Energy balance during treated acute respiratory exacerbation in Cystic Fibrosis.

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Submitted for the degree of MSc
to the University of Glasgow
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September 1998

Declaration

I certify that all the work presented and the writing in this thesis is specifically my own, and any assistance received is specified in the text. This thesis does not include work forming part of a thesis for a degree in this or any other University.

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ABSTRACT

Prior to the present study, there was a widespread perception that acute respiratory exacerbations result in negative energy balance, which leads to undernutrition in Cystic Fibrosis (CF). This study was the first of its kind to characterise the energy balance of subjects with CF, through the simultaneous measurement of all the components of energy balance in each child: energy intake (E_I); resting metabolic rate (RMR); total energy expenditure (TEE) and faecal energy losses (E_f), together with changes in body composition during a period of treated acute respiratory exacerbation, and a 'well', clinically infection free, period. The overall aims of this study were to (i) describe energy balance in a group of children and adolescents suffering from CF with a range of respiratory disease during a treated acute respiratory exacerbation, and (ii) to determine which component(s) of the energy balance equation underwent change during the treated acute respiratory exacerbation.

Methods

Fourteen children (6 girls, 8 boys, mean decimal age 9.9 years (SD 2.4)) were studied when 'well' and during an acute exacerbation treated with intravenous (i.v.) antimicrobial therapy. Seven subjects were treated with i.v. antibiotics at the time of experiencing acute respiratory exacerbation ('standard' i.v. therapy), and seven experienced acute respiratory exacerbation but were in the category defined as 'regular' i.v. therapy. These seven exhibited more severe lung disease and had a history of more frequent acute exacerbations.

A cross over study design was utilised and subjects were studied for 14 days during the treated acute respiratory exacerbation, and 'well' clinically infection free phase, respectively. Body composition was estimated using bioelectrical impedance (BIA) and skinfold thickness measurements, pulmonary function was evaluated using standard pulmonary function tests, Schwachman score was used to gauge disease severity, and growth and nutritional status were determined by means of height, weight and body mass index (BMI). Energy and fat intakes were assessed by means of dietary records, using household measurements (5 - 7 alternate days during the acute exacerbation, and 3 consecutive days when 'well'). Resting metabolic rate (RMR) was measured by open-

circuit indirect calorimetry (Datex Instrumentarium, Helsinki, Finland), over a 15 - 20 minute period after an overnight fast, or 5 hour fasting period. Total energy expenditure (TEE) was determined using the doubly labelled water technique over a 14 day period for both 'well' and exacerbation phases. Faecal fat output was calculated from a three day stool collection made during both phases, using a modified method of Van der Kamer *et al* (1949). Changes in faecal fat output were then standardised for fat intake using the co-efficient of fat absorption (CFA).

The mean paired difference for each energy balance variable between 'well' and exacerbation phases were calculated for each subject, together with descriptive statistics. T-tests and 95% confidence intervals were used to assess the significance of the paired differences.

Results

Overall the group were reasonably well nourished (mean BMI SD score -0.31 (SD 0.79); mean % body fat 21.8 (SD 5.6)), and had grown reasonably well (mean height SD score -0.54 (SD 1.08)), considering the fact they had Cystic Fibrosis. The group had managed to maintain a reasonably stable nutritional status over the one year period prior to the study (mean change in BMI SD score -0.01 (SD 0.61)). The treated acute respiratory exacerbation was associated with a statistically significant, but moderate reduction in energy intake (E_i), (mean paired difference 47 kJ/kg/d, $p< 0.05$). Changes in the other variables measured did not reach statistical significance, but the reduction in intake may have been offset by a modest decline in overall physical activity. No noticeable effect of acute exacerbation on RMR was apparent, and while TEE was lower during the acute exacerbation, this change did not reach statistical significance. Furthermore the absence of any significant changes in the body weight and composition of the group suggests no evidence of marked negative energy balance during the treated respiratory exacerbation.

Conclusion

Treated acute respiratory exacerbations appear now to represent less of a challenge to energy balance in CF than was previously thought. No marked negative energy balance was observed during the acute exacerbation. The findings are consistent with very recent studies which report that children with CF do not experience negative energy balance during acute respiratory exacerbations, irrespective of whether that subject is treated at home (Vic *et al* 1997), or within the hospital setting (Stallings *et al* 1998). It would appear that for certain children with CF, issues surrounding compliance with dietary treatment and pancreatic enzyme replacement therapy (PERT) may be regarded as more relevant factors in the maintenance of positive energy balance than acute respiratory exacerbation.

To conclude, this study did not support the view that a treated acute respiratory exacerbation results in negative energy balance in children with CF. However it is possible that an acute exacerbation may be more important to energy balance in older subjects with more severe lung disease.

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Abbreviations

AE	Acute exacerbation
ACS	Acute clinical score
ANS	Absolute neutrophil count
BIA	Bioelectrical impedance
BSIA	Bureau of stable isotope analysis
BMI	Body mass index
BMR	Basal metabolic rate
CF	Cystic Fibrosis
CFA	Co-efficient of fat absorption
CFTR	CF transmembrane conductance regulator
CI	Confidence interval
Cl^-	Chloride ion
CO_2	Carbon dioxide
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
CSS	Carstairs Scoring System
CV	Coefficient of variation
DIT	Diet induced thermogenesis
DLW	Doubly labelled water
EAR	Estimated average requirement
EBV	Epstein-Barr virus
EFA	Essential fatty acid
ESR	Erythrocyte sedimentation rate
E_d	Energy deposited
E_i	Energy intake
E_l	Energy losses
FEV_1	Forced expiratory volume in 1 second
FFM	Fat free mass
FM	Fat mass
FVC	Forced vital capacity
g	Gram
H	Height

hosp.	Hospital
IDECG	International Dietary Energy Consultancy Group
i.v.	Intravenous
kg	Kilogram
kJ	Kilojoule
kJ/kg/d	Kilojoule per kilogram per day
l	Litre
MAC	Mid arm circumference
MAMC	Mid arm muscle circumference
MIP	Maximal inspiratory pressure
mm	Millimetre
n	Number
Na^+	Sodium ion
O_2	Oxygen
PAL	Physical activity level
PERT	Pancreatic enzyme replacement therapy
RDA	Recommended daily amount
RHSC	Royal Hospital for Sick Children
RMR	Resting metabolic rate
RR	Respiratory rate
RQ	Respiratory quotient
SCFA	Short chain fatty acids
SD	Standard deviation
TBW	Total body water
TEE	Total energy expenditure
TNF - α	Tumour necrosis factor - α
TPN	Total parenteral nutrition
TSF	Triceps skinfold
VCO_2	Carbon dioxide production
VO_2	Oxygen consumption
W	Weight
^2H	Hydrogen isotope
^2H_2O	Deuterium oxide
$^2H_2\ ^{18}O$	Doubly labelled water

^{18}O Oxygen 18

Acknowledgements

I would like to thank:

All the children and their families who kindly took part, and made this study possible.

Dr J.J. Reilly for his help and guidance throughout this piece of work, and gratefully acknowledge the funding provided.

Dr James Paton for his financial input and guidance.

Dr Jane Wilkinson and Dr John Evans for their valuable input.

My Mum and Dad for their support throughout the undertaking of this work.

My fiance Brian Liddell for his patience and support.

My friend Rizwana Hamid for proof reading this work.

Dr C. Edwards, Jonathan Ventham, Nell Caine, Martin MacMillan, Linda Morrison, Linda Mason, and the CF Unit for their valued help throughout

1.0 Introduction

1.0 General Background

Cystic Fibrosis (CF) is the most common lethal genetic disease affecting those of Caucasian descent. It is transmitted as a severe autosomal recessive trait, with an incidence of approximately 1 in 2415 live births, and around 7000 patients have the disease in the United Kingdom (Dodge *et al* 1997).

Cystic Fibrosis (CF) is a multisystem disorder, with chronic pulmonary involvement as the major cause of morbidity and mortality. The basic genetic defect in CF causes abnormal ion transport regulation in epithelial cells (Rommens *et al* 1989). Life expectancy has gradually improved for patients with CF over the last 50 years, with antibiotic therapy appearing to be one of the prime reasons for the improved survival rates. The other main factor which has facilitated these improved survival rates, and quality of life is the considerable advances in other aspects of medical treatment and the acknowledgement of milder cases, with their inclusion within the survival data. Improved prognosis may also be attributed to earlier diagnosis, and subsequent early initiation of treatment (Jackson 1989; FitzSimmons 1993).

1.1.0 Genetics And Pathophysiology - The Basic Abnormality In CF

1.1.1 CFTR - The Gene Product

Recent genetic advances have enabled the CF gene itself to be localised to the long arm of chromosome 7 (Rommens *et al* 1989). Though the disease is known to present with various clinical manifestations, this genetic disorder is almost certainly the result of a single gene defect in the CF transmembrane conductance regulator (CFTR). Genetically, CF has been described by some as ' Defective CFTR' (Fiel 1993).

Recent work has shown that the CF gene product, CFTR, (a membrane-bound protein), is involved in the regulation of chloride (Cl^-) ion transport across epithelial cells (Kartner *et al* 1991). The epithelial linings of the nasal cavity, lower respiratory tract, pancreas,

gastrointestinal tract and the sweat glands are primarily involved (Kerem *et al* 1990). Research suggests that this gene defect, and the accompanying abnormality in ion transport, results in the altered composition of epithelial secretions (reduced water content) which cause blocked respiratory and pancreatic ducts (Wine 1991; Riordan *et al* 1989; Alton *et al* 1992). Defective Cl^- ion permeability is therefore responsible for the basic physiological abnormalities in CF, namely progressive lung disease, pancreatic dysfunction, and elevated sweat electrolytes.

1.1.2 Basic Mechanisms Of Respiratory Function

In the healthy respiratory tract, normal mucocilliary clearance is dependant on the upward flow of the mucous layer, which lies over the tips of the cilia, which move freely in the fluid (watery) layer beneath (Kendigs 1990). Ion transport is governed by absorption of Na^+ from the mucosal surface, over a favourable gradient, via Na^+ channels in the cell membrane. Water moves osmotically along with the Na^+. A suitable gradient also exists to allow Cl^- to leave the cell, via Cl^- channels (Alton *et al* 1992). This provides a balance between the secretion and reabsorption of both water and electrolytes, and prevents any dehydration which may impede the upward movement of the mucus layer (Fiel 1993).

In CF airway epithelial cells, Cl^- impermeability is present, as identified in CF sweat ducts . The current hypothesis is that this fluid contains less water due to the net effect of reduced water secretion into the airway, and increased water absorption from it. This is believed to be secondary to reduced epithelial cell chloride secretion, and increased sodium absorption (Knowles *et al* 1983; Nelsons Textbook of Paediatrics 1996).

1.1.3 Genotypes And Phenotypes In CF

The first, and most common, mutation described in CFTR is ΔF508. This represents the deletion of an amino acid, phenylalanine at position 508, in the encoded CFTR polypeptide (Zielenski & Tsui 1995; Shrimpton *et al* 1991). In 1994, a world-wide study carried out by the CF Genetic Analysis Consortium, screened 43,849 mutant allele-bearing chromosomes in

subjects with CF. This survey revealed that the ΔF508 mutation accounted for 66% of CF chromosomes (Kazazian 1994).

In 1991, Shrimpton *et al* carried out an extensive cohort study, within a predominantly Scottish population, to determine the frequency of 16 different mutations of the CFTR gene. It was shown that the ΔF508 mutation had a frequency of 71%, with 51% of CF cases presenting as homozygous for ΔF508. From this study it was also noticed that, in the same Scottish population, the G551D mutation was the second most common, with a relative frequency of 6%, with the G542X mutation having a frequency of 4%. Currently, in excess of 550 individual mutations of the CFTR gene have been reported, although there is uncertainty as to how many different mutations are in existence (Zielenski & Tsui 1995).

Extensive research has been undertaken to try and identify common clinical features among CF patients of the same genotype (Zielenski & Tsui 1995; Kerem & Kerem 1996). A more severe clinical presentation has been associated with CF patients homozygous for the ΔF508 mutation, (earlier disease onset, higher sweat Cl^- levels, more severe pancreatic insufficiency, and poorer nutritional status), compared with those who do not carry the ΔF508 mutation. However, severity of pulmonary disease has been found to vary considerably, even within homozygous ΔF508 patients. Overall, there is still a degree of uncertainty within the area of genotype-phenotype correlation, and substantial variation in phenotype is not readily explained by genotype (Kerem & Kerem 1996).

From a clinical perspective, CF is characterised by chronic obstructive pulmonary disease and bronchopulmonary infection, pancreatic exocrine insufficiency causing malabsorption, and elevated sweat electrolytes (namely Na^+) (Shepherd & Cleghorn 1989; Knowles 1983). Due to multiple organ involvement, patients with CF may also display gastrointestinal and hepato-biliary manifestations at some stage. The extent of this involvement has a major effect on the rate of progression of the respiratory disease (Park & Grand 1981). However, discussion here is limited to the pancreatic, and respiratory manifestations, because these are of particular nutritional significance.

In the CF pancreas, viscous secretions lead to inflammation, fibrosis and blockage of the pancreatic ducts. This in turn leads to pancreatic exocrine dysfunction and a reduction in delivery of enzymes to the duodenum. This causes fat and protein maldigestion/malabsorption, due to a severe deficiency of the digestive enzymes lipase (for the hydrolysis of fat), amylase (for the hydrolysis of starch), and trypsin (for the breakdown of proteins to amino acids in the duodenum), as well as chymotrypsin, and colipase. The resulting steatorrhoea, (foul smelling fatty stools), and the development of nutritional deficiencies, are due to the digestive and absorptive processes being greatly impaired (McPherson & Dormer 1992; Francis 1987; Sokol 1990). The latter are all common features in untreated CF.

1.2.0 Acute Respiratory Exacerbations

Lung infection is clearly a result of the basic defect in CF, and chronic bronchopulmonary infection is regarded as the main cause of advancing pulmonary disease in CF. An acute pulmonary exacerbation in the child with CF can be defined as an acute deterioration of symptoms and be clinically characterised by: increased cough and sputum production; change in sputum appearance; deteriorating lung function and X-ray changes; increased respiratory rate (or breathlessness); weight loss and poor appetite; impaired physical activity; rarely pyrexia; and treatment with i.v. antibiotics with hospitalisation, at least until recently (Vic *et al* 1997 ; Shepherd *et al* 1988 ; Dodge 1989; Nelson 1985; Naon *et al* 1993). The acute exacerbation is difficult to define and is known to be more easily recognisable, in terms of physical signs and symptoms, to an experienced CF clinician, than definable in a quantitative way. Currently there are no single or multiple clinical measures which objectively confirm the presence or absence of acute respiratory exacerbation (Rabin *et al* 1997). It is therefore usually defined as the presence of some or all of the clinical symptoms described above, and on the basis that i.v. antibiotic therapy is required. Varying combinations of symptoms are possible in any one CF child during what could be clinically defined as an 'acute respiratory exacerbation' (Rabin *et al* 1997). The precise cause of acute respiratory exacerbation is unclear because microbiological analysis of sputum samples from patients often indicates similar levels of pathogens, both during periods of remission and exacerbation (Nelson 1985).

It has also been suggested that primary Epstein-Barr Virus (EBV) infection may be associated with severe respiratory exacerbations, accompanied by a decline in clinical progress for CF patients (Winnie & Cowan 1992), and so the microbiology of the acute exacerbation is poorly understood.

Treatment of the acute exacerbation in CF involves a standard 14 day course of intravenous antibiotics, increasingly home intravenous antibiotic therapy. Administration of the appropriate anti-microbial therapy has been shown to improve pulmonary function, and clinical well-being, during an acute exacerbation (Smith *et al* 1988). Treatment is directed at the containment of Pseudomonas aeruginosa, (one of the main bacterial pathogens in CF respiratory infection), rather than its elimination, since permanent eradication of this pathogen in CF appears to be impossible (Szaff *et al* 1983).

In CF, the acute pulmonary exacerbation is therefore superimposed on the already present, chronic infection in the CF lung (Nelsons Textbook of Paediatrics 1996; Forfar & Arneils Textbook of Paediatrics 1992). A wide variety of pathogens have been associated with pulmonary infection in CF, both bacterial and viral in origin. The main bacterial pathogens, affiliated with chronic respiratory infection, in paediatric CF are Pseudomonas aeruginosa, Staphylococcus aureus, Pseudomonas cepacia, and Haemophilus influenzae. Historically, Staphylococcus aureus was the main bacterial pathogen associated with respiratory infection in CF. Initial chronic colonisation of the lungs by this pathogen is particularly damaging, directly inducing the process of lung injury in CF. Further colonisation, by Pseudomonas aeruginosa, occurs at a later stage (Hoiby 1982) and continues the process of lung injury (Kerem *et al* 1990). Pseudomonas eventually colonises the lungs of the majority of patients (Pitt 1986) and as the child advances in age, the incidence of this chronic infection is increased.

1.3.0 Clinical Management Of CF

1.3.1 Non-Nutritional Management

Treatment in CF involves a multidisciplinary approach, and compliance with medication, nutrition, and physiotherapy are fundamental to control of symptoms in CF. Gastroenterology, respiratory and dietetic management are all vital to successfully co-ordinated clinical care. However, all those therapies are subject to some degree of non-compliance, and are often used in circumstances which are difficult. Children with CF, and their families, often have a range of medical, social and psychological problems.

Chest physiotherapy continues to be a cornerstone of treatment, along with aggressive antibiotic therapy. The standard techniques of percussion and postural drainage assist the release of entangled mucus from the lungs (Fiel 1993). For most patients this takes 20-30 minutes, 2-3 times daily. Physiotherapy is thus very time-consuming, and is therefore subject to non-compliance.

Another health professional involved in CF care is the social worker. The social worker must provide both patient and family counselling, link in with the clinical team, and child psychologist, and be aware of the psychosocial implications of CF, for each individual patient and their family (Taub 1990). Due to the multiple social and psychological problems associated with having CF, for both the individual as well as the family, the social worker has an important role to play in the management of CF.

1.3.2 Nutritional Management

It has long been recognised that children with CF are at a greater risk of developing nutrient deficiencies due to maldigestion, malabsorption, and poor energy/nutrient intake (Shepherd *et al* 1980). Deficiencies of both fat soluble vitamins, and essential fatty acids (EFA's) are predominantly due to pancreatic dysfunction (Dowsett 1996). In 1996, Lloyd-Still *et al* stated that deficiency in EFA's may influence development of respiratory infection with Staphylococcus aureus, and Pseudomonas aeruginosa in the child with CF. Children with CF

typically exhibit poor linear growth and poor weight gain (Wootton *et al* 1991; Soutter *et al.* 1986). A decrease in respiratory muscle size is common (Hopkins 1984), as is reduced respiratory muscle strength (Arora & Rochester 1982) and each is associated with chronic undernutrition. A study carried out by Johannesson *et al* (1997), found that puberty was delayed in female CF patients, (17 females, mean age at peak height velocity 12.9 yrs (SD 0.8), and at menarche 14.9 yrs (SD 1.4), in spite of good nutritional, and clinical status. Research has demonstrated that enhanced nutritional status may slow the progressive decline in lung function in CF (Thomson *et al* 1995; Levy *et al* 1985), produce a significant decrease in infection rate, promote a steady improvement in lung function (Shepherd *et al* 1980), and improve the strength of respiratory muscles (Wilson *et al* 1986).

Improved survival rates in CF have been attributed to more effective targeting of nutritional management, and thus enhanced nutritional status (Levy *et al* 1985; Drury *et al* 1990). Due to maldigestion and malabsorption of both protein and fat, together with the resultant steatorrhoea, pancreatic enzyme supplementation (Eg Creon, Pancrease), is an ongoing essential part of overall treatment. However, a study carried out by Murphy *et al* (1991), suggested that pancreatic enzymes rarely resolved steatorrhoea completely, and faecal energy losses remained approximately 11% of gross energy intake (range 5 - 20%), in the group of 20 CF children studied, (mean age 11 years). This was compared to a group of 20 normal healthy children (mean age 9.0 years) with a mean of 3.5% (range 1 - 6%) of energy intake lost via stools.

Children with CF are encouraged to take a diet which provides 120-150% of the recommended intake for energy for age (Hubbard 1980; Pencharz *et al* 1989 ; Daniels *et al* 1987). However, in cross-sectional studies, dietary intake has frequently been reported to fall below such recommendations (Ramsey *et al* 1992; Bell *et al* 1984; Tomezsko *et al* 1992; Stark *et al* 1995; MacDonald *et al* 1991). Dietary fat (as the most concentrated source of dietary energy) should ideally provide approximately 40% of total intake (MacDonald *et al* 1991; Kawchak *et al* 1996).

1.4.0 Energy Balance In CF - Background

It is well known that negative energy balance causes malnutrition in patients with CF (Shepherd *et al* 1991) and that, as indicated previously, malnutrition is commonplace in CF (Chase *et al* 1979; Hopkins 1993). The energy demands of a child with CF are believed to be greater than those estimated for a child of the same gender, and similar age, typically by about 20 - 50%, as noted in the previous section. This is currently believed to be the result of the following factors: increased requirements for repair of tissue both during and after bouts of infection; the metabolic and immunological costs of infection; elevated energy costs of ventilation due to chronic pulmonary damage; increased faecal energy losses, due to pancreatic dysfunction (Dodge & O'Rawe 1996); and possibly an increase in cellular energy expenditure as a result of the defect in ion transport.

It has already been noted that the development of malnutrition and failing pulmonary function seem to occur together in CF, (Gurwitz *et al* 1979; Shepherd *et al* 1988; Thomson *et al* 1995; Zemel *et al* 1996) and that respiratory disease, as well as the patients general clinical status, may be improved with nutritional intervention (Shepherd *et al*, 1980; Levy *et al* 1985; Shepherd *et al* 1986). However, in clinical practice there are a few children who remain well nourished in the face of declining lung function. Management is still very much targeted at the prevention and treatment of the advancing pulmonary disease, malnutrition and malabsorption.

In CF the aim of nutritional management is therefore to establish a balance between the patients energy intake, requirements, and if possible achieve the small positive energy balance required for normal growth and development (Shepherd *et al* 1991; Shepherd & Cleghorn 1989; Lloyd-Still *et al* 1996; Shepherd *et al* 1984). Zemel *et al* (1996) found pulmonary function to be related to nutritional status in clinically well, mildly diseased CF children, and that pulmonary function (FEV_1 - the volume of air expired in 1 second, one of the main clinical markers of pulmonary function) deteriorated in those children who found it difficult to maintain

their percent ideal body weight (% IBW). Percent ideal body weight reflects current body weight for actual height.

In a review of CF energy balance literature by Reilly *et al* (1997) it was noted that the exact cause of malnutrition in CF remains uncertain, despite a great deal of research. That is, the relative contribution of impaired energy intake, increased energy expenditure, and increased faecal energy losses is unknown (Reilly *et al* 1997). It would therefore appear essential that in order to maximise survival and enhance quality of life in CF, the initial priority in nutritional research must be to identify the cause(s) of negative energy balance and malnutrition, in order to assist in the design of improved nutritional intervention.

1.5.0 Energy Balance In CF

Energy balance can be expressed by the following equation:

$$\Delta E = (E_i) - (TEE + E_l + E_d)$$

where E denotes energy balance, E_i is energy intake, TEE is total energy expenditure, E_l is energy losses, and E_d is energy deposited.

The challenge to maintenance of energy balance in CF presents itself as an unknown combination of reduced energy intake, elevated energy expenditure (resting metabolic rate, RMR and total energy expenditure, TEE), and impaired digestive and absorptive function resulting in excessively high energy losses. In order to investigate the problem it is necessary to consider all the components of the energy balance equation outlined above. This in turn will make it possible to establish the relative contribution of each of these components to the development of malnutrition in CF. In other words, which of the components of energy

balance are clinically significant in CF, to what degree they change as the disease progresses, and when they change. Since it is well established that growth and nutritional status become more compromised as CF progresses, and more specifically as lung disease itself progresses, the components of energy balance must also change progressively in such a way that negative energy balance becomes more likely.

1.5.1 Energy Intake In CF

The dietary goal of 120% - 150% of the estimated average requirement (EAR) for energy, in CF (Roy *et al* 1984; Littlewood & MacDonald 1987; Reilly *et al* 1997), has been perceived clinically as appropriate, although no studies have been carried out to verify the suitability of this target within CF (Reilly *et al* 1997). As noted above, evidence exists to suggest that children with CF rarely meet the current recommendation for energy intake, (Daniels *et al* 1987), which constitutes the specific aim in nutritional management of patients with CF. Many patients with CF fall short of this target even when clinically infection free, and severe lung disease is absent (Kawchak *et al* 1996; Morrison *et al* 1994.; Tomezsko *et al* 1992; Stark *et al* 1996; Wootton *et al* 1991). However, in some studies intakes have been reported to meet the CF-specific recommendations, (Salamoni *et al* 1996; Ramsey *et al* 1992).

Morrison *et al* (1994) reported that only nine out of fifty relatively well patients, (median age 7yrs), achieved > 120% RDA for energy. Energy intakes were determined using a 7 day weighed dietary intake method.

In 1991, Wootton *et al* found that the median E_i of the group of 30 children with CF examined when well, (median age 8.8yrs), only reached 92% of RDA for energy, even with regular dietetic input.

Tomezsko *et al* (1992) assessed 22 children with CF, (age 5-10yrs) who were clinically well, had mild lung disease, and who were pancreatic insufficient. A comparison was made with 23 healthy controls matched for age, gender and weight. The children with CF in this study had higher energy intakes than healthy peers, although energy intakes still fell below the CF nutritional recommendations.

Reilly *et al* (1997) cited that oesophagitis due to acid reflux, anorexia due to the respiratory disease itself (psychological suppression of appetite), and shortness of breath (dyspnoea), may all contribute somewhat, towards poor E_i. Energy intakes may be further compromised due to behaviourally related eating problems in CF. For example, Stark *et al* (1996) noted that the inclusion of an early behavioural intervention strategy within current treatment protocols, has been shown to help improve chronically poor eating habits, and nutritional status of children with CF. However, a fuller discussion of all these points is beyond the scope of this study (see Stark *et al* 1996; Stark *et al* 1995).

To summarise these studies, the E_i of children with CF appears not to achieve the current CF dietary recommendations, even when children are clinically well, and consuming, in some cases, greater quantities of energy than their healthy peers (Stark *et al* 1995). As well as the compromised E_i which occurs outwith periods of acute respiratory exacerbation, consideration must also be given to the likelihood of poor E_i during, and after such episodes.

1.5.2 Energy Expenditure In CF

Total energy expenditure (TEE) consists of three components: energy expended on physical activity; diet-induced thermogenesis (DIT); basal or resting metabolic rate (RMR). Figure 1a demonstrates each components contribution to TEE as a whole, in typical healthy children. The contribution of each of these separate components towards TEE might possibly be different in a child with CF, and might change within a child with CF as clinical status changes. Due to RMR

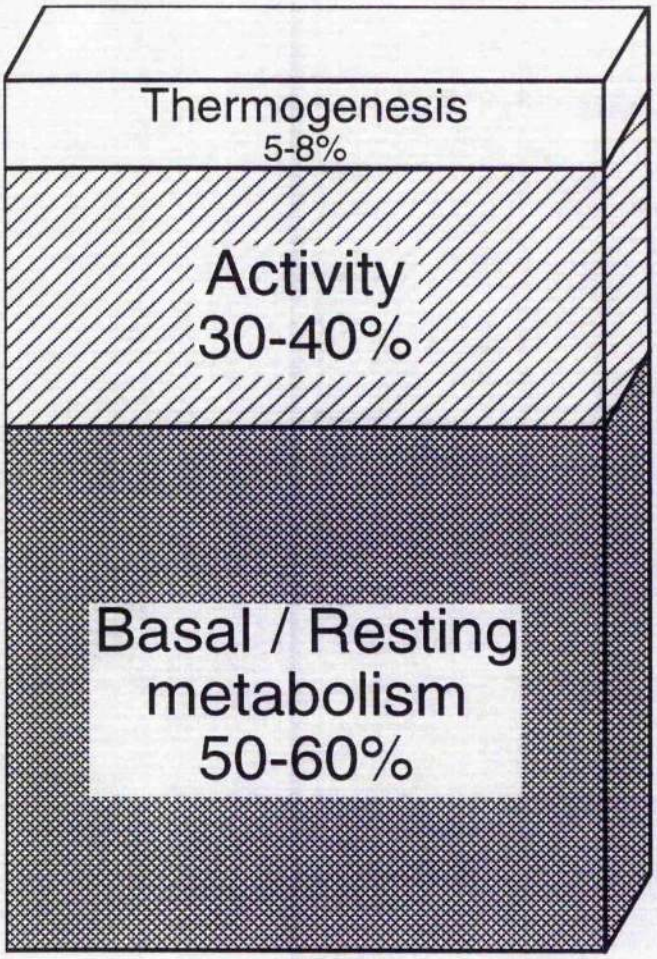


Figure 1a

Components of total energy expenditure in healthy children (taken from Davies et al 1991)

being easily measured, much research in CF has focused on measurement of RMR alone, and on the conventional meaning of hypermetabolism (that of increased RMR). Recently, TEE has become much more readily measured, due to the introduction of the doubly labelled water method (DLW). The measurement of TEE, the sum of all three components, is much more informative than simply measuring RMR. True hypermetabolism should be taken to mean an increased TEE (Reilly *et al* 1997). The conventional definition of hypermetabolism can be misleading, because in a disease in which RMR is increased, there can be some compensation by reduced physical activity, so that TEE is not increased, as in HIV infection, for example (MacAllan *et al* 1995).

Resting metabolic rate has been reported to be increased in patients with CF, relative to controls (Tomezsko *et al* 1994; Zemel *et al* 1996; Girardet *et al* 1994; Vaisman *et al* 1987a; Spicher *et al* 1991; Buchdahl *et al* 1988). The increase in RMR is typically 10-20% greater than predicted values, and patients with CF who have more severe lung disease have substantially raised RMR up to 40% above predicted values (Vaisman *et al* 1987a; Buchdahl *et al* 1988; Steinkamp *et al* 1989). An important point which has generated a great deal of attention to date is that of why RMR is increased in CF. More important, is the question of whether raised RMR is relevant in terms of causing increased TEE, and therefore a demand for greater energy intake (Reilly *et al* 1997), but this question has received less attention.

There has been suggestion of a possible link between the main gene mutation in CF and elevated RMR (O'Rawe *et al* 1990; O'Rawe *et al* 1992), together with a suggestion of an associated energy requiring mechanism based at a cellular level (Feigal & Shapiro 1979; Shepherd *et al* 1988). However Durie & Pencharz (1992) made the criticism that such studies failed to control for nutritional status or lung function, or adjust RMR for body composition in CF patients of different genotype and phenotype. This argument was further supported by research carried out in presymptomatic infants with CF (Bronstein *et al* 1995), and asymptomatic children with CF who have mild lung disease (Spicher *et al* 1991), where no

difference in RMR was found according to genotype when confounding variables were considered. Fried *et al* (1991) observed similar results in a study of patients with CF who had mild to moderate lung disease. Thus, it must be stated that the role of the primary genetic defect on hypermetabolism in CF is still unclear, but it probably does not make a major contribution to energy imbalance.

There are a number of likely mechanisms which may contribute towards an elevation in RMR in CF. It has frequently been postulated that it might arise from the increased work of breathing at rest experienced by patients with lung disease (Schols *et al* 1991; Donahoe *et al* 1989). However, work by Sridhar *et al* (1994), demonstrated that an elevation in RMR did not parallel the increased work of breathing, and that this was unlikely to explain the development of malnutrition in lung disease patients alone.

It is now appropriate to briefly consider what other causes may be involved in the increased RMR in CF. The relationship between elevated RMR in CF, and deteriorating lung function is one which must be given consideration. Fried *et al* (1991) documented in a study of males with CF (aged 7 - 39 yrs), that raised RMR strongly correlated with severity of lung disease. Resting metabolic rate was shown to rise significantly (125% (SD 14)), in those patients with advanced lung disease (FEV_1 35% to <70%), when compared to those with milder respiratory involvement (FEV_1 > 75%), who showed smaller increases in RMR. Resting metabolic rate rose in a curvilinear manner when predicted FEV_1 fell below 75% (Figure 1b). As noted earlier, a close relationship exists between changes in pulmonary function (FEV_1) over time, and nutritional status in CF (Zemel *et al* 1996). Thus it may be possible to continue good nutritional status until such times as more severe lung disease disturbs energy balance (Corey *et al* 1988). However it is currently unclear why lung function should be related to nutritional status in CF. One possibility lies in increased RMR, but there is a potential role for elevated cytokines, perhaps directly causing deterioration in nutritional status in patients with CF, either

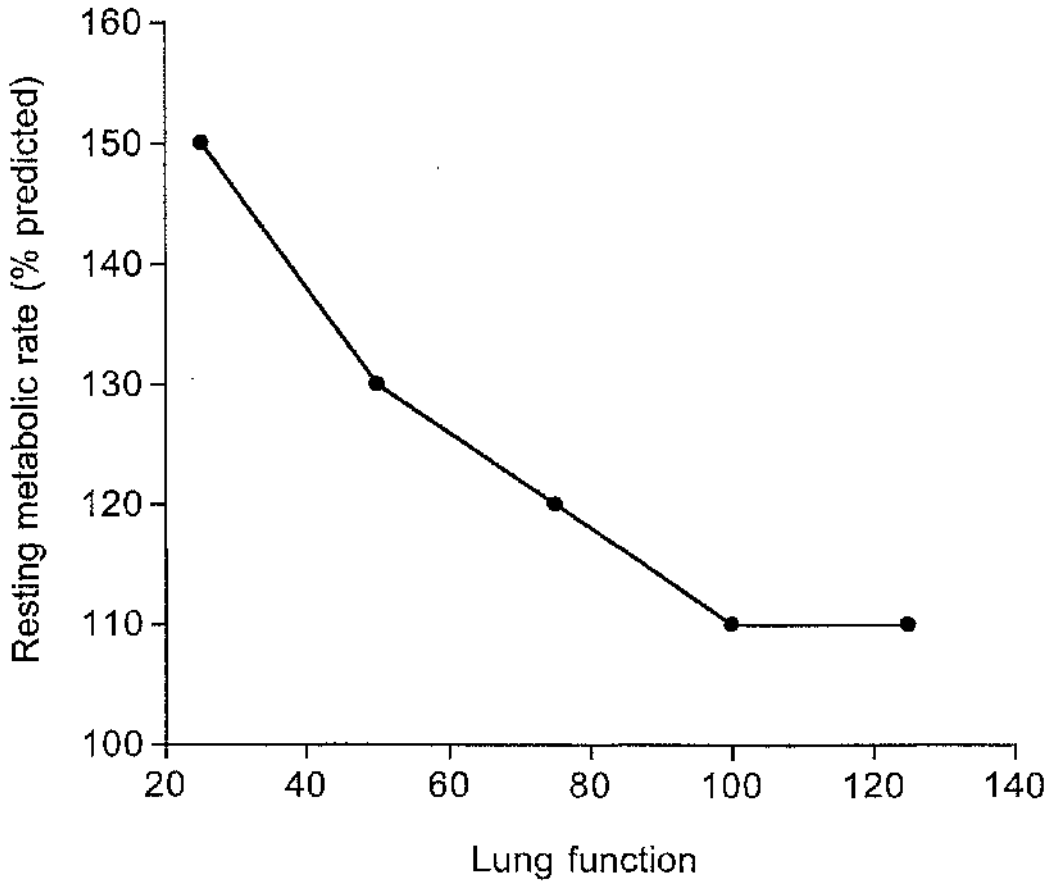


Figure 1b

Percent predicted resting metabolic rate, versus lung function (% predicted FEV_1) in normally nourished males with CF (adapted from Fried et al 1991).

by mediating muscle wasting (as in cancer cachexia), and/or by mediating anorexia (Stuart Elborn *et al* 1993; Reilly *et al* 1997).

At present, there are only four studies which have described total energy expenditure (TEE) in CF, three used the Doubly Labelled Water (DLW) technique (Shepherd *et al* 1988; Tomezsko *et al* 1994; Bronstein *et al* 1995), and were carried out in 9 asymptomatic infants with CF who have no evidence of chronic lung disease (Shepherd *et al* 1988), 25 clinically well subjects with CF (Tomezsko *et al* 1994), and 19 presymptomatic infants with CF (Bronstein *et al* 1995).

Shepherd *et al* (1988) investigated TEE and its role in disordered energy balance in CF. The group with CF, compared with the control data (16 retrospective controls matched for age and weight), had a 25% greater TEE. However, it was subsequently suggested by Durie & Pencharz (1992), that with further evaluation of more subjects, any differences between the two groups vanished. Certain aspects of the methodology used by Shepherd *et al* (1988) limited the results. Firstly, retrospective controls were used from a previous study matched only for age and body weight. No match was made for gender, or for body composition, between subjects, and controls. Insufficient numbers of subjects with CF were measured, in relation to the control numbers. Thus, this study provided no conclusive evidence that TEE was raised in asymptomatic infants with CF.

In 1994, Tomezsko *et al* found that both RMR and TEE were greater in those patients studied with CF, than in 25 age, gender and weight matched control children, and that TEE was related to CF genotype. Results showed that TEE was 15 - 18% greater than controls, (expressed as kcal/kg body weight), but this increase was found to be greater than the 6 - 9 % increase accounted for by RMR alone. Higher total energy expenditure (TEE) was only demonstrated in subjects who were homozygous for the ΔF508 deletion. Heterozygous subjects had TEE similar to controls (Tomezsko *et al* 1994). This could be regarded as evidence of hypermetabolism in clinically well children with CF. However, the explanation may simply be

that the children with CF were able to undertake unlimited physical activity due to the clinically well nature of the group. Definitively demonstrating increased TEE is not straightforward, and requires more evidence in CF (Reilly *et al* 1997).

Bronstein *et al* (1995) found no significant difference in TEE between 19 presymptomatic infants with CF, and controls through the use of the DLW technique. Spicher *et al* (1991), found similar results, using heart rate monitoring to estimate TEE, in 13 patients with CF aged 8 - 24 yrs, who were clinically stable. Here the subjects seemed to 'compensate' for any elevation in RMR by reducing physical activity. A comparison was made with 13 healthy subjects, matched for age, gender, height and weight, to provide control data.

In summary, there would appear to be no firm evidence of true hypermetabolism (i.e increased TEE) in children with CF at present. However, the studies which have measured total energy expenditure in CF concentrated on asymptomatic subjects, or infants and children who were clinically well. It is conceivable that TEE might increase during certain periods, such as acute exacerbations for example (Reilly *et al* 1997).

1.5.3 Faecal Energy Losses In CF

The energy content of stools are thought to be derived from 3 main constituents: maldigested and malabsorbed dietary residue; endogenous secretions and cellular debris (e.g. mucopolysaccharides); colonic bacterial microflora. The relative contribution of each of these components varies with dietary intake (Murphy *et al* 1991).

In CF, steatorrhoea, a condition characterised by stools which contain excess fat, as a result of the diminished pancreatic function, provides an indication of the degree of malabsorption/maldigestion occurring.

Habitual pancreatic replacement therapy is the mainstay of treatment, primarily aimed at controlling symptoms. If pancreatic hypofunction remains untreated, as much as 80% of dietary energy (in the form of fat), can be lost in stools (Reilly *et al* 1997). As noted earlier (p 8), even with regular enzyme therapy, malabsorption may not be completely eradicated (Murphy *et al* 1991). Thus, faecal losses are an important component of energy balance in the child with CF.

In 1991, Murphy *et al* reported that in their group of 20 children with CF (mean age 11.4 yrs), who were clinically well and on regular pancreatic replacement therapy, that stool energy losses averaged 10.6% (range 4.9 - 19.7%) of gross E_i. Stool lipid losses averaged 9.9g/day (SD 0.2) in the patients with CF, compared with 2.2g/day (SD 1.2) in healthy controls. However, some CF subjects lost up to 20g of lipid/day in the stool (equivalent to approximately 160kcal/day), which was approximately three times more than that of healthy children. Gross energy intakes of the healthy control group 9460kJ/day (SD 290), and the CF group studied 9930kJ/day (SD 790), were found to be very similar (Murphy *et al* 1991).

At this point it would seem appropriate to briefly look at the contribution of the other components of the energy balance equation. Unfortunately, no evidence is currently available on diet induced thermogenesis in CF. However, as Figure 1a demonstrated, this component contributes the least amount to TEE as a whole, and may therefore be of minor significance.

1.6.0 Energy Balance During Acute Exacerbations In CF

Maintenance of a positive energy balance in CF is widely believed to be seriously challenged during acute respiratory exacerbations. The overall effect of these may be cumulative in nature (Reilly *et al* 1997). As age and lung disease advances, and the patient with CF experiences more frequent acute respiratory episodes, then these adverse effects may become more

pronounced, causing positive energy balance to be more difficult to achieve/maintain. For the child with CF who is younger, has mild disease, good lung function and remains in reasonably good health, it might be easier to compensate for such challenges, provided energy intake is sufficient, and reasonably good growth and nutritional status can be sustained (Durie & Pencharz 1992), but the relevant importance of acute exacerbations in younger versus older patients is unclear at present.

To date, little attention has been paid to this specific area, and information on each individual component of the energy balance equation and its contribution towards energy balance during acute exacerbations in CF, is not available. Currently only three studies have been published which attempted to examine the effects of acute exacerbations in patients with CF (Naon *et al* 1993; Steinkamp *et al* 1993; Neijens *et al* 1985). However, these studies considered the effects of respiratory exacerbations in a limited way. Two of the studies measured only the effects of acute exacerbation on energy intake (E_i), and RMR. Naon *et al* (1993) recruited 13 patients with CF aged 2 - 26 years admitted for a mean hospital stay of 15 days; Steinkamp *et al* (1993) recruited 29 patients with CF aged 5 - 27 years, who were admitted for 15 days for i.v. anti-pseudomonal therapy. Total energy expenditure (TEE) and faecal energy losses were not measured in these studies. Neijens and colleagues (1985), concentrated solely on the influence of respiratory exacerbation on lung function variables (FEV_1 - % of predicted) and nutritional status (body weight and height) in CF, with no measurement of energy balance components. Furthermore, Naon *et al* (1993), Steinkamp *et al* (1993) and Neijens *et al* (1985) studied patients with relatively severe lung disease. Naon *et al* (1993) and Steinkamp *et al* (1993) also carried out measurements during hospitalisation for treatment of an acute exacerbation. In modern clinical management of CF, patients are usually treated at home during an acute exacerbation. These differences may be of potential relevance to energy balance during an acute exacerbation. Neijens and colleagues (1985) failed to state whether the patients with CF in the study were measured within the hospital setting, or at home, but it is likely that they were treated in hospital.

Some clinical evidence exists to support the view that weight loss can occur during acute exacerbations in CF. A study conducted by Shepherd *et al* (1988) over a fourteen month period, investigated the effects of short-term nutritional supplementation during treatment for acute pulmonary exacerbation in CF (15 patients with CF, aged 7 - 17 years of age who presented with acute respiratory exacerbation). Results suggested that short-term nutritional support can reduce the adverse effects of recurrent respiratory exacerbations, and reverse the weight loss during these episodes.

During an acute exacerbation, the literature suggests that energy intake is significantly lower (Naon *et al* 1993; Steinkamp *et al* 1993). Naon *et al* (1993) speculated that faecal energy losses may improve (reduce) during an acute exacerbation - no mechanism was suggested. However, Reilly *et al* (1997) suggested that faecal losses might be elevated during an acute exacerbation as a result of impaired colonic salvage of energy secondary to antibiotic therapy. There may also be a temporary increase (20% - 30%) in RMR during an acute exacerbation (Naon *et al* 1993; Steinkamp *et al* 1993; Reilly *et al* 1997; Fried *et al* 1991). Having summarised the possible effects of an acute exacerbation in CF, it is now appropriate to examine the evidence in this area in more detail.

1.6.1 Energy Intake During Acute Respiratory Exacerbation In CF

It has been shown that during and after acute respiratory infections, adult patients typically experience negative energy balance, weight loss, and periodic decline in nutritional status, mainly caused by inadequate E_i during acute illness (e.g. Klipstein-Grobusch *et al* 1995). The detailed processes that are involved in producing periods of malnutrition/anorexia during bouts of infection are, as yet, not fully understood.

As noted above, currently only two studies exist which have investigated energy intake during an acute respiratory exacerbation in CF. Steinkamp *et al* (1993) studied twenty nine patients

with CF, (19 male, 10 female, aged 5-27 years), with moderate to severe lung disease, who were hospitalised for a period of 2 weeks for treatment of acute respiratory exacerbation by means of i.v. antipseudomonal therapy. Energy intakes were recorded using 3 day prospective weighed dietary records, which were collected during the initial, and final three days of hospital stay only. These methods were similar to those employed by Naon et al (1993). Steinkamp *et al* observed a slight increase in daily energy intake towards the end of antipseudomonal treatment, (9573kJ (SD 2786kJ) at the start of treatment, rising to 10652kJ (SD 2259kJ) at the end of treatment). The slight rise in daily energy intake however did not account for an end of treatment mean weight gain of 1.7kg, nor did the changes observed in RMR provide an explanation. Within this study no attempt was made to compare daily energy intakes with recommended values for age and gender. Therefore, even though energy intake seemed to show a rise, no indication was provided of what the data represented in terms of either the recommended dietary guidelines for energy intake, or the current CF dietary recommendations. There was also no measure of daily energy intake during a period when the patients were defined 'clinically infection free' (e.g. post hospitalisation), to allow a more objective comparison of trends in energy intake to be made between a period of acute exacerbation and a 'clinically free of infection' phase (Steinkamp *et al* 1993).

In the same year, Naon *et al* (1993) assessed the energy intake of 13 patients with CF (mean age 11 years), by means of two, three day diet diaries (household measures technique). This was carried out at the beginning and end of a 15 day hospitalisation period for acute respiratory exacerbation. Energy intake was found to increase by 8% from baseline to post treatment (15 days). However, as with the Steinkamp study (Steinkamp *et al* 1993), no comparison was made with either the recommended energy intakes for age and gender, or the current CF dietary recommendations, nor were any energy intake data gathered relating to a period when the child was free of an acute respiratory episode. Therefore both these sets of results must be interpreted with caution as there are the following doubts about the energy intake data presented: there was no indication of the degree of increase in energy intake, and because

reference data were absent, it was difficult to interpret whether the levels of energy intake reported were high or low. The limitations of the two existing studies of energy balance during acute exacerbation will be discussed in a later Chapter (Section 4.0).

Recently the role of cytokines, and the inflammatory episodes occurring during acute infection, have received attention in relation to their role in malnutrition. It has been hypothesised that malnutrition, and weight loss in emphysema and chronic obstructive pulmonary disease (COPD) for example, may be cytokine mediated (Sridhar *et al* 1996; Sridhar 1995). These studies suggest that acute periods of respiratory illness may result in negative energy balance as a result of anorexia, and it is conceivable that this is also true in CF. Tumour necrosis factor - α (TNF - α) is known to function as a mediator in host inflammatory response, and raised circulating levels of this have been found to occur in patients with CF during chronic Pseudomonal aeruginosa infection (Stuart Elborn *et al* 1993). TNF - α levels reduce after anti-pseudomonal therapy, although levels still remain increased during infection free periods. (Norman *et al* 1991).

1.6.2 Resting Metabolic Rate During Acute Respiratory Exacerbation In CF

Only two published studies have reported RMR in CF during an acute respiratory exacerbation (Naon *et al* 1993; Steinkamp *et al* 1993). These two studies reported substantial increases in RMR (20-25%) in *some*, but not all of the patients studied, during the first few days of the exacerbation. Naon *et al* (1993) studied 13 patients (10 female, 3 male; mean age 11 yrs, range 2 - 26 yrs), during a 15 day hospital admission for acute respiratory exacerbation. Resting metabolic rate was measured twice over a period of 15 days, until discharge. During this period there was a significant reduction in RMR from admission (186 kJ/kg/day (SD 378)), to discharge (141 kJ/kg/day (SD 35)). Values for RMR, and RMR per kg lean body mass showed similar changes during this time. However no comparison was made with any literature values, therefore it was difficult to interpret exactly how high RMR was initially, and then

subsequently what it then fell to in terms of a reference population. Naon and colleagues noted that the changes observed in RMR paralleled improvement in the patients clinical status, and the amount of change observed was proportionate to the clinical parameters measured (acute clinical score, ACS; absolute neutophil count, ANC; respiratory rate, RR & maximal inspiratory pressure, MIP). From this it was suggested that acute respiratory infection may be linked with a significant rise in the metabolic needs of the patient. No indication was given as to whether RMR fell in all the subjects studied, by the end of the treatment period, or the number of patients who did not show changes in RMR during the study.

Steinkamp and colleagues (1993) studied 29 patients with CF during acute exacerbation (19 male, 10 female; aged 5 - 27 yrs). During the study eleven of these patients received regular prophylactic intravenous antibiotics, and eighteen patients experienced exacerbation of respiratory symptoms (increased cough/sputum and loss of appetite). Pre-antipseudomonal treatment the mean RMR for the group was 6462kJ/day (SD 1091), (119.0% (SD 14.4) of the predicted value) and by the end of therapy RMR had declined to 6218kJ/day (SD 1191), (112.8% (SD 11.5) of the predicted value). This represented a significant difference from initial values ($p<0.05$). The initial elevation of RMR was not present in all the patients studied. A cut-off point of 115% of predicted RMR was used by the author and, using this, 17 patients had raised RMR during the study period, while 12 had a 'normal RMR' (comparisons were made with the WHO predicted values). Clinical status improved post therapy with significantly better values for the clinical parameters measured (acute clinical score, ACS; respiratory rate, RR; and vital capacity). Steinkamp *et al* (1993) also noted that inflammatory indexes such as erythrocyte sedimentation rate (ESR), leukocyte count and C-reactive protein were significantly related to elevated RMR, and suggested that acute inflammation in lung disease may play an important role in determination of metabolic requirements. The elevations in RMR for both studies occurred initially prior to the start of antipseudomonal therapy. Resting metabolic rate remained high for the first 7 - 10 days hospitalisation and therapy, and eventually

declined towards the end of the hospitalisation period (15 days) (Naon *et al* 1993 ; Steinkamp *et al* 1993).

On the basis of these results Naon *et al* (1993) and Steinkamp *et al* (1993) suggested that for some patients at least, increased RMR contributed to negative energy balance during acute exacerbation.

1.6.3 Total Energy Expenditure During Acute Respiratory Exacerbation In CF

If hypermetabolism was to be a causal factor in the process of malnutrition, and subsequent poor growth, then TEE itself must be elevated. It may be the case that TEE may increase (or decrease) during episodes of acute exacerbation in CF.

As noted above, it is essential that measurement of this component (TEE) is made if we are to begin to understand the relationship between acute exacerbations and energy balance as a whole.

In patients with HIV infection, weight loss is episodic, and has a tendency to occur during bouts of acute 'AIDS-defining secondary infection' (MacAllan *et al* 1995). This process may have relevance for the study of acute exacerbations in CF. In HIV infection, raised RMR associated with the primary infection, (Grunfeld 1995; MacAllan *et al* 1995), was considered by some to be the main factor contributing to negative energy balance. More recent research has now shown that the episodes of acute infection cause weight loss as a result of anorexia (Grunfeld 1995). Poor E_i, and not increased energy expenditure, in HIV infection was the principal cause of weight loss during secondary infections (MacAllan *et al* 1995). MacAllan and colleagues (1995) noted that TEE was much lower (not higher) than expected in HIV infected patients (in comparison to the values for normal healthy patients). In many diseases weight loss might therefore occur when total energy expenditure (TEE) is low, not high. In the

MacAllan *et al* (1995) study, subjects who maintained weight had normal total energy expenditure (TEE), and energy intake. Reduction in TEE during secondary infection was probably due to reduced physical activity, but reduced physical activity did not adequately 'compensate' for reduced energy intake, and negative energy balance occurred in the weight losing patient. Evidence also exists to suggest that a decline in physical activity may occur in CF (Spicher *et al* 1991).

A very similar mechanism may conceivably be involved in patients with CF during acute respiratory exacerbations. Here, RMR may be significantly raised, with a reduction in physical activity. Investigation of TEE during acute respiratory exacerbations in CF would help resolve the uncertainty surrounding the question of which component of energy balance changes significantly during an acute exacerbation. To date, no studies of TEE during acute exacerbations have been reported.

1.6.4 Energy Losses During Acute Respiratory Exacerbation In CF

So far studies of energy losses in the stool in CF have only concentrated on periods when the child with CF was well, and research on faecal energy losses during acute respiratory exacerbations is lacking. Naon *et al* (1993) speculated about faecal energy losses being reduced during an exacerbation, and Reilly *et al* (1997) speculated, from reviews of current literature, that faecal losses may possibly be elevated.

Colonic bacterial microflora ferment carbohydrate food components which avoid digestion and absorption in the small intestine. These processes create short chain fatty acids (SCFA's). Normally SCFA production by this process will yield approximately 7% of daily energy (McNeil 1978; Cummings 1981). Due to the malabsorptive processes which take place in the CF small intestine, the level of carbohydrate, and fat, supplied to the colon is increased despite pancreatic replacement therapy. Under normal circumstances, the bacterial microflora present

within the colon should be able to recover much of the carbohydrate, and produce energy from this. Edwards *et al* (1986) stated that administration of prophylactic antibiotics, and use of large doses for acute infection, may decrease the potential of the microflora to carry out the carbohydrate fermentation. In turn this has been shown to cause an increased faecal output in healthy adults (Rao *et al* 1988), and heighten the possibility of diarrhoea linked with increased antibiotic usage (Vaisman *et al* 1992).

To date there have been very few studies which have looked at colonic fermentation in children with CF during a period of increased antibiotic usage. One small study by Parrett *et al* (1996), observed the *in vitro* fermentation capacity of a group of eight CF children, compared with eight healthy, age matched controls. Results demonstrated that the children with CF had reduced capacity of their colonic bacteria to ferment carbohydrates. Theoretically, a case could be put forward to suggest that during a period of acute respiratory exacerbation in CF, when increased doses of antibiotics are administered, fermentation capacity is poor, thus causing a reduction in the energy available from SCFA's (Reilly *et al* 1997).

To conclude the review of current literature on the effects of acute exacerbation on energy balance in CF, it would appear that there are still a number of important gaps in present knowledge. Research must therefore concentrate on: measurement of total energy expenditure (TEE) and faecal energy losses (E_l) (or a suitable proxy for E_l such as faecal fat losses) during treated acute respiratory exacerbation in children with CF; measurement of energy intake (E_i) and TEE in subjects with CF who have more severe lung disease. So far, no research has measured the effects of a treated acute pulmonary exacerbation on all the components of energy balance, (energy intake, E_i; total energy expenditure, TEE; resting metabolic rate RMR, and faecal energy losses, E_l), simultaneously in the same patient. As noted earlier, much of the research on CF has tended to concentrate on children with CF who are clinically infection free, with only one or two of the components of energy balance (usually E_i or RMR) being

investigated, which has provided a very incomplete picture of why negative energy balance occurs in CF.

Through the individual measurement of each of the components of energy balance in HIV infection, the cause of negative energy balance has been identified (MacAllan *et al* 1995). In view of the favourable results of this approach, these methods can be applied to the study of acute exacerbation in CF, to allow understanding of the clinical events which lead to poor nutritional status.

The present study was intended to identify and quantify the effects of a treated acute exacerbation on energy balance, through investigation of all the components of the energy balance equation, and to identify the causes of any negative energy balance observed (i.e. changes in intake, expenditure, or losses) during acute respiratory exacerbations. Thus the main aims within this study were: (i) to describe energy balance in a group of children with CF during a treated acute respiratory exacerbation, and (ii) to measure the effects of a treated acute exacerbation on each of the components of the energy balance equation. This was carried out through the comparison of a series of measurements made in the same child when well and during an acute exacerbation.

As has been shown in CF, and other diseases, malnutrition can result in a number of adverse effects, so an enhanced knowledge of what causes negative energy balance in CF could mean improved prognosis and quality of life (through the development of specifically targeted clinical treatment), for this patient group.

2.0 Methods

2.1.0 Cystic Fibrosis Sample Background

The study was conducted at the Royal Hospital for Sick Children (RHSC) in Glasgow, which is the largest paediatric centre in Scotland. Children who attend the RHSC for treatment are aged 0 - 15 years (Yorkhill NHS Trust Annual Report and Statement of Accounts. 1995 - 1996).

At the time of the study, the Cystic Fibrosis (CF) population for the hospital stood at 110 - 120 patients. In 1995, the UK had a CF population of 6300, with 587 of these patients resident in Scotland. The RHSC is the second largest CF centre in the United Kingdom. The gender distribution of the current UK population was 53% male, and 47% female in 1995 (UK CF Survey Report 1995).

2.1.1 Socio-Economic Status Of The Sample

Socio-economic status was determined using the Carstairs Scoring System (CSS) for Scotland (Carstairs & Morris 1991). This deprivation scoring system is based on the classification of postcodes on a scale of 1 - 7, where 1 is the most affluent, and 7 is the most deprived. The scale is based upon the values of an unweighted combination of four standardised census variables. Those variables are as follows: male unemployment; car ownership; overcrowded housing; social class.

This method of assessing the socio-economic status of the sample, was used as it was relevant to Scotland, and is currently widely used and accepted in Scotland. The alternative Registrar

Generals 1 - 5 scale uses occupation only as the standard to measure social class, and is now regarded by some as being out of date (Carstairs & Morris 1991).

Figure 2a (see page 32), represents the distribution of socio-economic status of the CF sample recruited. Although there would appear to be a fairly even spread throughout the categories, slightly more of the sample were in deprivation categories 4 -7 (from more deprived areas).

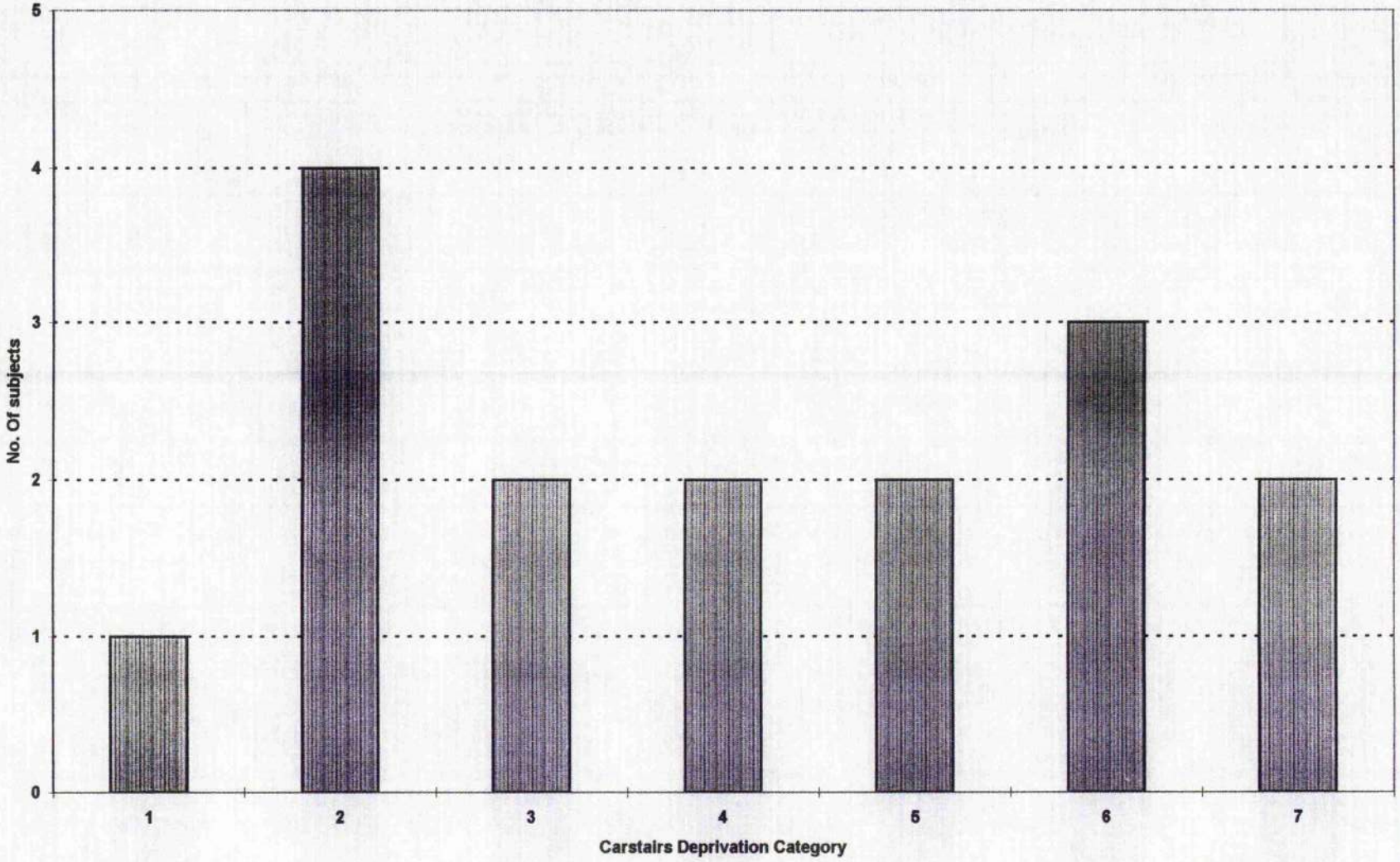
2.1.2 Genotype Of The Sample

The genotype of each subject was obtained from the annual review documents, in the medical notes. Recruits were then allocated one of the following genotype categories : ΔF508 / ΔF508 (homozygous for ΔF508) ; Δ F508 / Other (heterozygous for ΔF508) ; Other / Other. The 5 most common gene mutations occurring in the CF Trust 1995 Survey database are: Δ F508, G551D, G542X, 621 + 1 (G>T), 1898 + 1 (G>A). The incidence of the different CF genotypes within Scotland was discussed in the Section 1.0.

2.2.0 Experimental Design

The RHSC CF Team provided a list of suitable patients with CF, who met the requirements for participation. Entry criteria were as follows: amenable patients who could cope with the experimental protocol; aged 5 - 15 yrs; pancreatic insufficient; confirmed diagnosis of CF (sweat test). For inclusion as patients experiencing exacerbations, patients had to show evidence of increased respiratory symptoms, (e.g. increased cough, increased sputum volume,

Figure 2a - Distribution Of Socioeconomic Status In CF Sample



weight loss, wheeze) (Rabin *et al* 1997) as defined by the attending clinician, and treated using i.v. antibiotic therapy as a direct result of these symptoms.

Exclusion criteria were as follows: children under 5 years old; oxygen dependent patients; diabetic children; children on Total Parenteral Nutrition (TPN); families with a recent history of severe social and/or psychological difficulties.

Letters which detailed:

(i) what the study investigated

(ii) why the study was being carried out

(iii) what specific aspects were being looked at, and why

(iv) what participation would involve for both the child, and his/her family,

were mailed to all prospective recruits, to raise the awareness of the research and facilitate recruitment (See Appendix 5.1 & 5.1a). Contact was made with those families at regular clinic visits, prior to any recruitment.

Initially, it was decided that only patients with CF admitted to the RHSC for the 'standard' fourteen day intravenous (i.v) antimicrobial treatment, with a diagnosis of acute pulmonary exacerbation and moderate to severe lung disease, would be deemed eligible for inclusion in the study. However, over an initial recruitment period of three and a half months, it became apparent that insufficient patients with CF were presenting who met the entry criteria. Over a recruitment period of three and a half months, 9 routine CF clinics were attended, (136 patients in total were seen at those clinics), and from those clinics only two patients were recruited.

Routine clinics occurred once a week, and patients attended at regular intervals (e.g. once a month) according to their well-being, for frequent review. Six weekly emergency CF clinics were also attended during this initial period (24 - 30 patients seen at these clinics), and from those clinics only one patient was recruited over the same time period. Patients attended the emergency clinic if respiratory symptoms showed a deterioration and required medical attention immediately, (i.e outwith their routine clinic appointments).

In order to overcome this setback, the study design was altered slightly to also include those patients who received regular prophylactic i.v. antimicrobial therapy in order to reduce lung disease. Due to the regular nature of clinical treatment, within the latter patient group, availability for recruitment was more favourable and predictable. Patients with CF in this 'regular' group were recruited when they presented with increased respiratory symptoms requiring antibiotic therapy in advance of their regular clinic appointment for prophylactic antimicrobial treatment. In this thesis a distinction is made between these patients (referred to as the regular i.v. therapy group), and patients presenting with more 'classical' acute exacerbations (referred to as the standard i.v. therapy group). Comparisons of respiratory and other symptoms between the two groups are provided later in this thesis. In the RHSC CF Unit, a fourteen day i.v. antibiotic therapy was standard clinical practice for the treatment of acute respiratory exacerbation.

The guardians of suitable subjects were approached at the CF outpatient clinic (Monday morning Emergency clinic, or Wednesday morning routine CF clinic) regarding participation. Photographs of equipment and measurement techniques were used to provide a full description of the protocol. Informed written consent was obtained from each subject's parents, or

guardians, prior to beginning measurements. Approval was granted by the Ethics Committee of the Royal Hospital for Sick Children, for the study design and protocol.

Subjects were then studied for 12 -14 days, during a time when they could be described as clinically stable and infection free ('well' phase). It was initially intended that a minimum period of one month would be allowed to elapse between completing the exacerbation phase and commencing the 'well' phase of the plan, to ensure that subjects were clinically stable during the 'well' phase, and had recovered from the exacerbation. However in subjects 2, 5 and 13 (symptomatic subjects) a slightly greater period of time elapsed due to those subjects being asymptomatic for longer than one month.

However, these criteria had to be flexible, in order to fit in with the patients clinical routine. As a result, often more than a month elapsed between phases. In nine out of the sixteen subjects, measurements were started during a 'well' infection free phase, due to the timing of regular i.v. therapy, rather than measuring the child during an exacerbation phase first. Clinical data was consulted to monitor each patient's symptoms, and to guide measurements during both phases of the protocol. During the entire 2 x 2 week protocol, it was intended that each subject would act as his/her own control, and was expected to undergo the full programme of measurements in both phases.

2.3.0 Subjects And Study Protocol

To begin with it was hoped that each patient with CF would be studied, in the hospital setting, for a period of 12 - 14 days, during treatment for acute respiratory symptoms. However, since the initial design of the study, and the start of data collection, there was a marked trend towards home i.v treatment of respiratory exacerbations in patients with CF. In 1995, the total number of patients with CF treated by home i.v. therapy at RHSC was 49 (68%), with 23 (32%) being treated within the hospital setting (CF Nurses Data, January 1996). However, by the time the study began, hospital i.v. therapy was only used for those patients whose families could not have coped with home therapy, and the majority of patients were treated using home i.v. therapy. The CF Team were responsible for deciding the suitability of a patient, and his/her family, for home i.v. treatment. This decision was made after careful consideration of aspects such as the social, and psychological circumstances, which pertain to each individual case.

The protocol was then redesigned to (i) accommodate those patients on more regular i.v. regimens, so long as they presented with increased respiratory symptoms, and (ii) allow for the fact that a large number of patients with CF at the RHSC are now treated by means of home i.v. therapy. Therefore, home measurements had to be introduced into the design (See Appendix 5.2).

2.4.0 Techniques

2.4.1 Energy Intakes

Energy intake was assessed by means of dietary records, using standard household measurements (Gibson, 1990) (See Appendix 5.3) together with supplementary information collected by means of a 'dietary history' on completion of the food diaries in each phase (MacDonald 1996). Although this method of dietary assessment has been perceived as having inaccuracies (the accuracy and precision of this method of dietary assessment will be discussed in Section 4.0), it was felt that, in terms of the daily routine of the patients, the intensive nature of the measurement protocol, and subject compliance, that the methods employed gave as accurate and useful a guide to energy intake, as was practical (Sawaya *et al* 1996).

In 1996, a brief pilot study involving weighed dietary intakes in 4 patients with Acute Lymphoblastic Leukaemia, of similar age to the CF sample, revealed under-reporting, thought to be attributable in part to non-compliance with the weighing protocol (Ventham & Ralston, unpublished data; Livingstone *et al* 1990; Livingstone *et al* 1992). In addition, subjects and their families who took part in the pilot study reported that the weighing of food presented a substantial burden in addition to all the other measurements being made. Thus, as a result, the household measures method with dietary recall was used within the CF study, for the reasons stated earlier. From a practical view point, it was felt that the practical advantages of the method outweighed the disadvantages.

Each subject's parent was requested to document everything the child ate and drank for a period of 5 - 7 days alternately, during the exacerbation phase and 3 consecutive days when in the 'well' phase (including a weekend day). A slightly longer period of recorded food intake was employed in the exacerbation phase, as it was anticipated that food intake might vary more, as a result of clinical instability (see Section 4.0). To determine whether overall energy intake was reduced during the exacerbation period, and for how long this occurred, more frequent dietary records were requested (Goldstein *et al* 1987; Steinkamp *et al* 1993; Naon *et al* 1993; Chase *et al* 1979). Parents, and subjects received both verbal and written, comprehensive instructions/guidelines for collecting the data. A list of acceptable household measures was also provided (See Appendix 5.4).

Emphasis was placed on the child consuming his/her usual diet during the recording periods, with normal daily routine, and eating habits to remain unaltered. All foods and beverages were estimated in the edible form, and each item then recorded in grams by the parent. A full description of all foods taken was documented. Any left-over items were also noted by the subject's parent.

To ensure preservation of normal eating routine, foods consumed outwith the home were described as accurately as possible, and quantities estimated as instructed. Regular telephone contact was maintained with each subject during this time. Once each subject's record was complete, a detailed verbal dietary recall was carried out to ensure that all the required information had been collected and recorded.

Using the Food Portion Sizes Booklet (MAFF 1994), along with comprehensive, supplementary retail data, the weight of each food item, and any left-over food items, was determined. The total daily energy and nutrient intakes were calculated using the Comp-Eat 4.0 software package (Nutrition Systems, London, England), and the food tables by Paul & Southgate (1994). This method involved standard corrections for metabolisable energy, and this will be discussed further in Section 4.0.

From the analysis of each subject's food records, the following data were obtained for the exacerbation, and the 'well' phase, respectively: total mean energy intake (there was no evidence of systematic changes in E_i over time during either study period (repeated measures anova, calculated by Dr J. J. Reilly), thus the mean daily E_i was calculated from the 5-7 day food record during the acute exacerbation, and the 3 day food record during the 'well' phase, for each subject respectively, and this figure was then used as a summary measure for the energy balance determinations); mean % energy from protein, fat, and carbohydrate; mean energy intake in kJ/kg of body weight.

2.4.2 Co-efficient Of Fat Absorption

Standard methods were employed to determine the faecal fat content of CF stool samples, by means of Hydroxide Saponification & Petrolieum Ether Extraction (Van der Kamer *et al* 1949). In the study faecal fat output was used as a proxy for faecal energy losses, on the grounds that fat contributes most to the energy content of the stool (Murphy *et al* 1991), and measuring fat output was easier (practically) than measuring faecal energy output.

In order to determine the faecal fat content of the CF faeces, all stools were collected over a period of 3 days during the exacerbation, and 3 days during the 'well' phase. In certain subjects only a 2 day stool collection was possible, due to either non-compliance, or disturbance of normal bowel habit (constipation). The stools were collected into polythene bags, within commode collection pots, which could be sealed. Parents and subjects were provided with full written and verbal instructions to assist the collection.

Each batch of 2 -3 day stools were pooled, and the wet weight obtained (Oxford Series Scales, Oxford, England). The lipid content of the 2 - 3 day wet stool sample was determined by means of a modification of the method of Van der Kamer *et al* (1949). Values were noted in grams and mmols per day (See Appendix 5.5 for full modified method). The basic principle involved neutral fats (glycerides) being saponified with alkali, to yield glycerol and soaps; fatty acids were liberated from the soaps with acid extrapolated, and titrated against standard alkali. Values of > 17mmol lipid or 5 g / day are regarded as abnormal, in the CF literature, and by the hospital Biochemistry Department.

In order to take account of any alterations which may occur in dietary fat intake, changes in faecal fat output were standardised using the coefficient of fat absorption (CFA = (dietary fat intake in g - faecal fat in g) / dietary fat intake in g (Tomezsko *et al* 1992). This was achieved through the simultaneous measurement of fat intake from the dietary records, and the fat output from the 2 - 3 day stool collections. A CFA of > 0.93 is regarded as indicating good pancreatic enzyme replacement, and can be achieved by CF patients (Ramsey *et al* 1992).

2.4.3 Energy Expenditure : Resting Metabolic Rate

Resting metabolic rate (RMR) was determined by open-circuit indirect calorimetry (Deltatrac Metabolic Monitor, DATEX Instruments Corporation, Helsinki. Finland). Indirect calorimetry was carried out using this piece of equipment, which measured oxygen consumption (VO_2), and carbon dioxide production (VCO_2). From this was calculated (by Haldane transformation algorithm) the respiratory quotient ($RQ = VCO_2 / VO_2$), and resting metabolic rate (RMR).

To ensure the accuracy of all data, the gas sensors in the Deltatrac were calibrated prior to each measurement, using a calibration gas mixture (Datex Instrumentarium Corp., Helsinki. Finland), and alcohol burning used periodically as a check on the respiratory quotient.

A clear, perspex ventilated hood was placed over the head and shoulders of the subject, while quietly resting. Oxygen consumption and carbon dioxide production were measured in the supine position, over a 15 - 20 minute period, after an overnight fast, or 5 hour fasting period, for those subjects who would not tolerate an overnight fast. An initial 5 minutes or so was allocated to allow for environmental adjustment, or equilibration, by the subject, and were eliminated from the calculations. Measurements were taken every minute, until a steady state was reached (period of stable minute - minute measurements of RMR), for a minimum of 10-15 minutes (Steinkamp *et al* 1993; Naon *et al* 1993; Fried *et al* 1991). However, the period over which 0_2 consumption, and CO_2 production were measured, differs between many of the papers on resting metabolic rate in children (between 5 - 60 minutes). For the purpose of our study, the expired air collection time for the steady state was 10-15 minutes. This collection period was determined according to standard procedures carried out in our laboratory, documented by

Reilly *et al* (1996b) and Ventham & Reilly (1998). These are more practical for children than the classical conditions under which RMR has been measured in adults, and the coefficient of variation (CV) within subjects is <3% (Ventham & Reilly 1998). Fifteen minutes of steady-state sampling was found to be the maximum length of time acceptable to the majority of the children, after which a steady-state proved more difficult to maintain, and the quality of results decreased (i.e. the coefficient of variation (CV) of minute - minute measurements tended to increase). RMR was calculated as the mean of the minute - minute measurements after the steady state was achieved. None of the patients were allowed to fall asleep during the measurement period, and any gross movements that may have contributed to energy expenditure were prevented, by the author.

For each variable measured, mean standard deviation and coefficient of variation (CV) was calculated from the data, and this was used as a guide for data quality. A within measurement CV for minute to minute variability in RMR of >8% was deemed unacceptable, and data were excluded from the final analysis. The reproducibility of RMR measurement will be discussed fully in Section 4.0.

Care was taken to ensure that no routine bronchodilator medications (β_2-agonists) were administered during the fasting period, until the procedure was complete. Vaisman *et al* (1987b), found that inhaled Salbutamol, (a selective β_2- adrenergic agonist), temporarily increased RMR in CF patients from the U.S.A, although the exact mechanism of action (at that time), was unknown. Later, Naon *et al* (1993), stated that Salbutamol was not relevant to producing raised RMR, due to the much lower dose currently used in Europe. For the purpose of this study, however, it was decided that it was better to avoid use of inhaled β_2-adrenergic

agonists prior to any RMR measurement. However, in the case of routine i.v antibiotic therapy, no specification was made, although if possible the measurement was carried out prior to administration of i.v. antibiotic treatment for acute exacerbation. The frequency of measurements was different for both phases. Two were carried out during a 'well' phase, and 4 - 6 measurements during the exacerbation. In light of the findings of both the Naon *et al* (1993), and the Steinkamp *et al* (1993) papers, it was initially thought that during the exacerbation, RMR measurements should be carried out on a frequent basis. This was due to the instability of RMR observed in *some* patients during the initial stages of a pulmonary exacerbation, found to be present in those two studies (discussed in section 1.6.2). However in the present study, after measurements were carried out on 5 - 6 subjects, RMR was not found to be as unstable in nature, as was first thought, during the respiratory exacerbation. Thus, the frequency of RMR measurements was decreased to approximately 4 - 5, during the exacerbation. This point will be considered more fully in Section 4.0. There was no evidence of systematic changes in RMR over time during the exacerbation (repeated measures anova, calculated by Dr J. J. Reilly), so for each subject the mean RMR for each period was calculated and used as a summary measure in the energy balance determinations.

The RMR was measured, rather than basal metabolic rate (BMR), due to the following factors: for a BMR measurement, subjects should be measured in the postabsorptive state (no food eaten for at least 12 hours before measurements commenced). This was not always practical, within this study, and a minimum fast of 4 hours was agreed with the children. For a BMR measurement, subjects should be fully rested prior to any measurement (usually 30 minutes rest is used in adults), which again is usually not practical in children, especially in a clinical setting.

2.4.4 Prediction Of Resting Metabolic Rate

RMR for each subject was compared with the two prediction equations from Schofield (1985), one used weight (Scho-wt), and the other used weight and height (Scho-wtht). These estimates were converted to kJ using : 1kcal = 4.184kJ.

Schofield, weight only (kJ/d)

Males	3 - 10 yrs	22.7 W + 2071
	10 - 18 yrs	17.5 W + 2724
Females	3 - 10 yrs	22.5 W + 2088
	10- 18 yrs	12.2 W + 3121

Schofield, weight & height (kJ/d)

Males	3-10yrs	19.59W + 130.3H + 1736
	10-18yrs	16.25W + 137.2H + 2157
Females	3-10yrs	16.969W + 161.8H + 1327
	10-18yrs	8.365W + 465.0H + 837

Where W = weight; H = height.

To ensure clarity of results and presentation, only the Schofield-height weight equation is presented throughout this thesis, due to the predicted values derived from both Schofield equations proving very similar.

2.4.5 Energy Expenditure: Total Energy Expenditure

Doubly Labelled Water Technique

Total free-living energy expenditure (TEE), was determined using the doubly labelled water technique (DLW - $^{2}H_{2}$ ^{18}O), and was measured over a 14 day period, employing the two-point method. Briefly, the technique involved enriching the subject's body water with isotopes of hydrogen (^{2}H), and of oxygen (^{18}O), and then the wash-out kinetics of each isotope was established, as the concentrations fell exponentially towards the levels of natural abundance (Schoeller & van Santen 1982). The accuracy of the DLW method has been reported as 1-3%, with the precision of the technique between 2-8% in adults. (Schoeller *et al* 1986; Johnson & Halliday 1993; IDECG 1990; Prentice 1995). The doubly labelled water method is widely regarded as the only accurate means of measuring the TEE of individual children (Jones *et al* 1987; Jones *et al* 1988; Murgatroyd *et al* 1993)

Dose Preparation and Dosing Procedure

The dose was calculated from each individual subject's total body weight (kg). Subjects were dosed with 0.06ml/kg $^{2}H_{2}O$ (99.8% deuterium), and1.6ml/kg H_{2} ^{18}O, body weight (10% ^{18}O).

The reason for this dose ratio was to minimise the effect of changes in background levels of ^{18}O and ^{2}H in the body water (IDECG 1990).

Isotopes were delivered to a medical flat (150ml glass bottle: Gallenkamp UK), via a micropore filter. Each bottle was shaken, labelled appropriately, and autoclaved to sterilise the dose. The dose was then cooled to room temperature, and 2 x 1.5 aliquots were pipetted into bijou pots. The bottle was then weighed and refrigerated until required.

Prior to dose administration in each subject, a baseline sample of urine was collected, to enable determination of natural isotope abundance levels. Each subject ingested the whole dose using a straw, the procedure being abandoned if any dose was spilled. Bottle/dose, straw and bag were re-weighed to determine the actual dose consumed (to the nearest 0.01g).

Each subject was supplied with labelled Universal tubes (x3), inside a resealable plastic box. Due to the two-point methodology being used, subjects were requested to collect two 'spot' urine samples (not the first one of the morning) at the following set times (at least 10ml): one day post dose (day 1); two weeks post dose (day 14).

An extra urine sample was also collected at the midpoint (day 7), solely as a back up, however this sample was not analysed. Multipoint sampling was impractical, and the analyses would have been extremely expensive, thus the two point sampling method was more suitable for use in this study. The time of sample collection was documented by the subjects. All urine samples were stored in their box, in the freezer until collection by the investigator. The samples were

then thawed, and sub-divided into duplicate 4ml bijou containers (See Appendix 5.6 for description of dose preparation and administration protocol).

Urine isotope enrichments were measured commercially using isotope-ratio mass spectrometry (which measures absolute isotope ratios) at the Bureau of Stable Isotope Analysis (BSIA, Middlesex, England), following the analytical procedures described by Johnson & Halliday (1993).

Doubly Labelled Water Equations

To determine total energy expenditure (TEE), the calculation proposed and validated by Schoeller *et al* (1986; A6), was used. In this study 9 healthy male subjects were dosed, (6 subjects receiving a low isotopic dose 2.78g $^{2}H_2O$ 10.28g $H_2{}^{18}O$, and 3 subjects a moderate dose 5.5g $^{2}H_2O$ 13.0g $H_2{}^{18}O$) and saliva/urine samples were collected in accordance with the two-point method of sample collection. The doubly labelled water method was found to be valid when compared with near-continuous respiratory gas exchange, and the two-point method of sampling was adequate. The Schoeller equation (A6) provided the best agreement with CO_2 production rates measured by respiratory gas exchange methods, without any reduction in precision, relative to multi-point sampling methods (Wellc 1990).

A detailed description of the calculation of TEE is given in Appendix 5.7.

Physical Activity Level

For each subject, Physical Activity Level (PAL), was calculated as the ratio of total daily energy expenditure to the mean measured resting metabolic rate (RMR). This is a description of the subject's usual daily activity (Department of Health 1991).

2.5.0 Body Composition Estimation

2.5.1 Bioelectrical Impedance

Body composition was estimated using the bioelectrical impedance (BIA) method. The Bodystat 1500 monitoring unit (50kHz, 800 mAmp, Bodystat Limited, Isle of Man) was used to measure bioelectrical impedance in all subjects. This technique is based upon the principle that the electrical conductivity of the fat free mass (FFM), is far greater than that of the fat mass (FM). The accuracy and precision of bioelectrical impedance has been reported as acceptable in adults (Lukaski *et al* 1986; Lukaski *et al* 1985; Coward *et al* 1988), and in children (Ventham & Reilly 1998). Azcue *et al* (1993), stated that in CF BIA can be used to achieve a reliable measurement of TBW, as well as FFM.

One major issue which influences the accuracy of estimates of body composition is the population specificity of prediction equations. Reilly *et al* (1996a) concluded from the validation study carried out (using underwater weighing as the reference method) in 98 pre-pubertal children (mean age 9.0 yrs), that the Houtkooper equation (Houtkooper *et al* 1992)

for BIA predicted fat free mass (FFM), with the least bias and acceptable accuracy in Scottish children. The Houtkooper equation was intended for 10 - 14 year olds, but is valid in younger Glaswegian children (Reilly *et al* 1996a).

Spicher *et al* (1993), and Borowitz & Conboy (1994), supported the use of BIA in the estimation of fat free mass (FFM) in patients with CF. Thirty nine subjects with CF, and 39 healthy controls, aged 6 - 24 yrs were studied. Therefore, the clinical characteristics of CF should not rule out the use of the impedance technique.

Body composition was estimated after an overnight fast, or the 4 hour fasting period used for RMR measurements. This measurement was carried out in conjunction with the RMR measurement, to standardise the technique used. Detailed descriptions of the measurement technique are given in Appendix 5.8.

The formulae used to estimate FFM were as follows:

$$\text{FFM (kg)} = 0.61\ (\text{RI}) + 0.25\ (\text{Wt, kg}) + 1.31$$

$$\text{Where RI} = \text{Ht}^2\ (\text{cm}^2) \ /\ \text{Resistance (ohms)}$$

(Houtkooper *et al* 1992)

$$\text{FM (kg)} = \text{Wt (kg)} - \text{FFM (kg)}$$

$$\%\ \text{BodyFat} = \text{FM (kg)} \ /\ \text{Wt (kg)} \times 100$$

2.5.2 Skinfold Thickness

Skinfold thickness measurements were made by the one trained investigator (the author), at 2 sites in female subjects (triceps and subscapular), and 4 sites in male subjects (biceps, triceps, subscapular, and suprailliac). Each measurement was made to the nearest mm, using calibrated calipers (Holtain Ltd, Crymych, Dyfed, UK), with the subject in the standing position. In 1995, Reilly *et al* found that the equation chosen to predict body fatness can exert a large effect on the skinfold estimate obtained (population specificity). Ninety eight, healthy prepubertal children (64 boys, 34 girls) of mean age 9.1 yrs, were used to test the validity of 5 prediction equations, through the comparison of estimates from each of the 5 published prediction equations for skinfolds, with measurements of body fatness measured from hydrodensiometry (Reilly *et al* 1995). Within this validation study, the equations of Slaughter *et al* (1988) for girls, and Brook (1971) for boys, were recommended, as they predicted body fatness with negligible bias; other published equations were associated with substantial bias.

The Slaughter *et al* (1988) equation for girls is as follows:

Girls % fat = 1.33 (sum 2 skinfolds; mm) - 0.013 (sum 2 skinfolds^2; mm) - 2.5

This equation gives body fat directly. However in boys, the Brook (1971) equation works by predicting body density. In the boys, body density was therefore estimated in the following 2 steps :

Predicted density (kg/l) = 1.1690 - 0.0788 x (log sum 4 skinfolds; mm)

% fat from body density in prepubertal children (Lohman 1989), taking account of lower density of FFM in childhood.

Boys % fat = (5.30 / density - 4.89) x 100

The Brook equation (1971) employed all 4 skinfold sites, and the Slaughter equation (Slaughter *et al* 1988) employed measurement of triceps and subscapular skinfolds only.

At the start of the study, CF specific equations for the prediction of body fatness were considered. The equations of Johnston *et al* (1988) were found to underestimate body fatness and predicted unrealistically low estimates of body fatness, (e.g. a predicted density of greater than 1.1kg/l, which typically gave rise to estimates less than 0% body fat).

A standard skinfold measurement technique was used (Gibson 1990), and all measurements were repeated three times at each site, on the left hand side of the body. However, occasionally the right hand side had to be used in subjects, due to the fixed position of the i.v line, (mid upper arm) used for administration of antibiotic therapy during treatment of acute respiratory exacerbation in CF. The thumb and index finger elevate the skinfold, the calipers were applied at the marked point. Further details of these measurements are given in Appendix 5.9.

2.6.0 Anthropometric Assessment Of Nutritional Status

Anthropometric data were collected from each subject using standard methods (Gibson 1990). Height and weight are the two most commonly recorded anthropometric variables. They are reasonable indicators of nutritional status and growth (Gordon *et al* 1991).

Standing height was measured to the nearest 0.1 cm (Gordon *et al* 1991), using either a portable stadiometer (Child Growth Foundation, London), or fixed wall mounted Holtain stadiometer in the outpatient clinic. The subject was either barefoot, or wore thin socks. Each child was positioned with both feet flat on the base plate, heels together touching the vertical board of the stadiometer, or an upright wall, shoulders relaxed, and arms at sides. Care was taken to ensure that scapulae, buttocks and the posterior aspect of the cranium were touching the vertical board where possible. The head was held so that the subject looked straight forward, in the Frankfort plane. The moveable board was brought down to rest on the subject's head, with sufficient pressure to compress the hair. Throughout this procedure, constant checks by the investigator were required to ensure a fully erect position was maintained. Subjects body weight was measured in kilograms using digital scales, to the nearest 0.5kg, (Salter UK, 12kg - 136kg), in the standing position. All subjects wore light indoor clothing, and shoes were removed.

Body Mass Index (BMI), weight (kg) / height (m^2), was determined for each subject. BMI provides an objective index of 'protein - energy status' which adjusts weight for height, and is useful for assessing both under and overnutrition (Gibson 1990)

Standard deviation (SD) scores were calculated relative to reference data, from the measurements obtained for height and weight (Freeman *et al* 1995), BMI (Cole *et al* 1995), triceps and subscapular skinfolds (Davies *et al* 1993), in each subject, for both 'well' and exacerbation phases. The SD score can be applied to individual subject measurements, or to group/population data, and is a widely accepted statistic in nutritional assessment (Gibson 1990).

In all subjects, mid arm circumference (MAC) was measured in the standing position using standard methods (Gibson 1990) with arms hanging freely by the sides. The mid-point of the upper arm had been marked for the biceps and triceps measurements. The tape (Rabone Chesterman, Miniflex 6ft, England), was positioned horizontally around the circumference of the mid upper arm, at the marked mid-point. This measurement was employed to provide an index of body fatness, and fat free mass (FFM); useful in the clinical setting for assessing nutritional status (Gibson 1990)

Mid arm muscle circumference (MAMC) was also employed to describe muscle mass, and hence nutritional status. MAMC was derived from the measurements obtained from both MAC, and triceps skinfold thickness as follows:

$$\text{MAMC (mm)} = \text{MAC (mm)} - (3.14 \times \text{TSF mm})$$

This is a measure of the circumference of the inner circle of muscle mass, around a small central core of bone, and provides a more specific index of muscle mass in subjects (Gibson 1990).

Mid arm circumference (MAC) was also used to provide an indication of body fatness, and fat-free mass in subjects.

2.7.0 Clinical Data

2.7.1 Disease Status

For each subject, a range of clinical data were obtained to assist in the determination of overall disease severity/status. Standard, CF specific, methods were employed to obtain these data.

2.7.2 Schwachman Score

To gauge disease severity, the scoring system of Schwachman and Kulczycki (1958) was applied to each subject. The following four categories were assessed; 1. general activity; 2. physical examination; 3. nutritional status, and 4. X-ray findings. Each of these items was scored out of 25 points. The assessment and grading was carried out by the attending clinician. The status of a patient was regarded as 'excellent' if the score was over 85, 'good' if the score was between 71 and 85, 'mild' when between 56 and 70, 'moderate' between 41 and 55, and 'severe' when 40, or below (Schwachman and Kulczycki 1958). The Schwachman score was used to provide an indication of disease severity (Section 3.1.0, Table 1).

During the acute exacerbation it was also possible to collect data pertaining to the lung function, general condition, frequency of cough, and sputum volume/colour, of each subject, as determined by the attending clinician at examination. This was to form a more objective clinical picture, related to whether a subject was experiencing acute respiratory symptoms indicative of i.v antibiotic therapy.

2.7.3 Pulmonary Function

Pulmonary function was evaluated at the time of measurement in all patients recruited. Standard pulmonary function methods for spirometry, carried out by the RHSC Lung Function Laboratory, were used. Measurements included forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV_1). The latter were performed when each child was clinically stable ('well') and within the first two days of the acute exacerbation. Data were expressed as a percentage of reference values predicted for gender, age and height (Polgar & Promadhat 1971). In order to justify recruitment of a subject during an acute respiratory exacerbation, FEV_1 was used as one of the indicators of declining condition and respiratory function (Section 3.2.0, Table 2) and also of improved lung function during a the 'well' phase.

2.7.4 Bacteriology & X-Ray Data

Bacteriology, and x-ray data were also obtained at the time of a measurement period for each child. Bacteriology results were consulted at the time of the acute exacerbation to try to identify presence of signs indicative of infection; X-ray results were also examined in an attempt to determine whether there were any radiological changes consistent with active respiratory

infection (Section 3.2.0, Table 2 & Appendix 5.10), and similarly to ensure the absence of infection to allow 'well' measurements to commence.

Additional clinical data may have improved the definition of each measurement phase, but unfortunately it was not possible to collect any clinical data for c-reactive protein (CRP), leukocyte count, serum albumin concentrations, or a formal objective scoring of symptoms, as these data were not routinely collected at the RHSC.

2.8.0 Statistical Analysis

The Minitab (Release 10) statistical analysis package (Minitab Release 10 for Windows, 1994, Minitab Inc., U.S.A), was used.

As discussed in Sections 2.2.0 and 2.3.0, it was decided that a paired study design would be used, with each subject acting as his/her own control, since both 'well' and exacerbation data were available in the majority of subjects measured. However, due to a certain degree of non-compliance with experimental protocol (collection of faecal samples and urine collections), in one of the two study periods, loss of paired data occurred: it was not possible to obtain data from all children for all variables, during both phases. Therefore an unpaired analysis of the energy balance data was also carried out. Descriptive statistics (mean and standard deviation) are presented.

The mean paired difference for each energy balance variable between the 'well' and exacerbation phases were calculated, for each subject. The significance of paired differences was assessed using t-tests and 95% confidence intervals. From this it was also possible to obtain the p-value (this represents the probability of that result occurring by chance). A probability value of less than 0.05 was considered to represent statistical significance. Data were presented as kJ/kg/d for clarity of presentation, and comparability across patients of different age. As stated earlier (Sections 2.4.1 and 2.4.3) a repeated measures anova was carried out on the E_i and RMR data (by Dr John. J. Reilly) in order to justify the use of single summary measures for those variables in the energy balance determinations.

In one subject (no. 2) the treatment for the acute exacerbation included a prolonged period of enteral nutritional support which was not used when the child was 'well', and so for energy intake, subject no. 2 was excluded from the statistical comparison. All comparisons were expressed in the absence of subject 4 during the exacerbation phase because it became clear that this subject did not experience acute respiratory symptoms sufficient for inclusion within the acute exacerbation category, although a first growth of Pseudomonas aeruginosa was apparent, and the patient was treated with i.v. antibiotic therapy as for a typical acute exacerbation (Section 3.0).

3.0 Results

3.1.0 Clinical And Anthropometric Characteristics Of Subjects

The clinical and anthropometric characteristics of the subjects with CF (mean decimal age 9.9 years (SD 2.4)) studied are presented in Table 1. Seven subjects were categorised as 'standard' i.v. therapy, at the time of measurement, and had milder disease as indicated by better Schwachman scores (mean score 88 (SD 4)). Seven were within the category defined as 'regular' (received regular 2-3 monthly i.v prophylactic antibiotics), and had poorer Schwachman scores, (mean score 69 (SD 12)), which indicated more severe lung disease, but still mild - moderate disease status. Two subjects recruited (subjects 9 & 11) were measured when 'well' only, due to good health throughout the study period and inability to recruit them during an acute exacerbation. These have been included for presentation, but were not included in any of the statistical comparisons.

Overall, the group were reasonably well nourished (mean BMI SD score -0.31 (SD 0.79); (mean % body fat 21.8 (SD 5.6)), and had grown reasonably well (mean height SD score -0.54 (SD 1.08)), considering the fact they had Cystic Fibrosis. Also presented is the change in BMI SD score in the 12 months prior to the study (mean change in BMI SD score -0.01 (SD 0.61)), which showed that the group had managed to maintain a reasonably stable nutritional status, over the period of one year prior to the study. It would therefore appear that children with CF, and mild to moderate disease, have the ability to maintain adequate growth and nutritional status. This will be discussed in greater detail in Section 4.0.

Table 1 : Clinical And Anthropometric Characteristics Of Subjects.

Patient [1]	Age (years)	Category [2]	Genotype [3]	Carstairs [4] Score	Shwachman Score	BMI SD Score	BMI SDS [5] Difference	Height SD Score	% Body [6] Fat
1	9.1	S	2	2	90	-0.15	-1.33	-1.40	20.1
2	12.3	S	1	1	85	0.18	0.00	0.70	33.0
3	11.0	S	1	7	90	-1.32	-0.50	0.76	27.8
4	5.1	S	2	7	90	0.79	1.53	0.85	24.9
5	11.4	R	3	5	65	0.42	0.09	0.44	34.1
6	9.3	S	2	3	80	-0.98	-0.05	-0.56	17.0
7	9.3	S	1	2	90	0.26	0.05	-0.20	22.6
8	7.9	R	1	2	75	0.31	0.24	-1.60	15.9
9	8.2	W	1	6	90	-0.32	0.18	0.01	17.9
10	15.0	R	1	4	80	0.09	*	0.40	17.8
11	11.8	W	2	6	90	-1.48	-0.05	-0.40	22.4
12	10.8	R	1	3	45	-1.64	-0.13	-1.20	17.2
13	7.6	S	2	5	90	-0.54	-0.67	-1.20	18.4
14	8.9	R	1	2	85	0.66	0.04	-0.58	20.6
15	8.9	R	1	4	65	0.01	0.58	-2.98	22.7
16	12.5	R	2	6	65	-1.20	-0.13	-1.75	17.2
Mean	9.9				80	-0.31	-0.01	-0.54	21.8
SD	(2.4)				(13)	(0.79)	(0.61)	(1.08)	(5.6)

Footnotes :

[1] Patients 1 - 6 Female; 7 - 16 Male.

[2] Treatment category: S = 'standard' i.v.'s, R = 'regular' i.v.'s, W = measured when 'well' only.

[3] Genotype: 1= homozygous for Δ F508, 2= heterozygous, 3= other genotype.

[4] Carstairs Scoring System (CSS) for Scotland (Carstairs & Morris 1991). Deprivation scoring system used to provide an indication of socioeconomic status: 1= most affluent, to 7= most deprived.

[5] Change in BMI SDS calculated as, BMI SDS when 'well' minus BMI SDS value 12 months prior to commencing measurements.

[6] % body fat estimated by bioelectrical impedance.

3.2.0 Clinical Characteristics During Acute Exacerbation

Table 2 shows the clinical assessment of patient's symptoms, and lung function (% predicted FEV_1), at the time of acute exacerbation (see Appendix 5.10 for radiology results). The data shown in this table were routinely collected as part of each patient's clinical treatment, and were obtained from patient notes. The study did not interfere with any clinical treatment, and it must also be stated that any clinical decisions were made completely independently of the study itself.

The objective data in the table confirm a deterioration in lung function (mean paired difference in FEV_1 10% (SD 9; $p = 0.002$)) as a result of the acute exacerbation. The clinical assessments were made at the time of intravenous antibiotic therapy, and they confirm a general decline in condition, with increased cough frequency and volume of sputum. This was evident in children treated with 'regular' i.v. therapy, as well as those defined as receiving 'standard' i.v therapy (Table 2). Most of the subjects were treated with i.v antibiotics for the acute exacerbation within the home setting (all except numbers 2, 4, 5, 7, and 13), in contrast to previous research which studied subjects in hospital alone.

Subjects 4, 8 and 16 exhibited relatively stable % predicted FEV_1 values through both measurement periods. Subject 4 was treated for a first growth of Pseudomonas during the exacerbation phase, and was therefore effectively asymptomatic (i.e was not 'unwell' during the exacerbation phase): this accounts for the stable FEV_1 value. Analysis of all the paired differences between study periods was therefore carried out with the omission of subject 4. No

clinical evidence/explanation can be found as to why FEV_1 remained unchanged for subjects 8 and 16. In 'standard' i.v subjects (n=7), FEV_1 declined during the acute exacerbation in 6 of the subjects, with one remaining stable (subject 4, as explained above). In the group of 'regular' i.v subjects (n=7), FEV_1 declined in 4 of the subjects, in 2 subjects FEV_1 remained stable, (8 and 16, as explained above), and in 1 subject FEV_1 improved during the acute exacerbation (subject 5). In the case of subject 5 this apparent improvement in lung function during the acute exacerbation might be explained by the fact that she experienced recurrent respiratory infection over the 6 month period which followed the exacerbation, during which time 'well' measurements were attempted, and her poor clinical state may have been reflected in the 'well' measurements. However, in general clinical assessment (Table 2) tended to confirm a worsening of chest symptoms during the exacerbation in both groups, and to confirm the clinical impression of a deterioration, characteristic of the acute exacerbation.

The changes in body weight for the group during the 14 day period of the acute exacerbation, were small and not statistically significant (mean weight change +0.2kg over 2 weeks (SD 0.7)). Subjects were therefore able to maintain their weight relatively well during the acute exacerbation. Subject 7 showed a weight gain of +2.0kg during the exacerbation, and this is discussed in Section 4.0 of this thesis.

Overall, the data confirm that the subjects studied exhibited evidence of acute deterioration in symptoms during the acute respiratory exacerbation, and tend to justify the decision to treat them as such clinically.

Table 2 : Clinical Data During Acute Exacerbation

Patient	Treatment Location	FEV1 (% predicted)		Weight [2] Change (kg)	Condition [3]	Cough [4] Frequency	Sputum [5] Volume	Radiology [6]
		AE	'well'					
1	Home	80	83	+0.2	worse	+	+	+
2	Hosp.	51	67	+0.7	worse	+	+	+
3	Home	73	93	+0.2	worse	+	+	+
4 [7]	Hosp.	105	105	+0.7	similar	-	-	+
5	Hosp.	46	39	-0.3	worse	+	+	+
6	Home	67	78	-0.7	worse	+	+	-
7	Hosp.	71	82	+2.0	worse	+	+	+
8	Home	100	100	0.0	worse	+	+	-
9 [7]	N/A	*	87	*	*	*	*	*
10	Home	69	89	-0.5	worse	+	+	+
11 [7]	N/A	*	64	*	*	*	*	-
12	Home	33	44	0.0	worse	+	+	+
13	Hosp.	89	103	0.5	worse	+	+	-
14	Home	93	98	0.0	similar	+	+	-
15	Home	38	59	0.1	worse	+	+	-
16	Home	30	31	0.4	worse	+	+	-
Mean		64	74	+0.2				
SD		(23)	(24)	(0.7)				

Footnotes :

[1] Represents i.v. antibiotic treatment for acute exacerbation in hospital, or home setting during the study period.

[2] Body weight change during the acute exacerbation, calculated as day 14 minus day 1.

[3-6] Clinical assessments made at the time of antibiotic therapy (' + ' represents increased signs; ' - ' represents no change relative to the 'well' period; ' * ' represents data not applicable.

[7] Subjects 4, 9, & 11 omitted from calculations; the acute exacerbation phase for subject 4 was treatment for first growth of Pseudomonas, and patient was asymptomatic; patients 9 & 11 studied when 'well' only.

3.3.0 Changes In Body Composition And Nutritional Status During Acute Exacerbation

The changes in nutritional status during the acute respiratory exacerbation are presented in Table 3. The data shown in this table were specifically collected for the purposes of the study, and are not routinely collected for patients with CF at the RHSC. Data will be presented with subject 4 omitted, for the reasons given in Section 2.8.0.

Overall the group managed to maintain reasonably good nutritional status during the acute exacerbation, as suggested by the data presented pertaining to body weight during this period. The mean paired differences observed for fat free mass (FFM), % body fat and triceps skinfold were as follows : FFM (n=13) +1.2kg SD 2.6 (95% CI -0.3 to 2.8); % body fat (n=13) -1.1% SD 2.2 (95% CI -2.5 to 0.2); triceps skinfold (n=13) +0.6mm SD 1.8 (95% CI -0.5 to 1.7). These differences were not statistically significant. Only very slight differences were observed between day 1 to day 14 of the exacerbation.

In summary, there was no significant change apparent in the nutritional status of the group of subjects with CF when measured during the 14 day course of acute exacerbation.

Table 3 : Changes In Nutritional Status During Acute Exacerbation.

Patient	FFM (kg)[2]			% Body Fat[2]			Triceps (mm)		
	Day 1	Day 14	Difference[1]	Day 1	Day 14	Difference[1]	Day 1	Day 14	Difference[1]
1	19.1	18.9	-0.2	23.2	24.4	1.2	9.8	7.5	-2.3
2	29.5	32.5	3.0	34.4	29.0	-5.4	12.3	12.7	0.4
3	25.1	26.0	0.9	25.7	23.5	-2.2	8.4	9.4	1.0
4	15.1	15.3	0.2	24.1	24.3	0.2	9.5	9.6	0.1
5	27.2	26.0	-1.2	32.5	35.0	2.5	15.8	14.2	-1.6
6	20.2	20.2	0.0	21.2	19.2	-2.0	7.6	8.4	0.8
7	21.1	22.7	1.6	20.4	20.3	-0.1	5.3	5.4	0.1
8	10.5	19.5	9.0	14.9	15.2	0.3	4.5	5.5	1.0
9	*	*	*	*	*	*	*	*	*
10	48.5	49.8	1.3	18.5	15.5	-3.0	14.0	14.4	0.4
11	*	*	*	*	*	*	*	*	*
12	21.8	21.6	-0.2	15.5	16.2	0.7	5.2	6.5	1.3
13	19.3	18.8	-0.5	19.2	19.0	-0.2	5.7	5.8	0.1
14	23.1	23.1	0.0	19.0	19.0	0.0	5.2	11.0	5.8
15	16.8	17.4	0.6	21.8	19.2	-2.6	5.8	6.1	0.3
16	24.1	25.6	1.5	18.8	14.9	-3.9	3.5	3.8	0.3
Mean	23.0	24.8	1.2	22.0	20.8	-1.1	7.9	8.5	0.6
SD	(8.9)	(8.6)	(2.6)	(5.9)	(5.9)	(2.2)	(3.9)	(3.5)	(1.8)

Foootnotes:

[1] Difference is calculated as the day 14 measurement minus the day 1 measurement.

[2] Body composition was estimated by bioelectrical impedance.

3.4.0 CF Data Paired By Energy Balance Variable

Subject data paired by energy balance variable are shown in Table 4. Each variable is presented for both 'well' and acute exacerbation phases, and is expressed as kJ/kg/day. This format is used throughout Section 3.0. The paired difference between the two values is presented, together with the group mean data. Subjects 2 and 4 were omitted from paired comparisons where appropriate, as explained earlier in Section 2.8.0.

3.4.1 Energy Intake

Energy intake (E_i) (n=11) fell during the exacerbation period, mean paired difference was 47 kJ/kg/d (SD 49) (95% CI 14 to 80) $p < 0.05$. In four subjects, energy intake actually increased during the acute exacerbation. In one particular subject (subject 2), the increase was marked, and this was probably due to the fact that she was hospitalised during the exacerbation: she received substantial dietetic input while on the ward and additional enteral feeding (see Section 2.8.0) which was not given at home (and so was not included in the 'well' measurements).

Unpaired analyses of energy intake differences between the two phases indicated a trend towards reduced intakes during the exacerbation, but this did not reach statistical significance ($p = 0.09$).

Table 4 : Energy Balance Data (Mean & SD; kJ / kg / day).

Subject Code	Energy Intake			Total Energy Expenditure			Resting Metabolic Rate				Co-efficient of Fat Absorption (CFA)		
	'Well'	AE	Difference[1]	'Well'	AE	Difference[1]	'Well'	AE	Difference[1]	Predicted[2]	'Well'	AE	Difference
1	331	226	*105*	311	*	*	196	194	*2*	*164*	0.96	0.97	*-0.01*
2	164	253	*-89*	274	228	*46*	156	135	*21*	*149*	0.76	0.97	*-0.21*
3	195	194	*1*	237	270	*-33*	176	170	*6*	*119*	0.97	0.98	*-0.01*
4	436	471	*-35*	364	424	*-60*	224	224	*0*	*181*	0.98	*	*
5	251	261	*-10*	286	284	*2*	165	161	*4*	*124*	0.96	0.98	*-0.02*
6	382	264	*118*	250	242	*8*	157	174	*-17*	*166*	0.98	0.98	*0.00*
7	306	264	*42*	361	*	*	192	201	*-9*	*166*	0.86	0.92	*-0.06*
8	*	329	*	*	401	*	*	230	*	*180*	*	0.93	*
9	496	*	*	357	*	*	211	*	*	*179*	0.97	*	*
10	219	255	*-36*	*	196	*	138	122	*16*	*121*	0.98	0.97	*0.01*
11	333	*	*	333	*	*	169	*	*	*164*	0.98	*	*
12	431	406	*25*	294	274	*20*	218	221	*-3*	*181*	0.98	0.98	*0.00*
13	342	245	*97*	386	340	*46*	215	197	*18*	*195*	0.97	0.98	*-0.01*
14	330	267	*63*	*	307	*	165	205	*-40*	*166*	0.94	0.97	*-0.03*
15	409	353	*56*	*	*	*	206	219	*-13*	*192*	0.97	0.97	*0.00*
16	453	402	*51*	371	*	*	220	207	*13*	*167*	0.97	0.98	*-0.01*
Mean	344	289	*47*	314	282	*15*	184	187	*0*	*162*	0.95	0.97	*-0.03*
s.d.	(90)	(68)	*(49)*	(51)	(61)	*(30)*	(27)	(33)	*(18)*	*(24)*	(0.06)	(0.02)	*(0.06)*

Footnotes:

[1] Paired difference was calculated as 'well' minus AE, this difference is given in italics, (subject 4 excluded from all calculations and subject 2 excluded from the E_t comparisons).

[2] Predicted resting metabolic rate values calculated using the Schofield weight height equation, (Schofield 1985).

3.4.2 Total Energy Expenditure

Paired data for total energy expenditure (TEE) were only available for six subjects. There appeared to be a tendency for higher TEE when subjects were 'well', with a mean paired difference of 15 kJ/kg/d (SD 30); $p > 0.05$. This difference was not statistically significant (95% CI -17 to 46). In two subjects (3 and 4), there was evidence of a higher TEE during the acute exacerbation, but in subject 4 this may have reflected the absence of symptoms during the 'exacerbation' as explained earlier.

3.4.3 Resting Metabolic Rate

There appeared to be small and inconsistent differences in resting metabolic rate (RMR), (n=12), between 'well' and exacerbation phases. The mean paired difference for the 12 subjects was 0 kJ/kg/d (SD 18). Unpaired analysis of the complete data set, also suggested that changes in RMR during the acute exacerbation were small and statistically insignificant. Changes observed in RMR during the acute exacerbation were small, and likely to be related to measurement variability. The large increases in RMR observed during the acute exacerbation in *some* patients by other authors (Naon *et al* 1993; Steinkamp *et al* 1993), were not apparent here. Repeated measures anova (carried out by Dr John J. Reilly) on the daily RMR data showed no significant differences between days. Two subjects (2 and 6) had RMR values less than predicted. Within the present study, measured RMR exceeded predicted RMR by 19% on average, which was broadly in accordance with the degree of lung disease in the children studied (Fried *et al* 1991).

3.4.4 Co-efficient Of Fat Absorption

Co-efficient of fat absorption (CFA) tended to be lower when subjects were 'well'. Subject 4 was omitted from the analyses for reasons previously explained in Section 2.8.0. The mean paired difference (n=12) was -0.03 (SD 0.06), which was not statistically significant. The trend towards an improvement observed in CFA during the acute exacerbation was probably not of a clinically significant nature as the changes were probably too small to have any great effect on energy balance in CF.

Subjects 2 and 7 had very low CFA during the 'well' phase, and these improved substantially during the exacerbation. The latter may be explained by the fact that both subjects were suspected non-compliers with pancreatic enzyme prescription. The observed improvement during the exacerbation may be attributable to treatment of these subjects within the hospital setting during the acute exacerbation, where enzyme supplementation was supervised by nursing staff.

Overall the level of fat loss via the faecal route was not high, in comparison with either subjects with CF from the literature studied when 'well' (20 children with CF mean age 11.4 (SD 1.4), mean faecal fat output 9.9 g/d (SD 1.2); Murphy *et al* 1991), or subjects with CF when studied during an acute exacerbation (mean 6.7g/d); Parrett, unpublished data). However, any fat lost in CF faeces would be irretrievable energy for such children, thus energy available from dietary sources would be reduced.

3.5.0 Paired Energy Balance Data For Those Children With Complete Data For All Energy Balance Variables (n=6).

Table 5 shows the paired energy balance data for the six subjects in whom complete data were obtained in all variables during both measurement periods. All data are expressed as means (SD) in kJ/kg body weight /day. For reasons explained previously (Section 2.8.0), data will be described with and without subject 2, as appropriate. Subject 4 was omitted because she was asymptomatic during the 'exacerbation'.

3.5.1 Energy Intake

Energy intake (E_i) showed a decline from 'well' to exacerbation phases. If subject 2 was omitted the mean paired difference was 46kJ/kg/d (SD 58); $p > 0.05$. Subject 6 demonstrated a noticeable fall in Ei during the acute exacerbation phase (31%). Overall the data showed a trend towards a fall in Ei during the acute exacerbation (on omission of subject 2), (mean Ei 'well' 320kJ/kg/d (SD 96), mean Ei during the acute exacerbation 274kJ/kg/d (SD 79)).

Table 5 : Energy Balance Data For Patients With Complete Data (Mean & SD; kJ / kg / day).

Patient	Energy Intake			Total Energy Expenditure			Resting Metabolic Rate			Co-efficient of Fat Absorption (CFA)		
	'Well'	AE	Difference[1]	'Well'	AE	Difference	'Well'	AE	Difference[1]	'Well'	AE	Difference[1]
2	164	253	*-89*	274	228	*46*	156	135	*21*	0.76	0.97	*-0.21*
3	195	194	*1*	237	270	*-33*	176	170	*6*	0.97	0.98	*-0.01*
5	251	261	*-10*	286	284	*2*	165	161	*4*	0.96	0.98	*-0.02*
6	382	264	*118*	250	242	*8*	157	174	*-17*	0.98	0.98	*0.00*
12	431	406	*25*	294	274	*20*	218	221	*-3*	0.98	0.98	*0.00*
13	342	245	*97*	386	340	*46*	215	197	*18*	0.97	0.98	*-0.01*
Mean	320	274	*46*	288	273	*15*	181	176	*5*	0.94	0.98	*-0.04*
SD	(96)	(79)	*(58)*	(63)	(39)	*(30)*	(28)	(30)	*(14)*	(0.09)	(0.00)	*(0.08)*

Footnotes:

[1] Paired differences calculated as 'well' minus AE, given in italics.

3.5.2 Total Energy Expenditure

Total energy expenditure (TEE) tended to decline during the acute exacerbation (mean paired difference 15kJ/kg /d (SD 30); $p > 0.05$). In one subject (3), TEE was slightly higher during the acute exacerbation phase.

3.5.3 Resting Metabolic Rate

A small decline was observed in resting metabolic rate (RMR) during the acute exacerbation, (3%). The mean paired difference was 5kJ/kg/d (SD 14); $p > 0.05$. These changes were probably not clinically significant and did not reach statistical significance. Repeated measurement of RMR's during the acute exacerbation produced a between measurement coefficient of variation (CV) of 6% within the current study. This was similar to a recent study of reproducibility of RMR measurement in prepubertal girls (CV 6%), by Figueroa-Colon *et al* (1996). This will be discussed at greater length in Section 4.0.

3.5.4 Co-efficient Of Fat Absorption

As explained previously, the apparent improvement in CFA observed during the acute exacerbation indicated by Table 5 may be explained through better pancreatic enzyme compliance during this time in the less compliant subjects. Since subject 2 showed evidence of suspected non-compliance with pancreatic enzymes, analyses were carried out with and without her: it is clear from table 5 that any improvement in CFA during the exacerbation is due to the large change in this subject, and probably reflects the effect of hospitalisation on compliance

with pancreatic enzyme replacement therapy rather than the effect of the acute exacerbation on fat absorption.

3.6.0 Between Group Differences In The Effect Of An Acute Exacerbation On Energy Balance

Table 6a, 6b and 6c present a comparison of the subject data for each energy balance variable during the 'well' and the acute exacerbation phases, divided into subgroups of : 'regular' and 'standard' i.v antibiotic therapy; homozygous for ΔF508 and heterozygous for ΔF508; boys and girls, respectively. This was carried out in an attempt to examine whether the acute exacerbation might have had a different effect on energy balance in the different sub-groups examined. The data presented within the tables are merely descriptive in nature, and no statistical analysis was possible. This was due to the small size of the entire sample, and the small numbers which resulted once the group were split into sub-groups. The figures in brackets give the percentage change in each variable. Once again, for reasons previously explained (Section 2.8.0) data will be described for each subgroup without subjects 2 and 4 as appropriate.

Table 6a: Energy Balance Data In Subjects On 'Regular' And 'Standard' i.v. Antibiotic Therapy (Mean & SD; kJ / kg / day).

Subject	Energy Intake			Total Energy Expenditure			Resting Metabolic Rate			Co-efficient of Fat Absorption (CFA)		
	'Well'	AE	Difference[2]	'Well'	AE	Difference[2]	'Well'	AE	Difference[2]	'Well'	AE	Difference[2]
Regular[1]												
5	251	261	*-10*	286	284	*2*	165	161	*4*	0.96	0.98	*-0.02*
8	*	329	*	*	401	*	*	230	*	*	0.93	*
10	219	255	*-36*	*	196	*	138	122	*16*	0.98	0.97	*0.01*
11	333	*	*	333	*	*	169	*	*	0.98	*	*
12	431	406	*25*	294	274	*20*	218	221	*-3*	0.98	0.98	*0.00*
14	330	267	*63*	*	307	*	165	205	*-40*	0.94	0.97	*-0.03*
15	409	353	*56*	*	*	*	206	219	*-13*	0.97	0.97	*0.00*
16	453	402	*51*	371	*	*	220	207	*13*	0.97	0.98	*-0.01*
Mean	347	325	*25*	321	292	*11*	183	195	*-4*	0.97	0.97	*-0.01*
SD	(90)	(65)	*(40)*	(39)	(74)	*(13)*	(32)	(39)	*(21)*	(0.01)	(0.02)	*(0.01)*
Standard[1]												
1	331	226	*105*	311	*	*	196	194	*2*	0.96	0.97	*-0.01*
2	164	253	*-89*	274	228	*46*	156	135	*21*	0.76	0.97	*-0.21*
3	195	194	*1*	237	270	*-33*	176	170	*6*	0.97	0.98	*-0.01*
4	436	471	*-35*	364	424	*-60*	224	224	*0*	0.98	*	*
6	382	264	*118*	250	242	*8*	157	174	*-17*	0.98	0.98	*0.00*
7	306	264	*42*	361	*	*	192	201	*-9*	0.86	0.92	*-0.06*
9	496	*	*	357	*	*	211	*	*	0.97	*	*
13	342	245	*97*	386	340	*46*	215	197	*18*	0.97	0.98	*-0.01*
Mean	332	274	*73*	318	301	*17*	191	185	*4*	0.93	0.96	*-0.05*
SD	(112)	(90)	*(49)*	(58)	(81)	*(38)*	(26)	(28)	*(15)*	(0.06)	(0.02)	*(0.08)*

Footnotes:

[1] CF group divided into subgroups of 'regular' and 'standard' i.v.'s, in an attempt to examine whether the acute exacerbation may possibly have had different effects on energy balance.

[2] Paired differences calculated as 'well' minus AE phases of measurement, given in italics.

3.6.1 Differences Between Patients In The 'Regular' And 'Standard' Group

Table 6a shows that the 'regular' i.v group exhibited evidence of a decline in energy intake (E_i) (6%), and total energy expenditure (TEE) (9%) during the exacerbation, compared with the 'well' results for this subgroup. Resting metabolic rate (RMR), showed a slight increase during the exacerbation phase (7%), and CFA remained the same during both phases. As explained earlier in this section, this slight rise in RMR may be related simply to the small difference observed in repeated RMR measurements in children over a given period of time, and also due to the small sample size. Therefore the latter may not be attributable to a real effect of acute exacerbation. The 'standard' i.v group showed a larger decline in E_i (30%) during the acute exacerbation, with TEE showing a fall (13%) during the same period. RMR declined by 4%, during exacerbation, with CFA showing an improvement (4% on average).

Table 6b : Energy Balance Data In Subjects Homozygous And Heterozygous For Δ F508 (Mean & SD; kJ / kg / day).

Subject	Energy Intake			Total Energy Expenditure			Resting Metabolic Rate			Co-efficient of Fat Absorption (CFA)		
	'Well'	AE	Difference[2]	'Well'	AE	Difference[2]	'Well'	AE	Difference[2]	'Well'	AE	Difference[2]
Homozygous[1]												
2	164	253	*-89*	274	228	*46*	156	135	*21*	0.76	0.97	*-0.21*
3	195	194	*1*	237	270	*-33*	176	170	*6*	0.97	0.98	*-0.01*
7	306	264	*42*	361	*	*	192	201	*-9*	0.86	0.92	*-0.06*
8	*	329	*	*	401	*	*	230	*	*	0.93	*
9	496	*	*	357	*	*	211	*	*	0.97	*	*
10	219	255	*-36*	*	196	*	138	122	*16*	0.98	0.97	*0.01*
12	431	406	*25*	294	274	*20*	218	221	*-3*	0.98	0.98	*0.00*
14	330	267	*63*	*	307	*	165	205	*-40*	0.94	0.97	*-0.03*
15	409	353	*56*	*	*	*	206	219	*-13*	0.97	0.97	*0.00*
Mean	319	290	*25*	304	279	*11*	183	188	*-3*	0.93	0.96	*-0.04*
SD	(121)	(68)	*(37)*	(54)	(71)	*(40)*	(29)	(41)	*(21)*	(0.01)	(0.02)	*(0.08)*
Heterozygous[1]												
1	331	226	*105*	311	*	*	196	194	*2*	0.96	0.97	*-0.01*
4	436	471	*-35*	364	424	*-60*	224	224	*0*	0.98	*	*
5	251	261	*-10*	286	284	*2*	165	161	*4*	0.96	0.98	*-0.02*
6	382	264	*118*	250	242	*8*	157	174	*-17*	0.98	0.98	*0.00*
11	333	*	*	333	*	*	169	*	*	0.98	*	*
13	342	245	*97*	386	340	*46*	215	197	*18*	0.97	0.98	*-0.01*
16	453	402	*51*	371	*	*	220	207	*13*	0.97	0.98	*-0.01*
Mean	361	311	*72*	329	322	*19*	192	193	*4*	0.97	0.98	*-0.01*
SD	(69)	(100)	*(52)*	(49)	(79)	*(24)*	(28)	(23)	*(13)*	(0.01)	(0.00)	*(0.07)*

Footnotes:

[1] CF group divided into subgroups of those homozygous for Δ F508, and those subjects heterozygous, in an attempt to examine whether the acute exacerbation may have possibly had a different effect on energy balance.

[2] Paired differences calculated as 'well' minus AE phases, given in italics.

3.6.2 Differences In The Effect Of Acute Exacerbation Between Patients Homozygous And Heterozygous For ΔF508

The sub-group of homozygous and heterozygous subjects was also examined (Table 6b) to test whether the effects of the acute exacerbation might be affected by genotype differences. Similar trends could be seen within the homozygous subjects, and heterozygous subjects respectively. Overall in homozygous subjects, E_i appeared to decline during the exacerbation, (13% on average), TEE declined during the exacerbation, (8%), RMR increased slightly during the exacerbation, (3%), and CFA improved during the exacerbation phase (3%). For heterozygous subjects the findings were as follows : E_i declined during the exacerbation (20%), TEE showed a decrease during the exacerbation (11%), RMR and CFA remained static from 'well' to exacerbation phases.

3.6.3 Differences In The Effect Of An Acute Exacerbation Between Boys And Girls

In the girls E_i fell during the exacerbation phase (19%), TEE declined by 6% during the exacerbation. RMR fell by 2% during the exacerbation. Co-efficient of fat absorption improved during the exacerbation, by 4%. The boys gave the following results : E_i declined during a period of exacerbation (15%), TEE declined (13%) during the acute exacerbation, RMR showed a slight rise of 4% during the exacerbation, and CFA remained the same during this same period.

Table 6c : Energy Balance Data in Boys And Girls (Mean & SD; kJ / kg / day).

Subject	Energy Intake			Total Energy Expenditure			Resting Metabolic Rate			Co-efficient of Fat Absorption (CFA)		
	Well	AE	Difference[2]	Well	AE	Difference[2]	Well	AE	Difference[2]	Well	AE	Difference[2]
Girls[1]												
1	331	226	*105*	311	*	*	196	194	*2*	0.96	0.97	*-0.01*
2	164	253	*-89*	274	228	*46*	156	135	*21*	0.76	0.97	*-0.21*
3	195	194	*1*	237	270	*-33*	176	170	*6*	0.97	0.96	*-0.01*
4	436	471	*-35*	364	424	*-60*	224	224	*0*	0.98	*	*
5	251	261	*-10*	286	284	*2*	165	161	*4*	0.96	0.98	*-0.02*
6	382	264	*118*	250	242	*8*	157	174	*-17*	0.98	0.98	*0.00*
Mean	293	278	*53*	287	290	*6*	179	176	*3*	0.93	0.97	*-0.05*
SD	(108)	(98)	*(67)*	(46)	(78)	*(32)*	(27)	(30)	*(13)*	(0.08)	(0.00)	*(0.09)*
Boys[1]												
7	306	264	*42*	361	*	*	192	201	*-9*	0.86	0.92	*-0.06*
8	*	329	*	*	401	*	*	230	*	*	0.93	*
9	496	*	*	357	*	*	211	*	*	0.97	*	*
10	219	255	*-36*	*	196	*	138	122	*16*	0.98	0.97	*0.01*
11	333	*	*	333	*	*	169	*	*	0.98	*	*
12	431	406	*25*	294	274	*20*	218	221	*-3*	0.98	0.98	*0.00*
13	342	245	*97*	386	340	*46*	215	197	*18*	0.97	0.96	*-0.01*
14	330	267	*63*	*	307	*	165	205	*-40*	0.94	0.97	*-0.03*
15	409	353	*56*	*	*	*	206	219	*-13*	0.97	0.97	*0.00*
16	453	402	*51*	371	*	*	220	207	*13*	0.97	0.98	*-0.01*
Mean	369	315	*43*	350	304	*33*	193	200	*-3*	0.96	0.96	*-0.01*
SD	(86)	(66)	*(41)*	(33)	(76)	*(18)*	(29)	(34)	*(21)*	(0.04)	(0.02)	*(0.02)*

Footnotes:

[1] CF group divided into girls and boys subgroups in an attempt to examine whether the acute exacerbation may have possibly had a different effect on energy balance.

[2] Paired differences calculated as 'well' minus AE phases, given in italics.

In summary, through the examination of differences between the 3 sub-groups, it was apparent that the acute exacerbation did not seem to have any marked effect on the energy balance of children with CF, which was specific to their gender, genotype or treatment category, (i.e. 'standard' or 'regular' iv therapy). Throughout the 3 subgroups there appeared to be a very similar pattern of events occurring within the four energy balance variables measured, though a larger sample would be required to examine the subgroup differences adequately.

3.7.0 Comparison Of Energy Intake Data With Recommendations For CF And Other Data

The mean energy intakes (E_i) of the whole CF group, during 'well' and exacerbation phases, together with the mean data expressed for boys and girls separately, are shown in Table 7. Once again subject 4 was excluded from the descriptive analysis, as was subject 2 (Section 2.8.0). The size of the change in E_i is expressed as a percentage of the literature values and CF recommendations presented in Table 7.

Table 7: Comparison Of Energy Intake (kJ / kg / day) Data With Literature Values & CF Recommendations.

	CF Data		CF Recommendations[4]		DRV's	Healthy
	'Well'	AE	120% EAR	150% EAR	EAR for Energy[5]	Controls[6]
Boys[1]	369	315	331	413	275	251
Girls[2]	290	236	275	343	229	253
Group Mean[3]	344	289	303	378	252	252

Footnotes:

1. Boys n=9 'well'; n=8 AE.
2. Girls n=4 'well'; n=4 AE (calculations exclude subjects 2 & 4).
3. Group Mean n=13 'well'; n=12 AE (calculations exclude subjects 2 & 4).
4. 1.2 x EAR and 1.5 x EAR were the factors used to determine recommended energy intake in CF subjects. (Wootton *et al* 1991; McDonald 1996; Green *et al* 1995).
5. EAR for energy in healthy subjects of corresponding mean age and same gender. (DRV's for Food, Energy and Nutrients in the UK. DOH 1991).
6. Mean energy intakes of healthy controls of same mean age, studied using the same methods (n=20; boys n=9; girls n=11), taken from Reilly *et al* 1998.

3.7.1 Comparison With DRV's And CF Specific Recommendations

Overall E_i exceeded the minimum CF recommendation (Wootton *et al* 1991; MacDonald 1996; Green *et al* 1995) of 120% of EAR (estimated average requirement) for age and gender during the 'well' phase in the whole group, and in both boys, and girls. Thus the EAR for energy in healthy children was also exceeded (Department of Health 1991).

In contrast E_i fell below the minimum recommended value (120% of EAR), during the acute exacerbation for the CF group. However the mean group E_i, as well as the mean E_i for both boys and girls, failed to reach the maximum CF recommended target for energy intake of 150% of EAR, (Wootton *et al* 1991; MacDonald 1996), when 'well' or during the acute exacerbation. When subjects 2 and 4 were excluded from the analysis then the group mean E_i when 'well' was 344kJ/kg body weight (SD 90), 13% greater than the 120% minimum recommended E_i (DRV), and during the acute exacerbation phase, the group mean was 289kJ/kg body weight (SD 68), 5% less than the minimum EI goal children with CF.

3.7.2 Comparison with Other Relevant Literature Values

A final comparison of the data was made with healthy Glaswegian control children from a study carried out in Glasgow of 20 children, (9 boys, 11 girls, mean age 10.7 yrs (SD 2.9yrs) (Reilly *et al* 1998). The same method of household measures was employed in both studies to obtain E_i data. During the well phase the group mean, E_i exceeded the corresponding healthy control E_i, by 36% for the CF group, 47% for boys, and 15 % for girls, respectively.

In summary, self reported E_i data from these patients with CF exceeded those from a group of similar Glaswegian children studied at the same time with similar methods, as shown above. The CF group managed to consume greater than the minimum CF dietary recommendation of 120% EAR for E_i, when 'well' and relatively clinically stable. This must have contributed to the relatively good nutritional status of the group noted above. It was also noted that the exacerbation made it difficult to achieve an E_i of 120% of EAR.

3.8.0 Comparison Of Measured Resting Metabolic Rate With Literature Values

The mean measured RMR values both 'well' and during the acute exacerbation for the CF group, were shown in Table 8. All mean RMR values are expressed in kJ/kg body weight/day, and were compared with the Schofield-height weight (Scho-htwt) prediction equation (Schofield 1985). The data were also compared with the Schofield weight only (Scho-wt) prediction equation (Schofield 1985). However, as noted earlier the predicted RMR values derived from both Schofield equations proved very similar, so to ensure clarity of results and presentation only the Schofield-height weight equation is presented throughout (Kaplan *et al* 1995). As explained in section 2.8.0, subject 4 has been omitted from any calculation.

Table 8: Comparison Of Measured Resting Metabolic Rate (kJ / kg / day) Data With Literature Values.

	CF Data		Schofield Wgt Hgt Equation[4]		Predicted FEV_1	
	'Well'	AE	'Well'	AE	'Well'	AE
Boys[1]	193	200	164	164	73	66
Girls[2]	170	167	144	147	77	71
Group Mean[3]	184	187	154	156	75	69

Footnotes:

[1] CF Boys subgroup: n=9 'well'; n=8 AE.
[2] CF Girls subgroup: n=5 'well'; n=5 AE (subject 4 excluded).
[3] CF Group: n=14 'well'; n=13 AE.
[4] Schofield weight height prediction equation for resting metabolic rate (Schofield 1985).

Overall the mean measured RMR values in the CF group during both phases, in both the boys and girls, were greater than the predicted values of the Scho-htwt equation. Durie and Pencharz (1992) demonstrated that once FEV_1 dropped below about 75% of predicted values, then RMR increased quadratically, thus a link between declining lung function and RMR in subjects with CF was suggested (Figure 1b).

3.9.0 Comparison Of Total Energy Expenditure Data With Literature Values

A comparison of mean measured total energy expenditure (TEE), expressed as kJ/kg body weight per day, was made with literature values for healthy children taken from a recent review by Torun *et al* (1996), and the 20 healthy Glaswegian control children (described earlier), of similar age and gender to the CF group (Reilly *et al* 1998). Identical methods were used to carry out TEE measurements, by the Doubly Labelled Water (DLW) technique, in both groups of Glaswegian subjects.

Mean total energy expenditure was slightly higher than the mean value reported by Torun *et al* (1996), (Table 9) during both phases. Mean TEE also exceeded the mean values for healthy Glaswegian controls (Reilly *et al* 1998), for the 'well' phase, and matched the value (284kJ/kg/day) for the exacerbation phase. Although physical activity levels (PAL) were similar to the literature values from Torun *et al* (1996), they were lower than those of the healthy control group. It may be possible that there has been a 'compensatory' decline in

Table 9 : Comparison Of Total Energy Expenditure (kJ / kg / day) Data With Literature Values.

	CF Data				Literature Values			
	Total Energy Expenditure		Physical Activity Level		Healthy Control Values[3]		Torun et al Values[4]	
	'Well'	AE	'Well'	AE	TEE	PAL	TEE	PAL
Boys[1]	350	303	1.72	1.54	280	1.75	222	1.60
Girls[2]	271	256	1.58	1.60	287	1.80	232	1.58
Group Mean	314	282	1.65	1.57	284	1.78	227	1.59

Footnotes:

[1] Boys subgroup: n=6 'well'; n=5 AE.

[2] Girls subgroup: n=5 'well'; n=4 AE.

[3] Mean total energy expenditure and physical activity (PAL) calculated as TEE/RMR, taken from Reilly *et al* (1998)

[4] Estimated total energy expenditure and PAL of healthy children of similar age and gender (industrialised countries) taken from Toun *et al* (1996).

physical activity in the CF patients, and this seems more likely when the increased RMR of the patients relative to controls is considered.

3.9.1 Comparison Of Total Energy Expenditure With Other Relevant Literature Values

Finally, a comparison of the TEE data collected when subjects were 'well' was made with the findings of Tomezsko *et al* (1994), who studied 25 prepubertal children with CF using doubly labelled water to measure TEE, (13 females and 12 males, mean age 7.7yrs (SD 1.3)), with no evidence of acute pulmonary exacerbation at the time of the study, (or 6 weeks prior to the study). In the subjects with CF studied here, mean TEE ('well') was lower than that of Tomezsko *et al* (1994), (314kJ/kg/d compared to 351kJ/kg/d), and mean PAL was very similar to the group studied by Tomezsko *et al* (1.65 compared to 1.68).

In summary, average TEE was slightly higher in the group with CF when compared to the average literature values (Torun *et al* 1996), and the mean values for healthy Glaswegian control children, but levels of TEE were lower than those reported for a group of children with CF studied by Tomezsko *et al* (1994).

4.0 Discussion

The question of the effect of an acute respiratory exacerbation on energy balance in Cystic Fibrosis is an important one. Prior to the present study, there was widespread perception that acute exacerbations make a large contribution to undernutrition in CF (e.g. Reilly *et al* 1997). The overall aims of this study were to (i) describe energy balance in a group of children with CF who experienced a range of lung disease during a treated acute respiratory exacerbation, and (ii) to determine which component(s) of the energy balance equation underwent change during the acute respiratory exacerbation. Since the literature failed to provide answers to these questions, research must focus on such gaps in current knowledge. Thus, as stated earlier, much of the published research presented an incomplete picture of energy balance in CF. The study was intended to address the influence of a treated acute respiratory exacerbation on energy intake (Ei), resting metabolic rate (RMR), total energy expenditure (TEE), and fat absorption in 16 children and adolescents suffering from Cystic Fibrosis with a range of respiratory disease. A treated acute respiratory exacerbation was associated with a statistically, significant but moderate reduction in energy intake (Ei), but there was no evidence of marked negative energy balance. Changes in the other variables measured did not reach statistical significance, but the reduction in intake may have been offset by a modest decline in overall physical activity. No noticeable effect of the acute exacerbation on resting metabolic rate was apparent during the course of the study. From the energy balance and anthropometric data presented in this thesis, those children with CF studied not only presented with relatively good growth and nutritional status (Sections 3.2.0/3.3.0 Tables 2 & 3), but also managed to maintain this, by maintaining energy balance, (Section 3.4.0, Table 4) during the course of the treated acute exacerbation.

Previous studies carried out in hospitalised subjects with acute respiratory exacerbation or chest infections in adults, have suggested that a lowered Fi during the exacerbation may produce negative energy balance (Naon *et al* 1993; Steinkamp *et al* 1993; Shepherd *et al* 1988; Klipstein-Grobusch *et al* 1995). This, combined with the temporarily increased RMR during exacerbation suggested by Naon *et al* (1993) and Steinkamp *et al* (1993), has been thought to support the view that substantial negative energy balance can occur during such periods of acute clinical deterioration in CF (Reilly *et al* 1997; Fried *et al* 1991). It has therefore been suggested that the cumulative effect of these acute exacerbations (many patients with CF have 2 - 3 acute exacerbations per year) is to produce the undernutrition characteristic of the disease (Reilly *et al* 1997).

However, more recent evidence from this study, and notably from studies carried out by Stallings *et al* (1998), (published in the final preparation of this thesis), Vic *et al* (1997) and Shepherd *et al* (1988), which found that subjects with CF demonstrated improved weight gain and nutritional status following both home i.v. antibiotic therapy, and hospital i.v. antibiotic therapy, have suggested that the overall effect of an acute respiratory exacerbation on energy balance in children and adolescents with CF is smaller than expected. This small effect on energy balance seems to be independent of treatment location (hospital or home). It is apparent that in CF the relative contribution of each of the energy balance components to energy balance is uncertain (Reilly *et al* 1997). Thus, as in other chronic diseases characterised by malnutrition (e.g. HIV infection) research must focus not only on the periods of decline in clinical well-being, but also on the measurement of each component involved in overall energy balance, simultaneously in each subject. This comprehensive approach, adopted in the present study, is necessary for the understanding of exactly what triggers a state of negative energy balance in a

disease (Reilly *et al* 1997; MacAllan *et al* 1995). However, the present study does not support the view that acute exacerbations are critical periods of negative energy balance for children with CF, at least if the exacerbations are treated.

To date, this is the first study to measure all four components of the energy balance equation simultaneously in children and adolescents with CF, and to examine the potential effect of a treated acute respiratory exacerbation on each of those components, by comparing measurements made during a treated acute exacerbation with those made when the *same* child was 'well' and clinically stable. In order to begin identifying the effects of a treated acute exacerbation on energy balance in CF it was necessary to adopt a paired study design, i.e. to allow each child to be measured during a period of a treated acute respiratory exacerbation and again during a period of clinical stability, free of acute respiratory infection. Each child therefore acted as his/her own control. Unfortunately, as the study progressed it became apparent that certain of the energy balance variables could not be measured in all children due to the following reasons: (i) withdrawal from the study, (ii) poor measurement compliance. It was also noted that, even in those more compliant subjects, adherence to the faecal collection and urine sampling procedures was not perfect. This ultimately resulted in a loss of paired data. Therefore, as a direct result of the incompleteness of the paired data, an unpaired analysis (two sample comparison) was carried out. The latter suggested similar conclusions as that of the paired analysis (Sections 3.4.0/3.5.0, Tables 4 & 5), i.e. a treated acute exacerbation was associated with a trend towards a moderate reduction in E_i, a modest decline in physical activity, and no noticeable effect on RMR, or CFA.

It was also possible to examine the effects of a treated acute exacerbation in the following CF subgroups: (i) regular i.v. antibiotic therapy and standard i.v. antibiotic therapy, (ii) genotype, i.e. homozygous for ΔF508 and heterozygous for ΔF508, (iii) boys and girls; to examine whether or not the conclusions may alter any depending on the clinical characteristics of the subjects. There were no obvious differences in the effect of an exacerbation noted between these clinical groups, and even in those subjects on regular i.v. antibiotic therapy who suffered more severe lung disease, and more frequent respiratory exacerbations, no clear differences were apparent. However, due to the small sample sizes in these comparisons it was not possible to apply any statistical analyses, and there is, of course, a possibility that features such as severity of lung disease, genotype, and gender, might influence the effect of a treated acute respiratory exacerbation on energy balance. Further studies on larger samples would be necessary to test these possibilities. Initially the study aimed at recruiting 12-14 children, with paired measurements for each child. The reasoning behind this particular sample size was that this was deemed a sufficient number in which to identify a change of 25% in E_i, and 20% in TEE associated with the exacerbation (Reilly *et al* 1997). However once the exclusion criteria were applied, only eighteen subjects remained eligible for inclusion from the RHSC CF population, with only sixteen of those eighteen consenting to participation. The sample size may be perceived as small, but the volume and range of energy balance data gathered on each subject was greater than in any other comparable study of Cystic Fibrosis to date.

4.1.0 Energy Balance Variables

4.1.1 Energy Intake

As stated previously in section 2.4.1, it has long been recognised that the accuracy and precision of the currently used dietary assessment methodologies are open to much debate and criticism (Bingham 1987; Schoeller 1995; Black *et al* 1993; Sawaya *et al* 1996; Livingstone *et al* 1990; Livingstone *et al* 1992). The method of dietary assessment used in this study (Gibson 1990; MacDonald 1996) may be perceived by some as less accurate than the weighed dietary intake method. However recent evidence suggests that weighed dietary intakes are neither more accurate or precise than the estimation of energy intake using standard household measures, when compared with doubly labelled water measurements of total energy expenditure (Sawaya *et al* 1996). Weighed energy intake data need not exhibit any greater an advantage in terms of less error than energy intake records using estimated weights (Bingham 1987). Each method used to assess dietary intake is subject to its own sources of error within different population groups (Bingham 1987). Therefore, it was important to consider what degree of error could be tolerated within this study, and to what extent the method chosen would impose a burden on the subjects. For this study prospective food intake diaries (standard household measures), followed up with retrospective 'diet history', were used since they would provide a less labour-intensive method of data collection for the subjects. This was particularly important in light of the rigorous daily clinical care regimen of the child with CF, the intensive measurement requirements of the study protocol (discussed earlier), and the risk of non compliance with the protocol. It was felt that the requirement to weigh and record food

items would have jeopardised compliance with, and participation in the study, with only a limited gain in accuracy or precision.

Previously it was reported by Champagne *et al* (1996) that energy intake recorded by means of estimated dietary records have resulted in under-reporting of energy intake in biracial children, when verified by the doubly labelled water technique of measuring TEE. The suggestion of significant under-reporting of habitual energy intake is also supported by Bandini *et al* (1990) and Livingstone *et al* (1990), particularly in older children and adolescents. However, Maffeis *et al* (1994) found that non-obese children were reasonably accurate in the recording of food/energy intakes by means of 3 day weighed dietary record, and those values were similar to TEE estimates obtained. It must also be stated that no gross under-reporting of energy intake was obvious in this study: mean energy intakes fell below the 1.3 x measured RMR values used to define under-reporting (Goldberg *et al* 1991; Livingstone *et al* 1992; Black *et al* 1997), in only two subjects during the 'well' phase, and three subjects during the exacerbation.

The period of food intake recording was longer during the acute exacerbation than when 'well', due to the suggestion by previous authors of possible clinical instability during the exacerbation leading to increased variability in energy intake (Steinkamp *et al* 1993; Naon *et al* 1993), and because the greater the number of days recording, the more precise the data obtained (Bingham 1987). Thus it was proposed that in light of those points there may be a greater opportunity for any alterations in habitual energy intake to be observed by increasing the number of days of dietary assessment during the acute exacerbation, and the precision of the dietary assessment would be maximised. It is also of note that no other comparable study of CF has obtained this level of energy intake data. The available energy content of each subject's diet was calculated

using a computerised version of the standard food tables of McCance & Widdowson, Composition of Foods, 5th Edition (Comp-Eat 4.0 software package, Nutrition Systems, London). No changes in daily E_i over the course of the study were noted (repeated measures anova, calculated by Dr John J. Reilly), and this justified the use of a single summary measure of E_i during each study period.

In this study the mean energy intake of the group during a 'well' phase exceeded the minimum CF recommendation of 1.2 x estimated average requirement for energy (EAR). A modest fall in Ei (16% on average) was observed during a treated acute exacerbation. The value of 1.2 x EAR was a more realistic and practical value than the maximum CF recommendation of 1.5 x EAR, for this particular group of children (Section 3.7.0 Table 7). In general, the mean percentage of energy derived from fat in the diets of those subjects with CF studied was between 30 - 35% of dietary intake during 'well' and exacerbation phases. This shows that the diets of the children studied were not high in fat, and they were within the Dietary Reference Values suggested for a healthy daily fat intake, for the general UK population. The children within this study appear to have managed to consume an acceptable level of energy intake with a dietary fat intake of 30 - 35% during a 'well' phase. However, it has been suggested children with CF should be encouraged to consume nutritious high calorie foods with moderate to high fat contents in conjunction with appropriate pancreatic enzyme replacement therapy (PERT) (Ramsey *et al* 1992; Morrison *et al* 1994), and so the levels of fat intake observed might be of concern.

Overall, a treated acute respiratory exacerbation had a statistically significant but modest effect on the energy intake of the CF group studied (mean paired difference 47kJ/kg/d (SD 49);

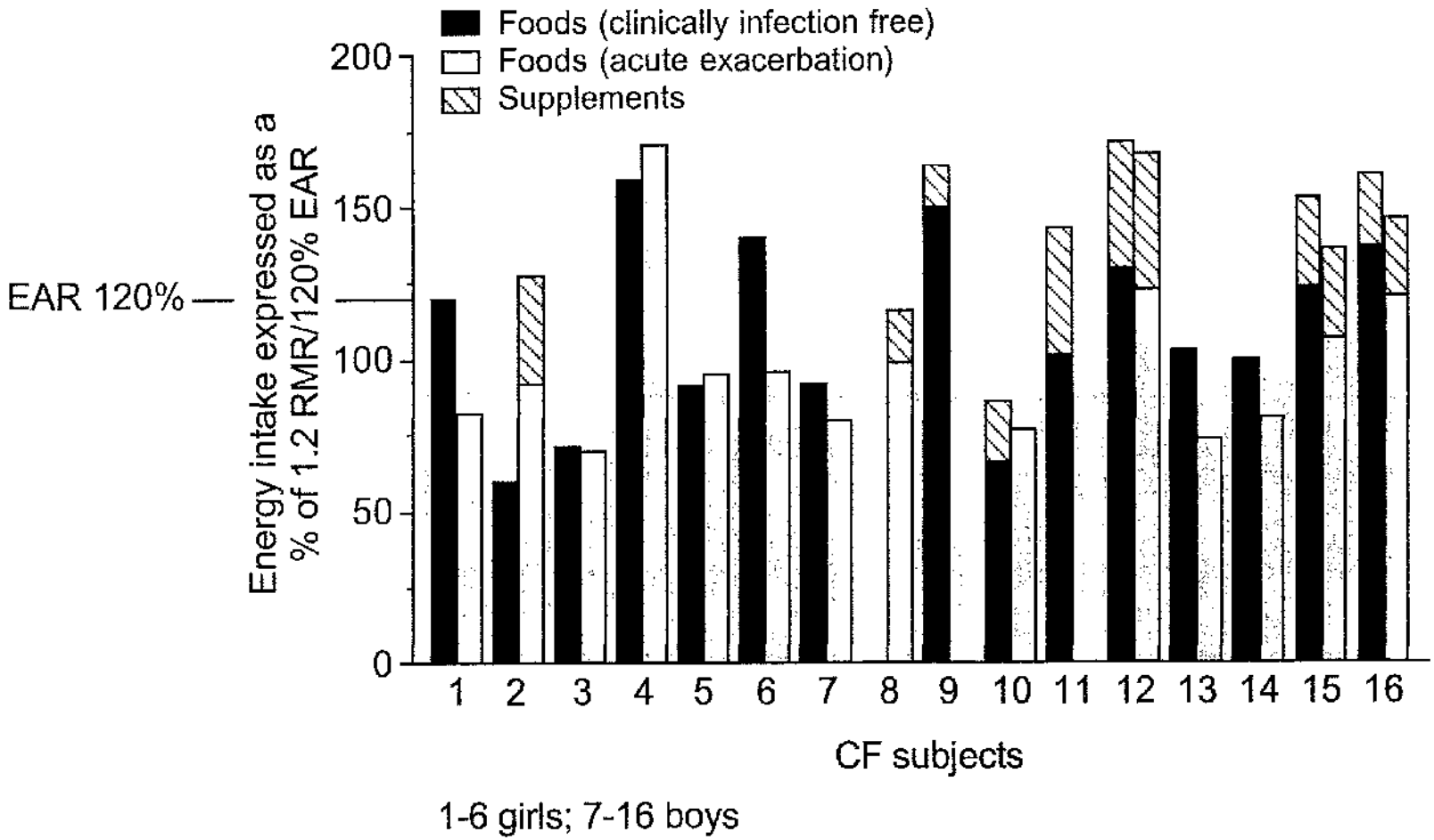


Figure 4a

Energy intakes of subjects with Cystic Fibrosis expressed as a percentage of the 120% of EAR for age, gender, and kJ/kg body weight

Figure 4b). There was an unexpected and sizeable increase in the energy intake of one subject (number two) during the acute respiratory exacerbation. In this subject there was known to be poor compliance with clinical treatment, and the improved energy intake during this phase was attributed to better compliance with the dietetic treatment regimen, and institution of enteral feeding in hospital which was not used at home, (treatment during the respiratory exacerbation took place within the hospital setting, which differed to the majority of other subjects in the study). This supports the exclusion of subject two from the energy intake analyses. Of those five subjects receiving nutritional support during an acute exacerbation, the percentage of mean energy intake provided by either sip feeds or enteral feeds was variable, but the minimum CF recommendation of 1.2 x EAR would not have been reached in the absence of the additional nutritional support (Figure 4a).

Poor compliance with the diet prescription in CF might therefore contribute to poor nutritional status in some patients at least. Subject numbers 2 & 7 (subject 7 was a suspected non-complier with pancreatic enzyme replacement therapy) illustrate that compliance with clinical treatment can be essential to the maintenance of energy balance, and thus good nutritional status. For these subjects it is possible that the effect of hospitalisation on compliance with dietetic regimen and PERT had a greater effect on energy balance that the acute respiratory exacerbation itself. This may also support the evidence of the large weight gain observed in subject number 7 during his hospitalisation for antibiotic therapy, and it is likely that the oral corticosteroid use increased while child number 7 was in hospital (explaining the large weight gain during the exacerbation), even though the oral corticosteroid prescription did not change during the acute exacerbation.

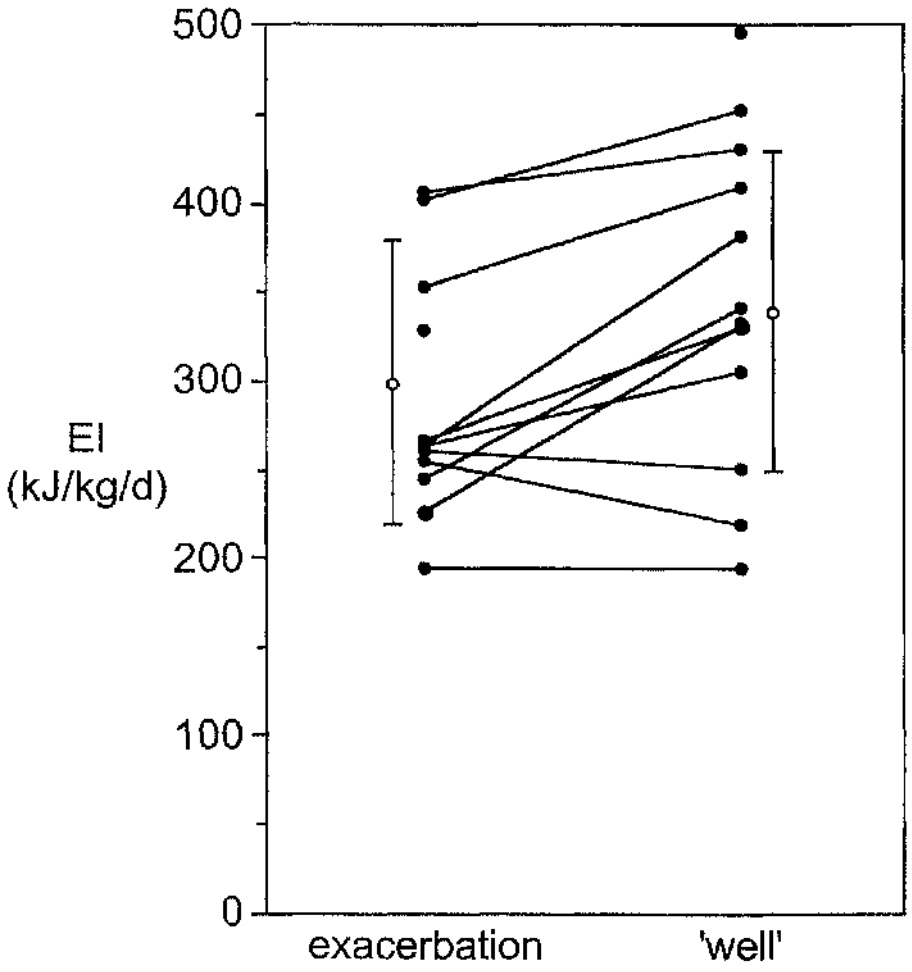


Figure 4b

Energy intake.

Footnote:
Mean paired difference between exacerbation and 'well' phases was statistically significant ($p = <0.05$)

4.1.2 Resting Metabolic Rate

An increased resting metabolic rate (RMR) in subjects with CF has been reported by many authors (Steinkamp *et al* 1993; Naon *et al* 1993; Reilly *et al* 1997). It has been hypothesised that an elevated RMR may contribute to a malnourished state in CF. One possible mechanism for this is the increased energy cost of basic cellular functions with the ΔF508 CF gene mutation (Feigal & Shapiro1979; O'Rawe *et al* 1990; O'Rawe *et al* 1992). It has also been suggested that an elevated RMR may be closely linked to a deteriorating pulmonary function (Section 1.5.2; Fried *et al* 1991; Vaisman *et al* 1987a). In the present study RMR was elevated relative to predicted values (Schofield Weight Height equation), and those increases were broadly consistent with the degree of pulmonary disease (Fried *et al* 1991; Table 8). Thus an elevated RMR was present in the subjects with CF, but there appears to be no noticeable influence of a treated acute respiratory exacerbation on RMR and this was consistent with one small previous study (Gamberara *et al* 1997). In the final preparation of this thesis, it was reported by Stallings *et al* (1998) that in children with CF who have mild to moderate lung disease, an acute respiratory exacerbation is not associated with an elevation in RMR. In contrast, both Naon *et al* (1993), and Steinkamp *et al* (1993) documented that certain subjects in their studies exhibited a markedly elevated RMR during an exacerbation. The marked instability of RMR in *certain* subjects during an acute exacerbation (Naon *et al* 1993; Steinkamp *et al* 1993) was, however, not found in the present study. The RMR measurement protocol was from the outset, designed to take account of any short-term variability which had been previously reported by both Naon *et al* (1993) and Steinkamp *et al* (1993), i.e. sharp elevations and declines in RMR during the course of an acute exacerbation, but these were not detected (Figure 4c). The conditions under which each individual child's RMR was measured

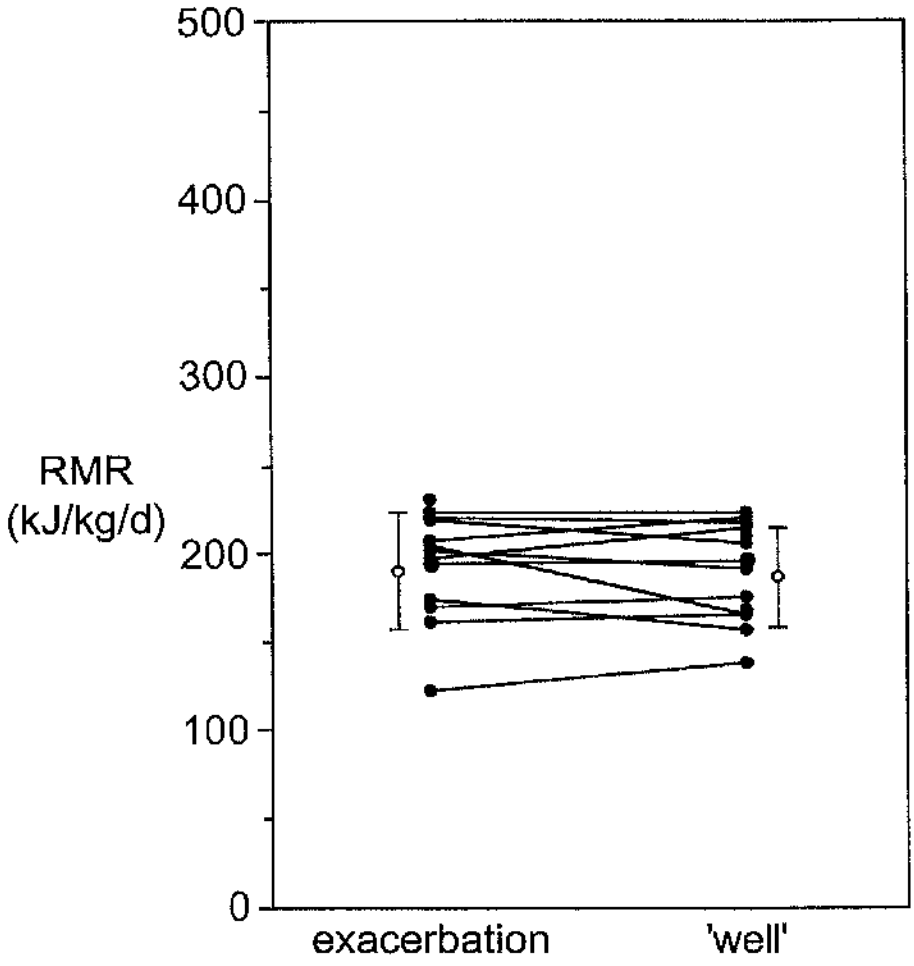


Figure 4c

Resting metabolic rate.

throughout the study period were standardised through the protocol explained in section 2.4.3 of this thesis, together with the standard procedures used by Reilly *et al* (1996b) for determining the expired air collection period. A minimum of ten to fifteen minutes 'steady-state' sampling was employed throughout the study to maintain data quality. Measurements of RMR were made on 4-5 occasions (alternate days) during the acute exacerbation, and on 2-3 occasions during the 'well' phase. RMR was calculated as the mean of the 4-5 measurements during an acute exacerbation, and the mean of the 2-3 measurements during the 'well' phase, in each subject studied. Repeated measures anova (calculated by Dr John. J. Reilly) on the RMR data revealed no evidence of systematic changes over time during the acute exacerbation, or 'well' phase, thus the use of a single summary measure of RMR was justified, for both measurement phases. Currently there is no obvious explanation for the apparent contradictions: observation of elevated RMR during acute exacerbation (in *certain* subjects) by some authors (Naon *et al* 1993; Steinkamp *et al* 1993), and no such evidence by the present study and more recently other authors (Gamberara *et al* 1997; Stallings *et al* 1998). It is possible to speculate that one reason for the difference is that Naon *et al* (1993) and Steinkamp *et al* (1993) studied older CF subjects with more severe lung disease. However, the results of the present study do not support this reasoning, as even those subjects with CF who were studied with more severe lung disease did not exhibit any obvious instability of RMR, such as 'spikes' in RMR, during a treated acute respiratory exacerbation. It is also recognised that RMR does not equate to TEE, and even if there was an elevation in RMR it does not necessarily imply either an increase in TEE, or an alteration in energy balance.

In our laboratory the co-efficient of variation of RMR measurements using our standard protocol has been documented at < 3% (Reilly *et al* 1996b; Ventham & Reilly 1998). The

standard protocol employed by our laboratory has shown that highly reproducible measurement of RMR is possible in prepubertal boys and girls (Ventham & Reilly 1998). Within the present study a between measurement co-efficient of variation (CV) of 6% was produced for repeated RMR measurements during the treated acute exacerbations. This value was identical to that reported by Figueroa-Colon *et al* (1996) of 6% for measurements of healthy pre-pubertal girls, and confirms the reasonably standardised nature of the RMR measurement protocol used throughout the study. However, the actual technical repeatability of such measurements during an acute exacerbation might be marginally better than is implied by a CV of 6%, due to the lack of clinical stability in the CF subjects studied here, which might increase the instability in RMR.

4.1.3 Total Energy Expenditure

It has become evident that the issue of whether or not TEE may be raised in childhood CF remains unanswered (Spicher *et al* 1991; Tomezsko *et al* 1994; Shepherd *et al* 1988). The subjects within this present study were noted to exhibit a slightly higher TEE when 'well' (mean paired difference 15kJ/kg/d (SD 30)). Overall TEE was reduced during an acute exacerbation (Figure 4d), but this trend did not reach statistical significance. This probably reflected the smaller sample size available for this particular energy balance variable. In order for the trend of reduced TEE during a treated acute respiratory exacerbation to have reached statistical significance, a much larger sample size of >35 paired comparisons would have been required (standardised difference of 0.40, power 0.80, at 5% level - calculated by Dr John J. Reilly). This would not have been practical within this group, and currently no other study of CF has reached this sample size using the doubly labelled water technique. The absence of any marked alterations in body weight/composition in the subjects suggests that the reduction in E_t

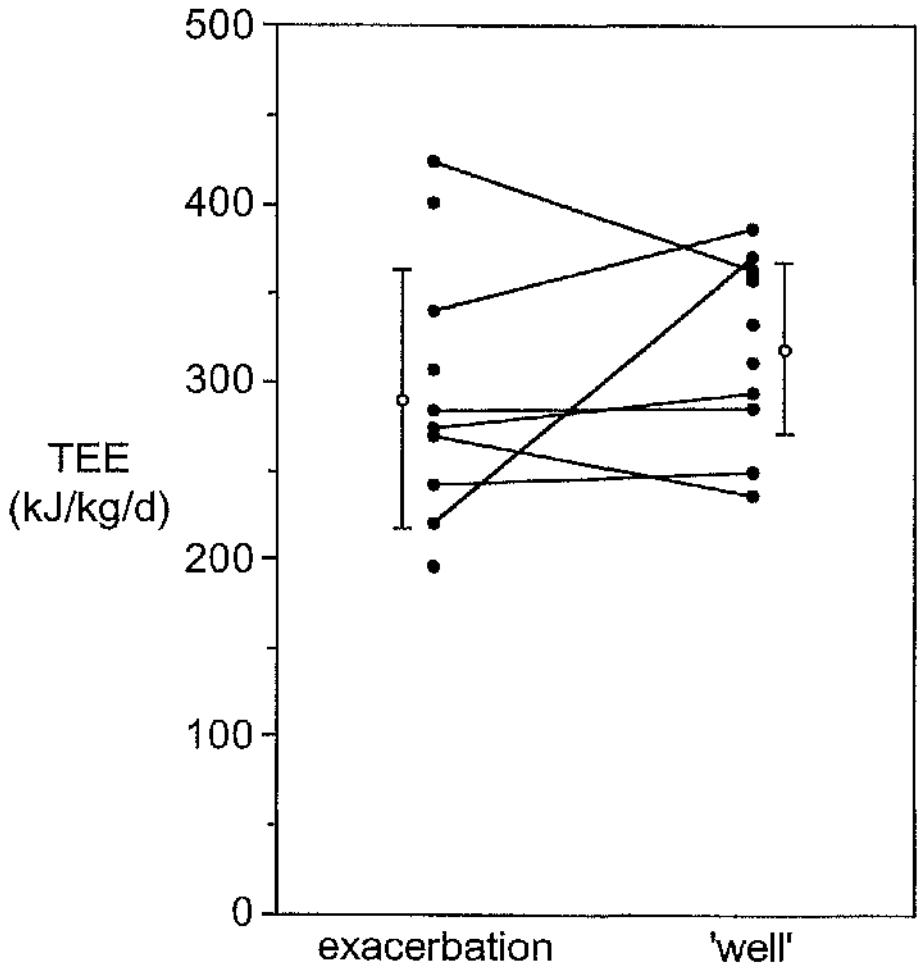


Figure 4d

Total energy expenditure.

must have been offset by a reduced TEE. However this particular variable could not be measured in all children with CF recruited due to poor compliance with the DLW protocol, and financial limitations on the study. Further research would be required in order to confirm any reduction in TEE during an exacerbation.

Overall, the levels of TEE observed in the subjects with CF studied 'well' were higher (314 kJ/kg/d) when compared to the UK Dietary Reference Values for children of the same age and gender (Department of Health 1991). Mean TEE was greater during both the acute exacerbation and 'well' phases, than the average requirements suggested by both Torun *et al* (1996), and those of the healthy Glaswegian controls respectively (Section 3.9.0 Table 9). However, it would appear unlikely that such levels of TEE observed in the subjects with CF within this study would create an energy imbalance. The latter is supported by the fact that the children had been nutritionally stable over the previous year (Section 3.1.0 Table 1). Data from the present study also suggested that the subjects might have compensated to a certain degree for a moderately reduced E_i during the acute respiratory exacerbation through a modest decline in energy requirements during the acute exacerbation. This process of 'compensation' by reduced activity has been demonstrated in other studies of acute illness/disease such as HIV infection (MacAllan *et al* 1995), and it has already been suggested that it is a feature of CF (Spicher *et al* 1991).

To date there have been few studies which have employed the doubly labelled water (DLW) method of measuring TEE in children with CF (Shepherd *et al* 1988; Tomezsko *et al* 1994). However, as previously stated in section 2.4.5 of this thesis, the DLW method of measuring TEE (Schoeller & Van Santen 1982; Schoeller *et al* 1986) is widely regarded as a highly

accurate means of determining the TEE of children (Jones *et al* 1987; Jones *et al* 1988; Murgatroyd *et al* 1993). This particular method has been reported to have an accuracy of 1-3%, and a precision of 2-8% in adults (Schoeller *et al* 1986; Johnson & Halliday 1993; IDECG 1990; Prentice 1995). The data from the present study could therefore be used in the future to aid the assessment of energy requirements in patients with CF.

4.1.4 Co-Efficient Of Fat Absorption

As stated earlier in this thesis, CF is a disease which is characterised by the malabsorption of fat, protein and carbohydrate due to pancreatic hypofunction, leading to a reduction in the availability of dietary energy (Murphy *et al* 1991). Despite regular, optimal doses of pancreatic enzyme supplements, the digestion of fat remains sub-optimal in these patients (Stead *et al* 1987; Reilly *et al* 1997). Furthermore, it has been previously reported that elevated stool lipid losses may contribute towards poor growth and nutritional status (Murphy *et al* 1991). The effect of an acute respiratory exacerbation on fat absorption was unknown, but had to be measured in view of its importance to energy balance in CF.

The present study did not support the view that fat absorption changed during the acute exacerbations: mean paired difference in CFA -0.03 (SD 0.06), which did not reach statistical significance (Section 3.4.4 Table 4). Subjects 2 & 7 showed distinct improvements in CFA during the acute respiratory exacerbation (Figure 4e). This may have been due to an incomplete 3 day faecal collection, or a disturbance of normal bowel habit (constipation). However, those subjects had also been suspected poor compliers with pancreatic replacement therapy (PERT), prior to commencing the study. Both subjects, in contrast to the other

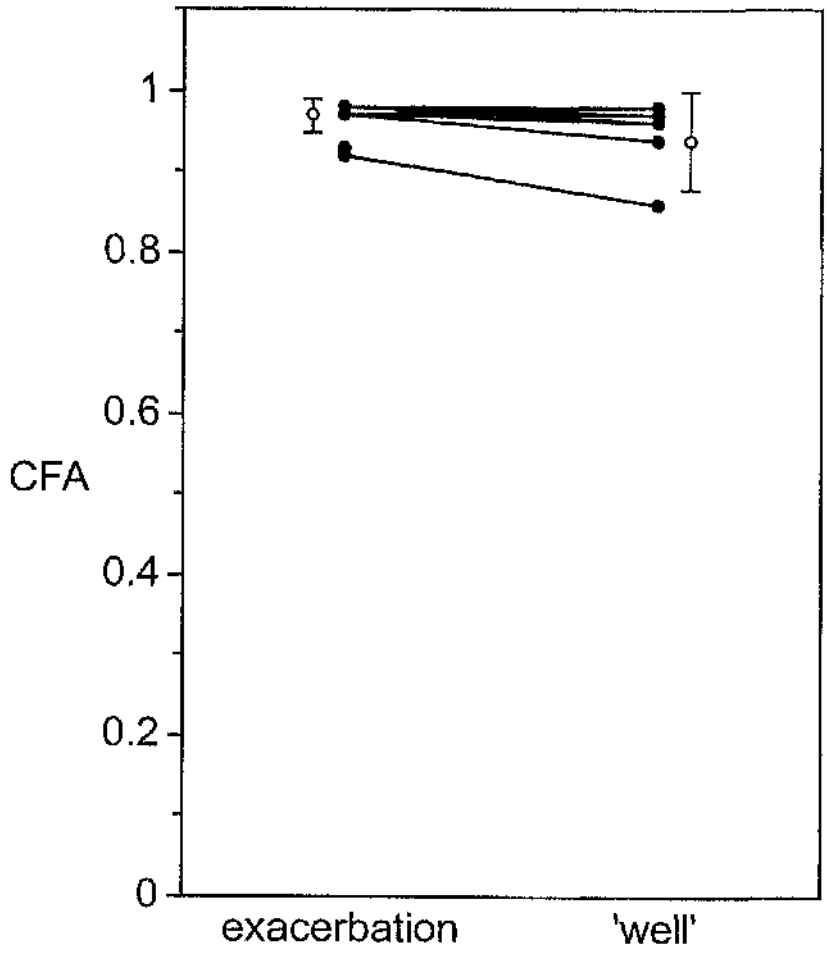


Figure 4e

Coefficient of fat absorption.

subjects in the study, had been hospitalised for a considerable part of their antibiotic treatment, and would have received a greater level of supervision in terms of their PERT during the acute exacerbation. The improvement observed in CFA in subjects 2 & 7 would therefore support the view that this change reflected improved compliance with therapy, and not the effect of acute exacerbation per se.

The levels of faecal fat lost were generally low in comparison to both subjects with CF studied when 'well' (Murphy *et al* 1991), or subjects with CF studied during an acute respiratory exacerbation (Parrett, unpublished data). When expressed as a % of dietary fat ingested, the level of fat/energy lost via the faecal route was not high in the subjects studied. Again, this may have been attributable to those same factors stated earlier in this section: incomplete faecal collection, constipation or improved PERT compliance. Compliance with these collections was not ideal, and the use of radio-opaque markers may have ensured complete collections (Morrison *et al* 1994). Most of the values of CFA exceeded 0.93 which is indicative of relatively normal fat absorption, which is not uncommon in CF (Ramsey *et al* 1992). Practically, faecal fat output was used as a proxy measure of faecal energy output within this study, as faecal fat output was considered a more straightforward measurement within this study protocol than measuring faecal energy output. Access to an automatic adiabatic bomb calorimeter (Gallenkamp) for the determination of faecal energy losses was limited.

4.2.0 Conclusion

The study set out to test whether a treated acute respiratory exacerbation represented a serious challenge to the maintenance of positive energy balance in children with CF, through the simultaneous measurement of all components of the energy balance equation in each child: E_i; RMR; TEE; E_l, together with changes in body weight and composition during a period of treated acute respiratory exacerbation and a period when the subjects were 'well'. The study was the first of its kind to characterise the energy balance of subjects with CF by measuring all components of energy balance in such detail. Treated acute respiratory exacerbations appear now to represent less of a challenge to energy balance in CF than was previously thought. From the data presented in this study it has become obvious that the main effect of an acute exacerbation in the subjects with CF studied is on the child's E_i during this period, (this may also have applied to a short period of time prior to them being recruited to the study). Overall this study has shown that during the period of the treated acute respiratory exacerbation, E_i falls significantly, changes in RMR and fat malabsorption/faecal fat losses were negligible, and TEE might decline. Changes in TEE did not reach statistical significance, but due to minimal changes in the body weight and composition (Sections 3.1.0/3.2.0 Tables 1 & 2), as well as the minimal changes observed in energy balance during the acute exacerbation, it seems likely that TEE fell. The findings are consistent with very recent studies which report that children with CF do not experience negative energy balance during acute respiratory exacerbations, this being irrespective of whether subjects are treated at home (Vic *et al* 1997), or within the hospital setting (Stallings *et al* 1998). In summary, the effects on energy balance of a treated acute respiratory exacerbation in CF are probably quite small, and it would appear that for certain subjects with CF, issues surrounding compliance with dietary treatment and PERT may be

regarded as far more relevant factors in the maintenance of adequate nutritional status, and positive energy balance.

In conclusion, this study did not support the view that a treated acute respiratory exacerbation results in marked negative energy balance in children with CF. It is however possible that the acute exacerbation might be more important to energy balance in older patients, with more severe lung disease, or to the rare occasions when patients present with unusually severe exacerbations (requiring intensive care).

4.2.1 The Future For CF Dietetic Management And Research

In this study we have demonstrated that the CF recommendation of 1.2 x EAR can be reached by the majority of those subjects studied when 'well' and clinically infection free, contrary to other studies (Tomezsko *et al* 1992; Kawchak *et al* 1996; Anthony *et al* 1998), and that the overall effect of a treated acute exacerbation on energy balance is smaller than was initially perceived. This study has also highlighted the importance of compliance with nutritional therapy (dietary prescription and pancreatic enzyme replacement therapy) to the maintenance of both nutritional status, and energy balance in some patients. However it remains important to recognise that, in terms of CF dietetic management, regular nutritional monitoring is still imperative before, during and after an acute respiratory exacerbation, and during puberty, as all children with CF and the acute exacerbations experienced are individual and unique.

Further studies are required to assess possible changes in energy balance, and nutritional status during treated acute respiratory exacerbations in larger numbers of subjects with CF (possibly a

multi-centre study to ensure an adequate sample size), with more severe respiratory disease and over significantly longer time-scales . Future research should also focus on when and why negative energy balance occurs in children with CF through the application of similar study techniques to the ones used in this study, perhaps using a longditudinal study design with the same child followed over longer periods than was possible here.

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Appendices

APPENDIX 5.1.

Pre-Recruitment Information

Below are samples of the letter mailed, and the information provided, to all prospective recruits, detailing the studies broad aims and requirements.

Date as postmark

«Title» «FAMILYNAME»
«Address1»
«Address2»
«City»
«PostalCode»

Dear «Title» «FAMILYNAME»

CYSTIC FIBROSIS STUDY

(Judith Ralston; Dr John J Reilly; Dr J Paton; Dr J Evans; Dr J Wilkinson)

The Department of Human Nutrition are presently carrying out a study investigating food intakes, and weight loss, during chest infections, in children suffering from Cystic Fibrosis.

Through these investigations it is hoped to:
(i) discover the main reason(s) for any fall in body weight during chest infections, and
(ii) how/when the childs weight begins to improve after such an infection.

The findings will help build a more complete picture as to how we can then achieve better nutritional management long-term, and also help improve body weight, nutrition, strength and growth in children with CF.

PLAN

We hope to collect information on 12 -14 CF patients **(during periods of chest infection only)**, aged 6 - 14 yrs, between November 1995, and March 1997.

Each child will be followed/measured for one month - through his/her hospital stay, home I.V. treatment (ie. **during chest infection)** , and 'recovery' from the chest infection at home.

This will allow us to identify any changes which may occur in;
(i) the amount of food eaten by the child
(ii) the amount of calories (energy), used up by the child, and
(iii) their body weight

***** THIS STUDY WILL NOT INTERFERE IN ANY WAY WITH YOUR CHILD'S TIME IN SCHOOL *****

HOW YOU CAN HELP

It would involve your child taking part in a series of simple measurements, which will be carried out by the investigator.
Two of those measurements will require the co-operation/participation of your child and yourself. For these, you will be given full guidance, and support.

Your sincerely

Judith M Ralston

IF YOU REQUIRE ANY FURTHER INFORMATION PLEASE CONTACT :

JUDITH RALSTON
DEPT. OF HUMAN NUTRITION
YORKHILL HOSPITAL
GLASGOW
0141 201 0712

APPENDIX 5.1a

Cystic Fibrosis Study Parent Information

The Department of Human Nutrition is presently carrying out a study investigating food intakes, and weight loss, during chest infections, in children suffering from Cystic Fibrosis. Through these investigations it is hoped to;

(I) discover the main reason(s) for this fall in body weight during chest infections, and
(ii) how/when the childs weight begins to improve after such an infection.

The findings will help build a more complete picture as to how we can then achieve better nutritional management long-term, and also help improve body weight, nutrition, strength, and growth in children with CF.

PLAN

To collect data/information on 12 - 14 CF patients aged 6 - 14 yrs, between November 1995, and March 1997.
Each child will be followed/measured for one month - through his/her hospital stay, and 'recovery' from the chest infection at home.
This will allow us to identify any changes which may occur in;
(I) the amount of food eaten by the child
(ii) the amount of energy/calories they use/burn up, and
(iii) their body weight

HOW?

It will involve your child taking part in a series of simple measurements, which will be carried out by the investigator; Part I - in hospital, and Part II - at home.
Two of those measurements will require the co-operation/participation of your child and yourself. These are;

- 3 day Diet diary
  - in hospital, and then at home every 2 days.

- 3 day stool collection
  - in hospital, and once again, for 3 days at home.

For both of these, there will be full guidance, and support.
In order to carry out Part 2, regular home visits, and telephone calls will be essential to gather all the information, and to ensure all collections & recordings go smoothly. This will be arranged after discussion with those concerned.
A 'Measurement Plan' / Schedule will be put together for you to refer to.

APPENDIX 5.2.

CF STUDY PROTOCOL

The redesigned study protocol to accommodate, (i) those subjects on 'regular' i.v. therapy, and, (ii) the large number of CF subjects treated by means of home i.v. therapy, is presented below.

Exacerbation Phase

Day 1	Days 2 - 4	Days 5 - 12	Day 13/14
Admission & Consent.	RMR days 2 - 4. Diet Record days 2 - 4.	Diet Record every 2nd day. RMR every 2nd day. Faecal Collection days 2 - 4. Skinfolds & Anthropometry.	RMR. Skinfolds & Anthropometry.

'Well' Phase

Day 1	Days 2 - 12	Day 13/14
RMR. Skinfolds & Anthropometry.	Diet Record for 3 days. Faecal Collection for 3 days.	RMR. Skinfolds & Anthropometry.

APPENDIX 5.3.

Food Record Diary Guide

Laid out below is the structure of the dietary record, with a guide for the standard household measurements, used throughout the study.

PLEASE USE THIS AS AN EXAMPLE ONLY

TIME	DESCRIPTION OF FOOD /DRINK	AMOUNT TAKEN	LEFT-OVER	WEIGHT
Breakfast 8.00am	Rice Krispies (Kelloggs)	31/2 Tablespoons		
	Semi-skimmed milk (Fresh n' Low)	1/4 pint		
	White bread, large loaf	1 medium slice		
	Butter (Anchor)	Spread thinly		
	Raspberry jam (Robertsons)	2 teaspoons		
	Orange Juice; unsweetened (Del Monte)	Small glass		
10.15am (snack)	Irn Bru	1 can		
	Kit Kat	4 fingers		
LUNCH 12.30	Heinz Tomato Soup	1 small bowl		
	Granary bread (medium loaf)	2 slices (medium cut)		
	Cheddar Cheese	3 thin slices		
	Tomato	4 slices		

	Butter (Anchor)	Spread thinly
	Ribena	Large glass
3.00pm SNACK	Cadburys Dairy Milk Chocolate	1/2 standard bar
	Ribena	Large glass
5.30pm DINNER	Chips (thick cut, fried in sunflower oil)	12 chips
	Carrots, boiled	2 Tablespoons
	Peas, boiled	2 Tablespoons
	Fish Fingers (Birds Eye) - grilled	2
	Mueller Fruit Corner Yoghurt (Strawberry)	1 pot (170g)
	Full cream milk (silver top)	medium glass
7.00pm SNACK	Digestive biscuit (McVities)	2
8.30pm SUPPER	Rice Krispies (Kelloggs)	3 tablespoons
	Full cream milk (silver top)	1/4 pint

APPENDIX 5.4.

Acceptable Household Measures Guidelines

Described below is a list of acceptable household measures that were used throughout the dietary record period, for each subject in the study.

A GUIDE TO "HANDY MEASURES"

Describe the portions in household measures.

Acceptable measurements include:

(i) **SPOONFULS:** Tablespoons, Dessertspoons, Teaspoons.
Please state whether flat/heaped/rounded, and the number.

eg. cereals, sugar, sauces, jams and powdered drinks such as Ovaltine, Horlicks.

(ii) **SLICES** Give indication of thickness of slice, and how many.

(iii) **LIQUIDS** Can be measured in teacups, or tablespoons, or measured in a measuring jug.

(iv) **BUTTER, MARGARINE & SPREADS:**

Indicate how thickly they are spread. State which brand used.

(v) **RICE & PASTA (COOKED):**

Record in tablespoons, or number of strands (for spaghetti).

(vi) **READY TO EAT MEALS:**

State the make or brand, and weight given on packet - then how much of the meal eaten.

APPENDIX 5.5.

Faecal Fats - Measurement Protocol

The following standard method was used to determine the lipid content of each batch of 2 - 3 day stool samples collected, for both exacerbation and clinically infection free phases, where appropriate.

Source: Van der Kamer, J.H., Huinink, H.T., and Wayers, A.A. (1949). J. Biol. Chem. 177; 347. (modified).

Reagents:

1 Stock potassium hydroxide KOH 60g/100ml in water.
2 Alcoholic potassium hydroxide 5g/100ml
- 25ml stock KOH to 300ml absolute alcohol (96%, Pharmacy Dept), including 1ml amyl alcohol.
3 Standard alcoholic sodium hydroxide (for titration) 0.1N.
- 4.00g NaOH dissolved in 100ml water and made up to 1 litre with absolute alcohol. Allow to stand overnight. Standardize against 0.1N oxalic acid (accurate) using phenolphthalein as indicator.
4 Oxalic acid 0.1N.
- 6.301g $(COOH)_2$. ^2H_2O to 1 litre with distilled water.
5 Thymol blue indicator.
- 0.1g thymol blue dissolved in a little water and made up to 100ml with absolute alcohol.
6 Hydrochloric acid, 33% v/v.
- 330ml conc. HCl to 1 litre with distilled water.
7 Petrolium ether 40° - 60° B.P.

Method:

1 The complete collection of faeces is made up to a known volume (500ml, 1litre, etc) with water and emulsified for 2 - 3 minutes in an electric homoginiser.
2 A 25ml aliquot of the fluid lying beneath the froth is transferred to a 250ml pyrex glass conical flask (with rubber stopper). Add 95ml alcoholic KOH. Boil for 20 minutes on a sand bath.
3 Cool the flask, add 30ml 33% (v/v) HCl, cool the flask, add 50ml petrolium ether (40° - 60°) and shake for a minute.
4 Allow contents of flask to settle and petrolium ether layer (top) to separate. Remove10ml petrolium ether extract to a small conical flask. Add 1ml absolute alcohol containing 0.4% (v/v) amyl alcohol. Titrate against 0.1N alcoholic sodium hydroxide with thymol blue as indicator.

Calculation:

Grams.

Faecal fat = (Titration x Normality x 5 x Total volume x 284) / 25 x 100
(fatty acids) (Volume NaOH Emulsion)

mmol.

Faecal fat = (Titration x Normality x 5 Total volume) / 25
(as stearic acid) (Volume NaOH Emulsion)
Express results per period of collection (e.g. 3 days), and per 24h.

APPENDIX 5.6.

Dose Preparation For Doubly Labelled Water

The standard protocols described below were followed during both dose preparation, and dose administration for each subject in the study.

a Current dose used is 0.06ml/kg $^{2}H_2O$ and 1.6ml/kg H_2 ^{18}O (10% enriched; normalised).
Doses made up on an individual basis per kg body weight for each child.

b Deliver approximate volume (nearest calculated ml) of each isotope into one medical flat via a micropore/acrodisc filter.

c Shake bottle well to mix the isotopes.

d Label bottle - subject name/code/date.

e Tighten bottle lid, then loosen slightly to break the seal - secure the lid with autoclave tape.

f Sterilse in the autoclave for a one hour cycle.

g Remove bottle, allow to cool - tighten bottle lid.

h Allow bottle to return to room temperature - shake bottle well to mix the dose. Add flavouring if required - shake bottle well to mix the dose. Pipette 2 x 1.5ml aliquots of dose into bijou pot - label bijou pots/subject code and freeze samples.

i Place dose/bottle in large resealable bag with 2 x flexible straws - label plastic bag with subject name/code/ & date.

j Weigh plastic bag/bottle/dose/ & straws - record the weight in the dose book.

k Provide subject with dose - see subject dosing sheet.

l Reweigh plastic bag/bottle/dose/ & straws - calculate mass of isotope consumed.

RECORD ALL SUBJECT DETAILS/CODES/WEIGHTS IN LAB DOSE BOOK

SUBJECT DOSING PROCEDURE

a Ensure collection of baseline urine sample.

b Make up dose - see dose preparation sheet.

c Weigh bottle/dose/straw/ & bag.

d Ask subject to ingest the whole dose using the straw provided.

e Abandon the procedure if any amount of the dose is spilled.

f Once all the dose is ingested place straw and bottle back in the bag and reseal. Reweigh to determine the dose consumed.

g **Urine samples**
Provide the subject with 3 x Universals with printed labels and their subject code in a large resealable bag.

Subject should collect 3 x 'spot' urine samples (at least 10ml, but do not overfill the Universal) at the following times:
i 24 hours/ one day after the dose.
ii 1 week after the dose.
iii 2 weeks after the dose.

It is essential that the subjects record the time of the sample collection on the Universal; that the urines have not been contaminated in any way by extraneous water during the collection; that the urines are stored appropriately (i.e. frozen if not being collected by the investigator).

Urine samples should not be collected first thing in the morning, but all other times are acceptable.

During DLW study subjects should be contacted to ensure the samples are being collected, stored, and labelled as necessary.

APPENDIX 5.7.

Total Energy Expenditure (TEE) Calculation Steps

Presented below is a detailed description of the calculation steps for the determination of each subjects TEE, during both exacerbation and clinically infection free phases.

STEP 1 Back extrapolate to determine ^{18}O and ^{2}H enrichments when time = 0
This step was equivalent to determining isotope enrichment if there had been instantaneous equilibration.

STEP 2 Calculate the point of interception using the following equation:

$$y = mx + c \qquad \text{for } ^{18}O \text{ \& } ^{2}H$$

STEP 3 Calculate the dilution spaces (Vo & Vh) using the following equation:

$$\text{Dilution Space} = (TA / a) \times (Ea - Et / Es - Ep)$$

T = amount of tap water used to dilute the dose (g).
A = dose given (g)
a = amount of dose used to make up the dilute dose (g)
Ea = enrichment of dilute dose (ppm)
Et = enrichment of tap water (ppm)
Ep = enrichment of pre-dose (ppm) - background enrichment
Es = enrichment of body water when t = 0 (ppm)

(IDECG 1990)

NB It was necessary in some cases to adjust the dilution spaces to a physiological value : 1.05. Deuterium ($^{2}H_2$) dilution space is always greater than ^{18}O. ^{18}O dilution space is approximately the same as body water, with $^{2}H_2$ being typically 4% greater than the subjects own body water (IDECG 1990). Coward, Ritz & Cole (1994) stated that the ratio of dilution spaces should be between 1.03 - 1.04, but noted that this is often not the case. Schoeller *et al* (1986) suggested that any difference in observed dilution space ratio is probably due to a technical error then the ratio must be adjusted to the more physiological value of 1.04 - 1.05. Coward *et al* (1994) did concede that specific groups have different ratios (possibly due to altered body composition), and that this average ratio of 1.04 - 1.05 may not apply in all cases. In this study of children with CF the observed ratios tended to be slightly higher than those quoted in the literature, and it is thought that the differences were technical (sample preparation for mass spectrometry), rather than biological in origin. Thus the dilution spaces were adjusted to make them more physiological (Coward *et al* 1994). The deuterium space ($^{2}H_2$) was adjusted to give the dilution space ratio 1.05 where necessary.

STEP 4 Calculate the rate constants (K_o & K_h)

$$K = (\ln c1 - \ln c2) / t$$

STEP 5 The mean daily Co_2 production (rCO_2 l/day), was calculated according to the equation (A6) proposed by Schoeller *et al* (1986) in his human validation study. This equation assumed isotopic fractionation of breath water only, and rather than employ TBW for the pool size the isotope dilution spaces were used. A further assumption made, was that ^{18}O and $^{2}H_2$ had been estimated as being 1% and 4% larger, respectively than total body water (TBW) itself (Johnson & Halliday 1993).

$$rCo_2 = (N / 2.078) \times (1.01K_o - 1.04 K_h) - 0.0246\ rGF \text{ moles/d}$$

where $rGF = 1.05\ N\ (K_o - K_h)$
where N = the average dilution space (moles)

NB : conversion to l/d from moles Co_2/d
($1g\ CO_2 = 510.2$ ml CO_2)

STEP 6 (i) Calculation of the correct energy equivalent per litre CO_2 produced, from dietary composition data.

Mean % Energy from protein
% Energy from fat
% Energy from Cho
(change each to a proportion of it's % energy value).

(ii) Calculate the energy equivalents for

Protein	5.56 kcal/l	x	
Fat	6.70kcal/l	x	above value.
Cho	5.00kcal/l	x	

ADD to get total kcal/l & convert to kJ/l.

(Schmidt-Nielsen 1980).

(iii) Calculate TEE (kJ/kg per day), by multiplying the answer obtained from Step 5 (rCO_2).

APPENDIX 5.8.

Body Composition Measurement - Bioelectrical Impedance Technique

Body composition, estimated by the tetrapolar whole body impedance (BIA) method, was carried out in each subject in accordance with the measurement techniques given below.

1 The subject was requested to lie flat, having removed the right shoe and sock.
2 Care was taken to ensure that no parts of the body were touching one another.
3 Self adhesive disposable electrodes were attatched to the right hand, and right foot.
4 The two main leads of the Bodystat were attached as follows:

Right hand:	Red lead:	Behind knuckle of middle finger.
	Black lead:	On wrist next to prominent ulnar bone.
Right foot:	Red lead :	Behind second toe, next to big toe.
	Black lead:	On ankle, level with, and between medial and lateral malleoli. (Large protruding bones on the side of the ankle).

5 The unit was switched to the ON position.
6 The enter key pressed. The screen then displayed the measurement on the LCD screen after 3 - 4 seconds.

The resistance figure (Ohms) displayed was subsequently entered into the Houtkooper et al equation (1992), which was used to predict FFM, FM and % body fat, in all subjects.

APPENDIX 5.9.

Body Composition Measurement - Skinfold Thickness Techniques

Described below are the standard skinfold measurement techniques employed throughout the study.

Triceps Skinfold

The reference point for this measurement is the midpoint of the distance between the tip of the acromion process (scapulae), to the olecranon process of the ulna. This was measured with the arm bent at right-angles (90°), and then the reference point marked. The skinfold was then measured 1cm above the marked level, with arms hanging loosely by the sides, on the posterior aspect of the arm (Weiner & Lourie 1969).

Biceps Skinfold

This skinfold was measured 1 cm above the marked line for the triceps measurement, at the front of the arm above the centre of the antecubital fossa (Weiner & Lourie 1969).

Subscapular Skinfold

The reference measurement was made 1 inch, just below the angle of the scapula, diagonally, and approximately 45° to the subjects spine, along the natural cleavage lines of the skin (Lohman *et al* 1988).

Suprailliac Skinfold

The point of measurement for this skinfold occured 2cm directly above the illiac crest (Lohman *et al* 1988).

APPENDIX 5.10.

Clinical Assessement of Symptoms During Acute Exacerbation

Presented below are the radiological results for each subject, corresponding to the time of acute exacerbation, (to be read in conjunction with Section3.2.0 Table 2).

Radiology Comments

Subject Name	Comments
1	"...appearances consistent with intercurrent infection..."
2	"...the appearances suggest intercurrent infection..."
3	" appearances in right upper lobe are worse than on previous film ,and they suggest superimposed infection "
4	No X-ray. Sputum sample showed Pseudomonas. Admitted for antibiotic therapy.
5	" increased mottled opacification & bronchial wall thickening of both bases, consistent with exacerbation."
6	" the lungs show no focal active infection"

7	" deterioration in appearance " " patchy changes are also evident in left lower lobe "
8	" no radiological features of active infection" " chronic infection in sinuses"
10	No radiology details available.
12	" more confluent shadowing in right upper zone compared with 16.6.96 film. Appearances suggest changes in left upper lobe "
13	" Allowing for technical differences, there is no radiological change and no features of active infection "
14	" lung fields show peribronchial thickening " " no evidence of consolidative change "
15	x-ray : 25.2.97 " no interval deterioration, & no evidence of active infection "
16	" no significant change in typical changes of CF. No definite areas of focal consolidation demonstrated"