Group A	24	13
	Probiotic July to Dec	Placebo Jan to June
Group B	14	15
Sum	**38**	**28**

The number of lung infections while off probiotic and probiotic was compared using both relative risk and repeated measures of ANOVA. The relative risk (exposed relative to unexposed) was 1.36 (95 % CI 0.83 to 2.21). This indicates that those exposed to the probiotic were at a 1.36 times higher risk of having a lung infection than those not exposed. However as the 95 % CI crossed over the null value of 1 the relative risk was not significantly greater than 1 at the 95 % level. The p value was 0.134, confirming that there was no significant association between the exposure to probiotic and an increase in lung infection rate.

Using the repeated measure of ANOVA there were no significant differences in the outcome of number of infections. There was no significant differences between the groups over each 6 month period (p=0.184). However, there was a trend for probiotic showing a reduction in the mean number of infections when on the placebo first compared with in the group which received the probiotic first (Figure 13). The group that received the placebo first remained relatively constant with mean number of infections over the two periods.

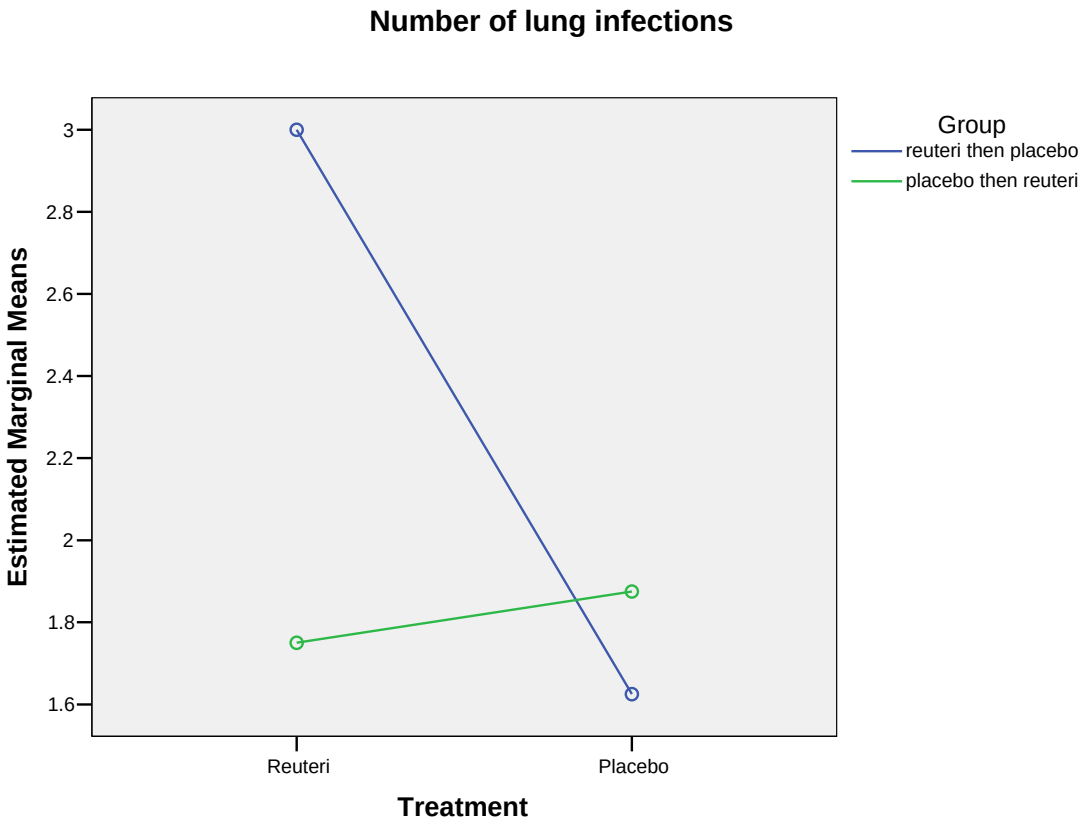

Figure 13 Profile plot of mean number of lung infections per treatment group

The duration on antibiotics was taken as a proxy measure for duration of lung infection. The total duration of days on antibiotics while taking the probiotic was 1828 days. This is a mean of 121.9 days per participant (SD 75.9) (Table 27). The mean duration on antibiotics while taking the placebo was 1488 days (Table 27). This was a mean of 99.2 days per person (SD 81.9). There was no significant difference between the group on or off probiotic (p=0.497)

Table 27 Duration on antibiotics by treatment group

No of days on antibiotics	Probiotic Jan to June (n=8)	Placebo July to Dec (n=7)
Group A	939	320
	Probiotic July to Dec (n=7)	Placebo Jan to June (n=8)
Group B	889	1168
Sum	**1828**	**1488**

There was a significant decrease in the use of antibiotics during the first 6 months (939 + 1168= 2107) of the year compared to the second 6 months (320+889=1209) (p=0.046) regardless of treatment (p=0.440). Both groups therefore decreased over time with regard to mean duration of antibiotics from the first 6 months to the second 6 months. This was not related to the treatment as the sequence of treatments cancelled this decrease out (Figure 14). Although more antibiotics were used in the first 6 months of the study there was no difference in antibiotic use in patients on or off the probiotic (p=0.497).

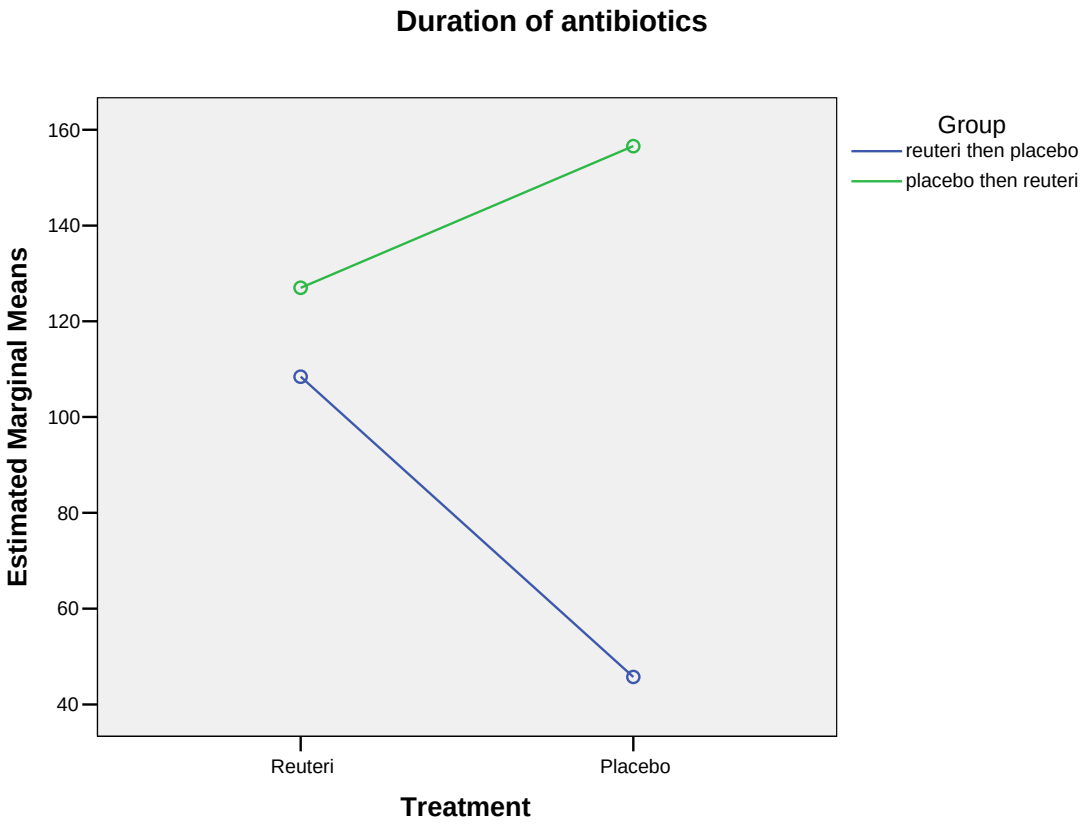

Figure 14 Profile plot of mean duration of antibiotics per treatment group

4.6 LUNG FUNCTION

FORCED EXPIRATORY VOLUME IN ONE SECOND

The sum of the total percentage change in FEV_1 while on the probiotic was 30%. This was a mean increase of 1.9% (Table 28). The sum of the total percentage change in FEV_1 while on the placebo was a decrease of -65%. This was a mean decrease of -4.1% (Table 28).

Table 28 Percentage change in Forced Expiratory Volume in one second for the treatment groups

Percentage change in FEV_1	Probiotic Jan to June (n=8)	Placebo July to Dec (n=8)
Group A	17	-50
	Probiotic July to Dec (n=8)	Placebo Jan to June (n=8)
Group B	13	-15
Sum	**30**	**-65**

There was a marginally non-significant treatment effect for FEV_1 ($p=0.079$). Both groups showed higher mean FEV_1 scores on the probiotic than on the placebo. Thus, while there was a trend in favour of the probiotic displayed for FEV_1 improvement, this could not be proved conclusively due to the non significant p value. Figure 15 shows the mean FEV_1 per treatment group.

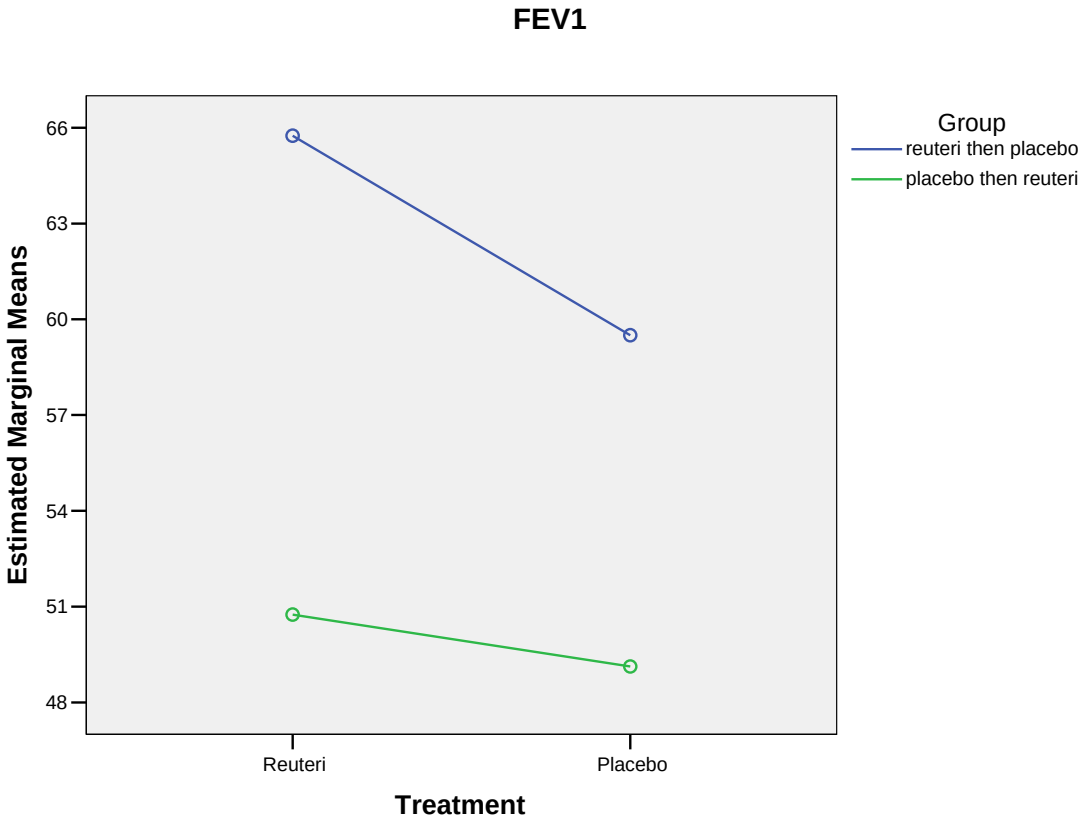

Figure 15 Profile plot of Forced Expiratory Volume in one second per treatment group

FORCED VITAL CAPACITY

The sum of the total percentage change in FVC while on the probiotic was 41%. This was a mean increase of 2.6 % (Table 29). The sum of the total percentage change in FVC while on the placebo was a decrease of -60%. This was a mean decrease of -3.75 % (Table 29).

Table 29 Percentage change in Forced Vital Capacity readings for the treatment groups

Percentage Change in FVC	Probiotic Jan to June (n=8)	Placebo July to Dec (n=8)
Group A	20	-40
	Probiotic July to Dec (n=8)	Placebo Jan to June (n=8)
Group B	21	-20
Sum	**41**	**-60**

The repeated measures ANOVA analysis for FEV_1 and FVC involved calculating the difference between end of probiotic of the first and end of probiotic of the second period values whilst controlling for starting values which were used as a covariate in the model.

The same trend of probiotic is evident with FVC. There was a marginally non significant treatment effect (p=0.094) which was favorable towards the probiotic rather than the placebo. Figure 16 shows the mean Forced Vital Capacity per treatment group.

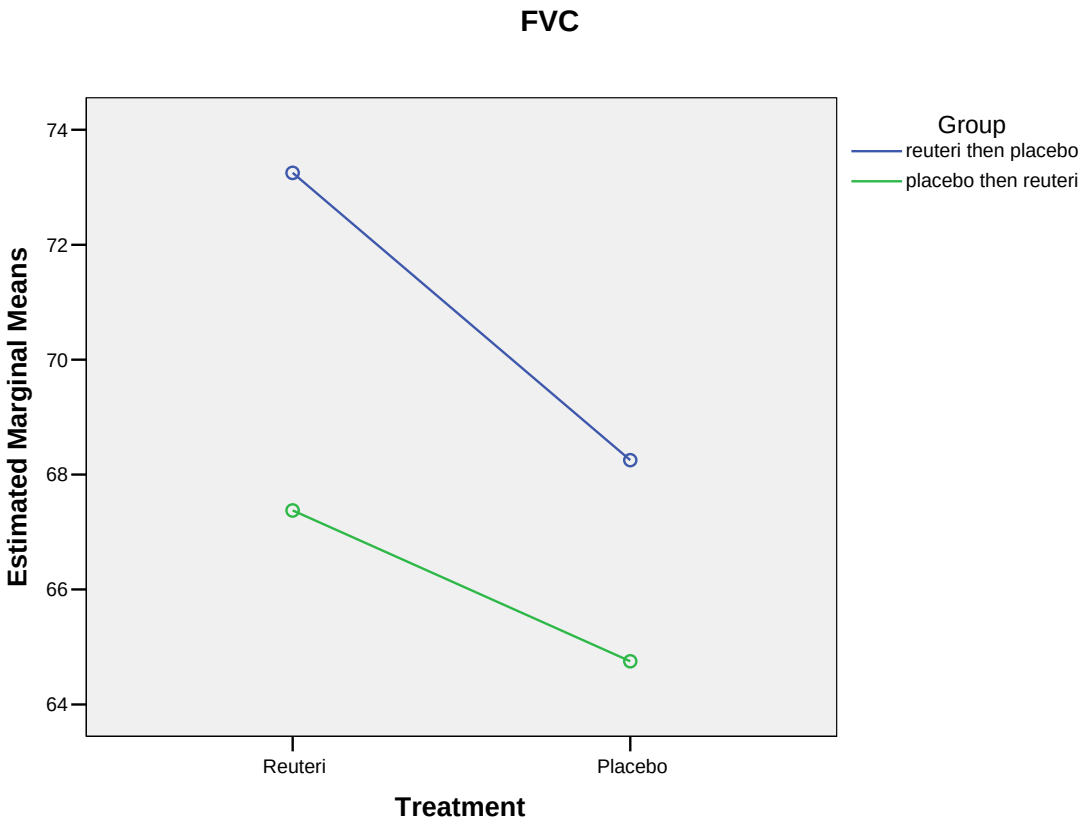

Figure 16 Profile plot of Forced Vital Capacity per treatment group

4.7 SPUTUM ANALYSIS

A total of 3 patients of the six from January to June and of the five from July to December did not culture *P. aeruginosa* during the 12 month study. Table 30 shows the figures for culturing *P. aeruginosa* from January to December.

Table 30 *Pseudomonas aeruginosa* culture figures

	Jan - June	July - Dec
Number of patients not culturing *P. aeruginosa*	6 (38%)	5 (32%)
Number of patients culturing *P. aeruginosa*	10 (62%)	11 (69%)

Of the common bacteria found in CF patients (Hart & Winstanley 2002) the three most troublesome pathogens were chosen (West *et al* 2002). These were *P. aeruginosa* (dry and muciod), *S. aurens*, and *B. cepacia*. Sputum score at start of probiotic (first period) and end of probiotic (second period) was calculated as the sum of responses (on a scale of 0 to 4) to the three selected bacteria. The sputum score while off probiotic was calculated in the same way. The higher the score, the more bacteria present. Change in sputum score was used as a measure of the change in bacterial infection during each period.

The total change in sputum score while on the probiotic was -5 (Table 31). This is a mean change of -0.38 indicating that the probiotic showed a mean decrease in sputum score. The total change in sputum score while on the placebo was 12 indicating a mean increase of 1.0 (Table 31).

Table 31 Change in sputum score by treatment group

Change in sputum score	Probiotic Jan to June (n=8)	Placebo July to Dec (n=8)
Group A	-1	0.0
	Probiotic July to Dec (n=5)	Placebo Jan to June (n=5)
Group B	-4	12
Sum	**-5**	**12**

Although there was a trend indicating that the probiotic resulted in slightly lower mean scores of bacteria in the sputum than the placebo, especially in the group that took the placebo first, this trend was not significant (p=0.453). Figure 17 shows the mean sputum score per treatment group

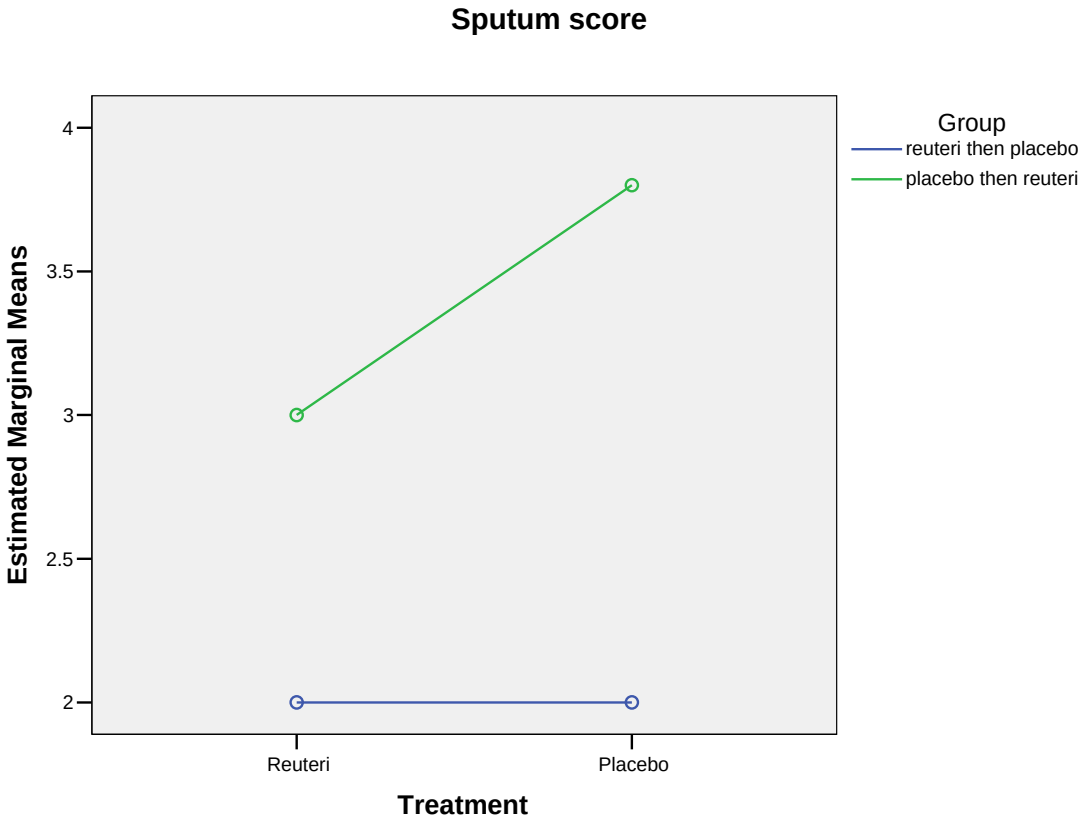

Figure 17 Profile plot of mean sputum score per treatment group

4.8 SYMPTOMS

Of the 16 participants only 8 subjects filled in the symptom diaries for both periods. Fever, runny nose, sore throat and ear ache where grouped together as they are similar conditions. The total number of symptoms was calculated on and off the probiotic (Table 32). There was no significant difference in the incidence of coughing, wheezing, thrush, diarrhea, constipation, vomiting and stomach ache when on or off the probiotic. There was a significantly higher mean number of episodes of fever, runny nose, sore throat and ear aches (p=0.016) while on probiotic (Table 32).

Table 32 Mean episodes of symptoms

Mean episodes of symptoms			**Probiotic**	**Placebo**
All Symptoms	Descriptive stats	Mean (n=11)	128.27	156.18
		SD	117.73	148.33
		Min	16	0
		Max	299	499
	Paired t-test (n=8)	Mean (n=8)	157.38	127.63
		SD	126.75	112.31
		Std. error mean	44.81	39.71
		p value (2-tailed)	**0.148**	
Mean episodes of symptoms			**Probiotic**	**Placebo**
Fever, runny nose, sore throat & ear ache	Descriptive stats	Mean (n=11)	23.73	19.91
		SD	20.39	23.91
		Min	0	0
		Max	66	82
	Paired t-test (n=8)	Mean (n=8)	28.00	10.38
		SD	21.94	11.17
		Std. error mean	7.76	3.95
		p value (2-tailed)	**0.016**	
Mean episodes of symptoms			**Probiotic**	**Placebo**
Wheeze	Descriptive stats	Mean (n=11)	14.27	30.64
		SD	31.11	46.69
		Min	0	0
		Max	105	159
	Paired t-test (n=8)	Mean (n=8)	17.75	15.25
		SD	36.30	20.79
		Std. error mean	12.94	7.35
		p value (2-tailed)	**0.776**	

Table 32 Mean episodes of symptoms continued

Mean episodes of symptoms			**Probiotic**	**Placebo**
Cough	Descriptive stats	Mean (n=11)	62.09	83.36
		SD	57.84	90.69
		Min	4	0
		Max	168	255
	Paired t-test (n=8)	Mean (n=8)	74.88	72.75
		SD	63.19	82.89
		Std. error mean	22.34	29.31
		p value (2-tailed)	**0.888**	
Mean episodes of symptoms			**Probiotic**	**Placebo**
Thrush	Descriptive stats	Mean (n=11)	4	2.73
		SD	12.62	8.08
		Min	0	0
		Max	42	27
	Paired t-test (n=8)	Mean (n=8)	5.50	3.63
		SD	14.77	9.47
		Std. error mean	5.22	3.35
		p value (2-tailed)	**0.360**	
Mean episodes of symptoms			**Probiotic**	**Placebo**
Diarrhea	Descriptive stats	Mean (n=11)	2.18	2.18
		SD	3.92	5.08
		Min	0	0
		Max	10	17
	Paired t-test (n=8)	Mean (n=8)	1.75	2.63
		SD	3.41	5.98
		Std. error mean	1.21	2.11
		p value (2-tailed)	**0.682**	
Mean episodes of symptoms			**Probiotic**	**Placebo**
Constipation	Descriptive stats	Mean (n=11)	0.45	0.18
		SD	1.51	0.60
		Min	0	0
		Max	5	2
	Paired t-test (n=8)	Mean (n=8)	0.63	0.25
		SD	1.77	0.71
		Std. error mean	0.63	0.25
		p value (2-tailed)	**0.351**	

Table 32 Mean episodes of symptoms continued

Mean episodes of symptoms			**Probiotic**	**Placebo**
Vomiting	Descriptive stats	Mean (n=11)	1.55	0.3 (n=10)
		SD	2.21	0.95
		Min	0	0
		Max	7	3
	Paired t-test (n=8)	Mean (n=8)	1.88	0.00
		SD	2.48	0.00
		Std. error mean	0.88	0.00
		p value (2-tailed)	**0.069**	
Mean episodes of symptoms			**Probiotic**	**Placebo**
Stomach ache	Descriptive stats	Mean (n=11)	20.00	18.60 (n=10)
		SD	33.49	31.15
		Min	0	0
		Max	92	89
	Paired t-test (n=8)	Mean (n=8)	27.00	22.75
		SD	37.35	33.89
		Std. error mean	13.21	11.98
		p value (2-tailed)	**0.372**	

4.9 FOOD FREQUENCY QUESTIONAIRE REGUARDING PRE AND PROBIOTIC USE

Forty four percent of the CF patients did not take any food supplements. Of the 56 % who did take a food supplement, the most commonly used was Nutren Activ (25%), Nesquik (12.5%), Ensure (6.25%) and others (12.5%). How often they were used ranged from once or twice a week (n=1), once a day (n=4), twice a day (n=2) and three times a day (n=1) and other (n=1). When mixing the supplements, 5 made it up according to directions; one made it weaker and 3 made it stronger than directed. Nutren Activ was the only commercial product being used that currently contains a prebiotic.

In an attempt to ensure that the patients were not receiving additional probiotics from other sources they were asked about their consumption of yoghurt and other products that may contain a probiotic. Yoghurt was consumed by 81% of which 25% were brand specific. These brands included Danone, Nutriday and Fairfields. Frequency of use ranged from once in 2 months (n=1), once or twice a month (n=2), once or twice a week

(n=7) or once a day (n=3). None of the brands mentioned contained a live culture with a known effect.

4.10 SUMMARY OF RESULTS

Table 33 shows the summary of the anthropometric data for the period prior to starting probiotic (while off probiotic) and while on the probiotic. While off probiotic there was more weight gained, a better increase in %EWFA, Z score (weight for age) Z score (height for age), % EHFA, %EWFH, BMI for ≤ 20 years and BMI Z score. % Expected weight for age and height were both better on probiotic. All the measurement were non significant. Table 34 shows the summary of all other results during the probiotic period and off probiotic period.

Table 33 Summary of changes in anthropometry during period prior to probiotic consumption and during probiotic consumption.

Change over 6 months* in	**Off probiotic (6 month period)**	**On probiotic (6 month period)**	**p value**
Weight (kg)	+ 2 kg	+ 1.2 kg	0.304
Z score (weight-for-age) for patients ≤ 20 years	+ 0.03	- 0.05	0.742
% Expected weight for age for patients ≤ 20 years	+ 0.4 %	+ 2.0 %	0.765
Height (cm)	+1.93cm	+2.03cm	0.838
Z score (height-for-age) for patients ≤ 20 years	+ 0.03	- 0.02	0.783
% Expected height for age for patients ≤ 20 years of age	+ 0.8 %	+ 0.3 %	0.254
% Expected weight for height for patients ≤ 20 years of age	+ 2.2 %	+ 0.3 %	0.448
Body Mass Index of adults (kg/m^2) (≥ 20 years)	0.6 kg/m^2	-0.1 kg/m^2	0.411
Body Mass Index of children (kg/m^2) (for ≤ 20 year olds)	0.4 kg/m^2	0.02 kg/m^2	0.651
Z score (Body Mass Index for age) of children (for ≤ 20 year olds)	+ 0.09	- 0.09	0.272

* Changes are for the whole group unless otherwise indicated

The number of lung infections was less off probiotic and less number of days on antibiotics. The FEV_1 and FVC, a lower microbiological sputum score and less episodes of all symptoms seemed to improve while on the probiotic. Again all measurements were non significant (Table 34).

Table 34 Summary of changes in lung infections, function, sputum and symptoms while on and off probiotic

	Off Probiotic	**On Probiotic**	**p value**
Number of lung infections	28	38	0.134
Duration on antibiotic (days)	1488	1828	
Forced expiratory volume in one second (% change)	-65	30	0.079
Forced vital capacity (% change)	-60	41	0.094
Change in sputum score	12	-5	0.453
Mean episodes of all symptoms	156.18	128.27	0.148
Fever, runny nose, sore throat, & ear ache	19.91	23.73	0.016
Wheeze	30.64	14.27	0.776
Cough	83.36	62.09	0.888
Thrush	2.73	4	0.360
Diarrhoea	2.18	2.18	0.682
Constipation	0.18	0.45	0.351
Vomiting	0.3	1.55	0.069
Stomach ache	18.60	20.00	0.372

Table 11 Weights by age group six months before start of probiotic, start of probiotic and end of probiotic

Age group	Weight (kg) 6 months before probiotic	Start of probiotic weight (kg)	End of probiotic weight (kg)	p value from start to end of study	Change in weight 6 months on placebo	Change in weight 6 months on probiotic	p value for placebo vs probiotic
6 to 9.99 years (n=2)	Mean 21.7	Mean 22.9	Mean 23.9	0.170	Mean 1.2	Mean 0.6	0.205
	SD 0.99	SD 0.99	SD 0.14		SD 0.00	SD 0.28	
	Range 21.0 – 22.4	Range 22.2 – 23.6	Range 23.8 – 24.0		Range 1.2- 1.2	Range 0.4 – 0.8	
10 to 12.99 years (n=2)	Mean 29.9	Mean 33.4	Mean 35.9	0.300	Mean 3.5	Mean 2.5	0.742
	SD 5.81	SD 6.22	SD 10.11		SD 0.42	SD 3.89	
	Range 25.8 – 34.0	Range 29.0 – 37.8	Range 28.7 – 43.0		Range 3.2 – 3.8	Range -0.3 – 5.2	
13 to 19.99 years	Mean 50.1	Mean 51.3	Mean 53.7	0.087	Mean 1.2	Mean 2.4	0.320
	SD 19.37	SD 16.83	SD 15.96		SD 2.88	SD 3.07	
	Range 30.0 – 89.0	Range 35.0 – 85.4	Range 37.3 – 84.0		Range -3.6 – 5.0	Range -1.4 – 8.7	
	n=8	n=8	n=6				
≥20 years	Mean 55.3	Mean 58.4	Mean 56.75	0.506	Mean 3.3	Mean -1.85	0.006
	SD 7.82	SD 8.61	SD 8.45		SD 2.62	SD 1.39	
	Range 43.9 – 61.8	Range 46.5 – 65.0	Range 45.9 – 64.0		Range 0.30 -6.70	Range -3.70 - -0.5	
	n=4	n=4	n=6				
Group as a whole (n=16)	**Mean 45.3**	**Mean 47.3**	**Mean 48.5**	**0.010**	**Mean 2.0**	**Mean 1.2**	**0.304**
	SD 18.28	SD 17.23	SD 16.57		SD 2.53	SD 3.04	
	Range 21.0 – 89.0	Range 22.20 – 85.4	Range 23.80 – 84.0		Range -3.60 – 6.7	Range -3.7 – 8.7	

Table 13 Weights for males by age group six months before start of probiotic, start of probiotic and end of probiotic.

Age group	Weight (kg) six months before probiotic	Start of probiotic weight (kg)	End of probiotic weight (kg)	p value from start to end of study	Change in weight 6 months on placebo	Change in weight 6 months on probiotic	p value for placebo vs probiotic
6 to 9.99 years (n=1*)	Mean 21.0	Mean 22.2	Mean 23.8		Mean 1.2	Mean 0.8	
10 to 12.99 years (n=1*)	Mean 25.8	Mean 29.0	Mean 28.7		Mean 3.2	Mean -0.3	
13 to 19.99 years	Mean 55.5	Mean 56.4	Mean 59.8	0.301	Mean 0.9	Mean 3.3	0.131
	SD 23.71	SD 21.07	SD 18.91		SD 3.04	SD 4.14	
	Range 33.2 – 89.0	Range 35.0 – 85.4	Range 37.9 – 84.0		Range -3.6 – 2.9	Range -1.4 – 8.7	
	n=4	n=4	n=2				
≥20 years	Mean 59.1	Mean 62.5	Mean 60.4	0.680	Mean 3.4	Mean -2.1	0.027
	SD 2.33	SD 3.91	SD 5.31		SD 3.4	SD -2.1	
	Range 57.7 – 61.8	Range 58.0 – 65.0	Range 54.3 – 64.0		Range 0.3 – 6.70	Range -3.7 - -0.5	
	n=3	n=3	n=5				
Groups as a whole (n=9)	**Mean 49.6**	**Mean 51.6**	**Mean 52.4**	**0.114**	**Mean 2.0**	**Mean 0.8**	**0.412**
	SD 20.89	SD 19.95	SD 19.11		SD 2.76	SD 3.68	
	Range 21.0 – 89.0	Range 22.2 – 85.4	Range 23.8 – 84.0		Range -3.60 – 6.7	Range -3.7 – 8.7	

*n=1 significance could not be calculated

Table 14 Weights for females by age group six months before probiotic, start of probiotic and end of probiotic

Age group	Weight (kg) 6 months before probiotic	Start of probiotic weight (kg)	End probiotic weight (kg)	p value from start to end of study	Change in weight 6 months on placebo	Change in weight 6 months on probiotic	p value for placebo vs probiotic
6 to 9.99 years (n=1 *)	Mean 22.4	Mean 23.6	Mean 24.0		Mean 1.2	Mean 0.4	
10 to 12.99 years (n=1*)	Mean 34.0	Mean 37.8	Mean 43.0		Mean 3.8	Mean 5.2	
13 to 19.99 years (n=4)	Mean 44.6	Mean 46.2	Mean 47.6	0.200	Mean 1.6	Mean 1.4	0.921
	SD 15.30	SD 12.20	SD 11.73		SD 3.14	SD 1.54	
	Range 30.0 – 64.6	Range 35.0 – 61.8	Range 37.3 – 63.6		Range -2.6 – 5.0	Range -0.8 – 2.6	
≥ 20 years (n=1*)	Mean 43.9	Mean 47.0	Mean 45.9		Mean 3.1	Mean -1.1	
Group as a whole (n=7)	**Mean 39.8**	**Mean 41.8**	**Mean 43.3**	**0.041**	Mean 2.0	Mean 1.5	**0.592**
	SD 13.83	SD 12.19	SD 12.02		SD 2.43	SD 2.19	
	Range 22.4 - 64.6	Range 23.6 – 61.8	Range 24.0 -63.6		Range -2.6 - 5	Range -1.1 – 5.2	

*n=1 significance could not be calculated

Table 18 Heights by age group six months before start of probiotic, start of probiotic and end of probiotic

Age group	Height (cm) 6 months before probiotic	Start of probiotic height (cm)	End of probiotic height (cm)	p value from start to end of study	Change in height 6 months on placebo	Change in height 6 months on probiotic	p value for placebo vs probiotic
6 to 9.99 years (n=2)	Mean 120.50	Mean 123.45	Mean 126.50	Std error of diff is 0	Mean 2.95	Mean 3.05	0.795
	SD 4.95	SD 4.74	SD 4.95		SD 0.21	SD 0.21	
	Range 117.00 – 124.00	Range120.10 – 126.80	Range 123.00 – 130.00		Range 2.8 – 3.10	Range 2.90 – 3.20	
10 to 12.99 years (n=2)	Mean 143.20	Mean 146.75	Mean 149.93	0.249	Mean 3.55	Mean 3.2	0.897
	SD 6.08	SD 9.54	SD 10.00		SD 3.46	SD 0.42	
	Range 138.90 – 147.50	Range 140.00 – 153.50	Range 142.90 – 157.00		Range 1.10 – 6.0	Range 2.9 – 3.5	
13 to 19.99 years	Mean 159.91	Mean 162.03	Mean 164.42	0.006	Mean 2.13	Mean 2.45	0.704
	SD 14.78	SD 14.18	SD 13.00		SD 1.96	SD 2.07	
	Range 135.20 – 182.00	Range 139.00 – 182.30	Range 143.90 – 182.30		Range 0 – 4.9	Range 0 – 4.9	
	n=8	n=8	n=6				
≥ 20 years	Mean 173.20	Mean 173.41	Mean 173.50	0.190	Mean 0.23	Mean 0.08	0.476
	SD 5.95	SD 6.09	SD 6.10		SD 0.33	SD 0.15	
	Range 164.50 – 178.00	Range 164.50 – 178.00	Range 164.50 – 178.00		Range 0 – 0.7	Range 0 – 0.3	
	n=4	n=4	n=6				
Group as a whole (n=16)	**Mean 156.22**	**Mean 158.14**	**Mean 160.17**	**0.001**	**Mean 1.93**	**Mean 2.03**	**0.838**
	SD 19.75	SD 18.93	SD 17.81		SD 1.98	SD 1.86	
	Range 117.00 – 182.00	Range 120.10 – 182.30	Range 123.05 – 182.30		Range 0 – 6.0	Range 0 – 4.9	

Table 20 Height for males in age groups six months before start of probiotic, start of probiotic and end of probiotic

Age group	Height (cm) 6 months before start of probiotic	Start of probiotic height (cm)	End of probiotic height (cm)	p value from start to end of study	Change in height 6 months on placebo	Change in height 6 months on probiotic	p value for placebo vs probiotic
6 to 9.99 years (n=1*)	Mean 117.00	Mean 120.10	Mean 123.00		Mean 3.10	Mean 2.90	
10 to 12.99 years (n=1*)	Mean 138.90	Mean 140.00	Mean 142.90		Mean 1.10	Mean2.90	
13 to 19.99 years	Mean 166.03	Mean 169.01	Mean 171.23	0.058	Mean 3.00	Mean 2.23	0.430
	SD 15.33	SD 14.58	SD 13.07		SD 1.93	SD 1.77	
	Range 146.60 – 182.00	Range 150.00 – 182.30	Range 153.70– 182.30		Range 0 – 4.9	Range 0 – 3.7	
	n=4	n=4	n=2				
≥ 20 years	Mean 176.10	Mean 176.40	Mean 176.50	0.225	Mean 0.30	Mean 0.10	0.510
	SD 1.65	SD 1.44	SD 1.320		SD 0.36	SD 0.17	
	Range 175.00 – 178.00	Range 175.20 – 178.00	Range 175.50 – 178.00		Range 0 – 0.7	Range 0 – 0.3	
	n=3	n=3	n=5				
All males (n=9)	**Mean 160.92**	**Mean 162.82**	**Mean 164.47**	**0.010**	Mean 1.90	Mean 1.66	**0.616**
	SD 22.14	SD 21.49	SD 20.37		SD 1.81	SD 1.62	
	Range 117.00 - 182.00	Range 120.10 – 182.30	Range 123.00 – 182.30		Range 0 - 4.9	Range 0 – 3.7	

*n=1 significance could not be calculated

Table 21 Height for females in age groups six months before start of probiotic, start of probiotic and end of probiotic

Age group	Height (cm) 6 months before probiotic	Start of probiotic height (cm)	End of probiotic height (cm)	p value from start to end of study	Change in height 6 months on placebo	Change in height 6 months on probiotic	p value for placebo vs probiotic
6 to 9.99 years (n=1*)	Mean 124.00	Mean 126.80	Mean 130.00		Mean 2.80	Mean 3.20	
10 to 12.99 years (n=1*)	Mean 147.50	Mean 153.50	Mean 157.00		Mean 6.00	Mean 3.50	
13 to 19.99 years (n=4)	Mean 153.80	Mean 155.05	Mean 157.73	0.128	Mean 1.25	Mean 2.68	0.343
	SD 13.24	SD 11.54	SD 10.11		SD 1.79	SD 2.59	
	Range 135.20 – 166.50	Range 139.00 – 166.50	Range 143.90 – 167.40		Range 0 – 3.8	Range 0 – 4.9	
≥ 20 years (n=1*)	Mean 164.50	Mean 164.45	Mean 164.50		Mean 0	Mean 0	
All females (n=7)	**Mean 150.17**	**Mean 152.14**	**Mean 154.63**	**0.022**	Mean 1.97	Mean 2.49	**0.577**
	SD 15.68	SD 14.31	SD 13.26		SD 2.33	SD 2.16	
	Range 124.00 - 166.50	Range 126.80 – 166.50	Range 130.00 - 167.40		Range 0 – 6.0	Range 0 – 4.9	

*n=1 significance could not be calculated

Table 35 Mid Arm Circumference and Triceps Skinfold Thickness measurements by age groups at the start and end of the study

Age group	**Mean MAC at start of the study**	**Mean MAC at end of the study**	**p value from start to end of study**	**Mean TSF at start of the study**	**Mean TSF at end of the study**	**p value From start to end of study**
6 to 9.99 years (n=2)	Mean 17.6	Mean 18.2	0.170	Mean 6.8	Mean 8.1	0.344
	SD 0.7	SD 0.49		SD 1.59	SD 2.65	
	Range 17.1 – 18.1	Range 17.8 – 18.5		Range 5.8 – 8.0	Range 6.3 – 10.0	
10 to 12.99 years (n=2)	Mean 18.3	Mean 20.1	0.317	Mean 7.1	Mean 7.9	0.742
	SD 1.8	SD 3.1		SD 0.53	SD 3.0	
	Range 17.0 – 19.6	Range 17.8 – 22.3		Range 6.8 – 7.5	Range 5.8 – 10.0	
13 to 19.99 years	Mean 23.6	Mean 24.8	0.008	Mean 10.2	Mean 9.8	0.321
	SD 4.7	SD 4.2		SD 4.1	SD 4.9	
	Range 18.5 – 31.7	Range 20.0 – 31.5		Range 6.3 – 19.5	Range 4.0 – 20.5	
	n=8	n=6		n=8	n=6	
≥ 20 years	Mean 25.1	Mean 24.6	0.398	Mean 6.4	Mean 4.7	0.239
	SD 2.6	SD 1.6		SD 4.3	SD 1.9	
	Range 21.4 – 27.4	Range 22.3 – 25.1		Range 3.5 – 12.5	Range 3.3 – 7.5	
	n=4	n=6		n=4	n=6	
Group as a whole (n=16)	Mean **22.6**	Mean **23.3**	0.027	Mean **8.44**	Mean **8.06**	0.405
	SD 4.5	SD 4.0		SD 3.8	SD 4.2	
	Range 17.0 -31.7	Range 17.8 – 31.5		Range 3.5 – 19.5	Range 3.3 – 20.5	

Table 36 Mid Arm Circumference and Triceps Skinfold Thickness measurements for males by age groups at the start and end of the study

Age group	Mean MAC at start the study	Mean MAC at end of the study	p value from start to end of study	Mean TSF at start the study	Mean TSF at end of the study	p value from start to end of study
6 to 9.99 years (n=1*)	Mean 17.1	Mean 17.8		Mean 5.8	Mean 6.3	
10 to 12.99 years (n=1*)	Mean 17.0	Mean 17.8		Mean 6.8	Mean 5.8	
13 to 19.99 years	Mean 24.51	Mean 25.78	0.106	Mean 8.0	Mean 7.0	0.144
	SD 5.2	SD 4.6		SD 2.0	SD 2.4	
	Range 19.5 – 31.7	Range 21.0 – 31.5		Range 6.3 – 11.0	Range 4.0 – 9.8	
	n=4	n=2		N=4	n=2	
≥ 20 years	Mean 26.36	Mean 25.33	0.136	Mean 4.33	Mean 3.8	0.336
	SD 1.1	SD 0.6		SD 1.4	SD 0.6	
	Range 25.2 – 27.4	Range 24.9 – 26.1		Range 3.5 – 6.0	Range 3.3 – 4.5	
	n=3	n=6		N=3	n=5	
Group as a whole (n=9)	**Mean 23.5**	**Mean 23.9**	**0.408**	**Mean 6.4**	**Mean 5.7**	**0.047**
	SD 4.9	SD 4.4		SD 2.2	SD 2.1	
	Range 17.0 -31.7	Range 17.8 – 31.5		Range 3.5 – 11.0	Range 3.3 – 9.8	

*n=1 significance could not be calculated

Table 37 Mid Arm Circumference and Triceps Skinfold Thickness measurements for females by age groups at the start and end of the study

Age group	**Mean MAC at start the study**	**Mean MAC at end of the study**	**p value from start to end of study**	**Mean TSF at start the study**	**Mean TSF at end of the study**	**p value form start to end of study**
6 to 9.99 years (n=1*)	Mean 18.1	Mean 18.5		Mean 8.0	Mean 10.0	
10 to 12.99 years (n=1*)	Mean 19.6	Mean 22.3		Mean 7.5	Mean 10.0	
13 to 19.99 years (n=4)	Mean 22.75	Mean 23.83	0.083	Mean 12.4	Mean 12.6	0.689
	SD 4.75	SD 4.2		SD 4.8	SD 5.4	
	Range 18.5 – 28.5	Range 20.0 – 29.1		Range 9.0 – 19.5	Range 8.0 – 20.5	
≥ 20 years (n=1*)	Mean 21.4	Mean 22.3		Mean 12.5	Mean 7.5	
Group s a whole (n=7)	**Mean 21.4**	**Mean 22.6**	**0.015**	**Mean 11.1**	**Mean 11.1**	**0.971**
	SD 3.9	SD 3.6		SD 4.1	SD 4.3	
	Range 18.1 -28.5	Range 18.5 – 29.1		Range 7.5 – 19.5	Range 7.5– 20.5	

*n=1 significance could not be calculated

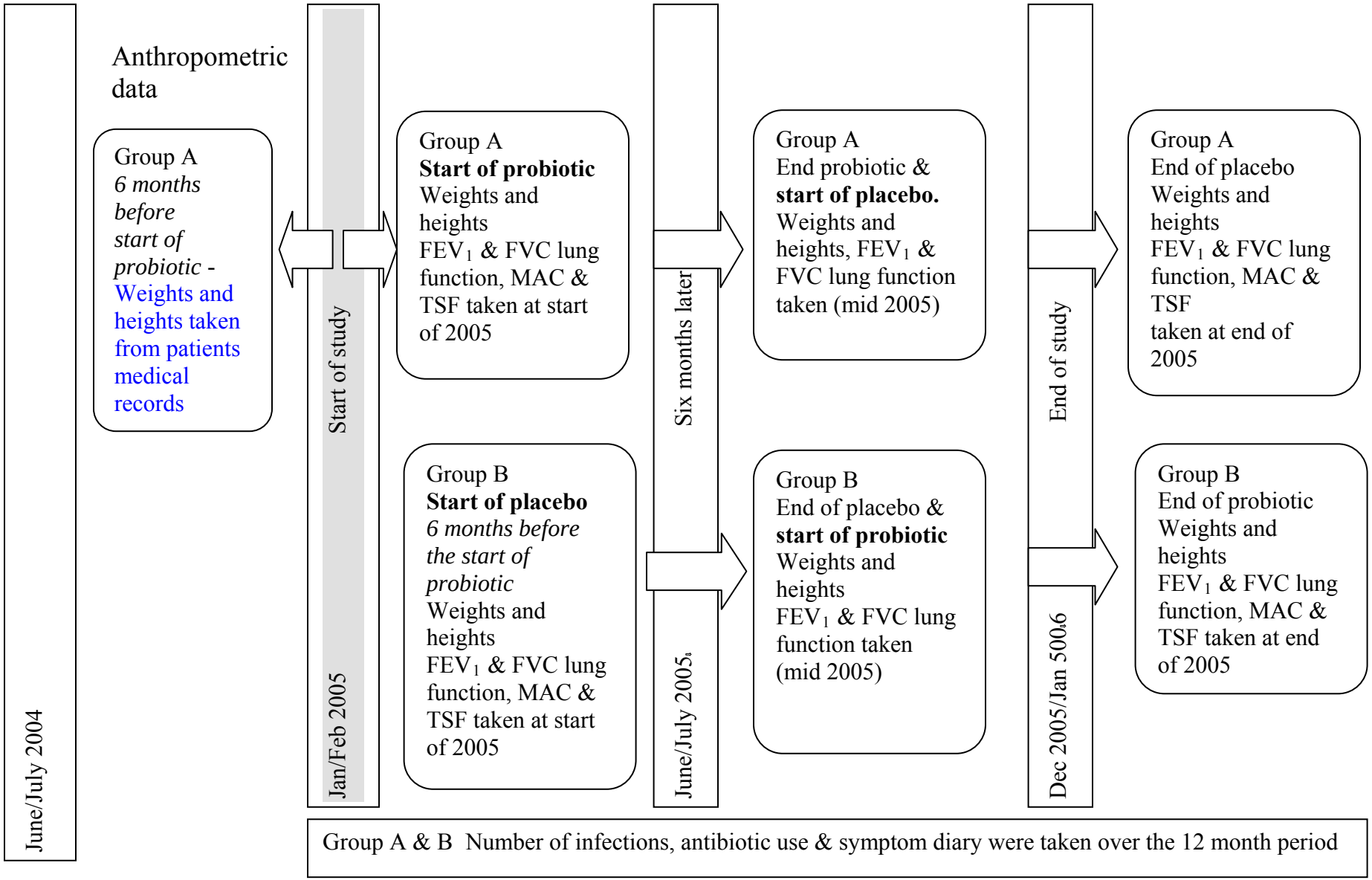

Figure 1 Time line showing different starts for the study for different measurements

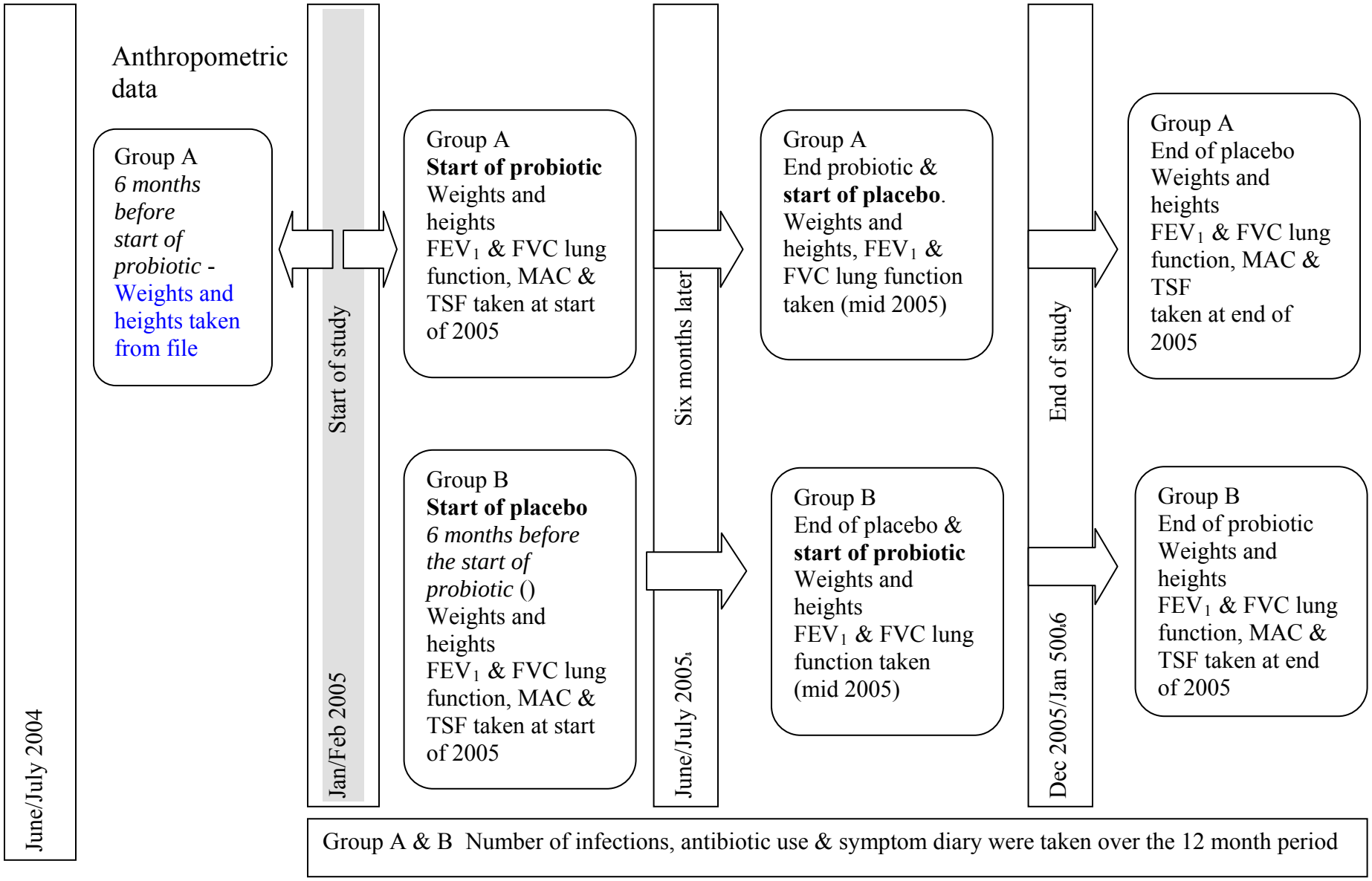

Figure 8 Time line showing different starts for the study for different measurements

Table 16 Z scores weight for age for those 20 years and under by gender and age

Z scores for weight for age	Z score 6 months before probiotic	Z score at start of probiotic	Z score at end of probiotic	p value for placebo vs probiotic	Changes in Z score 6 months on placebo	Changes in Z score 6 months on probiotic
≤ 20 year olds	**Mean -1.09**	**Mean -1.08**	**Mean -1.13**	**0.742**	**+0.01**	**-0.05**
	SD 1.28	SD 1.07	SD 0.98			
	Range -3.56 to 1.36	Range -3.05 to 1.09	Range -3.63 to 0.62			
	n=12	n=12	n=10			
Males	Mean -1.31	Mean -1.24	Mean -1.37	0.119	+0.07	-0.13
	SD 1.38	SD 1.16	SD 0.91			
	Range -3.56 to 1.36	Range -3.05 to 1.09	Range -3.63 to -0.68			
	n=6	n=6	n=4			
Females (n=6)	Mean -0.86	Mean -0.87	Mean -0.82	0.783	+0.01	+0.05
	SD 1.19	SD 1.00	SD 1.04			
	Range -2.88 to 0.80	Range -2.03 to 0.55	Range -1.93 to 0.62			
Non-adolescents (n=4)	Median -0.99	Median -0.95	Median -0.89	0.951	+0.04	+0.06
	SD 0.65	SD 0.78	SD 1.08			
	IQR -1.63 to -0.33	IQR -1.69 to -0.13	IQR -1.88 to 0.26			
Adolescents	Median -0.94	Median -1.76	Median -1.58	0.795	-0.82	+0.18
	SD 1.55	SD 1.26	SD 1.38			
	IQR -2.88 to -.020	IQR -2.03 to -0.02	IQR -1.93 to -0.24			
	n=8	n=8	n=6			

The interquartile range (IQR) is the distance between the 75th percentile and the 25th percentile. The IQR is essentially the range of the middle 50% of the data. Because it uses the middle 50%, the IQR is not affected by outliers or extreme values.

Table 17 Percentage expected weight for age for those less than and equal to 20 years by gender and age

Percentage expected weight for age	Percentage expected weight for age 6 months before probiotic	Percentage expected weight for age at start of probiotic	Percentage expected weight for age at end of probiotic	p value for placebo vs probiotic	Changes 6 months on placebo	Changes 6 months on probiotic
≤ 20 year olds	**Mean 82.6**	**Mean 83.0**	**Mean 85.0**	**0.765**	**+0.4**	**+2.0**
	SD 21.32	SD 18.85	SD 18.15			
	Range 57.3 – 127.0	Range 60.0 – 121.0	Range 57.0 – 119.0			
	n=12	n=10	n=10			
Males	Mean 81.4	Mean 80.8	Mean 83.3	0.492	-0.6	+2.5
	SD 25.00	SD 22.76	SD 21.04			
	Range 57.3 – 127.0	Range 60.0 – 121.0	Range 57.0 -119.0			
	n=6	n=4	n=4			
Females (n=6)	Mean 83.7	Mean 85.3	Mean 86.7	0.334	+1.6	+1.4
	SD 19.27	SD 15.85	SD 16.61			
	Range 61.0 – 115.0	Range 69.0 – 109.0	Range 69.0 – 112.0			
Non adolescents (n=4)	Mean 82.4	Mean 83.3	Mean 83.7	0.848	+0.9	+0.4
	SD 8.79	SD 10.01	SD 14.05			
	Range 72.3 – 88.0	Range 72.0 - 91.0	Range 69.0 – 97.0			
Adolescents	Mean 78.2	Mean 79.0	Mean 80.7	0.185	+0.8	+1.7
	SD 20.62	SD 18.49	SD 17.99			
	Range 57.3 – 115.0	Range 60.0 - 109.0	Range 57.0 – 112.0			
	n=8	n=6	n=6			

Table 23 Z scores for height for age for those 20 years and under and by gender and age

Z scores for height for age	Z score 6 months before probiotic	Z score at start of probiotic	Z score at end of probiotic	p value for placebo vs probiotic	Changes in Z score 6 months on placebo	Changes in Z score 6 months on probiotic
≤ 20 year olds	**Mean -0.86**	**Mean -0.83**	**Mean -0.85**	**0.783**	**+0.03**	**-0.02**
	SD 1.24	SD 1.14	SD 0.99			
	Range -3.39 to 1.75	Range -3.12 to 0.94	Range -2.62 to 0.99			
	n=12	n=12	n=10			
Males	Mean -0.91	Mean -0.94	Mean -1.09	0.989	-0.03	-0.15
	SD 1.21	SD 1.02	SD 0.73			
	Range -2.21 to 1.75	Range -2.42 to 0.77	Range -2.50 to 0.36			
	n=6	n=6	n=4			
Females (n= 6)	Mean -0.80	Mean -0.69	Mean -0.54	0.460	+0.11	+0.15
	SD 1.38	SD 1.34	SD 1.24			
	Range -3.39 to 0.68	Range -3.12 to 0.94	Range -2.62 to 0.99			
Non-adolescents (n=4)	Median -1.35	Median -1.37	Median -1.25	0.860	-0.02	+0.12
	SD 1.22	SD 1.36	SD 1.29			
	IQR -1.52 to 0.68	IQR -1.26 to 0.99	IQR -1.29 to 1.02			
Adolescents	Median -0.52	Median -0.55	Median -0.68	0.827	-0.03	-0.13
	SD 1.39	SD 1.43	SD 1.35			
	IQR -2.21 to 0.12	IQR -2.42 to 0.44	IQR -2.50 to -0.36			
	n=8	n=8	n=6			

The interquartile range (IQR) is the distance between the 75th percentile and the 25th percentile. The IQR is essentially the range of the middle 50% of the data. Because it uses the middle 50%, the IQR is not affected by outliers or extreme values.

Table 24 Percentage expected height for age of those 20 years and under and by gender and age

Percentage expected height for age	Percentage expected height for age 6 months before probiotic	Percentage expected height for age at start of probiotic	Percentage expected height for age at end of probiotic	p value for placebo vs probiotic	Changes 6 months on placebo	Changes 6 months on probiotic
≤ 20 year olds	**Mean 94.9**	**Mean 95.7**	**Mean 96.0**	**0.254**	**0.8**	**0.3**
	SD 5.78	SD 6.03	SD 5.87			
	Range 84.5 - 103.0	Range 86.0 – 103.0	Range 86.0 – 103.0			
	n=12	n=12	n=10			
Males	Mean 94.3	Mean 95.0	Mean 95.0	0.286	0.7	0
	SD 5.64	SD 6.19	SD 6.63			
	Range 89.0 – 103.0	Range 88.0 – 103.0	Range 86.0 – 103.0			
	n=6	n=6	n=4			
Females (n=6)	Mean 95.8	Mean 96.5	Mean 97.0	0.606	0.7	0.5
	SD 6.39	SD 6.35	SD 5.44			
	Range 84.5 - 102.0	Range 86.0 – 102.0	Range 89.0 – 102.0			
Non-adolescents (n=4)	Mean 95.33	Mean 95.67	Mean 96.00	Std error of diff is 0	0.3	0.4
	SD 4.16	SD 4.73	SD 5.29			
	Range 92.0 - 100.0	Range92.0 – 101.0	Range 92.0 - 102.0			
Adolescents	Mean 94.01	Mean 95.14	Mean 95.14	0.592	1.1	0
	SD 6.51	SD 6.99	SD 6.69			
	Range 84.5 – 10.2.0	Range 86.0 – 10.2.0	Range 86.0 – 102.0			
	n=8	n=8	n=6			

CHAPTER 5: DISCUSSION

The primary aim of the study was to determine whether supplementation with a probiotic (*L. reuteri*) could be shown to stimulate the immune system, possibly reducing the incidence and shortening the duration of lung infections while maintaining or preventing deterioration in lung function in CF patients attending the CF clinics in KZN Durban. The secondary aim of the study was to compare the nutritional status of CF patients attending CF clinics in KZN Durban with CF patients attending CF clinics in Cape Town as compared to the published data by Westwood & Saitowitz (1999). It was hypothesized that if the probiotic could stimulate the immune system and reduce the incidence and shorten the duration of lung infections this in turn would improve their anthropometric measurements. The improved nutritional status could enhance resistance to lung infections interrupting the malnutrition/infection cycle and reducing mortality.

5.1 STUDY COMPLIANCE

Compliance was a huge issue whether measured objectively by counting straws or subjectively by the use of the symptom diary. Very few patients took close to the correct number of straws and on average the symptom diaries were not filled in satisfactorily.

In this study it was found that two 14 year olds and one 20 year old were the most compliant with using the straws. This disagrees with the SA studies by Westwood & Saitowitz (1999) and Westwood & Ireland (2000) who showed that compliance was better in the younger child (under 10 years) than in the older CF patient. Anthony *et al* (1999) stated that it was well known that adolescents and young adults are generally poor compliers although this was not the case in this study.

In this study the parents of the more compliant children filled in the symptom diary and ensured that their child took the straws daily. This agreed with Powers *et al* (2002) who noted that compliance in younger patients is directly related to the commitment of the primary care givers and if the mother or care giver filled in the diary more information

was obtained. The Hatakka *et al* (2001) study had the care giver fill in the symptom diary as the subjects were younger (1-6 years). This may lead to better information being collected as an adult care giver is more able to give an objective comment. The study mentioned that they had good compliance but did not specify the compliance rate.

The poor compliance during the study as a whole could be explained by the fact that the probiotic did not offer an immediate benefit to the patient. Dodd & Webb (2000) found that if a treatment offered immediate effects then patients were more compliant. As the probiotic would not be expected to have an instant response and as the adults using the straws had the perception of not really taking any medication this in itself would led to a lower compliance rate. Compliance may have been increased if the adults had been given a probiotic tablet and the straws were used for the younger children only.

Reasons in this study given for non compliance were similar to those found by Abbott & Gee (1998) and fall into the 2 categories of time and health which they found to be the most common reasons for non-compliance amongst CF patients.

Cystic Fibrosis patients need to be compliant with enzyme therapy, daily physiotherapy, nebulisation, and early reporting of infections so that antibiotics therapy can be prescribed to manage the disease. It was assumed for this study that these general requirements would be met by the CF patients and that the CF patients themselves would be interested in using a new product that may help their condition. Apart form regular monitoring of the patients, at the CF clinics, there is no real way of measuring general compliance in these patients. It was found in this study that compliance was half of the required dose in taking the straw and poor regarding the symptom diary. Eating adequately involves frequent snacking during the day, taking additional food supplements and sometimes being fed enterally overnight. Enzymes need to be taken with all meals and snacks. Usually 1 to 4 enzyme capsules per meal/snack is the norm. CF patients at the Addington Clinic take up to 10 capsules per meal. Cystic Fibrosis patients use either singly, or a combination of, oral, inhaled or nebulized drugs to clear their lungs (Dodd & Webb 2000).

It has been demonstrated however that CF patients that attend CF clinics regularly have higher growth patterns and improved respiratory function (Anthony *et al* 1999). Clinic attendance at the Addington & St Augustine's Clinic involves taking the child out of school for the morning or day and their caregivers needing to take a day's leave or the adult CF patient needing to take the day off work to attend the clinic.

In general, the lack of compliance with using the straws meant that the therapeutic dose was seldom taken. In addition only 50% of the symptom diaries contained information that could be used, compromising the quality of the results. This in itself impacted on the rest of the study as it was hoped to show that supplementation with the probiotic would strengthen the immune system and decrease the number and duration of lung infections and in turn would lead to an improvement in nutritional status.

5.2 ANTHROPOMETRY

Because normal growth patterns vary by age and gender in order to compare weight and height changes in addition to using the weights and heights, anthropometric indices have been used in this study.

The use of anthropometric indices is complicated by the fact that there are a number of different anthropometric indices for example %EWFH, BMI and Z scores and reference standards for example British Growth Standards (Lai *et al* 1999) and the National Centre for Health Statistics (NCHS). Therefore in this study a range of anthropometric indices was calculated from the basic data of height, weight and age, to enable comparisons to the published data by Westwood & Saitowitz (1999). Midarm circumference and Triceps skinfold thickness readings were only taken at the start and end of the study. The end readings were then used to compare to the Cape Town data. Other than this the MAC and TSF readings were not used as anthropometric indices in this study. Due to the resulting volume of data some was placed in the Appendices for reference.

5.2.1 Weight

There was a significant average weight gain over the 12 month study period for all subjects. The majority of the weight gained was during the period on placebo and not while on the probiotic which was disappointing.

Over a similar period of time Guarino (2002) found a weight gain of 2.7% on placebo and 8.7% on the probiotic *LGG*. In this Durban study the average weight gain on placebo was 4.4% and on the probiotic 2.5%. The average age of Guarino's subjects was 9 years. In comparison the weight gain for the Durban study for the ≤ 10 year old age group was 5.5% on placebo and only 2.3% on probiotic. In fact in this study the only age group to have gained more weight while on the probiotic was the 13 to 19.99 year olds and because of the small sample size this did not reach significance. In addition those ≥ 20 years actually lost weight while on probiotic although this did not reach significance. The difference in outcome between the studies may be because Guarino's sample size was larger (n=30) resulting in greater statistical power. He also used *LGG* as the probiotic which may be more effective than *L. reuteri* and his subjects may have been more compliant resulting in the full therapeutic dose of the probiotic being taken. Compliance in his study was not discussed

Males (non significantly) and females (significantly) in all age groups gained weight across the 12 month study period. Females gained more weight than males during the whole study period but this was not significant (p=0.377). Although both males and females appeared to gain less weight while on probiotic this did not reach significance possibly due to the small sample size. This contradicts Anthony *et al* (1999) who claimed that males do better than females.

According to the WFA percentiles 50% of the subjects were under the 3rd percentile at the start and 42% at the end of the study[8]. As the SA study done at Red Cross hospital by

[8] End of study was used as this was the most recent data available

Hill *et al* (1988) found that 36% of their CF population were below the 3rd percentile for WFA, the extent of malnutrition experienced at the Durban clinics was higher in comparison.

These results were confirmed by the WFA Z-scores and the %EWFA. Although the clinic patients in the Durban study were gaining weight there was no increase in the WFA Z-scores or %EWFA therefore there was no improvement in nutritional status indicating that the prevalence of malnutrition was not decreasing in this clinic.

In this study there were an equal number of males and females under the 3rd percentile WFA which again contradicts what Anthony *et al* (1999) found in their review article which stated that male CF patients generally do better than their female counterparts.

The prevalence of malnutrition as defined by wasting (%WFH <75%) was higher in the SA CF patients than in the Spanish CF patients as Molina *et al* (2001) claimed that only 20% of the Spanish CF clinic suffered from frank malnutrition. On what basis frank malnutrition was determined was not clear in his article[9]. Westwood & Saitowitz (1999) found that the prevalence of malnutrition using %EWFH was related to age, in that the under 10 year olds were less malnourished than the older subjects. This was not the case in this study as no one group seemed to be at a higher risk.

It was hypothesized that it could be shown that patients on the probiotic would gain substantially more weight than those on the placebo and thus improve their nutritional status which in turn would have a positive effect on lung function and reduce the infection rate. Due to the small numbers and poor compliance this could not be demonstrated.

The hypothesis that there would be a greater weight gain, for the group as a whole, while taking the probiotic, would have to be rejected.

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[9] Frank malnutrition in this study was not defined

5.2.2 Height

As to be expected height in the ≥ 20 year old age group did not increase over the study period as this group should have reached their growth potential. For the group as a whole and in all age groups except the 10 to 12.99 year olds the increase in height was significant while the 10 to12.99 year olds experienced a non significant increase. Again this contradicts Westwood & Saitowitz (1999) who claimed that over 10 year olds were the higher risk group for growth failure.

For the group as a whole and in all age groups except the ≤ 9.99 year olds the increase in height was non significantly greater on the probiotic compared to the period on the placebo. Guarino's (2002) study did not appear to investigate the effect of probiotic on stature.

Both the males and females increased significantly in height across the study. All males except the 10 to12.99 years age group grew less on probiotic versus placebo although this was not significant. In contrast all females non significantly increased in height more while on the probiotic except the 10 to12.99 age group. In general the females appeared to be growing faster than and responding better to the probiotic than the males however this was not significant. This contradicts Anthony *et al* (1999) who claimed that males tended to do better than females.

In 1988 Hill *et al* reported that 24% of the CF patients at the Red Cross hospital were below the 3rd percentile for HFA and 11 years later in the same Red Cross CF clinics in 1999 Westwood & Saitowitz published that 16.2% were below the 5th percentile for HFA. It was suggested that this decrease in the prevalence of stunting could probably be attributed to the development of more effective enzymes, a more liberal high fat diet and other improved therapies (Westwood & Ireland 2000). In 2005 however, 42% of the children in this study in the Durban Clinics were below the 5th percentile for HFA in spite of the advancement in treatment for the CF patient suggesting low level of compliance, as can be seen in this study, regarding treatment regimes, nutritional status has not improve

to the level that would be acceptable. The KZN CF patients were experiencing a greater prevalence of stunting in comparison to the other SA clinics.

Height for age Z-scores were poor and improved marginally for the group as a whole but similarly to the WFA Z-scores the change was non significant indicating that there was no alteration in nutritional status over the study period. Although heights were increasing in both sexes over time the males were not growing as fast as their age dictated as can be seen by the non significant decrease in HFA Z-scores. In contrast the females HFA Z-scores increased non significantly indicating that their height was keeping pace with their age. For all age groups for %EHFA were non significant and followed a similar pattern to the HFA Z-scores confirming that their nutritional status was poor and was not improving.

The hypothesis that all subjects ≤ 19.99 year olds and younger, would improve in height more while taking the probiotic, would have to be rejected.

5.3 COMPARISON OF ANTHROPOMETRIC STATUS OF CYSTIC FIBROSIS PATIENTS IN DURBAN AND CAPE TOWN

Over half of the children in this study in the Durban clinics were underweight for their actual height compared to only one third of the Cape Town clinic. In this study the Durban clinics had more subjects (39%) experiencing underweight and mild malnutrition as shown as %EWFH compared to CT (19%) confirming the weight and height findings that the patients are not optimally nourished and not in proportion for weight for height. The greater prevalence of malnutrition in the Durban clinics was confirmed by the higher number with MAC and TSF readings below the 5^{th} percentiles. In other words the children in the Durban clinics were leaner with low fat stores.

The reasons for the differences between the Durban clinics and the Cape Town clinics were not investigated. The number of patients and age groups of the KZN Durban study were comparable to the CT study published by Westwood & Saitowitz (1999).

As there was no significant difference in the height and weight of the subjects whether on or off probiotics it was felt that the readings at the end of the study would be more recent to compare to the Cape Town 1996 data.

5.4 INCIDENCE AND DURATION OF LUNG INFECTIONS

No significant association was found between taking probiotic or placebo and the incidence of lung infections. Those taking probiotic in the first 6 months of the year showed a slight tendency to having less infections while on probiotic than placebo, while the group who took the placebo first had a similar number of lung infections whether on or off probiotic.

The hypothesis that supplementation with *L. reuteri* would reduce the incidence of lung infections in CF patients would have to be rejected.

More antibiotics were used in the first 6 month period compared to the second period and although the difference was significant it was regardless of whether they were on placebo or probiotic. It is expected that CF get sicker in the winter months but as the study was over 12 months (with winter in the middle) this was not showing the seasonal difference. It would be of interest to see if the study was run for another 12 months whether a similar pattern of antibiotic use was shown.

The use of antibiotics was not related to the use of either probiotic or placebo and there was no apparent effect of the probiotic on the number of infections as determined by the use of antibiotics.

The hypothesis that supplementation with *L. reuteri* would shorten the duration of lung infections in CF patients would have to be rejected.

5.5 LUNG FUNCTION

The results showed that while on probiotic the patients FEV_1 and FVC readings improved although significance could not be reached probably due to the small sample size. These are both direct objective measurements (Dodd & Webb 2000) so may more accurately reflect the effect of the probiotic rather than a subjective measurement such as days on antibiotics. It is encouraging that there was an indication that the probiotic may be effective and if the correct dose was taken and better compliance achieved statistical significant may have be reached.

The hypothesis was that CF patients taking *L. reuteri* would experience an improvement in lung function would have to be rejected.

5.6 SPUTUM ANALYSIS

In the Durban clinics 65.5% of the patients were infected with *P. aeruginosa* over the 12 months. These findings are in accordance with the South African data of Ventre *et al* (2000) who found more than two thirds of their patients at the Groote Schuur Clinic cultured *P. aeruginosa* and from overseas clinics where the infection rate ranged from 25 to 85% (Hart & Winstanley 2002).

The sputum score to measure bacteria was a direct objective measure to monitor the quantity of bacteria in the sputum for changes over time while on probiotic and while on placebo. There was a non significant trend indicating that while on the probiotic the patients had marginally lower mean scores of bacteria in the sputum. As *P. aeruginosa* has a negative impact on life expectancy these findings which showed a tendency towards the probiotic being effective are encouraging.

The hypothesis that CF patients while taking *L. reuteri* would experience a decrease in the count of *P. aeruginosa* (dry and mucoid), *S. aurens* and *B. cepacia* in the sputum would have to be rejected.

5.7 SYMPTOMS

The information from the symptom diaries should be viewed with suspicion. Only eight (50%) were filled in most of which was incomplete and poorly filled in. No significant difference was found in the incidence of coughing, wheezing, thrush, diarrhoea, constipation, vomiting and stomach ache whether on probiotic or on the placebo. There was a significantly higher mean number of episodes of fever, runny nose, sore throat and ear ache while on probiotic according to the limited data extracted from the inaccurately completed symptom diaries. Guarino (2002) found a reduction in stomach ache in 7 out of 9 subjects in his study. It is possible that those who experienced more symptoms and were sicker tended to not complete their symptom diary on both occasions. In addition the symptom diary is a subjective measure of compliance and has been reported by Dodd & Webb (2000) that when patients fill in a symptom diary they often overestimate their responses. This would in fact tie up with the burden of treatment for CF in that they have huge time commitments needed for health care and filling in the symptom dairies increased that burden (Dodd & Webb 2000).

The hypothesis that the mean episodes of symptoms would decrease while on the probiotic would have to be rejected.

5.8 FOOD FREQUENCY QUESTIONNAIRE REGARDING PRE AND PROBIOTIC USE

Generally just over 50% of the subjects used a food supplement from once or twice a week to three times a day. Nutren Activ was consumed the most (25%) and contains the prebiotic fructoligosaccrides in the amount of 8.6g per 100g powder. This amount of prebiotic once or twice week (n=1) to 3 times a day (n=1) was not considered to influence the study as the supplement was consistently taken prior and during the study. Nutren Activ is one of the feeds that are supplied to the CF patients at the clinics.

Yogurt was consumed by 81% of the subjects, 25% are brand specific. Although 62.5% consumed yoghurt at least 1 to 7 times per week none were using a brand containing a known live probiotic. Yogurt was not considered to have had any confounding affect in this study.

No subjects were taking other supplements containing either pre or probiotics so the additional intake of either probiotics and prebiotics was unlikely to influence the study.

5.9 SUMMARY

The study set out to show that supplementation with a probiotic (*L. reuteri)* could stimulate the immune system, possibly reducing the incidence and shortening the duration of lung infections while maintaining or preventing deterioration in lung function. A reduction in the number of lung infections in turn would decrease both nutritional requirements and nutrient losses of the patients with CF while increasing food intake. This would hopefully improve nutritional status, which would enhance resistance to lung infections in turn interrupting the malnutrition/infection cycle. It has been disappointing that because of a small sample size and poor compliance this could not be shown statistically. However as a pilot study a lot of useful information was gained. If the study is to be repeated it would be of interest to increase the dose and improve compliance of taking the probiotic, perhaps include other probiotics as a combination dose and maybe to extend the length of the study.

5.10 STUDY LIMITATIONS

Difficulties in carrying out this study included dealing with patients that already have a huge amount of treatment requirements to maintain their health and find it difficult to add yet another treatment option. The extent of non compliance was unexpected. After the payment was made to the subjects (at 6 months) their seemed to be a renewed awareness and more compliance was noted. As it was the first time any study has been carried out

in this patient group there was not a lot of interest or enthusiasm with some of the medical staff to drive the process. Study limitations have been listed below.

5.10.1 The total number of CF patients attending both the CF clinics in KZN is relatively small and not all patients agreed to participate in the study. Patient numbers were very small at the start of the study with a high drop off rate at the crossover.

5.10.2 Poor compliance was a major problem. Record keeping of the symptom diary was poor as was the use of the straws.

5.10.3 The probiotic and placebo were not identical as the probiotic had a date stamp on the package but the placebo did not. To overcome this, the person randomizing the probiotic/placebo put the straws into a brown paper packet so that the researcher was not aware which was being given out. In addition once the straws were opened one could see the oil stain where the probiotics were located in the straw. There was no oil stain on the placebo straw. If the patients had seen both straws at the same time this difference would have been obvious. This did not happen at the beginning of the trial as the straws were given out each six months. After 6 months once the second batch of straws was issued some of the patients noticed the difference. This meant that after the first 6 months some of the patients had a good idea of whether they were taking placebo or probiotic. At the end of the first 6 months a person not involved in the study was asked to count both the used and unused straws. It was obvious from the totals that the count had been done incorrectly. The researcher then had to recount the straws so breaking blinding. At this stage no record was kept by the researcher as to which patient was taking which straw and she could not actually remember who was on active and who was on placebo.

5.10.4 The study expected that all patients would be productive and be able to produce sputum samples at the start of the study. This did not happen so sputum samples

were taken as close to the beginning, middle and end of the study when the patient was able to produce sputum.

5.10.5 To standardize the sputum results permission was obtained from the state hospital to use their laboratory to process all the sputum. The laboratory staff however were unwilling to process the samples from the private sector and claimed that they were not able to run the few additional sputum samples as they were short staffed. Both the state and the private laboratory were therefore used.

5.10.6 It was assumed that standard laboratory practice would take place. After a few months the state laboratory changed the way that they processed the sputum results for the CF clinic without informing the researcher. They claimed that the new technique would detect the number and type of bacteria in the sputum more accurately.

5.10.7 It was anticipated that all patients would return to the clinic after the first 6 months. This did not happen. Straws and symptom diaries were posted to 3 patients that said that they could not make the clinic. Straws and the symptoms diary were either posted back or collected at the next clinic they attended. This meant that not all the anthropometric measurements were taken at the cross over.

5.10.8 On two occasions an antibiotic was prescribed by the doctor but was not issued by the state pharmacy. On another occasion a generic drug was given. The collation sheets were poorly filled in by the doctors and some data had to be collected retrospectively from the files. Patients who were prescribed antibiotics did not always complete the course.

5.10.9 There was no time period allocated for a wash out between taking the probiotic and placebo or vise versa in the study. The implication of this was that if there was a long term effect from the probiotic it would have had a carry on affect during the following six month period while the subjects were on the placebo.

5.10.10 Repeatability studies regarding anthropometric measurements between the two dieticians should have been carried out.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Compliance in this study was poor resulting in the average patient taking only half or less of the effective therapeutic dose of probiotic. Unlike the other studies, age did not seem to be a key determinant of compliance. What appeared more important was the lack of immediate benefit, the lack of time due to the burden of treatment as well as the perception that the straws were not real medication. If the study were to be repeated, using a more 'medicinal' approach such as probiotic tablets and offering immediate rewards for compliance, would be imperative.

Although all the subjects across the study period showed a significant increase in actual body weight the non significant decrease in WFA percentiles and WFA Z scores confirmed that the amount of weight being gained was not sufficient and that their nutritional status was not improving. As approximately half the clinic was wasted, malnutrition was a real issue in the Durban clinics. A higher percentage of CF patients were wasted in comparison to their Cape Town counterparts indicating that the Durban clinics are not being as effective in their weight management. Contrary to expectations the patients gained significantly more weight while on the placebo indicating that the probiotic may have unexplainably obstructed weight gain. No age group appeared to be more susceptible to malnutrition and females certainly did not do worse than the males and may even have performed slightly better.

Heights showed a similar trend to the weights in that overall their heights increased significantly across the study period. However the slight non significant increase in HFA percentiles and HFA Z scores again demonstrated that their nutritional status was not altering. Similarly to weight almost half the clinic were stunted confirming that malnutrition was a major challenge facing the clinic. The prevalence of stunting was almost three times that reported by the Cape Town clinic indicating that the existence of chronic malnutrition was greater. Again no particular age group was more susceptible

and females appeared to do non significantly better again contraindicating the belief that males are at a lower risk. Although it was non significant their increase in height appeared to be greater while on the probiotic possibly the probiotic was promoting an increase in height at the cost of an increase on weight. If taken at the correct therapeutic dose the increase in height might have reached significance.

The %EWFH, the MAC and TSF readings emphasized the enormity of the problem facing the Durban clinics. It has been well established therefore that malnutrition locally needs to be addressed and that Cape Town is managing the challenge more effectively probably because the clinic is better staffed and runs more frequently.

Findings were encouraging for the effect of the probiotic on the CF lung as *L. reuteri* showed a non significant trend towards the improvement of lung function as objectively measured by FEV_1 and FVC. The subjective measurements of lung infection rate and duration showed a non significant trend in favour of the placebo. However the objective measurement of sputum score again showed a non significant trend in favour of the probiotic. These trends were very interesting as they were noticeable even though the majority of the study sample seldom reached the correct therapeutic dose the compliance was so poor. A higher dose of probiotic might have been more appropriate due to the probability of the CF colonic microflora being seriously disturbed (Walters & Littlewood 1998: Roy *et al* 1979)..

The small sample size in combination with poor compliance lacked the power statistically. With the available evidence therefore it was impossible to conclude with any confidence whether the use of the probiotic was beneficial, had no effect or was harmful.

Alternative effective treatments to antibiotics are desperately needed in the treatment of CF patients. The trends found in this pilot study deserve further investigation using a larger sample size recruited from other CF clinics in SA, a higher dose of probiotic with compliance being encouraged by offering financial or other incentives. It is still hoped

that this could prove to be a novel, safe and affordable alternative to antibiotics for the CF patient.

6.2 RECOMMENDATONS FOR DIETETIC PRACTICE

Dieticians need to educate the CF patients on the dangers of malnutrition and put even more effort into encouraging both weight and height gain so that the prevalence of malnutrition decreases.

Good anthropometric data needs to be regularly collected and monitored and shared with the other clinics in South Africa.

As yet no recommendations can be made about the use of probiotics.

6.3 RECOMMENDATIONS FOR IMPROVEMENT OF THE STUDY

More subjects need to be included as numbers were too small on this study to reach significance. A larger population group would make the statistics more powerful as shown in the study by Hatakka *et al* (2001).

Stronger motivation is needed to keep the subjects compliant as this study group falls into the age range that is well documented as being non-compliant (Anthony *et al* 1999). Seventy-five rand each 6 months was issued on this study; if more money was available; subjects could be paid at each clinic visit for completed diaries and used straws.

The therapeutic dose could be increased (a medium dose was used) and as the CF population is likely to have an abnormal colonic microflora (Walters & Littlewood 1998) a high dose may be more appropriate. If the dose was increased taking into account a current compliance rate of 50% perhaps more would achieve the medium dose if not the high dose.

The form in which the probiotic is given could be changed from straws to chewable tablets for the older CF population. This may improve compliance as they may feel as though they were taking a medication.

6.4 IMPLICATIONS FOR FURTHER RESEARCH

Further measurements of the anthropometric status of the CF patients would allow for continued monitoring of the malnutrition status of these patients.

Although the study did not reach significance there was a positive trend to the probiotic playing a role in improving lung function and reducing the incidence of infections – it would therefore be important to investigate this further in a larger multi centre trial to determine whether the probiotic is an effective further treatment option.

Probiotics given as a straw for the older patients was not perceived as medication and a capsule/tablet may have been a better option and could be used in a larger study. Probiotics as a single strain was used in this study to show an effect of that particular strain. It has become popular to give 2 or more strains of probiotics and this would be of interest to see if a significant difference could be measured in this group of patients although one would not be able to attribute a change to any one probiotic. However these supplements are now available in South Africa and would be available for the patients to consume after the study was completed. As part of the KZN ethics requirements if a product is used in a study it has to be available for the patient to use after the study has been completed. *L reuteri* was available for the patients to use after the study had ended. Prebiotics are becoming popular with some products containing both the pre and probiotics. Further research could then include the use of pre and probiotics.

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APPENDIX A LETTER OF INTRODUCTION

Dear Clinic member

As many of you know, I am the dietician who works in the CF clinic at St Augustine's Hospital. I am currently completing my master's degree through the University of Kwazulu-Natal. I have decided to do a clinical trial exploring the potential of a natural product to both reduce the incidence and duration of lung infections in CF.

You could help contribute to the knowledge of cystic fibrosis by participating in a 12 month trial that I would like to conduct on all CF members of both clinics in Kwazulu-Natal. If the trial is successful the study would than expand to include all the other CF clinics in South Africa.

I intend to measure the effect of a probiotic, *Lactobacillus reuteri*, on both lung function and the number of lung infections in CF. To make the results of my study recognizable both locally and internationally it is very important to have an active and a non active treatment group. Both groups will receive identical looking product and neither myself, the rest of the medical team or you will know which group you are in. Each participant will receive active product for 6 months and placebo for six months. At the end of the trial you will be told which group you were in and the results of the study. If successful, the probiotic is available locally if you choose to continue treatment.

If you agree to be part of the study I will need you to take the probiotic/placebo daily for 12 months and keep a record of the number and duration of any lung infections that you experience during this period. Most of the information that I need is routinely done such as sputum samples, height, weight and lung function tests. In addition at the start and end of the study I will measure your mid arm circumference and triceps skin fold thickness and you will be asked a short questionnaire. There may be an extra clinic visit at both the start, in the middle, and at the end of the study if you were not due to attend the clinic in that month. You will be offered compensation for the extra petrol costs and a small gratuity for taking part in the trial. There will be no cost to you to participate in the study.

Lactobacillus reuteri has GRAS status in America, which means that it is Generally Recognized As Safe so there is no foreseeable risk to you as a consequence of participating in my study.

You will be free to withdraw from the study at any time without prejudicing any treatment that is required for existing or future medical conditions.

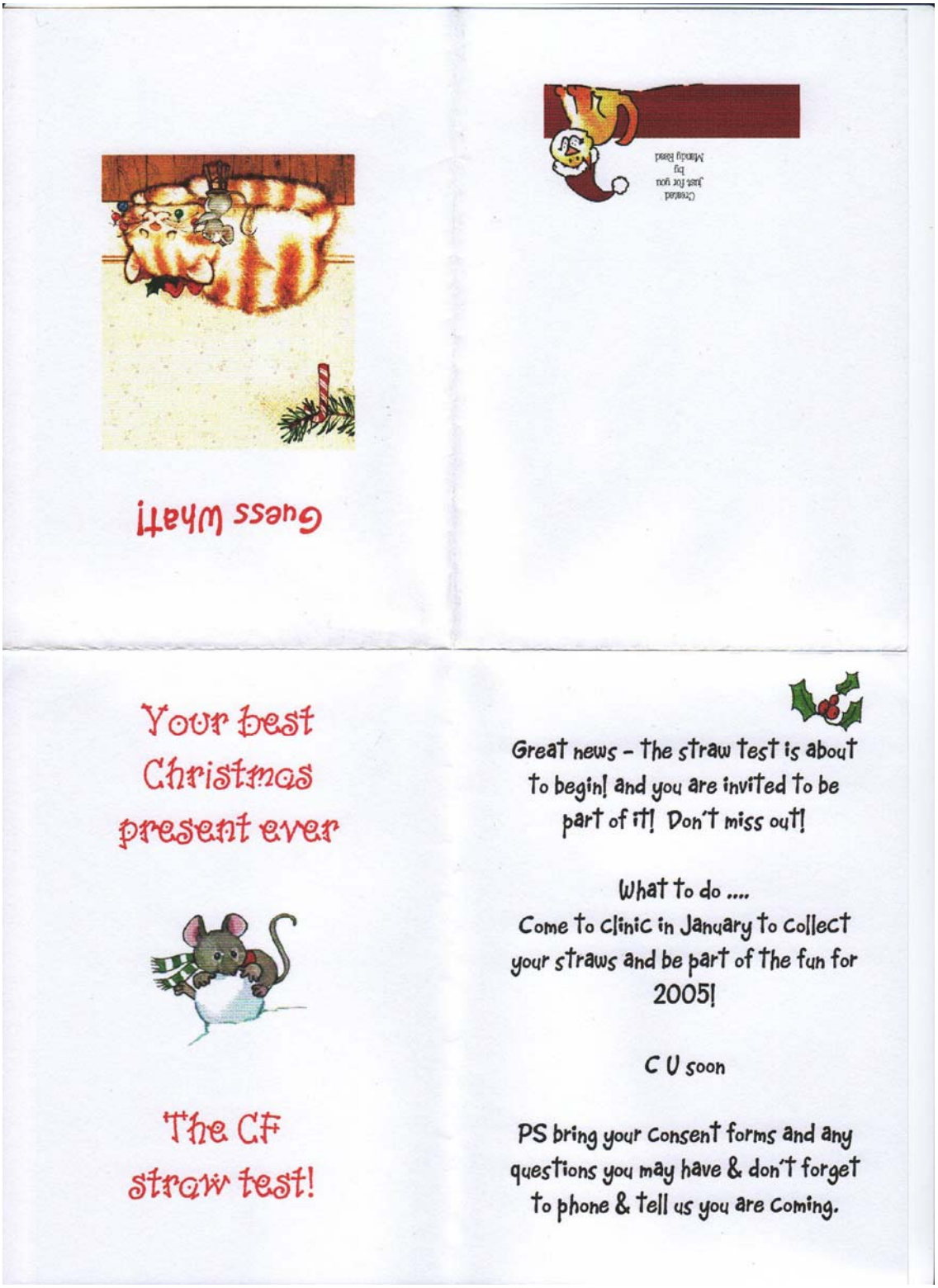
If you have any questions regarding the trial please do not hesitate to contact any of the CF doctors, physiotherapist or myself. We will be available at the next clinic to answer questions. At the start of the trial the trial will be explained to you in detail before the consent form is collected from you.

Ethics approval has been received from the University of Kwazulu-Natal, Nelson Mandela School of Medicine. If you have any queries or concerns regarding the ethics of this study you may contact the Medical Research Administration, telephone number 031 2604604, fax 031 2604410 or email ethicsmed@ukzn.ac.za.

Look forward to your participation.

Mandy Read
Dietician
Phone: (031) 2011154

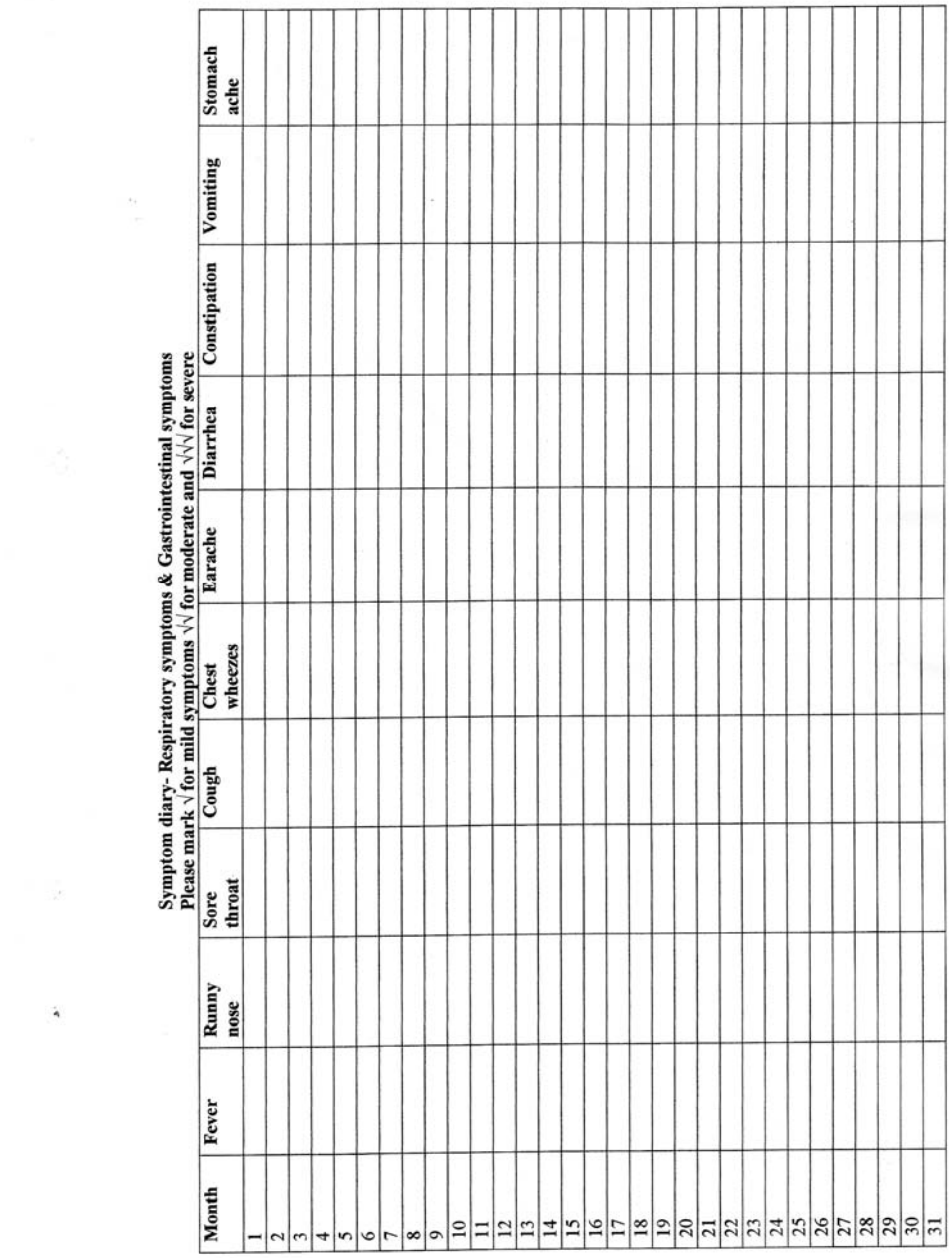
APPENDIX A LETTER OF INTRODUCTION continued



APPENDIX B DOCTORS COLLATION SHEET

Lung infection collation sheet					
Doctor Name:				Date	
Pt Name					
Organisms isolated:					
Pseudomonas		*Staphylococcus*		*Haemophilus*	
P. aeruginosa		*S. aureus*		*H. influenzae*	
Other					
Treatment prescribed			Length of treatment in days		
Antibiotic			Dose		
Antibiotic			Dose		
Other			Dose		
Signed					

APPENDIX C SYMPTOM DIARY



Symptom diary- Respiratory symptoms & Gastrointestinal symptoms
Please mark √ for mild symptoms √√ for moderate and √√√ for severe

Month	Fever	Runny nose	Sore throat	Cough	Chest wheezes	Earache	Diarrhea	Constipation	Vomiting	Stomach ache
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
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APPENDIX D 1 INFORMED CONSENT ADULTS

I hereby confirm that I have been informed by the research assistant about the nature, conduct, benefits and risks of the clinical study reference number Ref.:E048/04.

Ethics approval has been received from the University of Kwazulu-Natal, Nelson Mandela School of Medicine. If you have any queries or concerns regarding the ethics of this study or your rights as a research subject, you may contact the Medical Research Administration, telephone number 031 2604604, fax 031 2604410 or email ethicsmed@ukzn.ac.za.

- ❖ I have also received, read and understood the written information (letter of introduction and informed consent form) regarding the clinical study.
- ❖ I am aware that I am agreeing to participate in a research study where a treatment is under investigation, which may not personally benefit me.
- ❖ I am aware that the results of the study, including personal details will be anonymously processed into a study report and will remain confidential.
- ❖ I am willing to allow the Ethics committee full access to my medical records if it becomes necessary to verify study procedure and/or data.
- ❖ I am willing to allow the medical team (doctor, dietician and physiotherapist) full access to my medical records during and after the trial.
- ❖ I understand that I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- ❖ I understand I may contact Mandy Read at 031 2011154 any time if I have questions about the research.
- ❖ I declare myself prepared to participate in the study and have informed my doctor.
- ❖ I understand if I agree to participate, I will be given a signed copy of this document and the participant information letter, which is a written summary of the research.
- ❖ The research study, including the above information, has been described to me orally. I understand what my involvement in the study means and I voluntarily agree to participate.

PATIENT:

Printed Name	Signature	Date

APPENDIX D 2 INFORMED ASSENT FOR MINORS (under 18 years):

I hereby confirm that I have been informed by the research assistant about the nature, conduct, benefits and risks of the clinical study reference number (Ref.: E048/04).

Ethics approval has been received from the University of Kwazulu-Natal, Nelson Mandela School of Medicine. If you have any queries or concerns regarding the ethics of this study or the rights of your child or ward as a research subject, you may contact the Medical Research Administration, telephone number 031 2604604, fax 031 2604410 or email ethicsmed@ukzn.ac.za.

- I have also received, read and understood the written information (letter of introduction and informed consent form) and had an opportunity to discuss this study with the research assistant at the CF clinic.

- I am aware that I am agreeing for my child or ward to participate in a research study where a treatment is under investigation, which may not personally benefit my child or ward or me.

- I am aware that the results of the study, including personal details will be anonymously processed into a study report and will remain confidential.

- I am willing to allow the Ethics committee full access to my child or ward's medical records if it becomes necessary to verify study procedure and/or data.

- I am willing to allow the medical team (doctor, dietician and physiotherapist) full access to my child or wards medical records during and after the trial.

- I understand that I may, at any stage, without prejudice, withdraw my consent for my child or ward to participate in the study.

- I declare myself prepared for my child or ward to participate in the study and have informed the CF doctor.

- I understand if I agree to give consent as the parent/guardian for my child or ward to take part in the trial I will be given a signed copy of this document and the participant information letter, which is a written summary of the research.

- The research study, including the above information, has been described to me orally. I understand what the involvement of my child or ward in the study means and I voluntarily agree to his/her participation.

PARENT/GUARDIAN:

Printed Name Signature Date

On behalf of the PATIENT (minor):

Printed Name

APPENDIX E FOOD FREQUENCY QUESTIONNAIRE REGARDING PRE AND PROBIOTIC USE

Name:		Date of birth	
Male		Female	

Are you currently taking any of the following food supplements?		Yes	No
If yes which of the following?			
Build up		Sustagen T	
Ensure		Complan	
Nutren		Herbalife	
Sustagen		Other	
Other			
Other			
If yes, how often do you have a glass?			
Once a day		2 x day	
3 x day		4 x day	
1 or 2 x week		1 or 2 x month	
Other amount			
Do you make it up according to directions?	Yes / No	If not how do you make it up?	

Do you eat yogurt?		Yes	No
If yes, how often?			
Once a day		2 x day	
3 x day		4 x day	
1 or 2 x week		1 or 2 x month	
Other amount			
Any specific brand?		Yes	No
If yes, which brand/brands			
Do you take any **vitamin** supplements?		Yes	No
If yes, which brand/make?			
How often do you take them?			
Once a day	2 x day	3 x day	1 or 2 x week
1 or 2 month	Occasionally when you remember	Other	
How many do you take at a time?			

Do you take any **mineral** supplements?		Yes	No
If yes, which brand/make?			
How often do you take them?			
Once a day	2 x day	3 x day	1 or 2 x week
1 or 2 month	Occasionally when you remember	Other	
How many do you take at a time?			
Do you take any vitamin & mineral supplements?		Yes	No
If yes, which brand/make?			
How often do you take one?			
Once a day	2 x day	3 x day	1 or 2 x week
1 or 2 month	Occasionally when you remember	Other	
How many do you take at one time?			
Do you take any other food/herbal/alternative supplements?		Yes	No
Garlic & parsley		n-3 fatty acids	
Interflora		BioPro	
Other:			
Other:			
If Yes which brand/make?			
How many do you take at a time?			
If yes, how often do you take them?			
Once a day	2 x day	3 x day	1 or 2 x week
1 or 2 x month	Occasionally when you remember	Other	
When you take an antibiotic do you take any other supplements?		Yes	No
Eg Interflora/CoBiotic?Nutriflora?Kiddieflora?BioPro			
If you take a supplement, how many do you take at a time?			
If yes, how often?			
Once a day	2 x day	3 x day	1 or 2 x week
1 or 2 x month	Occasionally when you remember		
Other			
If any new products are started during this 6 months please inform the dietician			

APPENDIX F LETTER TO OTHER TREATING DOCTORS

Dear Doctor

This CF patient is currently taking part in a 12-month clinical trial investigating the efficacy of a probiotic on the occurrence and duration of lung infections in CF.

While they may need your assistance with a lung infections during this time please would you be so kind as to fill in the attached form to record the type and duration of treatment and fax it back to the phone number attached.

Due to the nature of this trial please could you refrain from scripting any probiotics or prebiotics including interflora during this time period. Should you have any queries regarding the scope of these please contact me at (031) 2011154.

Thank you for your assistance in this matter.

Yours sincerely

Mandy Read
Dietician
(031) 2011154

Lung infection collation sheet					
Doctor Name:				Date	
Pt Name					
Organisms isolated:					
Pseudomonas		*Staphylococcus*		*Haemophilus*	
P. aeruginosa		*S. aureus*		*H. influenzae*	
Other					
Treatment prescribed			Length of treatment in days		
Antibiotic			Dose		
Antibiotic			Dose		
Other			Dose		
Signed					

APPENDIX G SHWACHMAN SCORE

SHWACHMAN SCORE

To compute a Shwachman score, award up to 25 points for each of the four sections listed below, and total.

General activity

21 – 25	Full normal activity. Goes to school/work regularly.
16 – 20	Lacks endurance. Tires at end of day, but good attendance.
11 – 15	Tires easily after exertion. May rest during day. Fair attendance.
6 – 10	Dyspnœic after short walk. Rests a good deal. Poor attendance.
0 – 5	Confined to bed or chair. Orthopnœic.

General activity score: ☐☐ / 25

Physical examination

21 – 25	Normal. No cough. Clear lungs. No deformity. Respiratory rate normal.
16 – 20	Resting respiratory rate normal. Rare coughing. Minimal emphysema. Clear lungs.
11 – 15	Occasional cough. Mild emphysema. Respiratory rate slightly elevated. Rarely localized crepitations. Early clubbing.
6 – 10	Frequent cough usually productive. Moderate emphysema. May have chest deformity. Chest retraction. Crepitations present. Moderate clubbing.
0 – 5	Severe coughing spells. Tachypnœa with tachydardia. Extensive pulmonary changes. May have signs of right heart failure. Marked clubbing.

Physical examination score: ☐☐ / 25

Nutrition

21 – 25	Height and weight above 25th centile. Well formed stools.
16 – 20	Height and weight at 15 – 20th centile. Stools slightly abnormal.
11 – 15	Height and weight above 3rd centile. Abnormal stools. Poor muscle tone with reduced muscle mass.
6 – 10	Height and weight below 3rd centile. Abnormal stools. Abdominal distension. Flabby muscles.
0 – 5	Marked malnutrition. Protuberant abdomen. Rectal prolapse. Large, foul, frequent, fatty stools.

Nutrition score: ☐☐ / 25

Chest X-ray

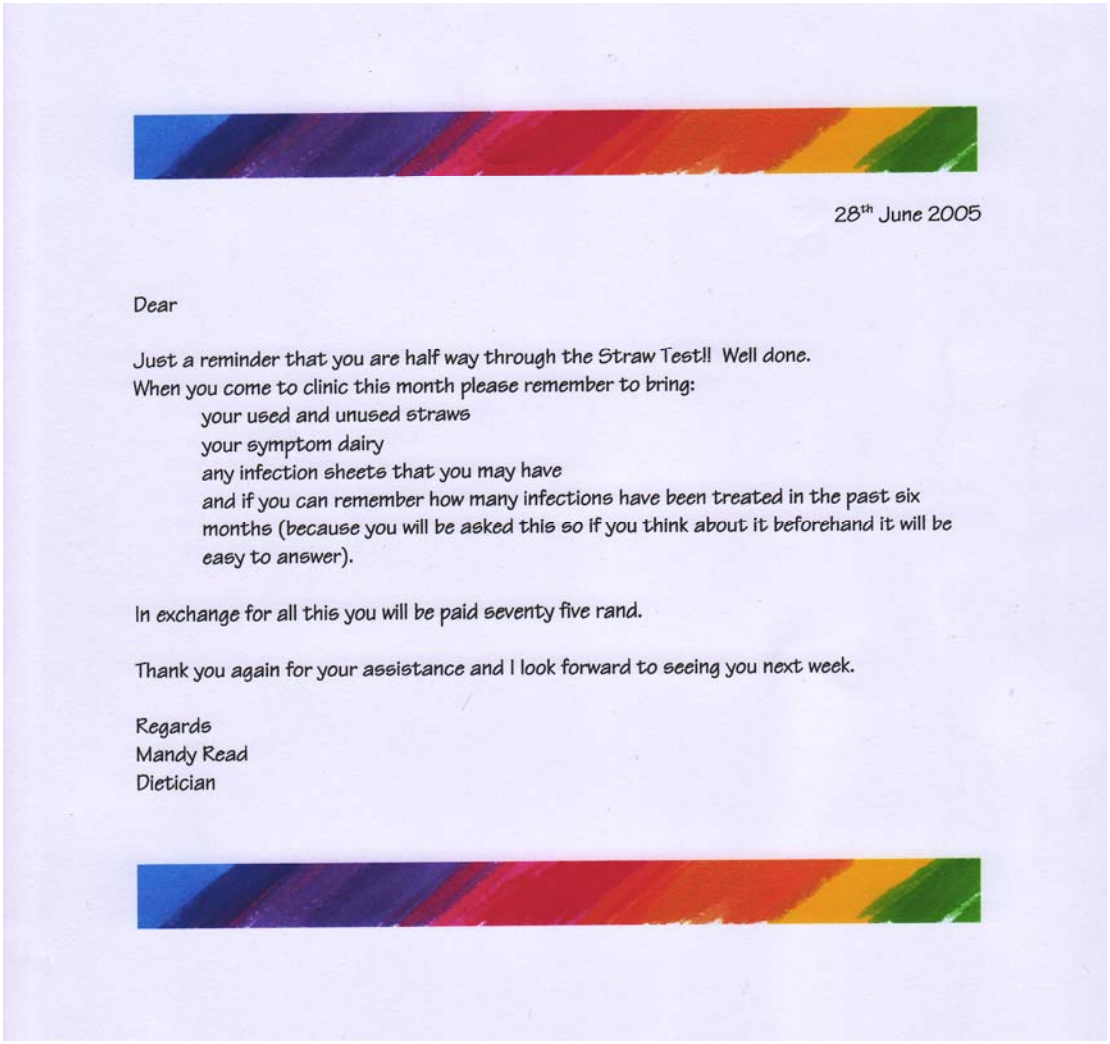
21 – 25	Clear lung fields.
16 – 20	Early emphysema. Minimal accentuation of bronchovascular markings.
11 – 15	Mild emphysema with patchy atelectasis. Increased bronchovascular markings.
6 – 10	Moderate emphysema. Widespread areas of atelectasis with superimposed areas of infection. Minimal bronchiectasis.
0 – 5	Extensive changes with pulmonary obstructive pneumonia and infection. Lobar atelectasis and bronchiectasis.

Chest X-ray score: ☐☐ / 25

Total Shwachman score: ☐☐ / 100

Guide to severity of Cystic Fibrosis indicated by Shwachman score:
Severe (0-40) Moderate (41-55) Mild (56-70) Good (71-85) Excellent (86-100)

APPENDIX H SIX MONTH REMINDER LETTER TO PATIENTS



28th June 2005

Dear

Just a reminder that you are half way through the Straw Test!! Well done.
When you come to clinic this month please remember to bring:

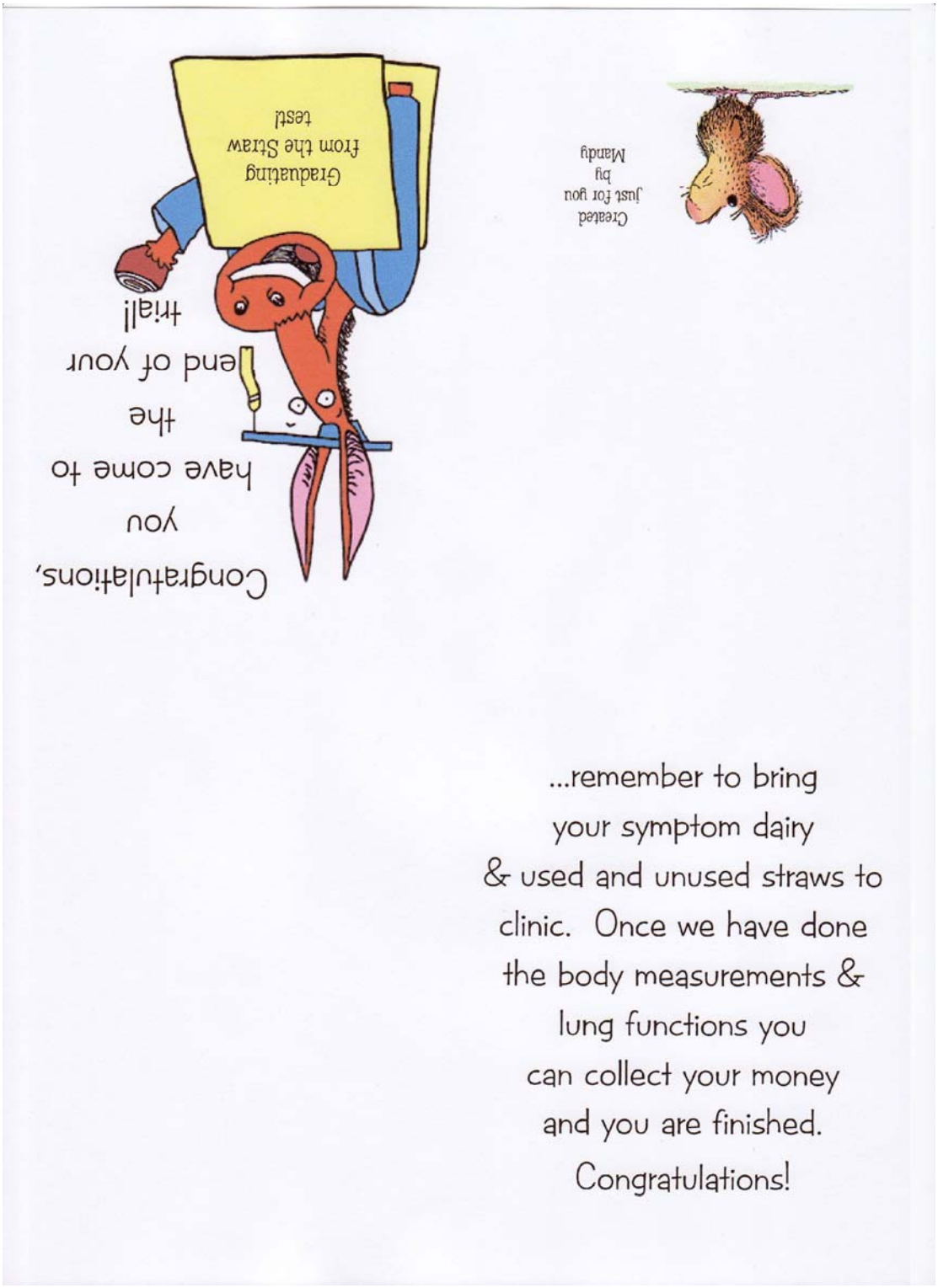
- your used and unused straws
- your symptom dairy
- any infection sheets that you may have
- and if you can remember how many infections have been treated in the past six months (because you will be asked this so if you think about it beforehand it will be easy to answer).

In exchange for all this you will be paid seventy five rand.

Thank you again for your assistance and I look forward to seeing you next week.

Regards
Mandy Read
Dietician

APPENDIX I END OF STUDY REMINDER TO PATIENTS



APPENDIX J RECORDING OF DATA SHEET

Check List
Patients Name or No:

Height measurement: Time:

First	Second	Third	Average:

Weight: Time:

First	Second	Third	Average:

Midarm circumference (MAC)

First	Second	Third	Average:

Triceps skinfold thickness (TSF)

First	Second	Third	Average:

Lung function FEV_1

First	Second	Third	Average:

Lung function FVC

First	Second	Third	Average:

Sputum:

sent to lab	Yes	no	
Addington number		Medical Aid	
		Medical Aid No	

Dietary Questionnaire: done
Shwachman Score: done
Collation sheet: given

6 x symptom dairy given

Consent form signed and collected: done

APPENDIX K1 ETHICS APPROVAL LETTER

UNIVERSITY OF
KWAZULU-NATAL

24 November 2004

Ms M Read
2 Wrenbury Place
GLENWOOD
Durban
4001

feathers@absamail.co.za

Dear Ms Read

PROTOCOL : The effect of supplementation with *Lactobacillus reuteri* on the incidence and duration of lung infections and lung function, and anthropometric measurements in a cystic fibrosis population in KwaZulu-Natal. M Read, Dietetics. Ref.: E048/04

The Research Ethics Committee considered the abovementioned application and made various recommendations. These recommendations have been addressed and the protocol was approved by consensus at a full sitting of the Research Ethics Committee at its meeting held on 9 November 2004 pending permission from the Hospital Managers. These documents have been received and the study may begin as at today's date : 24 November 2004.

This approval is valid for one year from 9 November 2004. To ensure continuous approval, an application for recertification should be submitted a couple of months before the expiry date.

Yours sincerely

PROFESSOR A DHAI
Chair : Research Ethics Committee

c.c. Professor E M W Maunder, Dietetics, UKZN - Pmb

Nelson R Mandela School of Medicine, Faculty of Health Sciences,
Head: Bioethics, Medical Law and Research Ethics

Postal Address: Private Bag 7, Congela 4013, South Africa

Telephone: +27 (0)31 260 4604 **Facsimile:** +27 (0)31 260 4529 **Email:** dhaia1@ukzn.ac.za **Website:** www.ukzn.ac.za

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

APPENDIX K2 ETHICS APPROVAL LETTER FOR HOSPITALS

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the Medical Superintendent/s / Hospital Manager/s for signature.

Once the document has been signed it should be returned to this office so that full ethical approval can be granted.

To: Chief Medical Superintendent / Hospital Manager

PROTOCOL : The effect of supplementation with *Lactobacillus reuteri* on the incidence and duration of lung infections and lung function, and anthropometric measurements in a cystic fibrosis population in KwaZulu-Natal. M Read, Dietetics. Ref.: E048/04

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 1 address: ADDINGTON HOSPITAL, P.O. BOX 977, DURBAN 4000

Investigator/s: Principal: MANDY READ; Co-investigator: CHIARA BIGGS; Co-Investigator: PROF ELENI MAUNDERS

Signature of Chief Medical Superintendent/Hospital Manager : [signature]

Date: 14/11/2004

Site 2 address: ST AUGUSTINE'S HOSPITAL, 107 CHELMSFORD ROAD, GLENWOOD DURBAN

Investigator/s: Principal: MANDY READ; Co-investigator: CHIARA BIGGS; Co-Investigator: PROF ELENI MAUNDERS

Signature of Chief Medical Superintendent / Hospital Manager : [signature]

Date: 21 OCTOBER 2004

NB: Medical Superintendent/s / Hospital Manager/s to send a copy of this document to Natalia

APPENDIX L DETAILED INFORMATION FOR MIDARM CIRCUMFERENCE AND TRICEPS SKINFOLD THICKNESS READINGS

The mean MAC at the start of the study was 22.6 cm and 23.3 cm at the end of the study (Table 35). This is a significant increase of 0.7 cm (p=0.027).

All age groups improved their MAC readings for the study period except the ≥ 20 year old who's MAC decreased. All were non significant findings except for the 13 to 19.99 year old group whose MAC increased significantly (p=0.008).

The mean MAC for males was 23.5 cm at the start and 23.9 cm at the end of the study (Table 36). This difference of 0.4 cm was not significant (p=0.408). The males MAC improved non-significantly for all but the ≥ 20 year olds whose MAC decreased over the study period.

The mean MAC for the female group as a whole was 21.4cm at the start of the study and improved to 22.6cm by the end of the study period showing a 1.2cm increase which was a significant finding of p=0.015 (Table 37). All other female age groups also improved their MAC readings non significantly over the study period.

When comparing the MAC for males and females there was a non significant difference between them at the start (p=0.388) and the end of the study (p=0.563).

The mean TSF for the whole group at the start of the study was 8.44 mm and 8.06 mm at the end of the study (Table 35). This was a non significant decrease of 0.38 mm (p=0.405). Only 4 (all ≤ 13 years) improved their TSF readings for the study period. Twelve patients (all 14 years to ≥ 20 years) decreased their TSF over the study period but these were non significant findings.

For the male group as a whole there was a significant (p=0.047) decrease in the TSF readings (Table 36) from the start to the end of the study. The only male group to improve was the ≤ 9.99 year olds, while all other age groups decreased their TSF over the study period. All the females increased their TSF over the study period with the exception of the 27 year old female who's TSF decreased. This was non significant (p=0.971) (Table 37). When comparing the TSF for males and females there was a significant difference between them at the start (p=0.011) and the end of the study (p=0.005).